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CELLULAR AND MOLECULAR PATHOPHYSIOLOGY OF INFLAMMATORY SKIN DISEASES

001
Linking circulating and tissue eosinophils through spatial transcriptomics in drug reaction with eosinophilia and systemic symptoms

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DRESS is a severe drug-induced hypersensitivity reaction with marked eosinophilia, yet the role of eosinophils (EOS) in skin remains debated. Whether circulating EOS transcriptional programmes reflect their tissue states is unknown. We aimed to integrate spatial and bulk transcriptomic data to match circulating and Skin EOS and characterize the local skin microenvironment. Paraffin-embedded skin biopsy was analysed using the Xenium *In Situ* Gene Expression platform (10X Genomics). Patient-matched bulk RNA sequencing was performed on purified circulating EOS. Integrative analyses used correlation, and pathway enrichment to assess concordance between circulating and tissue-associated EOS programmes. Bulk RNA-seq analysis demonstrates that EOS in DRESS exhibit a profoundly reprogrammed and high activated transcriptional state, dominated by IL-3/IL-5–associated signatures. Bulk–spatial integration revealed an overall positive correlation between global circulating and tissue EOS transcriptional profiles (Pearson's $r > 0.4$, P-value < 0.001), supporting partial matching across compartments. Tissue EOS were characterized by higher expression of *CLC, IL3RA, CCR3*, and *GATA2*, while *SIGLEC8* and *RNASE2* were relatively decreased, highlighting context-dependent modulation of key EOS markers within the skin microenvironment. Spatial analyses identified distinct EOS subpopulations with a *CCR3*^{high}/*IL5RA*^{low} and a *CCR3*^{low}/*IL5RA*^{high} cluster. The *IL5RA*^{high} subset exhibited increased expression of activation and cytokine-related genes (*CD69, IL4, IL1RL1, CCL2/5/17*), suggesting an active role within the tissue microenvironment. These findings support a heterogeneous and microenvironment-driven organization of EOS in DRESS skin.

002
HNRNPU is enriched in basal inflammatory epidermis and is required for epidermal development

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HNRNPU is a multifunctional nuclear protein involved in chromatin organization and transcriptional regulation, yet its role in epidermal homeostasis and inflammatory skin disease remains poorly understood. Here, HNRNPU expression was investigated using healthy and diseased human skin, 3D epidermal equivalents, public integrated spatial and single-cell

transcriptomic datasets, and epidermal-specific knockout mice.
HNRNPU was associated with basal and early epidermal states and declined during differentiation. This trend was confirmed in an independent 3D human epidermal equivalent model, where bulk RNA-seq showed reduced HNRNPU transcript levels upon differentiation. In normal human skin, HNRNPU protein showed strong nuclear staining predominantly in basal keratinocytes with weaker suprabasal expression. In atopic dermatitis lesional skin, immunofluorescence revealed increased nuclear HNRNPU burden along the basal epidermal interface compared with healthy skin. Spatial and pooled donor-level single-cell transcriptomic analyses across independent cohorts further supported basal-predominant enrichment of HNRNPU in diseased epidermis. In psoriasis, HNRNPU enrichment was similarly supported by increased progenitor-like basal keratinocytes features. To assess in vivo relevance, epidermal-specific Hnrnpu knockout mice were examined during skin development. Loss of epidermal Hnrnpu caused defective barrier formation, increased transepidermal water loss, and altered epidermal differentiation-marker expression, indicating that Hnrnpu is required for proper epidermal development and barrier integrity. Collectively, these findings identify HNRNPU as a basal-state-associated epidermal regulator enriched in inflammatory human epidermis and required for normal epidermal development, supporting a role for HNRNPU in basal keratinocyte remodeling, early differentiation control, and epidermal dysfunction in inflammatory skin disease.

003

IL-1R2 Acts as a Keratinocyte-Associated Regulator of Cutaneous Inflammation in Atopic Dermatitis

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Atopic dermatitis (AD) is a chronic relapsing inflammatory skin disease shaped by the interplay between epidermal barrier dysfunction and dysregulated immune activation. Beyond the well-recognised type 2 immune response, emerging evidence suggests that interleukin-1 (IL-1)-related pathways may contribute to the inflammatory landscape of AD. IL-1 receptor type 2 (IL-1R2) is a non-signalling receptor that limits IL-1 activity by binding IL-1α and IL-1β, thereby restricting their access to the signalling receptor IL-1R1.
In this study, we investigated the expression pattern and functional relevance of IL-1R2 in AD-associated epidermal inflammation, with a focus on keratinocyte-mediated immune regulation. Single-cell transcriptomic analysis of AD lesional skin revealed increased IL1R2 expression, predominantly within keratinocyte populations. By integrating human skin specimens, Il1r2-deficient mouse models, bone-marrow chimera experiments, reconstructed three-dimensional human skin equivalents and n/TERT keratinocyte cultures, we found that IL-1R2 constrains IL-1β-driven inflammatory activation and limits neutrophil-rich cutaneous inflammation.
In reconstructed 3D skin equivalents, stimulation with the type 2 cytokines IL-4 and IL-13 markedly induced IL-1R2 expression, suggesting that IL-1R2 may form part of a counter-regulatory programme within a Th2-skewed epidermal environment. Consistently, loss of Il1r2 aggravated MC903-induced AD-like dermatitis in mice, accompanied by enhanced inflammatory infiltration, particularly neutrophils. Complementary chimera experiments further supported a protective role for IL-1R2-expressing radioresistant skin-resident cells in restraining cytokine-driven dermatitis. Conversely, enforced IL-1R2 expression in keratinocytes dampened IL-1β-induced pro-inflammatory responses.
Together, these findings identify IL-1R2 as an epidermal negative regulator of IL-1β-mediated inflammation and highlight its contribution to maintaining cutaneous immune balance in AD.

004

Novel human organotypic model of hidradenitis suppurativa tunnel recapitulates disease wound healing phenotype

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Hidradenitis suppurativa (HS) is a devastating chronic skin condition affecting 1-4% of the US population, characterized by intradermal sinus tracts (tunnels), with poor response to current medical therapies and no representative animal or in vitro models. In this study, we engineered a novel 3D organotypic HS tunnel model with primary cells isolated from excised HS patient tunnels and matched control skin, recapitulating the wound healing phenotypes observed in vivo. We further characterized the cell-cell and cell-matrix interactions intrinsic to HS using comparative gene ontology (GO) and Ingenuity pathway analysis (IPA) of bulk RNAseq and spatial transcriptomics, as well as validation with immunohistochemistry (IHC), and qPCR, in HS tunnels, control skin, and corresponding 3D organotypics. RNAseq of HS tunnel tissue and age/sex/site matched skin controls showed enrichment in acute wound healing processes in tunnel tissue, confirmed by “wound activated” keratin 17 uniquely expressed in tunnel and not peri-lesional epidermis, by IHC and qPCR. HS tunnel fibroblasts also demonstrated a more contractile phenotype in 3D culture along with gene dysregulation associated with epithelial mesenchymal transition, which aligns with the clinical HS fibrotic scarring phenotype and tunnel histology. Bulk RNAseq of the 3D organotypic with HS fibroblasts and matched control fibroblasts was also conducted and compared to the tissue data. In conclusion, we developed a novel 3D *in vitro* human model of HS tunnels, which is the first lab model for this devastating disease. Using translational omics approaches and 2D/3D in vitro vs. in vivo validation, we demonstrate a wound healing phenotype with complex inflammation in HS tunnels, with the fibroblast phenotype recapitulated in a 3D tunnel organotypic model. Further development of this 3D tunnel model will pave the way for more targeted HS treatments.

005

Circulating MAIT cells correlate with disease severity and undergo a tissue type 17-to-cytotoxic type 1 switch linked to dysbiosis in Hidradenitis Suppurativa

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Hidradenitis Suppurativa (HS) is a chronic inflammatory skin disease whose pathophysiology remains poorly understood and is thought to involve a dysregulated immune response against skin microbiota. As Mucosal-Associated Invariant T (MAIT) cells regulate microbial flora, we hypothesized that their dysfunction contributes to HS pathogenesis.
We assessed MAIT cell frequency, phenotype, and function by spectral cytometry (n=35 HS patients, untreated or anti-TNFα-

treated; n=22 healthy donors, HD) and single-cell RNA sequencing (n=5 HS patients and HD), in peripheral blood and skin, with paired microbiome analysis on serial skin biopsies.
Circulating MAIT cells were decreased in HS vs HD, inversely correlated with disease severity and flare frequency, and were restored in anti-TNFα responders. In lesional skin, CD8+ MAIT progressively accumulated, whereas HD skin contained predominantly CD4+ resident MAIT cells. scRNA-seq revealed a phenotypic switch: skin-infiltrating MAIT cells displayed a chronic activation signature (LAG3, CTLA4, CD96) and shifted from a homeostatic type 17 tissue repair profile (HD) toward a type 1, cytotoxic profile (CD8A, GZMA, GZMB, PRF1, LYST, NKG7). Restimulation assays (PMA/ionomycin, 5-OP-RU: *MAIT ligand*) on circulating MAIT cells confirmed this switch, with impaired reactivation (lower CD69, CD25), defective IL-17A production, and increased granzyme B and IFN-g expression. HS lesion microbiome showed enrichment in the riboflavin pathway (precursor of MAIT agonists). Cobalamin and purine biosynthesis pathways correlated in opposite directions with MAIT activation markers (CD161, CD69, CD218), supporting a metabolic link between dysbiosis and MAIT phenotype.
Together, these data identify MAIT cells as integrators of microbial cues and disease activity in HS, undergoing a tissue type 17–to–cytotoxic type 1 switch coupled to dysbiosis affecting MAIT-relevant metabolic pathways. Circulating MAIT cell frequency may serve as a biomarker of severity and anti-TNFα response, while the MR1-MAIT axis emerges as a candidate therapeutic target to restore microbiota-immune homeostasis in HS.

006
A Distinct Circulating T-cell Signature in Dupilumab Induced Lymphoid Reactions
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In a small subset of atopic dermatitis patients, dupilumab treatment can cause a reversible and local adverse reaction, clinically mimicking cutaneous T-cell lymphomas (CTCL). These are found to represent a distinct histopathological entity, called Lymphoid Reactions (LR). In this study we aimed to characterize circulating (skin homing) T-cells of patients who developed LR. Using spectral flow cytometry, PBMCs of patients who developed a LR during dupilumab treatment (n=6) were compared to patients with adequate response to dupilumab (DR) (n=6), CTCL patients (n=6) and healthy controls (HC) (n=8). Unbiased clustering resulted in 20 T-cell clusters, of which 7 expressed skin-homing markers (CLA+ CCR4+). Notably, a population of CD4+CD45RO+CD26+CLA+CCR4+CD30+CD103+ T cells, “recent skin emigrants”, was significantly expanded in LR patients. This population increased in 5/6 LR patients just before LR diagnosis, and exhibited elevated expression of IL-13 and IL-22. CD45RO+CD26dimCLA+CCR4+CD30dimCD103- skin homing CD4+ Th memory cells were increased in both LR and CTCL patients, with the highest IL-4 production in LR patients. Skin homing FOXP3+ regulatory T-cells (Tregs) were also significantly expanded in both LR and CTCL patients, and in a subset of DR patients, confirming enhanced counter-regulation in the skin homing T-cell compartment during inflammatory skin diseases. A similar trend was observed for CD103+ “recent skin emigrant” Tregs, which were most abundant in LR patients. The expression of chronic activation markers PD-1, CTLA-4 and LAG-3, as well as the proportion of proliferating cells within skin homing T-cells were comparably increased in LR and DR patients. In conclusion, LR is characterized by an expansion of activated, cytokine-producing skin-emigrant CD4+ T cells and Tregs. Despite its clinical resemblance to CTCL, LR lacks features of sustained malignant-like activation and instead reflects a transient, dynamic immune redistribution.

007
IL-26+ Tissue-Resident T Cells Represent a Shared Precursor Pool for Th17 and Th2 Immunity
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IL-26 is a cytokine with antimicrobial activity derived from Th17 cells. In psoriasis, IL-26+ T cells reside in uninvolved skin as tissue-resident memory T cells (TRMs). These IL-26+ T cells can differentiate into IL-17A+ pathogenic Th17 cells via IL-26 receptor (IL-26R)-mediated induction of TGF-β. Interestingly, we found that IL-26+ T cells are also present as TRMs in the uninvolved skin of patients with atopic dermatitis (AD). In AD lesions, IL-26+ T cells are associated with the expression of the Th2 cytokine IL-13 rather than the Th17 cytokine IL-17, as demonstrated by single-cell RNA sequencing (scRNA-seq) and spatial transcriptomics analyses indicating cellular proximity. Pseudotime analysis of scRNA-seq data revealed that IL-26+ T cells differentiate into IL-26+IL-13+ and IL-13+ Th2 cells, rather than Th17 cells. This differentiation occurs at the clonal level, as shown by single-cell TCR sequencing (scTCR-seq). In vitro, culturing naïve T cells under Th2-polarising conditions induced IL-26+ T cells, which subsequently differentiated into IL-13-producing Th2 cells. Collectively, these findings suggest that IL-26+ T cells serve as precursors for both Th17 and Th2 lineages, with their differentiation trajectory determined by the local microenvironment. It remains to be determined whether IL-26+ T cells are entirely plastic or pre-committed to a Th2 or Th17 lineage. While the role of IL-26 in promoting Th17 differentiation via IL-26R-mediated induction of TGF-β has been established, its role in Th2 differentiation remains under investigation.

008
The skin-enriched lncRNA TMEM99/KRT10-AS1 promotes epidermal differentiation and is downregulated in atopic dermatitis
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Proper keratinocyte differentiation is essential for a functional skin barrier. Epidermal barrier dysfunction is a hallmark of atopic dermatitis (AD), the most common chronic inflammatory skin disease. Long non-coding RNAs (lncRNAs) are emerging as important regulators of epidermal homeostasis, yet their functional roles in epidermal barrier formation and AD remain poorly understood. Here, we investigated the skin-enriched lncRNA TMEM99 (KRT10-AS1), as a potential regulator of epidermal differentiation.
Transcriptomic profiling of 3D epidermal equivalents across different development stages revealed increased expression of TMEM99 during stratification and barrier formation. RNAscope® single molecule *in situ* hybridization analysis demonstrated that TMEM99 is virtually absent in the submerged growth phase, but it becomes highly enriched in the uppermost viable layers

of fully matured 3D models. Consistent with these *in vitro* findings, TMEM99 localized specifically to the stratum granulosum of healthy human epidermis.
Functionally, siRNA-mediated knockdown of TMEM99 in 3D epidermal equivalents led to reduced expression both of its neighboring gene keratin 10 (*KRT10)* and the late differentiation marker filaggrin. Furthermore, single-molecule in situ hybridization and qRT-PCR revealed that TMEM99 is downregulated in lesional AD epidermis compared to non-lesional or healthy epidermis.
Taken together, these findings identify *TMEM99* as a previously unrecognized non-coding regulator of keratinocyte differentiation and barrier function. Its downregulation in AD suggests it may play a pathogenic role in the barrier dysfunction characteristic of the disease.

009

Fli1 Haploinsufficiency Extracts Systemic Sclerosis-Relevant Transcriptomic Signatures from a Bleomycin-Induced Mouse Model

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Reduced expression of the transcription factor FLI1 is implicated in the pathogenesis and severity of systemic sclerosis (SSc), but standard bleomycin (BLM)-induced fibrosis models also contain broad injury-associated responses. To identify transcriptomic components more selectively linked to an SSc-like disease-prone state, we compared BLM-treated *Fli1*$^{+/-}$ mice with BLM-treated wild-type mice by bulk RNA-seq analysis of skin and lung. This design was intended to subtract the common BLM-driven injury/fibrosis program and highlight pathways biased by *Fli1* haploinsufficiency. Differentially expressed genes showed marked organ specificity, with 463 genes in the skin and 80 in the lung, and minimal overlap. In skin, *Fli1* haploinsufficiency enhanced a COL8A1-high activated fibrogenic program, including increased *Col8a1*, *Cthrc1*, *Acta2*, *Timp1,* and *Snai1*, consistent with contractile and matrix-remodelling fibroblast activation. The skin signature also suggested vascular perturbation, with reduced *Apold1* and increased *Vcam1* and *Angptl4*, and a senescence-like remodelling state marked by strong *Cdkn2a* (p16) induction and a trend toward increased *Serpine1* (PAI-1). In contrast, the lung showed coordinated downregulation of canonical B-cell maturation genes, including *Cd19*, *Cd79a/b*, *Pax5*, *Pou2af1*, *Fcer2a*, *Ighd,* and *Ms4a1*, together with increased genes associated with vascular wall or perivascular matrix remodelling, such as *Myh11*, *Fbln2*, *Timp3,* and *Sox7*. TLS-associated chemokines and ligands were not clearly enhanced, and changes in *Serpine1* expression and senescence-related signatures were not prominent in the lung. These findings suggest that *Fli1* haploinsufficiency does not merely amplify a uniform fibrotic program but rather biases BLM-induced tissue repair toward organ-specific, SSc-relevant modules. Comparative profiling of BLM-treated *Fli1*$^{+/-}$ and wild-type mice may therefore provide an exploratory screening platform for prioritizing SSc-relevant genes, pathways, and potential therapeutic targets.

010

New insights into the aetiology of keratin-associated inflammatory skin disease: Thymic keratin 76 shapes skin-specific central tolerance

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Inflammatory skin conditions associated with keratin mutations — including pachyonychia congenita and palmoplantar keratodermas — are widely attributed to disrupted epidermal barrier function and secondary microbial invasion. Whether a primary autoimmune mechanism contributes to their pathology has remained unexplored. We investigated whether loss of keratin 76 (KRT76), expressed in both skin and thymic epithelium, disrupts central immune tolerance to skin-specific antigens, with broader implications for the management of keratin-associated skin disease.
We employed single-cell RNA sequencing, thymic grafting in vivo models, immunofluorescence, flow cytometry and serum autoantibody profiling to characterise medullary thymic epithelial cell (mTEC) biology and T cell selection in the absence of Krt76.
Transfer of Krt76-/- thymic lobes was sufficient to drive skin inflammation, CD45+ immune cell infiltration and tissue-specific autoantibody reactivity directed exclusively against skin and oral mucosa, in the complete absence of epidermal barrier disruption. Mechanistically, Krt76 loss disrupts the differentiation of keratinocyte-like mimetic mTECs (corneoTECs) and ablates a Krt76-dependent, skin- and oral mucosa-restricted tissue self-antigen (TSA) gene signature. This impairs negative selection of skin-autoreactive T cells, evidenced by reduced thymic T cell apoptosis and an altered TCR repertoire.
These findings reveal a previously unrecognised thymic mechanism underlying skin-directed autoimmunity, challenging the prevailing paradigm that keratin-associated skin pathology is solely barrier-driven. Patients with keratin gene mutations may harbour an autoimmune component to their disease that is refractory to conventional antimicrobial or barrier-repair strategies. This work opens new avenues for therapy in keratin-associated disorders and underscores the importance of thymic central tolerance in the aetiology of inflammatory skin disease — a framework applicable to a broad spectrum of keratin-linked conditions in which T cell involvement has been reported.

011

Multi-omics uncovers keratinocyte–melanocyte–immune crosstalk driving UV-induced hyperpigmentation

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Ultraviolet (UV) irradiation induces a broad spectrum of cutaneous responses, including inflammation, immune modulation, and pigmentation. While UV-induced tanning is a well-recognized adaptive process, the spatial and cellular crosstalk underlying this response remains incompletely understood. Here, we investigated these mechanisms using a murine model of repeated UVB exposure.

Mice were subjected to a short chronic irradiation regimen (three times per week over two weeks; seven exposures total) at 0.5 minimal erythemal dose. To capture spatially resolved responses, we leveraged the intrinsic gradients of UV exposure across the ear, defining regions with low, intermediate, and high irradiated regions based on distance from the ear base, epidermal thickness, and cyclobutane pyrimidine dimer (CPD) levels.
Using the Xenium 5K spatial single-cell transcriptomics platform, we generated high-resolution maps of gene expression, revealing pronounced spatial gradients in transcriptional responses. Pathway enrichment analyses demonstrated coordinated, distance-dependent activation of stress response, inflammatory signaling, and tissue remodeling pathways. Notably, pigmentation-associated pathways, cell cycle regulation, and cellular activity were differentially modulated across regions, accompanied by a localized increase in melanocyte and immune cell abundance.
Consistently, proteomic data confirmed modulation of pigmentation-related proteins alongside robust activation of immune and inflammatory pathways, particularly in highly irradiated regions. Ultrastructural analysis by electron microscopy further revealed increased melanosome accumulation in melanocytes and enhanced transfer to keratinocytes.
Together, this integrative and spatially resolved study highlights the critical role of keratinocyte–melanocyte–immune cell crosstalk in shaping UV-induced hyperpigmentation. These findings provide new insights into the spatial organization of tanning responses and identify coordinated molecular programs linking inflammation, tissue remodeling, and pigmentation.

012

Inflammatory memory of psoriatic resolved keratinocytes

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Immune cells can develop an inflammatory memory that enables a faster and stronger response to new stimuli. We previously showed that epigenetic changes persist in resolved psoriatic epidermis. Here, we aimed to study the functional abnormalities behind these changes by examining responses to repeated inflammatory stimuli. Punch biopsies were collected from treated, asymptomatic psoriatic patients from never-lesional (PS-NL) and previously lesional, resolved (PS-RES) skin. The epidermal and dermal parts were treated separately with an inflammation-inducing cytokine cocktail (CC) and the effect of the treatment was tested by cytokine/chemokine protein array followed by Reactome over-representation test on differentially expressed proteins (DEPs) with twofold expression. CC-treatment resulted in a more pronounced cytokine/chemokine expression in PS-RES epidermis and dermis than PS-NL samples, and activation of interleukin signaling was characteristic in all treated groups. CCL2 expression increased in all samples after CC-treatment, which was confirmed by ELISA. DEPs that were upregulated only in PS-RES epidermis (21) were mainly associated with the TNF receptor-mediated non-canonical NFκB pathway, while those in PS-NL epidermis (4) were linked to IL-4 and IL-13 signaling. The CC-treatment led to stronger psoriasin expression, particularly in PS-RES keratinocytes, using immunofluorescence staining. Moreover, CC-treatment was also applied on keratinocytes cultured from PS-NL and PS-RES skin, which resulted in higher 5-methylcytosine levels and reduced proliferation in both PS-NL and PS-RES keratinocytes. Finally, our study highlighted that repeated inflammatory stimuli in the PS-RES epidermis resulted in a more intense response, suggesting the presence of training type, while the observed functional differences in cultured keratinocytes indicate tolerance-type inflammatory memory.

013

Modelling the Breakdown of Hair Follicles in the Inflammatory Skin Disease Hidradenitis Suppurativa

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Hidradenitis suppurativa (HS) is thought to originate in hair follicles (HFs), but the early pathological events in human tissue are not well understood. We present an ex vivo HF organ culture platform using scalp sections and microdissected HFs cultured in Williams' E medium. Scalp HFs from hair transplant surgeries were microdissected to preserve whole scalp, epidermal and follicular structures. Quality was assessed through morphology and viability, including monitoring of hair shaft elongation and outer root sheath expansion. This platform builds on previous alopecia areata organ culture work, enabling controlled exposure to soluble HS-associated factors such as interleukin 17A (IL-17A) and interferon gamma (IFN-γ) to assess proliferation, morphology and immune changes across different HF regions. After culture, samples were preserved for downstream analysis by optimal cutting temperature embedding, protein extraction and single-cell RNA sequencing (scRNA seq). Initial findings show region-specific changes within the HF unit: IL-17A increased Ki-67 expression in specific areas, indicating hyperproliferation, while IFN-γ increased major histocompatibility complex class I expression without immediate structural collapse. Single-cell analysis was performed using the Parse Biosciences Evercode WT Mini kit and identified multiple HF-specific cell clusters, supporting the suitability of this platform for resolving compartment-associated transcriptional responses. This model aims to define early changes preceding HF collapse and identify factors associated with immune dysregulation in HS.

014

Sebaceous gland dysregulation in Parkinson's disease

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Pathological aggregation of α-synuclein (αSyn) is a hallmark of Parkinson's disease (PD). Several evidence indicates that αSyn deposits extend from the central nervous system to peripheral tissues, including the skin. In PD patients, cutaneous αSyn accumulation has been associated with seborrheic dermatitis (SD), a chronic inflammatory condition linked to sebocyte dysfunction and altered sebum production. Sebum composition differs between PD patients and healthy controls. We aimed to investigate whether pathological αSyn could contribute to dysregulated sebum production and composition by assessing key markers of sebocyte differentiation and lipogenesis in human sebaceous gland cells and skin biopsies from PD patients and matched controls. Exposure to oligomeric αSyn (O-αSyn) of immortalized sebaceous gland cells (SZ95) altered the transcriptional programs controlling sebocyte differentiation, inflammation, metabolism, and lipogenesis. Consistently, protein

markers of mid-to-late sebocyte differentiation were increased. Upregulation of PLIN2 together with elevated PPARγ, SREBP1, SCD1, and FADS2 indicated progression toward a mature and lipid-producing phenotype characterized by enhanced accumulation of neutral lipids within lipid droplets. Lipidomic profiling revealed remodeling of multiple lipid types, with increases in triglycerides, ceramides, and phospholipids. Increased PLIN2 and PPARγ expression in sebaceous glands from PD skin biopsies supports a functional link between αSyn accumulation and lipid droplets genesis. These findings identify αSyn as a potential driver of sebaceous gland dysfunction in PD and provide mechanistic insight into the association between PD and SD, further supporting the potential of skin-based molecular and lipid biomarkers as accessible tools for early PD detection.

015 [WITHDRAWN]

016

A Novel Mouse Model of Hidradenitis Suppurativa Based on Hair Follicle Stem Cell-Specific Nicastrin Deletion

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Hidradenitis suppurativa (HS) is a chronic, severely debilitating inflammatory skin disease with limited therapeutic options and unpredictable treatment responses. The scarcity of reliable preclinical models has critically hampered mechanistic understanding and drug development. The observed dysregulation of hair follicle stem cells (HF-SCs) in HS, combined with the identification of NCSTN — encoding the γ-secretase component Nicastrin — as a candidate causative gene, prompted us to develop a model of conditional Nicastrin deletion specifically in HF-SCs. Building on a previous model that exhibited off-target immune effects, we established a refined, locally restricted approach by generating *Ncstn*$^{fl/fl}$*Krox20*^{CreERT2}*R26*^{tdTomato} mice, in which NCSTN is selectively deleted in Krox20-expressing HF-SCs upon local tamoxifen application. Two weeks after induction, tdTomato expression was confined to epithelial cells with no labelling in lymph nodes, spleen, or thymus, confirming local specificity and absence of off-target immune effects.

Immune cell infiltration was detectable at week 4, preceding the emergence of macroscopic skin lesions at week 8, suggesting that the inflammation is an early and primary consequence of HF-SC Nicastrin loss. To characterize this pre-lesional inflammatory window, scRNA-seq of skin immune cells was performed at week 6, revealing prominent neutrophil infiltration and a distinct myeloid population with high IL-1β expression, consistent with a myeloid-driven inflammatory cascade. Flow cytometry confirmed these findings and additionally revealed an expansion of regulatory T cells. Histological analysis at week 8 revealed marked hyperkeratosis and keratinous plugs in hair follicles, recapitulating the follicular occlusion thought to initiate HS.

Collectively, this model faithfully reproduces follicular-driven HS pathogenesis, specifically in genetic forms linked to γ-secretase mutations. It supports the concept that HF-SC Nicastrin loss directly triggers an early myeloid-dominated inflammatory cascade preceding overt follicular disease, and provides a powerful platform for dissecting disease mechanisms and evaluating targeted therapies.

017

From Floating to Sleeping Melanocytes: Redifferentiation as a New Paradigm in Vitiligo Therapy

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Vitiligo is widely regarded as an autoimmune disorder associated with melanocyte loss. However, the precise fate of melanocytes within lesional skin remains incompletely understood. In this study, we provide evidence that a subset of melanocytes may persist in vitiligo skin in a dedifferentiated state rather than being entirely eliminated.

We observed changes in basement membrane-dependent adhesion mechanisms between normal and vitiligo melanocytes. Whereas normal melanocytes preferentially interacted through dystroglycan-laminin-211 signaling, vitiligo melanocytes showed increased reliance on integrin α3β1-laminin-332 binding. This adhesion shift was associated with activation of Rho-F-actin signaling and downstream Hippo/MAPK pathways, accompanied by c-Jun induction and features consistent with melanocyte dedifferentiation. These cells also displayed neural crest-like characteristics and broader differentiation potential.

Importantly, this altered melanocyte state appeared to be at least partially reversible. Pharmacological modulation of the pathway promoted repigmentation in animal models and human skin explants. In addition, JAK inhibitors showed the capacity to enhance melanocyte differentiation beyond their established immunomodulatory effects. Combination with excimer phototherapy may further enhance therapeutic responses by promoting melanocyte activation and migration.

Together, our findings suggest that basement membrane niche remodeling may contribute to melanocyte state changes in vitiligo. This model complements conventional concepts of melanocyte loss and highlights the possibility that restoration of melanocyte identity and function could represent a therapeutic opportunity.

018

Activation of the NLRP3 inflammasome induces type 1 inflammation and neutrophil activation in pyoderma gangrenosum across human and murine models

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Pyoderma gangrenosum (PG) is a rare neutrophilic dermatosis, with poorly defined molecular mechanisms. We aimed to identify conserved pathogenic pathways by integrating analyses of human PG and a brequinar-induced murine model. In analyses of human samples, skin biopsies from PG patients showed increased myeloperoxidase-positive neutrophil infiltration

and neutrophil extracellular traps (NETs) formation, with a dominant type 1 inflammatory profile confirmed by transcriptomic analysis of published RNA-sequencing data. Next, we newly developed a modified murine PG model using topical application of brequinar to full-thickness excisional wounds under antibiotic prophylaxis. Brequinar-treated wounds exhibited significantly delayed healing, increased neutrophil infiltration, enhanced NET formation, and elevated expression of pro-inflammatory cytokines, thereby closely recapitulating the features of human PG. Moreover, murine RNA-sequencing analysis revealed enrichment of immune system processes, defense responses, and chemotaxis pathways, along with upregulation of type 1 cytokines and Nrf2-dependent oxidative stress–related genes. Cross-species transcriptomic integration identified NLRP3 as a key commonly upregulated gene across species, along with five additional genes. Immunofluorescence further demonstrated increased expression of NLRP3, activated caspase-1, and cleaved IL-1β in both human PG lesions and brequinar-treated murine wounds. Temporal analysis in brequinar-treated mice revealed that infiltrating neutrophils co-express these inflammasome markers, suggesting that they represent a principal cellular source. Finally, we evaluated the therapeutic effects of prednisolone and avacopan, a selective complement C5a receptor antagonist, in brequinar-treated mice. Both agents suppressed inflammatory cytokine expression; however, only avacopan significantly improved wound healing. We demonstrated that NLRP3 inflammasome activation is a core pathogenic mechanism shared between human PG and the murine model, and that pharmacological inhibition of the C5a pathway represents a potential novel therapeutic strategy for PG.

019

Galvokimig, a multispecific antibody-based therapeutic, attenuates key immune pathways in atopic dermatitis: transcriptomic insights from a Phase 2a trial

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Inflammatory pathways beyond T helper (Th)2 play key roles in atopic dermatitis (AD). Interleukin (IL)-13, IL-17A, and IL-17F have been shown to act in combination to amplify downstream inflammatory mediators associated with AD. Galvokimig is a multispecific antibody-based therapeutic that inhibits IL-13, IL-17A, and IL-17F. In a Phase 2a trial (NCT04643457) in moderate-to-severe AD (N=47), the ≥75% improvement in Eczema Area and Severity Index (EASI75) response rate was 64.9% with galvokimig vs 12.3% with placebo; the EASI90 response rate was 46.6% vs 3.5%, respectively. Here, we report the transcriptomic signature of tissue from patients treated with galvokimig in this trial.
Non-lesional (n=46) and lesional (n=47) biopsies at baseline and at week 12 were collected; bulk mRNA sequencing was performed.
At baseline, principal component analysis showed marked differences between lesional and non-lesional skin; lesional skin showed dysregulation of Th2-/Th17-/Th1-associated gene signatures and other AD-associated inflammatory genes. At week 12, galvokimig treatment attenuated Th2/Th17/Th1 immune pathways vs baseline, including reduction of *IL13* expression and normalisation of expression of *IL17A*, *IL17F*, *IL31*, and skin barrier integrity genes (*FLG*, *LORICRIN*, and *IVL*).
The modulation of Th2/Th17/Th1 inflammatory signatures together with the depth of clinical responses observed in the trial support the therapeutic potential of simultaneously targeting IL-13, IL-17A, and IL-17F in AD. A Phase 2b dose-ranging clinical trial to assess the efficacy, safety, and pharmacokinetics of galvokimig in participants with moderate-to-severe AD is underway (PossibILity; NCT07277660).

020

miR-335–Mediated HDAC Inhibitor Therapy for Atopic Dermatitis

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Atopic dermatitis (AD) is a chronic inflammatory skin disease driven by epidermal barrier disruption and persistent immune activation. Effective therapies that concurrently restore barrier integrity and suppress inflammation remain limited. We investigated the therapeutic potential of Belinostat, a broad-spectrum histone deacetylase (HDAC) inhibitor, and newly developed analogues as dual-action treatments for AD. Previously, we identified microRNA-335 (miR-335) as a key regulator of keratinocyte differentiation and cornification through repression of SOX6. In AD skin, reduced miR-335 disrupts epidermal maturation and barrier formation. Epigenetic profiling implicated aberrant HDAC activity in this defect, suggesting HDAC inhibition as a strategy to restore miR-335 and epidermal homeostasis. Human keratinocyte cultures, three-dimensional organotypic skin models, and an MC903-induced murine AD model were used to assess effects on barrier repair, inflammation, and miR-335 signaling. Structural analogues were synthesized to improve safety, stability, and suitability for topical delivery. Treatment with Belinostat restored miR-335 expression, enhanced keratinocyte differentiation, and improved epidermal barrier architecture. Mechanistically, miR-335 suppressed caspase-7, a mediator of AD-associated inflammation, reducing secretion of pro-inflammatory chemokines and cytokines including CCL3, CCL5, IL-4, IL-13 and IL-31. Newly synthesized analogues retained efficacy while demonstrating improved safety profiles across preclinical models. Our findings support a dual-action therapeutic strategy targeting both barrier dysfunction and inflammation in AD. HDAC inhibitor analogues with enhanced safety and efficacy represent promising candidates for next-generation topical therapies and highlight the translational potential of epigenetic approaches in inflammatory skin disease.

021

Contribution of ultra-rare variants in inflammasome sensors to hidradenitis suppurativa immunopathology

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Hidradenitis suppurativa (HS) is a debilitating inflammatory skin disease affecting 1-4% of the population globally. Like many chronic inflammatory diseases, the etiology of HS is poorly understood and highly complex, suggesting the need for personalized treatment approaches. Although inflammasomes are central to innate immunity, excessive inflammasome activation is highly pathogenic and has been reported in HS. Moreover, while pathogenic gain of function (GoF) variants in inflammasome sensors have been reported, the contribution of these variants to the immunopathology of HS remains unclear. Understanding how inflammasomes contribute is critical, as inflammasome overactivation is a key therapeutic target.
Whole exome sequencing (WES) from 270 HS participants identified 25 ultra-rare variants in inflammasome sensors and inflammasome related genes. Using Time of Flight Inflammasome Evaluation (TOFIE), we functionally characterized five variants in *MEFV* (encoding pyrin), and two *NLRP3* variants. Despite the known role of *NLRP3* in autoinflammatory diseases, our data suggest the identified *NLRP3* variants do not contribute to inflammasome overactivation. Conversely, one *MEFV* variant was identified as a likely GoF mutation. This finding suggests the harboring participant may benefit from targeted off-label treatment with the IL-1 receptor antagonist therapy.
In summary, this work underscores the importance of human genetic studies and functional characterization to uncover HS disease mechanisms. By identifying novel variants like this *MEFV* mutation, we can prioritize personalized treatment strategies and improve clinical outcomes for HS patients.

022

Spatial Single-Cell Mapping of the Hair Follicle Microenvironment in Alopecia Areata using CosMx™ Spatial Molecular Imager

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Alopecia areata (AA) is a common non-scarring hair loss disorder. The detailed mechanisms of the hair follicles (HF) microenvironment remain unclear. The CosMx™ Spatial Molecular Imager enables direct detection of 1,000 RNA transcripts in tissue at single-cell resolution, allowing high-resolution analysis of both cell types and their spatial organization. This research aims to characterize the spatial distribution of cell populations and gene expression of HF microenvironment in AA patients, constructing a spatial single-cell map of the disease pathophysiology. Scalp biopsy samples (4-mm punch) were obtained from the active lesion margins of six AA patients (5 females, 1 male; mean age 27 ± 12.01 years), including multifocal (n=4), totalis (n=1), and universalis (n=1) subtypes, with a mean SALT score of 49.03 ± 27.12. By applying the CosMx™ Human 1000-plex RNA panel across 70 fields of view centered on the peribulbar region, we successfully generated a comprehensive dataset of 32,614 high-quality single-cell profiles. Unsupervised clustering identified 18 distinct clusters across 9 major cell lineages. Spatial mapping revealed a highly organized, layered distribution of keratinocytes, fibroblasts, T cells, and antigen-presenting cells extending from the central HF bulb to the periphery. Furthermore, sub-structural niches, including sweat glands and perivascular regions, were reconstructed at subcellular resolution. UMAP and cell-proportion analyses suggested that this spatial composition was largely comparable across the various clinical subtypes, hinting at a potentially shared pathological basis for AA. This first subcellular-resolution spatial map identifies a universal immune infiltrate pattern, offering new insights into AA immunopathology and supporting the development of location-specific therapeutic interventions.

023

IL-4/IL-13 blockade alters the inflammatory landscape of the conjunctival epithelium in atopic dermatitis

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This study aimed to investigate the transcriptional mechanisms underlying ocular surface disease (OSD) following atopic dermatitis treatment with the IL4/IL13 signaling inhibitor dupilumab or the selective IL13 inhibitor tralokinumab in non-immune conjunctival epithelial cells. Conjunctival samples were collected by impression cytology from six dupilumab-treated patients, six tralokinumab-treated patients (at baseline and after four weeks), and six non-atopic controls. These samples were analyzed using single-cell RNA sequencing. The results revealed three main epithelial populations: basal cells, superficial epithelial cells, and immune-activated superficial epithelial cells. No significantly enriched pathways were observed between non-atopic controls and baseline patients in either of these populations, although weak immune activation was indicated by increased HLA-DQA1 and IL6R expression in the superficial epithelial cells. Following dupilumab treatment, immune-activated superficial epithelial cells expanded and displayed an IFNγ-driven type 1 immune signature, characterized by increased CXCL1, CXCL6, CXCL9, CXCL10, CCL20 and HLA-DR expression. These results suggest increased activation and recruitment of immune cells. In comparison tralokinumab treatment induced a similar but less pronounced response. Concluding, IL4/IL13 signaling inhibition induces a conjunctival type 1 immune response, which is more pronounced with dual IL-4/IL-13 blockade than with selective IL-13 inhibition by tralokinumab. These findings align with the clinical manifestations of ocular surface disease observed in treated patients and provide translational insights that may inform therapeutic decision-making.

024

Novel anti-diabetic drugs may modulate pro-inflammatory cytokine production of human dermal fibroblasts

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Obesity and type 2 diabetes mellitus are key health challenges in industrial countries. While several new therapeutic agents have recently been introduced in the management of these conditions, little is known about the potential cutaneous effects of these drugs. Thus, in the current study we aimed to investigate the effects of the glucagon-like peptide-1 receptor (GLP-1R) agonist liraglutide (LIR), the GLP-1R and gastric inhibitory polypeptide receptor (GIPR) dual agonist tirzepatide (TIR), the GLP-1R, GIPR and glucagon receptor triple agonist (investigational) retatrutide (RET), the sodium/glucose cotransporter 2 inhibitor dapagliflozin (DAP) and the “traditional” oral antidiabetic drug metformin (MET) using primary human dermal fibroblasts of healthy adults (HDFs) cultured in a “high glucose” (~23 mM) medium.

First, we showed that all compounds can be applied without the risk of cytotoxicity (24-h treatments, EZ4U assay). Next, we assessed the effects of selected concentrations (TIR and RET: 100 nM; DAP and LIR: 1 µM; MET: 1 mM) of the drugs on the spontaneous and tumor necrosis factor (TNF)-α (10 ng/ml)-induced release of interleukin (IL)-6 and IL-8 (24-h treatments). We found that TIR, DAP and RET suppressed the spontaneous, untriggered release of IL-6 from HDFs, while MET and LIR had no such effects. Interestingly, however, all investigate compounds could significantly decrease the TNF-α-induced release of IL-6. Quite surprisingly, this was not the case for IL-8. Indeed, while TIR tended to suppress both the spontaneous as well as the TNF-α-induced release of IL-8, these alterations did not reach the level of statistical significance, and MET was found to slightly, but significantly increase spontaneous IL-8 release.
Collectively, our data argue that DAP, RET, and especially, TIR may modulate immune phenotype of HDFs, indicating that they may beneficially influence fibroblast activity in case of diabetes-associated fibroblast dysfunction.

025
Identification of a Pruritus-Specific Molecular Signature in Chronic Inflammatory Skin Diseases
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Pruritus is a burdensome symptom of chronic inflammatory skin diseases such as atopic dermatitis (AD). Despite recent advances, the mechanisms underlying itch remain poorly understood.
In this study, we aimed to identify a pruritus-specific molecular signature by comparing atopic and psoriatic inflammation and investigating electrically induced itch in both conditions. We obtained clinical data and skin biopsies from patients with AD (n = 61), Psoriasis (Pso) (n = 50), and healthy volunteers (HV, n = 17). We applied slowly depolarizing electrical stimulations (ES) to lesional and non-lesional skin of all participants.
Transcriptomic analysis revealed inflammatory heterogeneity in the AD cohort, but not in Pso. We thus stratified AD patients into those with heightened (AD^{hi}) and lower (AD^{lo}) atopic inflammation. Differential expression analysis between AD^{hi}, Pso, and HV, revealed a set of upregulated genes, which were enriched for neuroinflammatory pathways. Overlapping genes in lesional and non-lesional AD skin revealed atopy markers such as IL-13, as well as prostaglandin receptors and cysteinyl leukotrienes. During ES, pain was reported by all participants, whereas pruritus was preferentially reported among AD patients. Within the AD group, transcriptomic comparisons between responders and non-responders to ES-itch revealed genes enriched for inflammatory and neuronal pathways. Taken together, we demonstrate that, electrically induced itch differentiates pruritus in AD and Pso, suggesting distinct underlying mechanisms in both conditions. Moreover, we identified novel atopic inflammation-related genes and potential therapeutic targets for pruritus in AD.

026
Intrinsic differences among keratinocytes cultured from mild and severe psoriatic non-lesional and resolved skin.
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In psoriasis, cytokine and chemokine expression patterns distinguish the non-lesional (PS-NL) and previously lesional, resolved skin, reflecting distinct inflammatory stages. To study whether keratinocyte-intrinsic features can affect these changes, we analyzed cultured keratinocytes derived from PS-NL skin of patients with mild and severe disease, and from resolved skin. Our newly developed, DAPI staining-based microscopic analysis revealed lower average cell density in the resolved group, in paralell cell cycle profiling analysis demonstrated S phase predominance in severe, and G1 phase enrichment in mild PS-NL keratinocytes. Scanning electron microscopic analysis supported the observed cell number differences, and morphologically, mild PS-NL and resolved cells exhibited prominent membrane protrusions, including lamellipodia and filopodia, while severe PS-NL cells showed a more elongated morphology with visible nuclei. Proteomic analysis identified the lowest number of differentially expressed proteins (DEPs) between mild and resolved PS-NL cells, mainly associated with cornified envelope formation and keratinization. In contrast, the highest number of DEPs was detected between severe PS-NL vs. resolved cells, with proteins mostly linked to interferon signaling and IL-12–mediated JAK-STAT signaling pathways. Overall, our results indicate that PS-NL keratinocytes retain intrinsic differences *in vitro*, where severe PS-NL cells may reflect a more proliferative, pre-psoriatic state, while mild PS-NL and resolved cells appear activated but less proliferative.

027
Mitochondrial alterations in the psoriatic non-lesional keratinocytes
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The enhanced stress sensitivity of psoriatic non-lesional (PS-NL) skin has previously been reported. Our recent findings suggested that stress-related proteins, which are expressed differently in PS-NL skin, may affect mitochondrial function. Thus, we aimed to determine whether PS-NL keratinocytes contribute to these alterations. Using high-resolution respirometry, we observed no significant differences in oxidative phosphorylation capacity, ATP-independent respiration or residual oxygen consumption between PS-NL and healthy (H) keratinocyte cultures, or between PS-RES and H keratinocyte cultures. However, maximal mitochondrial respiratory capacity was reduced in both PS-NL and PS-RES keratinocytes compared to H controls. Production of hydrogen peroxide, an indicator of reactive oxygen species generation, was significantly elevated during both

oxidative phosphorylation and leak respiration in PS-NL and PS-RES keratinocytes compared to H controls. Mitochondrial staining intensity was higher in keratinocytes derived from PS-NL and PS-RES compared to H skin, as assessed by immunofluorescence staining (IFS). Additionally, enhanced mitochondrial staining intensity was observed by IFS, and less structured mitochondria were detected by transmission electron microscopy in PS-NL, PS-L, and PS-RES epidermis on skin sections. Finally, our findings indicate an increased number of mitochondria, as well as their potential dysfunction, in PS-NL, PS-RES and potentially PS-L keratinocytes. These alterations may contribute to the heightened stress sensitivity of psoriatic keratinocytes.

028

Reduced RNA acetyltransferase NAT10 activity is associated with increased cellular stress in keratinocytes and enhanced inflammatory responses in mouse model of psoriasis

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RNA modifications represent an important layer of post-transcriptional gene regulation, particularly in the context of mRNA. N4-acetylcytidine (ac4C), catalyzed by NAT10, enhances mRNA stability and translational efficiency and has been implicated in cellular stress responses, proliferation, and immune regulation. Here, we investigated whether ac4C is required for epithelial homeostasis and whether it plays a role in psoriasis.
We show that NAT10 expression is increased in lesional skin from psoriasis patients at both mRNA and protein levels. To delineate NAT10 function, we performed siRNA-mediated knockdown (KD) of NAT10 in primary human keratinocytes, followed by transcriptomic, mRNA, and protein-level analyses. Transcriptome analysis revealed that NAT10 KD alters multiple inflammatory signaling pathways and induces a pronounced epithelial stress response. Specifically, NAT10 KD led to suppression of MYC signaling, alongside activation of TGF-β, IL-6, and STAT3 signaling pathways, and upregulation of genes associated with epithelial–mesenchymal transition.
Pharmacological inhibition of Nat10 in mice using remodelin resulted in stronger skin inflammation in the imiquimod-induced mouse model of psoriasis. This was associated with increased expression of Ccl20, Cxcl1, and Il1b in the skin, and elevated Il6, Cxcl2, and Il17a mRNA expression in lymph nodes. Together, these findings identify NAT10-mediated RNA acetylation as a critical regulator of epithelial homeostasis and suggest that enhancing NAT10 activity may represent a potential therapeutic strategy in psoriasis.

029

HuR-mediated RNA stabilization drives keratinocyte hyperproliferation and Th17/Th1 activation in psoriasis models

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Psoriasis is a chronic inflammatory skin disease driven by keratinocyte hyperproliferation and sustained Th17/Th1-mediated inflammation. We investigated the role of the RNA-binding protein HuR in the cellular and molecular pathophysiology of psoriatic skin inflammation and explored the impact of targeting HuR-mRNA binding with a Tanshinone-mimic compound (TM11). HuR activity was assessed in HaCaT cells, patient-derived keratinocytes and lymphocytes, and HuR-target mRNAs were analyzed by enhanced Cross-link ImmunoPrecipitation sequencing (eCLIP-seq). In parallel, cellular responses to TM11 were evaluated in vitro, ex vivo, and in vivo. In HaCaT cells, eCLIP-seq revealed new HuR-target transcripts in a psoriasis-like context and confirmed HuR-dependent stabilization of mRNAs encoding key inflammatory and proliferative factors. By HuR silencing experiments, keratinocyte proliferation and lymphocyte activation and differentiation were shown to depend on HuR function. Treatment with TM11 reduced keratinocyte proliferation and modulated the expression of Cyclin B, Survivin, S100A7 and eFABP5. In lymphocytes, TM11 decreased T-cell activation ($CD25^+CD69^+$), Th17/Th1 populations, and the production of IL-17AF and IFN-γ. In Imiquimod-treated mice, topical TM11 reduced psoriasis-like symptoms, including PASI scores, systemic inflammation, and histological alterations. In ex vivo InflammaSkin models, TM11 restored epidermal morphology, lowered Ki-67 expression, and reduced IL-17AF and IFN-γ secretion. These findings identify HuR-mediated RNA stabilization as a central mechanism in the cellular and molecular pathophysiology of psoriasis and support HuR-targeted modulation as a strategy to restore skin homeostasis.

030

Using immune-competent 3D human skin equivalents to evaluate the immune microenvironment adapting under JAK inhibition in Atopic Dermatitis

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Atopic dermatitis (AD) is a chronic inflammatory skin disorder characterized by a dominant Th2/Th22 immune response. Recent 3D human skin equivalents lack a functional immune compartment, which limits their translational value. Current *in vitro* AD skin models rely on exogenous cytokine stimulation, which fails to capture the dynamic crosstalk between infiltrating immune cells and the skin epithelium that drives disease progression. Incorporating peripheral blood mononuclear cells (PBMC's) directly into the dermal compartment offers a more physiologically relevant way to model these interactions. In addition, previous studies of JAK inhibition have mainly focused on clinical efficacy and safety endpoints, rather than profiling of the immune microenvironment. Therefore, we developed a novel immunocompetent full-thickness model (FTM) incorporating PBMC's to recapitulate immune driven AD and evaluate JAK inhibitor response.
FTMs were generated using healthy fibroblasts, keratinocytes and PBMC's. AD-like inflammation was induced using IL4, IL13 and IL31, followed by Upadacitinib treatment. FTMs were evaluated for phenotypic and immunological changes using flowcytometry. Epidermal morphology, barrier integrity and stress level were analyzed by hematoxylin and eosin staining and immunohistochemistry. Cytokine secretion profiles were quantified using Luminex. Additionally, differences in barrier function were assessed through qPCR.

Flowcytometry confirmed viable immune cells within FTMs after 14 days, validating successful PBMC incorporation. PBMC-containing models displayed an differential AD phenotype versus immune cell-free controls, and marked reduction of filaggrin and loricrin by IHC. JAK inhibition strongly attenuated cytokine secretion in PBMC-FTMs with restoration of filaggrin and loricrin expression, effects greater than in immune cell-free FTMs.
Immune-competent 3D human skin equivalents enable the assessment of immune microenvironment dynamics in atopic dermatitis under JAK inhibition.

031

LINC01605 is induced in atopic dermatitis and suppresses keratinocyte differentiation

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Atopic dermatitis (AD) is a chronic inflammatory skin disorder characterized by impaired epidermal barrier and predominantly type 2 immune activation. Long non-coding RNAs (lncRNAs) are >200-500 nt non-protein-coding transcripts that regulate gene expression. The contribution of lncRNAs to epidermal differentiation and barrier function in AD has remained largely unexplored.
To identify lncRNAs with potential roles in epidermal dysfunction in AD, we performed RNA sequencing in epidermis from lesional and non-lesional AD skin and healthy controls. In our analyses, LINC01605 emerged as one of the most upregulated lncRNAs in AD epidermis. This upregulation was confirmed by qRT-PCR and by RNAscope® single-molecule *in situ* hybridization, which showed strongest LINC01605 expression in the basal layer and lower expression in suprabasal layers. *In vitro*, LINC01605 expression decreased during calcium-induced keratinocyte differentiation. In contrast, IL-4 and IL-13 significantly induced LINC01605 expression in both monolayer keratinocyte cultures and three-dimensional (3D) epidermal equivalents.
siRNA-based knockdown of LINC01605 led to G1/S phase cell cycle arrest and increased expression of the terminal differentiation markers filaggrin and loricrin both in monolayer cultures and in 3D epidermal equivalents. Transcriptomic analysis of LINC01605-depleted keratinocytes revealed significant upregulation of genes involved in keratinization" and cornified envelope formation, and downregulation of genes involved in unfolded protein response and G2-M checkpoint pathways.
Collectively, our findings identify LINC01605 as a regulator of the keratinocyte proliferation-differentiation balance. Its upregulation in AD, induced by type 2 cytokines, may contribute to impaired epidermal differentiation and barrier dysfunction.

032

Increased expression and activity of ADAM17 in skin keratinocytes is linked to atopic dermatitis pathogenesis

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Atopic dermatitis (AD) is a chronic inflammatory skin disease that affects millions of people worldwide. Despite the prevalence of AD, there are still gaps in our understanding of the mechanisms involved in AD pathogenesis. A disintegrin and metalloprotease 17 (ADAM17) is a protease that cleaves inactive proteins involved in inflammation, keratinocyte proliferation, differentiation and skin barrier integrity into their active form. Previous studies showed that ADAM17 knock-out mice display an AD-like phenotype. This study aimed to understand how ADAM17 contributes to the pathogenesis of AD.
Using publicly available RNAseq data (GSE121212), *ADAM17* gene expression was found to be increased in patient AD lesions compared to healthy control tissue. ADAM17 protein abundance and proteolytic activity was also similarly increased in AD lesions. Overexpression of ADAM17 in N/TERT-2G keratinocytes elevated both gene and protein levels of transglutaminase-1 in a protein kinase C-dependent manner, which may lead to hyperkeratosis frequently seen in AD. ADAM17 overexpression was also associated with increased gene expression of *TNF-α*, *cytokeratin-16* and *cytokeratin-17*, molecules also seen elevated in AD lesions. ADAM17 overexpression also led to an increased secretion and cleavage of transforming growth factor-α, resulting in phosphorylation and downstream activation of the epidermal growth factor receptor (EGFR). Blocking EGFR signalling with cetuximab or erlotinib prevented the ADAM17-dependent increase in transglutaminase-1. Current studies are extending these findings to 3D engineered skin models to more accurately mimic AD in patients. These results indicate that ADAM17 and its downstream pathways may contribute to AD pathogenesis and could serve as suitable targets for therapeutic intervention.

033

Early divergence of Th1/Th2 signaling in African American and White non-Hispanic skin organoids defines disease-specific risk trajectories

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African American (AA) individuals exhibit a higher risk of atopic dermatitis, whereas White non-Hispanic (WNH) populations are more prone to psoriasis; however, the molecular basis of these disparities remains poorly understood. Here, we investigated whether intrinsic properties of healthy AA and WNH skin bias inflammatory signaling toward disease-specific trajectories at early stages of activation by cytokines. We used 3D human skin organoids (HSO) generated from AA and WNH neonatal keratinocytes and treated with Th1/Th17 cytokines (TNF-α + IL-17) or Th2 cytokines (IL-4 + IL-13) for 72 hours. While both AA and WNH HSO developed morphological features consistent with pro-psoriasis (Th1/Th17) or pro-AD (Th2) responses, transcriptomic profiling revealed pronounced ethnicity-dependent divergence in signaling programs. WNH HSO exhibited a stronger response to Th1/Th17 cytokines, whereas AA HSO showed heightened sensitivity to Th2 stimulation, as reflected by both the number and magnitude of induction of differentially expressed genes (DEGs). Consistent with these patterns, psoriasis-associated genes (S100A7A/koebnerisin, PI3K, DEFB4B) were preferentially induced in WNH HSO, whereas AD-

associated genes (CA2, TSLP, TNFAIP6, CH25H) were more robustly upregulated in AA HSO. In addition, cytokine treatments differentially affected epidermal differentiation markers: filaggrin and loricrin expression were preserved or increased in AA HSO but markedly reduced in WNH HSO. Furthermore, Th2 cytokines selectively activated STAT6, STAT3, and NF-κB signaling in AA HSO, indicating enhanced engagement of canonical pro-AD pathways. Together, these findings reveal early, intrinsic skewing of inflammatory signaling and differentiation programs in AA versus WNH skin, providing a framework to define molecular drivers of differential disease risk in these populations.

034

Spatial transcriptomic profiling reveals shared and distinct fibrotic programs across localized scleroderma subsets

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Localized scleroderma (LS) is a unique model to study fibrosis, as different disease stages can be observed within same lesion. Skin biopsies from patients with plaque morphea (PM), generalized morphea (GM), eosinophilic fasciitis (EF), and healthy controls were analyzed using spatial transcriptomics, followed by clustering, differential expression, pathway analysis and fibroblast deconvolution. In PM, spatial trajectory analysis detected molecular changes and gradients from clinically uninvolved skin across inflammatory to fibrotic areas.
Across all subtypes, inflammatory regions surrounding sclerotic lesions revealed strong immune activation with increased expression of antigen presentation and immune response genes (e.g. HLA-DRA, CD72 and C1QB), while fibrotic centers showed low cellularity and limited transcriptional activity. Extracellular matrix-related genes (e.g. COL1A1, COL3A1, TNXB, FBLN1), were most strongly expressed not in fibrotic core but in inflammatory regions and adjacent clinically unaffected skin, suggesting fibrotic processes may be initiated prior to visible tissue changes. PM and GM showed highly similar spatial and transcriptional patterns, including epidermal activation marked by increased keratin gene expression (e.g. KRT6A, KRT14 and KRT17).
In contrast, EF displayed strongest inflammatory signature, with enrichment of interferon-related pathways and increased expression of genes (e.g. CXCL9, IFI27, IFI30, STAT1), as well as chemokines (e.g. CCL8, SERPINE1), particularly in deeper dermis. Fibroblast deconvolution identified shared populations across all subsets, including inflammatory and fascia-like myofibroblasts, while immune activation was most pronounced in EF.
Altogether, these findings suggest LS subsets share common underlying mechanisms but differ in intensity and localization of inflammation-associated genes within tissue. Our data show fibrosis-specific signatures are activated in clinically uninvolved areas.

035

Oxidative Phosphorylation Represents a Therapeutically Targetable Pivot of Pathophysiological Macrophage Programs in Granulomatous Skin Disease

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Granuloma annulare (GA) and cutaneous sarcoidosis (cSAR) represent prototypic diseases of non-communicable granulomatous skin inflammation. Recent “omics“-methods have provided novel insights into their pathophysiology and illuminated IFN-γ instruction of macrophages as a key driver of disease. However, mechanistic understanding of druggable pathways in granuloma macrophages is lacking. Here, we used published single-cell RNA sequencing (scRNAseq) data of GA and cSAR, human *ex-vivo* skin samples, *in-vitro* human monocyte-derived macrophages (MDM) and an *in-vitro* human granuloma model to identify a druggable immunometabolic macrophage program. Re-analysis of scRNAseq data revealed significant up-regulation of oxidative phosphorylation (OXPHOS) in granulomatous IFN-γ-activated macrophages. Likewise, IFN-γ treatment of MDM showed STAT1-dependent increase in OXPHOS. Pharmacologic inhibition of electron transport chain (ETC) complexes broadly attenuated IFN-γ-driven macrophage activation downstream of STAT1 and enabled the identification of an OXPHOS-dependent inflammatory granuloma program that critically involves guanylate-binding proteins and CXCL10. Importantly, pharmacological interference with IFN-γ signaling and ETC function impaired granuloma formation in the human *in-vitro* model. In this all-human approach, we propose new therapeutically targetable molecules and immunometabolic pathways that can be explored in drug-repurposing strategies.

036

Matricellular Protein Mindin Orchestrates Fibroblast Heterogeneity in Organ Repair and Fibrogenesis

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Fibroblast activation is essential for wound healing and tissue repair, but when deregulated, it can lead to tissue or organ fibrosis and contribute to tumor stroma formation in cancer. “Activation,” however, is an overarching term encompassing diverse fibroblast functions, including proliferation, migration, contraction, cytokine production, and secretion of extracellular matrix (ECM) proteins by specialised fibroblast subtypes/subpopulations that uniquely contribute to tissue repair, fibrogenesis, and the tumour microenvironment. Despite growing insights into fibroblast heterogeneity, the upstream molecular mediators that dictate subtype-specific fibroblast responses in homeostasis, repair, and pathology remain inadequately defined. Leveraging a novel mouse model of systemic sclerosis as a discovery platform, we identified the matricellular protein Mindin as a crucial driver of fibroblast heterogeneity in organ repair and fibrosis. This matricellular protein, Mindin is elevated in multiple mouse models of organ fibrosis (skin, lung, liver, kidney), human fibrotic tissues, and diverse cancers (skin, gastric, prostate).

Mindin selectively activates distinct dermal fibroblast subpopulations via its N-terminal F-spondin domain, enhancing the release of inflammatory cytokines and chemokines (CCL5, IL-6, IL-17A, IL-1β, CXCL12, CXCL10, CXCL5, CXCL3) and migration in reticular fibroblasts, while promoting contractility and ECM secretion in papillary fibroblasts. In vitro, Mindin treatment reprograms primary reticular fibroblasts into inflammatory cancer-associated fibroblasts (CAFs) and papillary fibroblasts into myofibroblastic CAFs. Through an integrated computational and biochemical approach, we identified specific pattern recognition receptors that, in coordination with Src-family kinases, Rho GTPases, and the NF-kB pathway, orchestrate distinct subtype-specific fibroblast responses downstream of Mindin. In summary, our findings position Mindin as a key driver of fibrogenesis, offering mechanistic insights into fibroblast heterogeneity across tissue repair, fibrosis, and tumour stroma, with implications for targeted therapies.

037

Induction of a vitiligo-like phenotype in healthy human skin *ex vivo*: a preclinical model for therapeutic development

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Vitiligo is a chronic autoimmune skin disease characterized by depigmentation due to melanocyte loss, driven by oxidative stress, activation of damage-associated molecular patterns (DAMPs), and melanocyte-specific autoreactive $CD8^+$ T cell responses. With few effective therapies available, preclinical models that recapitulate disease complexity are essential for identifying novel therapeutic targets. While we have previously established an organ culture model using (peri)lesional vitiligo skin, limited biopsy availability constrains its throughput. Here, healthy human skin *ex vivo* was treated with a proprietary compound cocktail designed to induce Th1/type I IFN inflammation, oxidative stress, and melanocyte death, generating a vitiligo-like phenotype as a medium-throughput preclinical platform. Skin punches from one to three healthy donors were treated for two days with vehicle control or the cocktail, then analyzed by histochemistry, immunofluorescence, and LegendPlex. Cocktail-treated skin showed a donor-dependent reduction in melanin content and decreased microphthalmia-associated transcription factor (MITF) expression, indicating impaired melanogenesis and melanocyte survival. Protein secretion analysis revealed a proinflammatory milieu with trends toward elevated TNF-α, IFN-γ, and IL-6, alongside increased granulysin and enhanced secretion of Fas receptor and Fas ligand. Elevated epidermal $CD3^+$ and $CD3^+/CD69^+$ T cells confirmed induction of T cell activation and an inflammatory response. These findings demonstrate that a vitiligo-like phenotype can be induced in healthy human skin *ex vivo*, recapitulating key hallmarks of disease pathogenesis including melanocyte loss and immune dysregulation, and providing a valuable tool for preclinical evaluation of novel vitiligo therapeutics without requiring patient-derived tissue.

038 [WITHDRAWN]

039

Spatial transcriptomics reveals SERPINE1^{high} endothelial cells with mesenchymal transition underlying purpura associated with disease severity in drug-induced hypersensitivity syndrome/drug reaction with eosinophilia and systemic symptoms (DIHS/DRESS)

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DIHS/DRESS is a severe, life-threatening cutaneous adverse drug reaction characterized by multiorgan involvement and sequential herpesvirus reactivation. We previously demonstrated that the extent of purpura significantly correlates with disease severity, suggesting a key role of vascular pathology in DIHS/DRESS. We aim to investigate mechanisms of purpura formation to gain insight into the pathogenesis of DIHS/DRESS.
We performed spatial transcriptomic analysis of purpuric lesions in DIHS/DRESS (n=3) and erythematous lesions in erythema multiforme (EM) (n=3), an ordinary cutaneous drug reaction. Affected endothelial clusters in DIHS/DRESS exhibited marked upregulation of SERPINE1, a TGF-β responsive gene, compared with EM (log2FC = 3.50, FDR = 0.01). Gene set enrichment analysis showed enrichment of mesenchymal transition–related signatures along with TGF-β signaling in DIHS/DRESS. Consistently, serum proteomic profiling (11,083 proteins) identified 195 differentially expressed proteins in DIHS/DRESS, among which SERPINE1 and TGF-β were significantly elevated compared with EM and maculopapular exanthema (SERPINE1: 20.2 vs 13.5×10^3 RFU, p=0.03; TGF-β: 4.94 vs 3.43×10^3 RFU, p=0.02) with a strong positive correlation (r=0.747, p=0.0001). Immunofluorescence analysis demonstrated increased expression of the mesenchymal marker TAGLN in endothelial cells with a reduction in CD31 expression, supporting a shift toward a mesenchymal-like phenotype. In vitro, stimulation of HUVEC with sera from patients with DIHS/DRESS, followed by quantitative PCR analysis, induced upregulation of SERPINE1 compared with sera from those with EM.
These findings suggest that purpura in DIHS/DRESS is driven by TGF-β–mediated endothelial reprogramming toward a SERPINE1^{high}, mesenchymal-like state, promoting erythrocyte extravasation, rather than simple endothelial injury.

040

Keratinocytes initiate cytokine cascades leading to food allergen sensitization by sensing moisture levels in the stratum corneum

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Although several proinflammatory mediators are known to play a role in the immunopathogenesis of food allergy (FA), their role in the induction of FA remains unclear. We previously reported that epicutaneous exposure of BALB/c mouse footpad skin to ovalbumin (OVA) induces OVA sensitization and anaphylaxis when sweating is suppressed during OVA exposure.
We sought to determine whether reduced moisture content in the stratum corneum induces keratinocyte-derived cytokine responses and whether these responses contribute to sweat suppression–mediated epicutaneous food allergen sensitization. We used a human 3D epidermal model to assess whether low-humidity exposure induces IL-6 and IL-1β mRNA expression and whether this response is affected by high-humidity conditions or moisturizer application. We also examined whether sweat suppression–mediated OVA sensitization and anaphylaxis in mice could be attenuated by neutralization with an anti-IL-6

antibody or prevented under high-humidity conditions.
In a human 3D epidermal model, low-humidity exposure increased IL-6 and IL-1β mRNA expression. This response was attenuated under high-humidity conditions and cancelled by moisturizer application. In the mouse model, sweat suppression during epicutaneous OVA exposure induced OVA sensitization and anaphylaxis. This response was attenuated by administration of an anti-IL-6 antibody and abolished under high-humidity conditions.
These findings suggest that reduced moisture content in the stratum corneum triggers keratinocyte-derived cytokine responses, particularly IL-6, contributing to epicutaneous food allergen sensitization. Maintenance of stratum corneum hydration may therefore represent a potential strategy for reducing the risk of epicutaneous food allergen sensitization.

041

Restoration of Keratinocyte Homeostasis Drives Resolution of Skin Inflammation

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Chronic skin inflammation is sustained by reciprocal interactions between epidermal dysfunction and immune activation, yet whether epithelial state actively governs restoration of tissue homeostasis remains unclear. Using a murine model of inflammatory skin disease, we modulated epidermal lipid metabolism and examined its effects on tissue organization. Transcriptomic profiling revealed coordinated reversal of inflammatory, metabolic, and structural gene programs accompanied by normalization of epidermal architecture. Single-cell RNA sequencing showed that this remodeling was concentrated in differentiated keratinocytes, with suppression of IL-17 and neutrophil-associated responses and restoration of barrier and mitochondrial-lipid programs, while stromal and myeloid compartments displayed secondary adaptation. Cross-species analysis demonstrated that resolution-associated gene networks are inversely regulated in human psoriasis. Integrated proteomic and transcriptomic analyses further identified a conserved epithelial regulatory triad whose concordant regulation in psoriasis and atopic dermatitis, and whose in vivo silencing, establish mechanistic control of disease severity. Together, these findings indicate that inflammatory resolution reflects reorganization of epidermal transcriptional networks and position epithelial state as a determinant of inflammatory persistence.

042

Stromal immune circuits distinguish cutaneous from ileal Crohn's disease despite shared inflammatory programs

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Crohn's disease (CD) affects multiple barrier tissues, yet mechanisms underlying tissue-specific inflammation remain unclear. Here, we aim to define shared and tissue-specific signatures in CD using single-cell and spatial RNA sequencing. We analyzed datasets from ileal and cutaneous CD together with matched control ileum and skin. Analysis of cell proportions revealed expansion of TNC+ myofibroblasts, neutrophils, M1 macrophages, CD4 memory T cells and B cell subsets including plasma cells in CD. In the skin, we found pronounced increase of B cells and plasma cells, suggestive of tertiary lymphoid structure (TLS) formation.
Pathway analysis showed that both ileal and cutaneous CD exhibited a shared inflammatory program defined by interferon (STAT1/IRF pathway), TNF/NFκB activation, and myeloid programs suggestive of IL-1 activation, together with chemokine-mediated recruitment (*CXCL9/10/11, CCL2/20).* Ileal CD showed epithelial dysfunction, with inflammatory M1-like macrophages (*IL1B, IL6, PTGS2*) and stressed epithelial cells (*LCN2*, CXCL chemokines), consistent with a barrier-centric axis. In contrast, cutaneous CD showed an organized fibroinflammatory network. TNC+ myofibroblasts and inflammatory pericytes expressed neutrophil chemokines, matrix-remodeling enzymes (MMPs), and lymphoid-organizing signals (*CXCL13, CCL19, LTB*). Furthermore, cutaneous CD exhibited macrophage activation (*IL1B, CHI3L1, SPP1*) and a signature in keratinocytes indicative of stress responses (*KRT6/16, S100A8/A9*).
Together, CD skin and ileum share inflammatory programs but diverge into tissue-specific circuits. Our results suggest that cutaneous CD is driven by a stromal, lymphoid-organizing niche, whereas ileal CD reflects myeloid–epithelial barrier dysfunction.

043

Single-cell transcriptomics reveals a distinct molecular signature of the follicular phenotype in hidradenitis suppurativa

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Hidradenitis Suppurativa (HS) is a chronic inflammatory disease affecting skin areas rich in apocrine glands, such as the armpits and groin. Yet, it presents with significant clinical heterogeneity, including a follicular phenotype associated with earlier onset, longer disease duration, and greater severity. In this phenotype, lesions frequently occur on in the back or face. Here, we aimed to investigate the pathogenesis of follicular HS by analyzing follicular HS lesions and comparing them with typical HS lesions and healthy skin. We sampled 4 inflammatory follicular HS lesions from the back, 6 early typical HS lesions, 2 peri-lesional skin samples from typical HS patients, and skin from 6 healthy donors (3 from apocrine regions and 3 from back). We then performed scRNAseq using the Flex/Chromium technology platform (10x Genomics). The final dataset after filtering comprised 141,552 cells and 19 clusters were identified across all samples. Pseudo-bulk aggregation of gene expression profiles revealed a clear separation between typical HS, follicular HS, and control skin. The comparison between typical and follicular HS showed an increased proliferative signature in follicular HS. Sub-clustering analysis of keratinocytes showed that bulge, hair follicle progenitors, isthmus and outer root sheath cells displayed upregulation of genes involved in interferon

signaling and DNA damage. Overall, our study suggests that the phenotypic differences arise from specific transcriptional differences and emphasizes the need for a deeper understanding of the molecular mechanisms of HS endotypes.

044 [WITHDRAWN]

045

A cytotoxic plasmacytoid dendritic cell subset contributes to interface dermatitis in lichen planus

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Plasmacytoid dendritic cells (pDCs) are known for their dual role in immunity: they contribute to innate responses through robust type I interferon (IFN) production and support adaptive immunity by activating T cells. These functions are thought to be carried out by distinct subsets that emerge following pDC activation, although the mechanisms driving this functional divergence remain unclear.
In this study, we used single-cell RNA sequencing to characterize pDC activation and differentiation after stimulation with TLR7 and TLR9 ligands. We identified 3 transcriptionally distinct activated pDC populations: IFN-producing pDCs (IFN-pDCs) and two non–IFN-producing subsets defined by differential MHC class II expression, termed MHCII^low and MHCII^high pDCs. While IFN-pDCs and MHCII^high pDCs align with previously described subsets associated with innate and adaptive functions, respectively, the MHCII^low subset has not been reported before. Moreover, MHCII^low pDCs produced high levels of granzyme B upon stimulation, suggesting a potential cytotoxic role.
Analysis of patient-derived skin samples from inflammatory skin diseases revealed an enrichment of MHCII^low pDCs in lichen planus lesions. These cells were frequently observed in direct contact with apoptotic keratinocytes in the basal epidermal layer, indicating that pDCs may directly contribute to keratinocyte death.
Overall, our findings uncover previously unrecognized functional diversity within activated pDCs and identify a cytotoxic subset that may play a key role in the pathogenesis of interface dermatitis in lichen planus.

046

Spatial Transcriptomics Reveals the Senescence-Immune Crosstalk driven by Senescence-Associated Secretory Phenotype and the $p16^{INK4a}$ Pathway in Vitiligo

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Vitiligo is an acquired depigmenting disorder characterized by the loss of melanocytes, involving CD8 T cell-mediated destruction and oxidative stress. Despite these recognized factors, the spatially localized gene expression patterns and the complex crosstalk among various skin cells within the tissue context remain poorly defined. To evaluate the spatial patterns of gene expression and cell-to-cell interactions underlying vitiligo, we performed spatial transcriptomic profiling on skin samples from patients with vitiligo (n=3), psoriasis (n=3), and healthy controls (n=3). Direct comparison revealed that while psoriasis was characterized by epidermal keratinization and upregulated IL-17 signaling, vitiligo was uniquely defined by the enrichment of extracellular matrix-related genes and significantly elevated *APOD* expression, which spatially correlated with Tc cells and fibroblasts. Cell type deconvolution identified distinct cellular recruitment patterns revealing that keratinocytes correlated with Th cells, whereas melanocytes showed strong spatial associations with Tc cells and Langerhans cells in vitiligo. The vitiligo inflammatory landscape was further typified by a prominent senescence-associated secretory phenotype (SASP) pathway that positively correlated with Tc cell infiltration. Meanwhile, the $p16^{INK4a}$ pathway showed a negative correlation with melanocytes. Crucially, the CCND2–NFκB gene pair emerged as a consistent spatial interactor across all vitiligo samples. This molecular axis was reinforced by the broad co-localization of CXCL chemokines with immune receptors, specifically CXCL1 with CCR7/CXCR3 and CXCL8 with CCR7. In conclusion, this study provides a high-resolution spatial map of the vitiligo immune landscape, highlighting that melanocyte loss is driven by distinct spatial networks involving SASP signaling and CCND2–NFκB interactions.

047

Prenatal Enhanced Immunological Experience Protects Against Atopic Dermatitis

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While atopic dermatitis (AD) frequently begins in infancy, its precise developmental drivers remain unclear. We aimed to identify early immune signatures associated with the condition and assess maternal influences on the fetal immune environment. Using the Munich Atopy Prediction Study (MAPS), a birth cohort of 407 mother-infant pairs, we analyzed cord blood at birth and peripheral blood at age three.
We employed a multi-omics approach that integrated the measurement of over 30 cytokines and chemokines with metabolomics, specifically focusing on short-chain fatty acids (SCFAs) in cord blood and maternal stool. Additionally, we performed functional assays on peripheral blood mononuclear cells (PBMCs), exposing them to microbial stimuli including TLR2/6 ligands, patient-derived Staphylococcus aureus, and skin commensals to gauge early-life responses to dysbiosis.
Our results demonstrated an unexpected upregulation of the inflammatory response in the cord blood of healthy infants compared to those who later developed AD. This protective signature was a robust, mixed Th2/Th1 response characterized by elevated levels of IL-13, TNF-α, IFN-γ, CXCL9, CXCL10, and CXCL11. By age three, children with AD had shifted toward a classic, isolated Th2 signature. Microbial stimulation of control infant PBMCs confirmed the presence of this robust, protective mixed response. Although maternal gut microbiome composition was similar across groups, the functional SCFA profile differed and was directly traceable to the infant's cord blood.
Ultimately, these data suggest that an enhanced prenatal immunological experience, potentially primed by maternal microbial metabolites, may protect against the development of AD, providing a new framework for future biomarker and prevention strategies.

048

Lower levels of brain-derived neurotrophic factor is characteristic for psoriatic non-lesional epidermis and modulate keratinocyte proliferation and immune response

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Stress-related regulation and distinct cytokine and chemokine profiles characterize the psoriatic non-lesional (PS-NL) skin of patients with plaque-type psoriasis, and can contribute to preventing lesion formation. We previously identified that brain-derived neurotrophic factor (BDNF) shows a psoriasis severity-dependent decreased expression in PS-NL epidermis. In this study, we examined the possible role of the reduced BDNF levels in the behaviour of keratinocytes. BDNF expression was assessed by immunofluorescence staining (IFS) in healthy vs. lesional skin of untreated and never-lesional vs. resolved PS-NL skin of treated patients and in different cell cycle phases of HaCaT keratinocytes. Reduced BDNF levels of the PS-NL epidermis were modelled in HaCaT cells by siRNA silencing and its effect was tested by MTT- and BrdU cell proliferation assays, CytoSmart time-lapse microscopic analysis, FOXO1 and p27 Kip1 IFS and cytokine/chemokine protein array followed by analysis. Reduced expression of BDNF was detected in never-lesional vs. resolved, and not observed in the lesional vs. healthy epidermis. BDNF expression of resting HaCaT keratinocytes was lower compared to growing cultures. BDNF silencing resulted in reduced cell proliferation and increased expression of FOXO1 and p27 Kip1 . BDNF silencing increased TNFSF6, FGF2, CXCL1, IL-6, and IL-18 levels, mainly affecting IL-4/IL-13 and IL-10 signaling, while reduced IL-10, IL-19, IL-22, IL-23A, IL-24, and IL-27 expression, with primary effects on IL-20 family and IL-12 signaling pathways. These findings highlight BDNF as a novel factor that contributes to preventing lesion formation in PS-NL epidermis by attenuating keratinocyte proliferation and modulating the local tissue cytokine environment.

049

Early intervention with secukinumab prevents epigenetic scar development in new-onset psoriasis – STEPIn Mechanistic Sub-study results

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Psoriasis is a common chronic inflammatory skin disease characterized by recurrent flares at previously affected locations. Although biologics targeting IL-17 or IL-23 effectively suppress active disease, they rarely achieve long-term remission, suggesting that clinically resolved skin retains molecular changes predisposing to relapse.
The STEPIn Main Study indicated that early intervention with secukinumab, an anti-IL-17A monoclonal antibody, may modify the course of psoriasis. The accompanying Mechanistic Sub-study presented here sought to define cellular and molecular changes in psoriatic skin lesions during treatment.
Psoriatic skin was sampled at baseline and up to 52 weeks of secukinumab treatment in new-onset (≤1 year) and long-standing (≥5 years) cohorts. Biopsies were evaluated histologically by immune profiling, global gene expression, and genome-wide DNA CpG methylation analyses.
Treatment with secukinumab resulted in marked molecular differences between new-onset and long-standing psoriasis despite similar clinical improvement. Gene expression normalized faster in new-onset disease but ultimately resolved in both cohorts. In contrast, disease-associated CpG methylation detected at baseline persisted despite treatment but only in long-standing psoriasis. Baseline methylation differences were enriched for AP-1 transcription factor binding sites in long-standing disease and persistent methylation changes mapped to genes enriched for small GTPase pathways. Notably, AP-1 components were found to amplify IL-17A and tumor necrosis factor-α responses in keratinocytes and a subset of these persistent methylation sites overlapped with accessible chromatin regions in psoriatic keratinocytes.
Early secukinumab intervention may prevent formation of an epigenetic “scar” that persists in long standing psoriasis even after clinical resolution.

050

IL-13Rα2 mediates intracellular signaling and JNK activation in human keratinocytes

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IL-13Rα2 has long been considered a “decoy” receptor due to its short cytoplasmic tail and the apparent lack of a signaling domain. However, recent evidence suggests that IL-13Rα2 can actively participate in intracellular signaling pathways involved in cell proliferation and disease progression. In particular, the IL-13/IL-13Rα2 axis has been linked to activation of AP-1 in cancer and is upregulated in skin disorders such as atopic dermatitis in response to scratching and positive regulation induced by IL-13, IL-4, and TNF-α. Despite these findings, the precise role of IL-13Rα2 in healthy keratinocytes remains unclear.
This study aimed to investigate whether IL-13Rα2 functions as a signaling receptor in human keratinocytes. Primary keratinocytes from donors of different ages were analyzed, showing that IL-13Rα1 increases with age, while IL-13Rα2 is higher in younger cells. This pattern suggests a potential role for IL-13Rα2 in enhancing non-canonical signaling pathways such as JNK/AP-1, which may support cellular adaptability and proliferation. As a confirmation of these findings, cell proliferation assessed by MTT assay showed increased proliferation in young keratinocytes stimulated with IL-13, as well as in

keratinocytes with IL-13Rα1 silencing. Functional experiments showed that IL-13 stimulation induces JNK phosphorylation (p-JNK), which decreases when IL-13Rα2 is silenced and increases when it is overexpressed. Even when IL-13Rα1 was silenced, IL-13Rα2 overexpression still enhanced p-JNK levels, indicating that IL-13Rα2 can independently drive signaling.

051

Non-communicable inflammatory skin diseases comprise clinically meaningful distinct endotypes as identified by non-hypothesized integration of phenotypic and transcriptome data

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Non-communicable inflammatory skin diseases (ncISDs) comprise over 100 conditions with overlapping clinical features. Current classification systems are largely based on morphology and do not adequately reflect underlying molecular mechanisms, limiting the implementation of precision therapies.
Here, we aimed to establish a data-driven, molecular framework for stratifying ncISDs independent of conventional diagnostic categories. We integrated 727 skin transcriptomes with clinical metadata from 390 patients across 22 ncISDs and applied unsupervised and machine-learning approaches to identify molecular endotypes and develop a predictive classifier. Thirteen distinct molecular endotypes were identified, which segregated into two major groups: seven driven by immune response programs and six by metabolic and epidermal structural pathways. Common diseases such as psoriasis and eczema distributed across multiple endotypes, highlighting substantial molecular heterogeneity. Psoriasis-dominated endotypes showed differential responses to IL-23-, IL-17-, and TNF-targeted therapies, underscoring clinical relevance. A multi-endotype classifier achieved 81.4% accuracy and was validated in independent cohorts. These findings define a molecularly informed framework for disease stratification that transcends traditional diagnostic boundaries and enables more precise, mechanism-based therapeutic decision-making across inflammatory diseases.

052 [WITHDRAWN]

053

Nanoplastics in Atopic Dermatitis: Effects on Barrier Function and Th2-Associated Signaling

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Micro- and nanoplastics (NPs) are ubiquitous environmental contaminants increasingly recognized for their capacity to interact with epithelial and immune systems. However, their contribution to skin inflammation and barrier dysfunction, particularly in atopic dermatitis (AD), remains poorly defined. Here, we investigated the impact of NPs on epithelial activation, barrier integrity, and Th2 polarization using complementary in vivo, ex vivo, and in vitro models.
An AD-like murine model was established in Balb/c mice by tape stripping followed by topical application of NPs and/or Staphylococcus aureus. Barrier function was assessed by transepidermal water loss (TEWL). Immune responses were characterized by flow cytometry, while transcriptional and protein expression of Th2 cytokines, epithelial alarmins, and barrier-related markers were analyzed by qPCR and multiplex assays. Human skin explants were cultured under air–liquid interface conditions with optional mechanical barrier disruption and IL-4/IL-13–induced Th2 polarization prior to NP exposure. Primary human keratinocytes were exposed to increasing NP concentrations under basal or Th2-skewed conditions.
NP exposure in vivo significantly increased TEWL, indicating barrier impairment, and was associated with downregulation of barrier-related genes as well as upregulation of epithelial alarmins, Th2-associated cytokines and transcription factors. These effects were amplified by *S. aureus*. In human skin explants, NPs induced epithelial alarmin and Th2-associated chemokine expression independent of barrier disruption, with no additional effect under established Th2 polarization. In keratinocytes, NPs triggered a dose-dependent epithelial stress response coupled to Th2-associated signaling and reduced barrier gene expression.
Together, these data position NPs as epithelial stressors that lower the threshold for type 2 immune activation and exacerbate barrier vulnerability, supporting a role for environmental plastic exposure as a disease-modifying factor in AD rather than a mere bystander.

054

Therapeutic Target of Interleukin-15 and Role of LAMP3+CCR7+IL-15+ Dendritic Cells in Atopic Dermatitis

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Interleukin-15 (IL-15) is a key regulator of immune responses implicated in inflammatory disorders such as celiac disease and eosinophilic esophagitis. Its role in atopic dermatitis (AD) remains unclear. We investigated IL-15's contribution to AD-associated inflammation and whether targeting IL-15 could reduce disease severity. *IL15* mRNA and protein levels were quantified in serum, lesional and non-lesional AD skin, and healthy controls. Public AD transcriptomic datasets were analyzed to assess IL15 expression and cellular sources. IL-15 transpresentation impact on cytokine production by activated T-cell was evaluated, and the effect of IL-15 blockade was assessed. Efficacy of an anti–IL-15 antibody was evaluated in ex vivo AD skin and a murine AD model. IL-15 levels were elevated in serum and lesional AD skin, primarily produced by LAMP3+CCR7+ dendritic cells. IL-15 transpresentation to T-cells induced an AD-relevant cytokine profile including IL-13. IL-15 blockade reduced inflammation ex vivo and disease severity in the murine AD model, supporting IL-15 as a potential therapeutic target in AD. This study highlights the key role of IL-15 in the physiopathology of AD and its potential as a therapeutic target.

055

Tapinarof restrains IL-17-producing human memory T cells through metabolic reprogramming

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Psoriasis often returns in the same areas after treatment stops, suggesting that long-lived immune cells in the skin preserve a local memory of disease. Here we show that tapinarof, a topical aryl hydrocarbon receptor agonist notable for inducing prolonged remissions after treatment cessation, directly restrains pathogenic human memory T cell responses through metabolic reprogramming. In skin from patients with psoriasis, tapinarof preferentially reduced interleukin-17 production by skin-resident $CD4^+$ T cells. In human memory T cells, it induced a shared transcriptional program that suppressed fatty acid oxidation and cholesterol biosynthesis, with the strongest functional effects in T_H17 cells. These cells were particularly sensitive because they relied more strongly on fatty acid-supported mitochondrial metabolism and on sterol-dependent support of the T_H17 program. In human skin explants, topical tapinarof recapitulated these immune effects while also enhancing epidermal differentiation and attenuating psoriasiform inflammation. Together, our findings identify a metabolic mechanism by which aryl hydrocarbon receptor activation constrains pathogenic T cell responses in psoriasis and may contribute to durable disease control.

056

Single cell spatial profiling of lichen planus

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Lichen planus (LP) is a chronic autoimmune mucocutaneous disease characterized by pruritic skin lesions, painful mucosal ulcers, cicatricial alopecia and nail dystrophy that affects 1-2% of the global population. Currently, no FDA-approved or EMA-approved therapies exist, and conventional treatments are often inadequate. Recent single cell and spatial transcriptomics studies have identified $CD8^+CXCL13^+$ T cells as unique, potentially pathogenic T cells in cutaneous and mucosal LP. Here we extend these findings in a larger cohort of patients, performing Xenium in situ transcriptomics using 5K human immunology gene probe panel on 86 patient biopsies. Specifically, cutaneous LP (cLP, n=63), lichenoid drug eruption (LDE, n=12), lichen planus-like keratoses (LPLK, n=6), and control skin (n=5) were included in this study, and a total of 771,300 cells were spatially identified. Immunohistochemistry did not show any significant differences in number of $CD3^+$ T cells among cLP, LDE, and LPLK. However, we identified colocalization of CD8+ T cells with CXCL13 in both cLP and LDE, but not in LPLK or control skin, suggesting that drug-induced lichenoid eruptions may share a pathogenic cytotoxic T cell subset. In both cLP and LDE, there is robust transcriptional dysregulation of type I and II interferon downstream genes, including MX1, IFITL, IFI44L (type I) and IFNG, GBP1, IDO1 (type II) as well as chemokines CXCL9, CXCL10, CXCL11. In summary, our data provides a comprehensive single cell spatial atlas of cLP and allows for comparative analysis of other lichenoid dermatoses, including LDE.

057

Type 2 cutaneous inflammation licenses vagus nerve-dependent oral anaphylaxis

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Food allergy is a life-threatening allergic reaction driven mast cell activation. It is widely believed that gastrointestinal (GI) mast cells react to orally ingested antigens via cell surface IgE, resulting in the release of effector molecules that promote anaphylaxis. However, the precise mechanisms that promote susceptibility to food allergy remains poorly understood. It is well appreciated that atopic dermatitis (AD) predisposes to food allergy. Thus, we employed a murine model of AD in which mice are epicutaneously exposed to the model food antigen ovalbumin (OVA) to interrogate mechanisms by which anaphylaxis is facilitated in the GI tract. Strikingly, we found that the induction of AD-like disease with the topical irritation MC903 alone reduced the threshold for anaphylaxis in association with the accumulation of gastric mast cells. We also found that gastric challenge with OVA resulted in the activation of sensory neurons within vagal ganglia. Further, selective denervation of vagal sensory neurons with resiniferatoxin (RTX) abolished AD-associated, food allergic anaphylaxis. Collectively, these findings suggest that AD-like type 2 inflammation in the skin activates a GI-vagal axis that enhances susceptibility to anaphylaxis.

058

Endoplasmic reticulum aminopeptidase 2 (*ERAP2*) genotype selectively remodels the HLA class I immunopeptidome of primary human epidermal keratinocytes

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Endoplasmic reticulum aminopeptidase 2 (ERAP2) trims endogenous peptides before loading onto HLA class I molecules. Single nucleotide polymorphisms (SNPs) in *ERAP2* are associated with HLA class I-associated inflammatory diseases, including psoriasis. A common SNP (rs2248374) leads to nonsense-mediated decay of *ERAP2* mRNA, resulting in loss of protein expression. We hypothesized that differences in ERAP2 expression alter the HLA class I-bound peptide known as the

immunopeptidome. This study investigates how distinct *ERAP2* genotypes affect HLA-C expression and the immunopeptidome in primary human epidermal keratinocytes (HEKs). HEKs were established from skin of 34 healthy donors and genotyped for *ERAP2* (rs2248374) and *HLA-C* (rs4406273) using real-time PCR allelic discrimination plots. HLA-C surface expression was assessed by flow cytometry. The immunopeptidome was isolated by immunoaffinity purification using the pan-HLA antibody W6/32 and analysed by liquid chromatography– mass spectrometry (LC–MS). Peptides were identified using *de novo* sequencing and database-assisted workflows. A total of 52,228 immunopeptidomes (8–14-mers) were identified. Across *ERAP2* genotypes, global immunopeptidome features, including peptide yield, length distribution, and overall amino acid composition, were comparable. However, *ERAP2* genotype was associated with selective differences in the peptide repertoire, including genotype-specific peptides and distinct abundance patterns among peptides shared across genotypes. Genotype-specific peptides exhibited subtle differences at the P1 position. Restricting analyses to *HLA-C*06:02*–positive donors revealed a higher proportion of genotype-specific peptides. These findings support a role for ERAP2 in shaping keratinocyte antigen presentation at multiple levels by influencing both peptide identity and relative peptide abundance, with effects modulated by HLA background.

059

From West to East for Atopic Dermatitis: Comprehensive molecular profiling of Asian populations identifies novel pathways with therapeutic potential for Atopic Dermatitis

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Immunological and phenotypic profiles of Atopic Dermatitis (AD) in Asian populations differ from those reported in Western cohorts, with variation in disease severity and molecular signatures. We hypothesised that genetic predisposition and environmental exposures contribute to distinct molecular endotypes in Asian AD patients that may inform disease stratification and therapeutic intervention.
We comprehensively profiled N=300 subjects (160 AD, 140 Controls) from ADEPT (Atopic DErmatitis research Program for patienTs) cohort in Singapore using clinical history, immune and skin barrier profiling. We performed immune profiling using high-dimensional flow cytometry, single-cell & circulatory inflammatory panels. Disease severity and barrier measurements were integrated with immune signatures to identify biomarkers and candidate pathways associated with AD.
NULISA profiling identified 48 markers associated with disease severity, including key cytokines and chemokines. Significantly increased SIGLEC-8 expression on eosinophils and elevated FCER1A and IgE expression on basophils was observed in AD patients. Single-cell analysis identified innate immune pathways associated with AD and highlighted granulocyte-associated signatures, complementing flow cytometric findings. IL-18- and IL-1-associated pathways emerged as prominent inflammatory signatures alogn with type 2 pathways in the Asian AD population with increased levels associated with disease severity and poor disease control. Skin barrier dysfunction was significantly increased in AD patients, with elevated transepidermal water loss and reduced hydration levels.
Comprehensive molecular profiling of Asian AD patients identified novel inflammatory pathways with therapeutic relevance. Systemic inflammatory signatures correlated with skin barrier dysfunction, supporting interactions between immune dysregulation and barrier impairment. Our findings validate the importance of eosinophils and basophils while identifying additional therapeutic opportunities through in-depth molecular profiling of innate immunity.

060

The newly identified NAMPT–TLR2 axis orchestrates inflammation and epidermal dysregulation across *in vitro* and *in vivo* models of psoriasis

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Nicotinamide phosphoribosyltransferase (NAMPT) is a pivotal pro-inflammatory mediator in psoriasis, consistently elevated in lesional skin and circulation. Although Toll-like receptor 4 (TLR4) has been regarded as its primary receptor, NAMPT-driven effects are not fully accounted for by TLR4 signaling, indicating additional pathways.
Here, we identify Toll-like receptor 2 (TLR2) as a functional NAMPT receptor in psoriatic disease. Transcriptomic analyses of publicly available datasets validated by qPCR, reveal marked TLR2 upregulation in psoriatic lesions. Immunohistochemistry and primary human keratinocyte studies show TLR2 enrichment in hyperproliferative, aberrantly differentiated cells expressing high Keratin16.
Mechanistically, NAMPT-TLR2 engagement amplifies IL-22-driven inflammatory gene expression and keratinocyte proliferation via STAT3 and ERK activation, while also inducing TLR2 expression. TLR2 silencing significantly blunts these responses, confirming pathway specificity. Proximity ligation assays further demonstrate a direct NAMPT-TLR2 interaction in keratinocytes.
In vivo, the imiquimod-induced psoriasis model exhibits increased cutaneous TLR2 expression with concomitant STAT3 and ERK activation, paralleling epidermal hyperplasia and inflammation. NAMPT neutralization mitigates inflammation and proliferation, restores differentiation markers, and reduces TLR2 expression, consistent with disruption of an autocrine NAMPT-TLR2 loop.
Collectively, these findings establish TLR2 as a previously unrecognized NAMPT receptor and define the NAMPT-TLR2 axis as a central, druggable driver of psoriatic inflammation and epidermal remodeling.

061

Differential disease signatures in baricitinib-treated alopecia areata patients accentuate persistent Th1 inflammation and compensatory cytotoxic/IFN effector activity in non-responders versus responders

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Alopecia areata (AA) can improve under baricitinib treatment, yet 20–35% of patients exhibit primary non-response. This pilot study aimed to delineate molecular and immunological disease signatures distinguishing baricitinib responders from non-responders. 12 AA patients (SALT 50–100) received baricitinib 4 mg/day for 24 weeks; 10 responded (4F/6M; age 22–58 years) and 2 did not (2F; SALT 97.9 and 100). Scalp biopsies were analyzed by RNA-seq, ALADIN disease activity scoring, Th profiling, and immunofluorescence for CD3-, CD8-, and NKG2D-positive infiltrates and MHC class I expression. Innate immune/inflammatory genes (*CCL24*, *FCGR3*) and extracellular matrix/cell adhesion genes (*COL11A2, THBS4*) were differentially regulated in non-responders. ALADIN scores were higher at baseline in non-responders; only responders showed post-treatment reduction in cytotoxic T-lymphocyte and interferon signaling alongside increased keratin activity. Th profiling showed decreased Th1/Th2 and increased Th17/Th22 in responders; non-responders maintained Th1 with reduced Th2 and rising Th17/Th22. Immunofluorescence confirmed greater CD3-, CD8-, and NKG2D-positive infiltration at baseline in non-responders; only responders showed post-treatment decline in intra- and perifollicular T-cells, particularly NKG2D-positive effectors. Only responders showed elevated baseline MHC class I expression, which decreased predominantly in the follicular epithelium after treatment. These preliminary findings suggest JAK inhibitor resistance could arise from persistent Th1 inflammation and compensatory cytotoxic/interferon effector activity, providing mechanistic basis for patient stratification and combination therapeutic strategies.

062

Proteomic signatures of cardiovascular risk are amplified in psoriasis: a UK Biobank proteomics study

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Psoriasis confers increased risk of major adverse cardiovascular events (MACE), yet the biological pathways linking the two remain incompletely defined. Large-scale proteomics enables identification of molecular signatures of cardiovascular risk across disease states.

We analysed UK Biobank Pharma Proteomics Project participants with 2,920 baseline proteins and longitudinal follow-up for MACE. Psoriasis and MACE were defined using linked hospital, questionnaire, procedural, and mortality data. Two populations were studied: prevalent psoriasis (n=936; 132 events, 14.1%) and psoriasis-free (n=39,923; 4,514 events, 11.3%), with ~15 years of follow-up. Cox models with LASSO regularisation identified 28 proteins associated with incident MACE in psoriasis, which were then evaluated in both cohorts using multivariable Cox models adjusted for age, sex, ethnicity, body mass index, and smoking.

Of these, 14 proteins were associated with incident MACE in both cohorts after multiple testing correction (FDR<0.05), including markers of cellular stress (GDF15), immune activation (LAMP3), lipid metabolism (FABP3), and neuroaxonal injury (NEFL). Associations were consistently stronger in psoriasis, with lower FDR-adjusted p-values. Five proteins, including LEO1, SCN2A, CNTNAP2, COPE, and MORN4, were associated only in psoriasis (FDR<0.10), while five were specific to psoriasis-free individuals. Between-cohort heterogeneity was observed for selected proteins, including SCN2A (p=0.018) and CNTNAP2 (p=0.031), with directionally discordant effects. Pathway analysis showed enrichment in immune, cellular stress, and metabolic pathways.

Proteomic associations with incident MACE are stronger in psoriasis, with several proteins demonstrating psoriasis-specific or amplified effects. These findings support a model in which psoriasis modifies protein-cardiovascular risk relationships, providing mechanistic insight into excess cardiovascular risk.

063

***NLRP7* promoter methylation affects epidermolysis bullosa simplex severity**

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Epigenetic mechanisms have recently emerged as potential modifiers of clinical variability in inherited skin diseases. We investigated whether epigenetic modulation of *NLRP7* expression contributes to disease severity in *KRT14* associated epidermolysis bullosa simplex (EBS). Reduced representation bisulfite sequencing (RRBS) in 39 epidermolysis bullosa (EB) patients identified a differentially methylated region spanning the *NLRP7* promoter and first non coding exon in individuals with a severe phenotype. Within this region, two CpG sites (CpG1: 54966285 and CpG2: 54966302) showed consistent hypermethylation in severe *KRT14* associated EBS. To dissect their functional impact, we engineered *NLRP7* promoter first exon constructs in which either one, both or none of the methylation sensitive CpG dinucleotides were preserved, and manipulated methylation using DNMT1/3 with S adenosyl methionine or the hypomethylating agent 5 aza 2′ deoxycytidine. Hypermethylation at either CpG site suppressed *NLRP7* promoter activity and markedly reduced *NLRP7* mRNA and protein expression, whereas hypomethylation rescued these effects. Using luciferase reporter assays, RT qPCR, ELISA and western blotting, we showed that *NLRP7* silencing driven by targeted CpG methylation augments interleukin 1β (IL 1β) and IL 18 secretion and activates NF κB signaling. Neutralization of IL 1β abrogated NF κB activation despite unchanged CpG methylation, indicating that promoter methylation enhances NF κB signaling indirectly via a cytokine dependent amplification loop. Collectively, our findings indicate that *NLRP7* promoter methylation can act as an epigenetic modifier of EBS severity by

amplifying cutaneous inflammation and suggest that therapies targeting IL 1β may be particularly beneficial in EB patients with *NLRP7* promoter hypermethylation.

064

Tapinarof modulates cutaneous dendritic cell populations in human skin

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Tapinarof is a naturally derived small molecule approved for the topical treatment of plaque psoriasis in adults. It restores the epidermal barrier function and reduces inflammation through activation of the Aryl Hydrocarbon Receptor (AhR), a ligand-dependent transcription factor expressed across multiple skin cell types, including keratinocytes, dendritic cells (DCs), and Langerhans cells (LCs).
While AhR activation in keratinocytes promotes terminal differentiation, barrier repair, and anti-inflammatory responses, its effects on cutaneous immune cells, particularly LCs and DCs, remain poorly understood.
This study aimed to characterize the functional and transcriptional responses of LCs and DCs to Tapinarof-mediated AhR activation using human skin explants treated with either topical or soluble Tapinarof. LC and DC populations were analyzed by bulk RNA sequencing, immunofluorescence and flow cytometry.
Tapinarof treatment induced sustained AhR pathway activation, as evidenced by the upregulation of canonical target genes *CYP1A1* and *CYP1B1*. Notably, this was accompanied by marked downregulation of LC-associated markers *CD1A* and *CD207*. Immunofluorescence analysis revealed a reduction of $CD1a^+CD207^+$ LCs in the epidermis, alongside a relative increase in the dermis. Consistently, flow cytometry demonstrated a decrease in $CD1a^+$ cells, suggesting redistribution and phenotypic reprogramming of $CD1a^+$ antigen-presenting cells in the skin.
Collectively, these findings indicate that Tapinarof-driven AhR activation modulates LC distribution and reshapes $CD1a^+$ antigen-presenting cell dynamics in human skin. This highlights a previously unrecognized role for Tapinaorf in cutaneous immune regulation, which may contribute to its anti-inflammatory effects and therapeutic efficacy in psoriasis.

065

Identification of Inflammatory Genes in Acrodermatitis Continua of Hallopeau Compared with Palmoplantar Pustulosis Through Bulk RNA Sequencing

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Acrodermatitis continua of Hallopeau (ACH) and palmoplantar pustular psoriasis (PPP) are localized forms of Pustular Psoriasis (PP), a group of immune mediated skin diseases characterised by a sterile, predominantly epidermal neutrophilic infiltrate. Due to its rare occurrence ACH is the least well understood entity of PP variants. To analyze molecular characteristics and to identify potential therapeutic targets in ACH, punch biopsies of lesional and healthy skin from patients with ACH and PPP were collected and expression profiles were compared.
RNA sequencing identified 72 differentially expressed genes (p value < 0.05, |log2FC| > 0) with 44 upregulated and 28 downregulated in lesional vs. healthy ACH skin. Direct comparison between lesional ACH and PPP skin showed 14 highly regulated genes (p value < 0.05, |log2FC| > 0) of which 5 were upregulated and 9 downregulated. Upregulated genes in lesional ACH skin were located in the IL-36 pathway including IL36RN, IL36G and IL36A. Further, upregulation was noted for genes involved in the JAK-STAT pathway which is part of the IL-23/IL-17 pathway: SOCS3, STAT3, IL6R and IL23A. In direct comparison IL17C and IL17F showed lower expression in ACH compared to PPP lesional skin.
Our results indicate involvement of multiple inflammatory pathways including innate and adaptive immune pathways as therapeutic targets for ACH. Our data show an upregulation of the IL-36 signaling axis and activation of the JAK-STAT pathway. This suggests that Spesolimab, an IL-36 receptor antagonist, could be a promising option for ACH. IL-17 blocking biologics may be less promising as the IL-17 axis was found to be less prominent in ACH compared to PPP. However, JAK-inhibitors or IL-23 biologics may affect the disease.
In conclusion our data provides hints on which drugs, well established in immune-mediated diseases, may help to treat the rare severe forms of ACH.

066

Differential modulation of IL-17 and IL-1 signaling by bimekizumab across hidradenitis suppurativa lesion subtypes in an *ex vivo* model

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Hidradenitis suppurativa (HS) is characterized by follicle-associated nodules and fistulas. Bimekizumab, a humanized monoclonal antibody dually neutralizing interleukin (IL)-17A, IL-17F, and IL-17AF, is approved for moderate-to-severe HS, but its molecular effects across lesional subtypes remain to be clarified. Here, a validated lesional HS skin organ culture model was used to characterize the transcriptomic and proteomic effects of bimekizumab on nodular- and fistulous-lesional as well as perilesional tissue from five patients (age 25-58 years; Hurley stages II-III), cultured for 24 hours with bimekizumab (10 µg/mL) or vehicle control. RNA-seq and multiplex protein secretion analyses were performed on tissue and conditioned media, respectively. Bimekizumab downregulated IL-17-dependent markers (*LCN2*, *CCL20*, *CXCL1*) and IL-1 pathway members (*IL36A*, *IL36G*), along with antimicrobial peptide-encoding genes (*S100A7A*, *CAMP*, *DEFB4A*, *DEFB4B*), predominantly in fistulous-lesional and perilesional skin. Transcriptomic response rates (3-4 of 5 patients) aligned with reported clinical efficacy of 50-75%. At the protein level, bimekizumab reduced IL-1 family cytokines (IL-1β, IL-18, IL-33), IL-6, IL-8, MCP-1, CCL20, hBD2, and Th1/Th17 mediators (IFN-γ, IL-12p70, IL-23) in fistulous-lesional skin, without affecting TNF-α, while nodular lesions were mainly unaffected. These findings demonstrate that bimekizumab suppresses IL-17- and IL-1-driven inflammatory and antimicrobial peptide responses preferentially in fistulous-lesional tissue, consistent with the predominance of IL-17-producing T

cells in chronic HS lesions. This work validates the HS organ culture model as a patient-responsive preclinical platform and supports lesion subtype-specific therapeutic targeting in HS.

067 [WITHDRAWN]

068

Skin barrier dysfunction and stratum corneum ceramide alterations in early-stage mycosis fungoides during topical chlormethine treatment

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Mycosis fungoides (MF) is the most common subtype of primary cutaneous T-cell lymphoma, in which malignant T lymphocytes infiltrate the skin, resulting in patches and plaques with a reduced barrier function. The skin barrier function depends on the stratum corneum lipid matrix, which is largely composed of ceramides. Alterations in the ceramide profile have been associated with the impaired barrier function in benign inflammatory skin diseases and might also be an underlying factor in MF. This study aims to characterize the stratum corneum ceramide profile and barrier function in lesional and non-lesional skin of MF patients compared to healthy volunteers, and to assess the effect of treatment with chlormethine gel.
21 early-stage MF patients and 10 healthy volunteers participated in the study. Ceramides were collected by tape stripping at baseline and after 16 weeks of treatment and analyzed using liquid chromatography-mass spectrometry. Barrier function was assessed by measuring transepidermal water loss.
The ceramide profile of lesional skin in MF was significantly different from non-lesional and healthy skin and correlated with the reduced barrier function. Specifically, lesional skin showed an altered ceramide subclass composition and reduced average ceramide chain length. These changes were partially normalized with chlormethine treatment. Importantly, ceramide characteristics correlated with barrier function and clinical lesional severity over time, highlighting their interdependence.
To conclude, MF lesions exhibit a distinct and altered ceramide profile compared to non-lesional and healthy skin, which can partially be restored with topical chlormethine treatment.

069

Boosting Endocannabinoid Signaling: FAAH Inhibition Improves Psoriasis-Like Skin Features in 2D and 3D *in vitro* models

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The endocannabinoid system (ECS) has emerged as a key lipid signaling network in the skin, orchestrating keratinocyte proliferation and differentiation, barrier integrity, and cutaneous immune responses. Dysregulation of ECS signaling may contribute to psoriasis, a chronic inflammatory skin disease marked by Th1/Th17-driven immune activation and aberrant keratinocyte behavior. In this study, we explored whether enhancing endocannabinoid tone through inhibition of fatty acid amide hydrolase (FAAH), a central enzyme in endocannabinoid degradation, could mitigate psoriasis-associated alterations. To this end, we employed advanced human-based models, including 3D full-thickness psoriatic skin constructs, 3D epidermal equivalents, and primary human keratinocytes stimulated with psoriasis-related cytokines, in the presence of the selective FAAH inhibitor URB597. Integrated transcriptomic, lipidomic, and cellular analyses revealed that URB597 markedly attenuates cytokine-induced psoriatic features by restoring keratinocyte differentiation programs and rebalancing key enzymes involved in epidermal lipid metabolism. Notably, FAAH inhibition also exerted pronounced anti-inflammatory effects, reducing the levels of bioactive lipids and signaling mediators implicated in disease progression. Overall, our findings highlight the ECS as a crucial regulator of skin homeostasis and FAAH inhibition may therefore represent an innovative and effective approach for the treatment of psoriasis and other inflammatory skin disorders.

070

A scRNA-seq atlas of chronic inflammatory skin diseases

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Inflammatory skin diseases (ISDs) impact roughly a quarter of the global population, yet differential diagnosis can be challenging, and effective targeted therapies are lacking for many diseases. Although single-cell RNA sequencing (scRNA-seq) has revolutionized our understanding of individual diseases, the molecular diversity and common pathogenic pathways across ISDs remain largely unexplored. Most scRNA-seq studies to date have concentrated on comparisons within a single disease (lesional vs. non-lesional or healthy skin), with limited studies comparing multiple skin conditions simultaneously.
To bridge this gap, we combined in-house and publicly available scRNA-seq datasets covering 27 ISDs from 50 studies, totaling more than 2 million cells from 441 samples. The resulting Inflammatory Skin Disease Atlas (ISDA) allows for the differentiation of universal inflammatory patterns from those specific to individual diseases. The reliability of our integrated dataset was confirmed by reproducing previously established findings. Furthermore, our analysis uncovered new insights, including the transcriptional upregulation of CXCL13, a chemokine traditionally associated with B cell recruitment, in T cells across almost all ISDs. In contrast, only a few diseases showed an increase in B cell numbers within lesions, indicating a context-specific function for CXCL13.
This discrepancy demonstrates the capability of the ISDA to distinguish between shared and distinct immune mechanisms, offering a solid basis for investigating skin disease pathogenesis at single-cell resolution.

071

Targeting type 2 cytokines in atopic dermatitis includes upregulation of immune signatures of resolution

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Atopic dermatitis (AD) is a chronic, recurrent inflammatory skin disease characterized by barrier disruption (BD), *Staphylococcus aureus* (SA) predominance, and a type 2 immune response. Current treatments for AD, which target the key type 2 immune cytokines IL-4 and IL-13, are effective at suppressing inflammation; however, the role of these cytokines in the early phase of AD has not been fully elucidated, as does the contribution of BD and SA to their modulation. This study aimed to identify how BD and SA drive AD and how intervention modulates AD inflammation. To this end, we established a preclinical model mimicking early AD by applying BD and SA to healthy skin. Application of BD and SA alone induced only partial inflammatory features, whereas the combination of both provoked AD-like dermatitis, characterized by increased transepidermal water loss, epidermal spongiosis, increased type 2 immune cell counts, and cytokine expression. Cutaneous IL-13-producing cells appeared early, and deletion of IL-13 significantly reduced AD-like dermatitis and simultaneously enhanced signatures of immune resolution, characterized by increased proportions of regulatory T cells and IL-10 protein levels. These findings were confirmed in mice that received an anti-IL-4Rα antibody blocking IL-4 and IL-13. Conversely, cellular exposure to IL-13 significantly suppressed IL-10 and TGF-β. To evaluate these findings in humans, we reanalyzed data from clinical trials of dupilumab, a human anti-IL-4Ra antibody, and confirmed an upregulation of signatures of immune resolution in skin and peripheral blood upon effective treatment.
Therefore, targeting IL-13 alone or IL-4 and IL-13 restores endogenous signatures of immune resolution, which may be critical for long-term AD control and disease modulation.

072

Prurigo nodularis with poor response to dupilumab presents keratinocyte-dominant gene expression in skin.

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Prurigo nodularis is a chronic inflammatory dermatosis with a high response rate to dupilumab. However, patient stratification with reliable biomarkers to identify non-responders are lacking. We aimed to identify transcriptomic features associated with treatment response. Lesional skin biopsies from 20 untreated prurigo nodularis patients and 20 healthy subjects underwent transcriptomic profiling by bulk mRNA sequencing, followed by differential expression and gene set enrichment analysis (GSEA). Among patients, response status to dupilumab, defined by pruritus reduction at 9 months, was available for 9 individuals (5 responders = R and 4 non-responders = NR).
Lesional skin from patients showed clear separation from healthy controls, while R and NR exhibited partial segregation. NR lesions were characterized by upregulation of genes related to epidermal differentiation and barrier function, particularly keratinization. In contrast, R lesions displayed increased expression of inflammatory genes, notably interferon-induced and immune activation pathways. GSEA confirmed a predominance of inflammatory and antiviral responses in R, whereas NR showed enrichment of epidermal development pathways. These differences were not explained by cell composition, as deconvolution showed similar cell proportions between R and NR, including keratinocytes.
To assess whether these transcriptional programs extended beyond patients with known response status, correlation analyses were performed across all prurigo nodularis samples. This approach identified two major and inversely regulated gene clusters, one associated with epidermal differentiation and barrier function and the other with inflammation and proliferation, indicating that these programs are conserved across the broader disease cohort.
These findings reveal two distinct molecular programs underlying prurigo nodularis, associated with differential response to dupilumab. A predominantly inflammatory signature appears to predict better therapeutic response, whereas a keratinocyte-driven profile is associated with poor response, supporting a role for molecular stratification in personalized treatment approaches.

073

Comparative Transcriptomic Analysis Across Atopic Dermatitis Severities and Implications for Clinical Trials and Therapeutic Development.

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Transcriptomic data from mild to moderate atopic dermatitis(AD) skin biopsies were compared with the TREATgermany moderate to severe cohort to evaluate similarities across disease severity.
In total, 72 matched lesional and non-lesional skin biopsy samples were collected from 39 patients participating in a Dutch clinical trial. Lesional and non-lesional skin biopsies were collected across disease severities. The samples were profiles using bulk RNA sequencing and jointly analyzed with samples from the TREATgermany cohort (GEO ID GSE157194). The clustering of samples was inspected using PCA, followed by differential gene expression and pathway analysis using GSEA to compare lesional vs. non-lesional samples.
The Dutch study population had a mean age of 22.6 years (SD 3.8) and a mean body mass index of 22.3 kg/m2 (SD 3.1). Based on the Investigator's Global Assessment (IGA) and objective SCORAD (oSCORAD), most patients were classified as having mild AD (69.2% and 51.3%, respectively), compared with moderate disease (30.8% and 48.7%, respectively). The

transcriptomic profiles of the study population were consistent with previously reported eczema datasets. Within the Dutch cohort, transcriptomic features were largely comparable when the samples were stratified by global severity measures (EASI, IGA, and SCORAD). In contrast, stratification by local SCORAD, enriched with a more severe population from the TREATgermany cohort reflecting lesional severity, revealed distinct transcriptomic differences.
When stratified by global severity measures, patients with mild to moderate AD showed transcriptomic profiles comparable to moderate to severe AD, whereas local severity measures were more strongly associated with transcriptomic variation. These findings support the inclusion of AD patients with limited overall disease but moderate to severe local involvement in clinical trials of biological and systemic therapies. This may facilitate recruitment in early phase studies, where enrollment of treatment naïve moderate to severe AD patients is increasingly challenging

074

Epidermal distribution of the interleukin-1 cytokine network may explain therapeutic resistance to biologics in atopic dermatitis

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Despite the established pathogenic role of interleukin-33 (IL-33) in atopic dermatitis (AD), clinical trials evaluating its blockade showed limited efficacy. We hypothesized that this therapeutic failure is driven by intense post-transcriptional regulation and spatial pharmacokinetic barriers within the epidermal IL-1 family network.
We generated a novel layer-resolved spatial mRNA map using public single-cell RNA sequencing data, integrated with in-house spatial immunofluorescence protein profiling of keratinocytes. To distinguish AD-specific hallmarks from general inflammation, we compared normal skin, non-lesional AD (ADNL), lesional AD (ADL), and psoriatic lesions (PSL).
While *IL33*, *IL1A*, *IL1B*, *IL18*, and *IL36B* mRNA lacked any disease-specific induction in keratinocytes, IL-33 protein emerged as the sole AD-specific alarmin, accumulating early in subclinical ADNL skin and peaking in ADL. Significantly elevated IL-1α, IL-1β, IL-18, and IL-36β protein levels in ADL represented a generalized inflammatory response shared with PSL. Other family members (IL-1RA, IL-36α, IL-36γ, IL-36RA, IL-37, and IL-38) were not induced at the protein level in ADL. High-resolution spatial mapping identified distinct anatomical niches: IL-33, IL-1α, IL-1β, and IL-36β were strictly localized to the suprabasal and upper layers. In contrast, IL-18 uniquely peaked only in the basal layer.
The therapeutic failure of anti-IL-33 monoclonal antibodies in AD may be driven not only by a temporal mismatch of targeting an early-phase alarmin in chronic disease but also by profound pharmacokinetic diffusion barriers that shield suprabasal targets from these large biologics. Future interventions should account for these spatial limitations by utilizing small-molecule inhibitors that can penetrate the epidermis. Additionally, despite lacking AD-specificity, the basal localization of IL-18 can make it a promising therapeutic target in this disease as well.

075

Emerging function of tissue-resident memory T cells in chronic allergic contact dermatitis

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Allergic contact dermatitis (ACD) is a CD8+ T cell–driven inflammatory skin disease that often progresses to chronic forms due to repeated exposure to allergens. Chronic ACD is characterized by profound tissue remodeling, including epidermal hyperplasia and mild papillary dermal fibrosis, yet the immunological mechanisms underlying this transition remain poorly understood.
Here, we explored whether repeated hapten exposure drives a functional reprogramming of CD8+ T cells, uncoupling effector function from pathogenic activity.
Using a chronic DNFB-induced ACD model combined with adoptive transfer, spectral cytometry, histological analysis, and single-cell RNA sequencing, we demonstrate that hapten-specific CD8+ T cells progressively acquire a hyporesponsive state marked by sustained expression of inhibitory receptors (TIM-3, PD-1, CTLA-4, LAG-3) and impaired production of effector molecules (IFN-γ, CCL3, CCL4, XCL1, granzyme B). Despite their accumulation in the tissue, these cells are dispensable for the maintenance of chronic inflammation.
Strikingly, chronically stimulated CD8+ T cells adopt non-canonical functions and actively contribute to tissue remodeling, promoting dermal fibrosis through collagen III deposition. These findings identify a previously unrecognized functional switch in CD8+ T cells, from cytotoxic effectors to tissue-remodeling cells.
Collectively, our results redefine the role of CD8+ T cells in chronic ACD and highlight their contribution to fibrotic remodeling, providing new insights into disease chronicity and paving the way to the discovery of new therapeutic targets.

076

Loss of epidermal miR-149 exacerbates atopic skin inflammation

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Atopic dermatitis (AD) is characterized by a dysfunctional interplay between environmental factors, the epidermal barrier and the immune system. The intrinsic mechanisms regulating keratinocyte responses to environmental triggers and cytokines in AD remain poorly defined. Previously, we identified miR-149 as a miRNA regulating keratinocyte immune responses by linking the IFN-g and TWEAK pathways.
Here, we show that miR-149 is significantly downregulated in AD skin lesions as compared to healthy skin. To explore the potential role of epidermal miR-149 in atopic skin inflammation, we performed the ovalbumin (OVA)-induced AD model in our previously generated mice with keratinocyte-specific deletion of miR-149 (*Mir149^{EKO}*). Following epicutaneous sensitization and challenge with ovalbumin (OVA), *Mir149^{EKO}* mice developed significantly exacerbated skin inflammation compared to wild-type controls. Loss of epidermal miR-149 led to increased skin thickening and elevated transepidermal water loss in OVA-challenged skin. Furthermore, *Mir149^{EKO}* mice exhibited increased serum OVA-specific IgE levels.
Transcriptomic analysis of OVA-challenged skin revealed a distinct pro-inflammatory signature, with significant upregulation of

the TWEAK/TWEAKR axis and IL-6. Gene ontology enrichment highlighted pathways associated with leukocyte migration and innate immune activation. Consistent with these findings, *Mir149^{EKO}* skin showed markedly increased immune cell infiltration, in particular an expansion of mast cells as confirmed by flow cytometry and toluidine blue staining. Notably, mast cell numbers were elevated even in untreated *Mir149^{EKO}* skin as compared to wild-type controls, suggesting a state of baseline immune priming.
Collectively, our findings identify miR-149 as a homeostatic brake in keratinocytes limiting atopic skin inflammation.

077

Lipid signature of epidermal barrier impairment in erythematotelangiectatic rosacea

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Erythematotelangiectatic rosacea (ETR) primarily affects fair-skinned women in the central face areas densely populated with sebaceous glands. Although the epidermal barrier dysfunction is central to rosacea pathophysiology, ETR-specific metabolic and lipidomic alterations remain under-investigated. Given the role of lipids in the barrier integrity and inflammation, this study investigated the lipid profile in both sebum and the stratum corneum (SC) of ETR patients to identify potential biomarkers and mechanism-based therapeutic targets.
The study involved 28 ETR female patients and 26 age-matched healthy controls. Sebum excretion rates (SER) and transepidermal water loss (TEWL) were measured on the forehead and cheeks. Sebum and SC samples, collected via Sebutape™ and Corneofix®, respectively, underwent comprehensive lipidomic profiling using liquid chromatography and gas chromatography coupled with mass spectrometry.
ETR patients exhibited significantly higher TEWL, confirming impaired barrier function, whereas SER was not significantly different. Both groups showed site-specific lipid distribution between the forehead and cheeks. Sebum analysis in ETR revealed mild alterations across most lipid species, regardless the site. In the SC, the overall NDS, NS, NP, NH, and AS ceramides were markedly reduced, particularly long-chain species (C≥40). Two classes of acylceramides, such as the Cer[EODS] and Cer[EOP] classes, displayed significantly lower levels.
Epidermal-type free fatty acids (FFAs) were significantly altered; notably, the ultra-long chain FA 29:0 and FA 30:0, which are essential for the synthesis of acylceramides, were depleted. FA 30:0 correlated positively with ceramide EOS (Cer[EOS]64), suggesting functional interdependence between these species.
This study provides evidence that ETR associates with alterations of distinct FFAs and ceramides supporting the disruptions of specific pathways involved in the skin lipid metabolism that likely contribute to the observed epidermal barrier dysfunction.

078

Visfatin, TNF-α and IFN-γ as key mediators linking psoriasis and obesity: implications for therapeutic response to anti–IL-23 and anti–IL-17 biologic therapies

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Psoriasis is a chronic inflammatory skin disease frequently associated to obesity, which impairs therapeutic response. Obesity is characterized by increased levels of pro-inflammatory adipokines, specifically visfatin and leptin, produced by adipocytes, which promote IL-23/Th17 axis activation - key driver of psoriasis pathogenesis targeted by anti-IL-17 and anti-IL-23 therapies. In psoriatic patients, obesity-associated low-grade inflammation and adipokine dysregulation may compromise the therapeutic efficacy.
This study aimed to investigate the molecular effects of anti-IL-23 and anti-IL-17 biologics on the immune-adipose interplay in psoriasis and *vice versa* the impact of adipose tissue immunomodulation on therapeutic efficacy, in order to identify a therapeutic target for obese patients.
The immunophenotyping of Th1, Th17 and T-reg subsets was performed on circulating PBMCs from 40 psoriatic patients before and after 16 weeks of anti-IL-17 or anti-IL-23 treatments. Lymphokines production and mRNA expression, as well as serum cytokines and adipokines levels were assessed and correlated with clinical outcomes. In parallel, adipocytes derived from stem cells of obese donors were stimulated with inflammatory molecules resulted associated with reduced therapeutic response. Both therapies reduced visfatin in serum and PBMCs and decreased CD4+/TNF-α+ and CD4+/IFN-γ+ lymphocytes. Notably, patients with reduced response to biologics exhibited higher BMI, no decline in visfatin and TNF-α after therapy and higher baseline IFN-γ levels. Both therapies increased T-regs whereas only anti-IL-23 decreased Th17 and CD4+/IL-17A+ cells. In parallel, visfatin, TNF-α and IFN-γ triggered adipocytes to produce IL-23, IL-22, TNF-α, IL-6 cytokines, CCL-20, CXCL-8 chemokines, and visfatin. These data identified visfatin, TNF-α and IFN-γ as key mediators of immune system/adipose tissue axis in psoriasis and visfatin as a potential therapeutic target for treatment-resistant obese patients.

079 [WITHDRAWN]

080

Investigating the Skin-Joint Axis: Spatial Neighbourhood Analysis Reveals Immune Cell Escape Mechanisms in Psoriatic Arthritis

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Psoriasis and psoriatic arthritis involve interconnected pathologies of the skin and joints, yet the mechanisms driving the transition from cutaneous to joint inflammation remain unclear. This study aims to characterize the skin-joint axis by spatially profiling immune populations in psoriasis and psoriatic arthritis lesional skin. We utilized imaging mass cytometry to assess broad lineage and functional markers, enabling the precise resolution of heterogeneous immune populations and their spatial architecture. All three major innate lymphoid cell subtypes were detectable in psoriatic skin lesions. Furthermore, we successfully defined Interleukin-17A (IL-17A)-expressing T cells and tissue-resident memory T cells (TRMs). Crucially, through spatial neighborhood analysis, we observed an increased interaction of IL-17A-producing cells with vessels in psoriatic arthritis skin compared to psoriasis skin. Additionally, skin-limited psoriasis samples exhibited a higher frequency of TRMs and a trend toward stronger expression of the CXCL16/CXCR6 axis. Specifically, CXCR6 expression on CD4+ TRMs was notably reduced in psoriatic arthritis compared to skin-limited disease. The increased perivascular localization of IL-17A-producing cells in psoriatic arthritis lesional skin suggests an enhanced escape tendency of these immune cells from the cutaneous microenvironment. We conclude that spatially resolved profiling uncovers distinct disease-driving factors, shedding light on the mechanisms of psoriatic arthritis and providing a strong foundation to identify biomarkers predictive of joint involvement.

081

Partial deletion of the filaggrin gene compromises epidermal differentiation and alters keratinocyte proliferation.

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Filaggrin deficiency resulting from a pathogenic loss-of-function variant in the *FLG* gene is a major genetic risk factor for atopic dermatitis (AD). The most common *FLG* pathogenic variants introduce a premature stop codon, terminating translation and resulting in a truncated, nonfunctional protein. In keratinocytes, reduced filaggrin levels are associated with impaired differentiation, resulting in a defective epidermal barrier. The aim is to investigate the effect of partial filaggrin deletion on keratinocyte proliferation and differentiation.
In this study, we used N/TERT-2G keratinocytes in which the desired part of the *FLG* gene was knocked out (KO) using a CRISPR/Cas9 approach. Homozygous clones were either stimulated with 1.5 mM calcium chloride in 2D culture or terminally differentiated while generating 3D epidermal models. Keratinocytes after the calcium switch were analysed by flow cytometry to determine their cell growth. Proliferation capacities of 3D epidermal models were assessed by Ki67 staining. Epidermal differentiation was characterised by the thickness of the living nucleated keratinocyte layers (LK), the stratum corneum (SC) and total thickness of the epidermal model (E), along with staining for filaggrin, loricrin, involucrin and cytokeratin 10.
Partial filaggrin KO decreased the total thickness of epidermal models, including LK and SC, as well as reduced expression of filaggrin and loricrin, without affecting involucrin and cytokeratin 10. Proliferation capacities assessment showed increased mean fluorescence intensity of Ki67 in KO keratinocytes, without an increased number of Ki67-positive cells. Flow cytometry analysis revealed that the KO keratinocytes after calcium switch exhibit altered cell growth.
Our study shows that partial filaggrin deletion in keratinocytes compromises epidermal differentiation and alters keratinocyte proliferation, indicating that filaggrin potentially controls both keratinocyte proliferation and differentiation. Further studies are needed to fully understand the underlying mechanism.

082

T cells with overlapping phenotypes of circulating and resident memory T cells in chronic skin lesions

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Human skin contains both circulating memory T cells and sessile resident memory T cells (T_{RM}). Although skin T cells are implicated in the pathogenesis of chronic cutaneous disorders, the specific contributions of these T-cell subsets to disease initiation, persistence, and expansion remain unclear.
We isolated T cells from lesions of fixed drug eruption (FDE, n=4), discoid lupus erythematosus (DLE, n=4), psoriasis (n=5), chronic eczema (n=3), and from control skin (Ctl, n=5), and compared the expression of the T_{RM} marker CD69 and the T_{CM} marker CCR7 by flow cytometry. A trend toward increased $CD69^+CCR7^+$ cells was observed in both CD4 and CD8 T-cell fractions in chronic cutaneous disorders compared with controls (CD4: $p < 0.01$ in DLE vs Ctl, $p < 0.05$ in psoriasis vs Ctl; CD8: $p < 0.05$ in DLE vs Ctl, $p < 0.05$ in psoriasis vs Ctl).
Spatial transcriptomic analysis of three DLE lesions further confirmed the increase in *CD69*$^+$*CCR7*$^+$ T cells, which were rare in six Ctl (DLE: 14.9 ± 4.8% in CD4, 15.0 ± 2.9% in CD8; Ctl: 1.8 ± 1.5% in CD4, 2.0 ± 1.8% in CD8). These cells expressed additional T_{CM}- and T_{RM}-associated genes and exhibited an activated phenotype. They prominently expressed *GPR183*, which regulates T-cell positioning via oxysterol sensing, and *BIRC3*, which enhances T-cell survival by inhibiting apoptosis and modulating NF-κB signaling. *GPR183*$^+$*BIRC3*$^+$ T cells localized within dense dermal lymphoid infiltrates, with potential close interactions with macrophages and B cells, and *BIRC3* expression was also detected in scattered stromal cells.
These findings indicate that $CD69^+CCR7^+$ T cells emerge in chronic inflammatory skin disorders and may be guided to specific dermal niches where they participate in activation and cellular interactions.

083

Fibroblast-MMP14 Regulates Delayed-Type Hypersensitivity in Skin

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Inflammation is a biological process protecting against external threats while coordinating repair and immune balance. In skin, immune system dysregulation leads to disorders, driven by alterations in a variety of proteins, including matrix metalloproteinases (MMPs). Among MMPs, in skin inflammatory diseases, MMP14 is highly expressed in the immune infiltrate and stromal cells, including fibroblasts, but its role remains unknown. To analyze the function of MMP14 in fibroblasts during inflammation, we used mice with induced MMP14 deletion in dermal fibroblasts (*Mmp14*$^{Sf-/-}$) and two models of inflammation:

allergic contact dermatitis (ACD) and irritant contact dermatitis (ICD). Whereas *Mmp14*$^{Sf-/-}$ mice displayed no differences in the ICD response, the ACD response was enhanced in *Mmp14*$^{Sf-/-}$ compared to wild-type (WT). At 24 hours post-elicitation, transcripts for inflammatory mediators, such as IL6, IL1, and IFN-γ, were significantly higher in *Mmp14*$^{Sf-/-}$ compared to WT mice. In isolated *Mmp14*$^{Sf-/-}$ fibroblasts, stimulation with IFN-γ led to enhanced IL6, CXCL1, and CXCL9 synthesis compared to WT cells, suggesting that these cells are the source of IL6 *in vivo*. Analysis of the elicited ears shows comparable enhancement in CD4+ cells in both genotypes, but CD3+ and CD8+ effector cells were significantly increased in *Mmp14*$^{Sf-/-}$ mice, as detected in the tissue and quantified by flow cytometric analysis of isolated cells. This was due to enhanced CD8+ proliferation in challenged skin, rather than altered clearance, since apoptosis was unchanged, but the numbers of double-positive proliferating CD8/Ki67 cells were increased. Enhanced CD8 also led to increased neutrophil numbers in *Mmp14*$^{Sf-/-}$ mice, but not macrophages. Together, these findings indicate that fibroblast-derived MMP14 limits CD8+ T cell–driven inflammation in allergic contact dermatitis.

084

***ZC3H12A* variants modify the severity of epidermal differentiation disorders**

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CARD14 and *SLURP1* pathogenic variants result in 2 types of epidermal differentiation disorders (EDD): *CARD14* nEDD and *SLURP1* pEDD, both with increased NF κB activity and proinflammatory cytokine expression. Here, we studied two families with *CARD14* nEDD and *SLURP1* pEDD with an unusually severe phenotype, suggesting a genetic modifier. We used whole exome and Sanger sequencing, RT qPCR, luciferase assay, protein modelling, immunofluorescence confocal microscopy, immunoblotting and organotypic skin equivalents. Whole exome sequencing identified two heterozygous variants in *ZC3H12A* encoding Regnase 1: c.779T>C (p.Ile260Thr) in an individual with *CARD14* nEDD carrying a homozygous *CARD14* c.458G>C variant, and c.1378G>A (p.Glu460Lys) in an individual with *SLURP1* pEDD carrying a heterozygous c.82delT *SLURP1* variant. Regnase 1 is an endoribonuclease that maintains epidermal homeostasis through degradation of pro inflammatory cytokine mRNAs. Bioinformatic analyses and protein modelling supported the pathogenicity of both *ZC3H12A* variants. Luciferase assays demonstrated increased NF κB activity in primary human keratinocytes (KCs) expressing mutant *ZC3H12A* compared to wild type. This was associated with upregulation of NF κB target genes (*IL6*, *IL1B* and *TNFA*) and reduced desmoglein 1 (DSG1) expression at both mRNA and protein levels. *ZC3H12A* knockdown in primary KCs recapitulated these findings, with increased *IL6* levels and reduced *DSG1* expression. Moreover, *ZC3H12A* knockdown in organotypic skin equivalents resulted in reduced DSG1 expression and acanthosis. Our findings suggest that *ZC3H12A* variants exacerbate the severity of inherited EDDs by up regulating NF κB, leading to cytokine associated downregulation of *DSG1*, a key driver of EDD pathogenesis.

085

Interrupted Autophagy Flux and Diminished TGFß1 Activity as Novel Molecular Mechanisms in Stiff Skin Syndrome

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Stiff Skin Syndrome (SSS) is a rare inborn disease with only 140-200 cases described. The skin of SSS patients is characterized by progressive hardening of connective tissue in the absence of immune cell infiltration. In some patients, mutations in *fibrillin 1* gene were described, however, very little is known about the pathophysiological consequences.
We here describe a patient with typical clinical symptoms lacking the described mutations, analyzed skin biopsies and primary fibroblasts by immunohistochemistry and multi-omics approaches. Surprisingly, skin analysis revealed markedly reduced elastin fibers and altered supramolecular collagen structures. Proteoglycans and fibrillin 1 were strongly diminished. Gene signature and mass spectrometric analyses revealed an overall downregulation of matrix components like elastin, collagens, fibronectin, fibrillin 1, LTBP-1, fibulin 1, TSP 1 and biglycan, in accordance with a TGFß1 and ECM remodeling signature. Western blot and ELISA supported reduced TGFß1 expression, altered processing and less activatability. Expression and secretion of collagen I, fibrillin 1, biglycan and LTBP-1 were restored by treatment of SSS fibroblasts with recombinant TGFß1, clearly pointing towards defective TGFß1 availability.
Further studies indicated reduced Wnt signaling at the translational level, whereas heat shock family proteins, unfolded protein response mediators and chaperone-mediated autophagy markers were enriched. However, the data demonstrated that autophagy was initiated in SSS fibroblasts but autophagic flux was interrupted, suggesting defective lysosomal fusion or function.
Taken together, we suggest that the characteristic clinical phenotype of SSS is based on highly heterogeneous mechanisms involving TGFß1 activity and the macromolecular architecture of the ECM.

086

Prurigo nodularis exhibits miRNA dysregulation driving inflammation

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Prurigo nodularis (PN) is a chronic inflammatory skin disease characterized by intensely pruritic nodules, with poorly understood molecular mechanisms. While a TH2-skewed neuroimmune axis has been implicated, the role of posttranscriptional regulation by microRNAs (miRNAs) remains unexplored.
Our objective was to characterize miRNA expression in PN skin and investigate their contribution to disease-associated molecular pathways.

Skin biopsies from 10 patients with moderate-to-severe PN and 10 healthy donors were analyzed using RNA sequencing. Differentially expressed miRNAs (miDEGs) and mRNAs (mDEGs) were identified, followed by integrative bioinformatics analyses to determine miRNA–mRNA interactions and enriched pathways.
PN lesions exhibited marked miRNA dysregulation, with 206 miDEGs identified. Key upregulated miRNAs included miR-31-5p, miR-155-5p, and miR-223-3p, whereas miR-143-5p, miR-133b, and miR-145-5p were significantly downregulated. Integrated analysis with 2,664 mDEGs revealed that miRNAs potentially regulate 6.48% of dysregulated genes, including inflammatory mediators (e.g., CXCL13, CCR5) and matrix metalloproteinases. Pathway analysis showed enrichment of inflammatory (IL-4/IL-13 signaling), neuroinflammatory (synaptogenesis), and keratinization pathways, alongside relative downregulation of profibrotic processes. A limited subset of miRNAs appeared to drive key pathogenic pathways. In silico target prediction revealed potential targeting of broader pathways implicated in PN pathophysiology by this small dysregulated miRNA subset. This study identifies widespread miRNA dysregulation in PN and highlights their role in modulating inflammatory, neuroimmune, and fibrotic pathways. These findings provide novel insight into PN pathophysiology and suggest miRNAs as potential therapeutic targets.

087

Dermal Fibroblasts, Not Keratinocytes, Dominate IL-17A/TNF-Driven Inflammation

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The aim of this study was to define and compare the transcriptional responses of primary human dermal fibroblasts and keratinocytes in IL-17A- and TNF-driven inflammation. Primary human dermal fibroblasts and keratinocytes were stimulated *in vitro* with IL-17A, TNF, or their combination, followed by analysis via bulk RNA sequencing and Western blotting. Dermal fibroblasts exhibited a broader and more robust proinflammatory response than keratinocytes, particularly following TNF and combined TNF/IL-17A stimulation. This was characterized by enrichment in immune signaling and chemotaxis pathways, with robust induction of *CCL20*,*CXCL8*, and *IL6*. In contrast, keratinocytes primarily upregulated genes associated with epithelial differentiation and barrier function (*IL36G*, *S100A7A*, *DEFB4A*). This fibroblast-specific responsiveness correlated with increased TNF sensitivity and significantly higher TNFR2 (*TNFRSF1B*) expression and signaling compared with keratinocytes. These findings demonstrate that dermal fibroblasts are major contributors to TNF- and IL-17A-driven inflammatory responses in human skin and highlight their important role in shaping cutaneous immune signaling.

088

Corticosteroid-mediated regulation of complement activation at the dermal–epidermal junction in pemphigoid diseases

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Pemphigoid diseases (PD) are autoantibody mediated blistering disorders in which pathogenic antibodies bind structural proteins of the dermal-epidermal junction (DEJ), triggering complement activation, linear deposition of complement component 3 (C3), and subepidermal blister formation. While corticosteroids are effective in suppressing disease activity, the molecular mechanisms underlying their anti-inflammatory effects in PD remain incompletely defined. We analyzed the impact of the corticosteroid methylprednisolone (MP) on complement activation using an *ex vivo* human skin model. In an indirect complement fixation assay, recombinant PD antibodies triggered linear C3 deposition along the DEJ, enabling quantitative assessment of corticosteroid interference with complement activation. Incubation with MP inhibited C3 deposition in a dose dependent manner (0.3mM, 1mM, 3mM). This effect was reproducible in skin sections from six healthy donors incubated with recombinant PD antibodies and MP at 3mM. Moreover, sera from three PD patients induced robust complement deposition at the DEJ, which was significantly reduced upon MP incubation. To assess pathway specificity, complement activation products were analyzed in assay supernatants. MP treated samples showed a significant reduction in C5a levels, whereas factor B and C4d levels remained unchanged, indicating preserved upstream pathway initiation. Similar inhibitory effects on C3 deposition were observed with dexamethasone, prednisolone, and hydrocortisone, supporting a class specific corticosteroid mechanism. These data suggest that corticosteroids directly interfere with C3 activation at the DEJ, limiting downstream complement effector mechanisms and highlighting complement modulation as a therapeutic target in PD.

089 [WITHDRAWN]

090

The role of sweat-derived IL-1β in modulating inflammatory responses and PD-L1 expression in human dermal fibroblasts

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Sweat is essential for maintaining skin homeostasis through thermoregulation, hydration, and innate immune defense. In atopic dermatitis (AD), sweat leaks into the dermis due to impaired tight junctions in sweat glands, triggering abnormal immune responses. We previously reported that this leaked sweat stimulates dermal fibroblasts to produce pro-inflammatory cytokines, mainly via sweat-derived IL-1β. Given the emerging role of PD-1/PD-L1–mediated immune regulation in allergic and inflammatory skin diseases, we investigated how sweat affects this regulatory system. Normal Human Dermal Fibroblasts (NHDFs) were stimulated with sweat and recombinant IL-1β, and PD-L1 (CD274) expression was evaluated at the mRNA and protein levels by quantitative PCR, ELISA, and flow cytometry. Transcriptomic changes were further analyzed by bulk RNA sequencing.
In this study, sweat stimulation increased PD-L1 expression, with a nearly 4-fold increase in MFI and an approximately 10-fold upregulation of CD274 mRNA. This effect was closely mimicked by recombinant IL-1β, but not IL-1α, and was completely abrogated by an IL-1 receptor antagonist, indicating mediation via the IL-1β/IL-1 receptor axis. RNA-seq analysis of sweat-

stimulated NHDFs revealed a robust inflammatory signature characterized by the upregulation of multiple cytokines and chemokines, with a consistent increase in CD274 expression, supporting the PCR and protein findings.
These findings suggest that while sweat-activated fibroblasts drive dermal inflammation, the simultaneous induction of PD-L1 is likely to function as a compensatory immunoregulatory mechanism, potentially modulating local T cell responses. In conclusion, sweat-derived IL-1β exerts a dual role in the dermis by promoting inflammation while simultaneously inducing PD-L1-mediated immunoregulatory feedback in human dermal fibroblasts. This mechanism links barrier disruption–induced sweat leakage to dermal immune regulation and may contribute to the persistence of inflammation in AD. To our knowledge, this is the first study demonstrating that sweat-derived IL-1β regulates PD-L1 expression in dermal fibroblasts.

091

Duodenal Inflammatory Infiltrates and Signs of Gut Barrier Dysfunction in Atopic Dermatitis Patients

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Atopic dermatitis (AD) frequently co-occurs with food allergy (FA), affecting 24% of adult AD patients. Although gut barrier dysfunction has been linked to FA pathogenesis, there is limited direct tissue-level evidence of intestinal involvement in AD. We explored whether AD patients show duodenal immune remodeling and barrier dysfunction, and whether these characteristics differ between AD, FA, and comorbid AD+FA patients.
We obtained duodenal biopsies from 23 adults: healthy controls (HC, n=6), AD without FA (n=5), FA without AD (n=9), and comorbid AD+FA (n=3). Imaging mass cytometry (IMC) was used to characterize cellular composition, spatial organization, and intraepithelial lymphocyte (IEL) density. NanoString gene expression profiling assessed pathway-level transcriptomic signatures.
All disease groups showed shared features of barrier damage, mucosal inflammation and disturbed antigen processing/presentation. This was evidenced by increased epithelial-to-epithelial cell distance, expanded cytotoxic T cells and eosinophils, upregulation of epithelial stress markers (XBP1, SOCS3), reduced MHC class II expression, and a disrupted M1 macrophage–DC/NK co-localization and spatial redistribution. However, we also identified disease-specific patterns: AD exhibited the highest cytotoxic T-cell and M1 macrophage densities with transcriptomic features suggesting barrier repair (upregulated MUC2, MUC4, TFF3, claudins) alongside dampened innate immune sensing (downregulated TLRs, MHC-II). FA showed active inflammatory priming with NLRP6 inflammasome and chemokine upregulation. AD+FA displayed pronounced type 2-skewed polarization (elevated IL13, CCL2).
AD patients exhibit duodenal immune remodeling and epithelial barrier dysfunction, substantiating the gut-skin axis concept. However, rather than a uniform type 2-driven allergic gut pathology, each condition displays a distinct mucosal immune signature. These findings suggest therapeutic strategies targeting mucosal immunity and barrier function could complement skin-directed AD therapies.

092

Early OX40–OX40 ligand signaling imprints relapse-driving skin tissue-resident memory T cells in atopic dermatitis

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Recurrent flares at sites of prior inflammation suggest that atopic dermatitis (AD) is driven by local immune memory in the skin, but when and how this pathogenic memory is established remain unclear. Using an oxazolone-induced AD model, we tested whether OX40–OX40L signaling during the initial inflammatory phase imprints relapse-driving tissue-resident memory T cells (T_{RM}). Mice were sensitized and challenged on days 0–12, allowed to resolve, and re-challenged on day 47. OX40L blockade during days 0–12 significantly suppressed recall dermatitis, whereas blockade during the resolution phase (days 33–40) or at re-challenge had no significant effects, suggesting a narrow disease-modifying window during which pathogenic skin memory is programmed. Flow cytometry showed reduced skin $CD4^+$ T_{RM} ($CD44^+CD69^+$) and $KLRG1^-$ T_{RM} precursor–enriched populations after early blockade, indicating that early OX40 signaling is required to generate a relapse-driving T_{RM} pool rather than to sustain it. To assess human relevance, we analyzed single-cell RNA sequencing data from lesional AD skin (n = 30) together with Xenium spatial transcriptomics (n = 2). *TNFRSF4* (OX40) was enriched in *CD4*$^+$ T_{RM}, and *OX40*$^+$ *CD4*$^+$ T cells exhibited a Th2-skewed effector program marked by *IL13*. Ligand–receptor analysis suggested dendritic cell-derived CCL17–CCR4 input to *OX40*$^+$ *CD4*$^+$ T cells, and spatial transcriptomics localized *OX40*$^+$ *CD4*$^+$ T cells in close proximity to *CCL17*$^+$ dendritic cells in the superficial dermis, consistent with a conserved T_{RM} niche. These findings identify a temporally restricted window that imprints a relapse-driving memory reservoir in the skin, rather than merely amplifying active inflammation. Early targeting of the OX40-OX40L pathway may prevent the establishment of pathogenic immune memory and enable durable disease control in AD.

093

Characterisation of tissue-resident memory T cells in psoriatic disease

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To advance the understanding of tissue-resident memory T (T_{RM}) cell biology in psoriatic disease, we investigated the prevalence of key survival markers (interleukin 7 receptor [IL7R] and IL15RA) and pathogenic drivers of disease, including IL17A, IL17F and IL23R, in this cell population.
Ex vivo lesional skin samples from patients across 8 independent single-cell transcriptomic studies (7 psoriasis; 1 psoriatic

arthritis) were processed using standard workflows (CellRanger and Seurat). *In vitro* T_{RM}-like cells were generated by stimulating untouched pan T cells from healthy peripheral blood mononuclear cells with cluster of differentiation (CD)3/28 activation beads, IL2 and transforming growth factor (TGF)-β. Cells were restimulated using plate-bound anti-CD3 and soluble CD28 with TGF-β.
Across single-cell datasets, a small subset of T_{RM} cells (CD103+ T cells) expressed *IL23R*. T_{RM} cells could express *IL17* (*IL17A, IL17F* or both) in the absence of *IL23R*; a larger proportion expressed *IL17* alone vs *IL23R* alone. Similarly, functional *in vitro* assays showed IL17+ T_{RM} cells could be induced in the absence of IL23. Across T_{RM} cells, *IL7R* was the predominant survival factor, while *IL15R* was expressed in lower proportions. The majority of IL23R− T_{RM} cells showed expression of IL7R. Additionally, our prior study showed a higher proportion of IL7+ cells in lesional vs non-lesional psoriatic tissue among keratinocytes and stromal cells, supporting the role of *IL7R* in T_{RM} cell survival. *IL7R* expression was higher on IL17F+ T_{RM} cells vs IL17A+ T_{RM} cells, which may support their role in chronic pathogenicity. Multiplex RNAscope imaging of lesional skin from psoriasis patients supported these results.
In conclusion, IL17-producing T_{RM} cells represent an important pathogenic population in psoriatic disease that can function and produce IL-17 independently of *IL23R* expression. The reliance of these T_{RM} cells on alternative survival pathways, particularly the IL7 pathway, provides a mechanistic basis for T_{RM} cell persistence, recurrence and psoriatic disease chronicity.

094
Persistent Th2 elevation predicts non-response to biologic therapy in psoriasis
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The pathogenesis of psoriasis is driven by a Th17-dominated immune response. However, targeting this pathway results in heterogeneous clinical outcomes, the reasons for which are not fully understood. It can be hypothesized that, at patient level, the Th17 immune response may be accompanied by additional immune mechanisms that are currently not adequately targeted. Given the variability in treatment responses, there is an urgent need for biomarkers predicting therapeutic success. We aimed to characterize longitudinal changes in circulating immune cell subsets and cytokine production during biologic therapy in psoriasis (PsO) and psoriatic arthritis (PsA). Peripheral blood was collected longitudinally (t0, baseline; t1, week 12; t2, week 24) from patients with PsO (n = 56), PsA (n = 8), and healthy controls (n = 17). Whole blood staining and ex vivo stimulation of peripheral blood mononuclear cells were performed. Spectral flow cytometry using a 34-marker panel enabled comprehensive profiling of cellular phenotypes and intracellular cytokine production (response >PASI75; non-response <PASI50 at t1). At baseline, both PsO and PsA patients showed elevated Th17/Treg ratios compared to controls (1.6- and 1.73-fold, respectively). PsO patients also exhibited increased Th2 frequencies compared to healthy controls (8.76% vs. 7.16%, $p < 0.05$), which normalized under therapy (7.84% at t2, n.s.). Notably, non-responders showed persistently elevated Th2 proportions at early follow-up (t1) compared to both responders and controls (8.91% vs. 7.12% vs. 7.16%, $p < 0.05$). This was accompanied by increased IL-4 production by T cells across all time points compared to responders (1.25-fold, $p = 0.01$). Biologic therapy induces dynamic immune rebalancing beyond the targeted pathways. Persistent Th2 elevation and increased IL-4 production in non-responders suggest that these parameters may serve as early predictors of treatment response. Future studies are warranted to validate their predictive value for personalized therapy selection.

095
Mitochondrial messenger in Vitiligo: the FGF21 interface
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Research over the last few decades has shown that mitochondrial dysfunction may significantly influence the pathogenesis of vitiligo. More specifically, the impairment of the organelle may provide the biochemical basis for the onset of the disease. In this study, we investigated whether vitiligo signals mitochondrial impairment through local and systemic expression of the mitokine fibroblast growth factor 21 (FGF21). Reanalysis of publicly available scRNA-seq datasets revealed a marked metabolic reprogramming in fibroblasts from vitiligo patients, which was evident even in non-lesional skin. Pathway enrichment analysis identified oxidative phosphorylation as one of the most significantly dysregulated processes. Primary fibroblast cultures and dermal compartment of nonlesional skin of patients with vitiligo showed increased levels of FGF21. Ultrastructural analysis confirmed mitochondrial abnormalities, providing morphological evidence that reinforces metabolic impairment in dermal cells. Plasma measurements showed higher FGF21 levels in vitiligo patients compared to healthy controls, which correlated with the inflammatory markers CXCL10 and IFN-γ, linking mitochondrial stress to immune activation. Overall, the current study reinforces the central role of mitochondrial dysfunction in vitiligo pathogenesis and identify FGF21 as a local and systemic mitokine that could serve as a disease-specific biomarker. More importantly, FGF21 could facilitate the early detection of mitochondrial stress before extensive depigmentation becomes clinically apparent. This would provide opportunities for preclinical diagnosis, patient stratification and the monitoring of metabolism-targeted therapies.

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eIF4E as a Novel Diagnostic Marker for Mycosis Fungoides
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Parapsoriasis is a rare inflammatory skin disorder that may progress to cutaneous T-cell lymphoma, most commonly Mycosis fungoides (MF). Distinguishing parapsoriasis from early-stage MF remains a significant clinical and histopathological challenge. In MF, chronic inflammation is associated with the overexpression of eukaryotic translation initiation factors (eIFs), which play essential roles in regulating key cellular processes such as proliferation, apoptosis, and differentiation. Despite this, the specific role of eIF4E in MF pathogenesis and its potential diagnostic value remain poorly understood. This study aimed to investigate the involvement of eIFs - particularly eIF4E - in parapsoriasis and MF, with a focus on their potential as diagnostic biomarkers. In this translational observational study, paraffin-embedded skin biopsy samples from patients with MF and parapsoriasis, as well as frozen MF tissue samples, were analyzed. The expression levels of the proinflammatory cytokines IL-4, IL-5, IL-13, IL-22, IL-23, and IL-31, as well as the transcription factors STAT3, STAT5, and the translation initiation factor eIF4E, were assessed using immunohistochemistry and quantitative real-time PCR (qRT-PCR).

A marked overexpression of eIF4E was observed in MF samples, in contrast to parapsoriasis samples, where no significant change in eIF4E expression was detected. Elevated eIF4E levels in MF correlated with increased cellular proliferation (Ki-67 expression) and heightened levels of proinflammatory cytokines and transcription factors, including STAT3 and STAT5. These findings suggest a dysregulation of translational control in MF and highlight the pivotal role of eIF4E in its pathophysiology. eIF4E may serve as a promising diagnostic or prognostic biomarker to differentiate MF from parapsoriasis, offering new insights into the diagnosis and clinical management of inflammatory skin diseases.

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Spatial characteristics of ciliated keratinocytes as pro-inflammatory state in psoriasis and atopic dermatitis

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Chronic inflammatory skin diseases, including psoriasis and atopic dermatitis, are characterized by recurrence, potentially driven by incomplete molecular remission despite clinical improvement. Keratinocytes (KCs) are regulators of cutaneous inflammation, yet the mechanisms that maintain their inflammatory responsiveness remain unclear. Primary cilia, microtubule-based sensory organelles that integrate extracellular signals, remain poorly characterized in this context. Notably, primary cilia were rare in healthy epidermis but markedly increased in atopic dermatitis and psoriasis, and persisted after phototherapy in psoriasis despite clinical improvement.
Here, we investigated the molecular features of ciliated KCs in chronic inflammatory skin diseases by integrating immunofluorescence with spatial transcriptomics at single-cell resolution. We analyzed 6 healthy, 10 atopic dermatitis, and 14 psoriatic skin collected before and after phototherapy. Ciliated KCs were mainly found in stem/progenitor-like KC populations. Compared with layer-matched non-ciliated KCs, ciliated KCs showed enhanced inflammatory and stress-response programs, including MAPK-related signaling and cytokine-responsive pathways. Consistently, ATAC-seq analysis of ciliated KCs revealed increased chromatin accessibility at multiple inflammatory gene loci even in the absence of inflammatory stimulation, accompanied by enrichment of AP-1 family transcription factor motifs, indicating chromatin priming.
Together, our findings identify ciliated KCs as a disease-associated epidermal cell state characterized by AP-1–linked inflammatory readiness and persistent chromatin accessibility. Primary cilia may act as signaling platforms that promote KC inflammatory memory and contribute to relapse-prone skin in chronic inflammatory diseases.

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PARP2 regulates the differentiation of human sebocytes

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PARP2 is increasingly recognized as an important regulator of lipid metabolism. Its deletion impairs lipid storage in white adipose tissue, and we demonstrated that PARP2 loss promotes mitochondrial biogenesis, lipid oxidation, and cholesterol biosynthesis in liver and skeletal muscle. Moreover, genome wide association studies linked PARP2 variants to circulating lipid traits, indicating a broader role for PARP2 in systemic lipid homeostasis.
Sebaceous glands are holocrine skin appendages that secrete the lipid rich sebum, yet the role of PARP2 in sebocyte biology has not been characterized.
In this study, we generated PARP2 knock-out (KO) SZ95 human sebocytes and analyzed consequent transcriptomic and lipidomic alterations. RNAseq identified almost 5000 differentially expressed genes, with strong enrichment in lipid metabolic processes. Principal component analysis of the lipidomic dataset demonstrated a clear segregation between control and PARP2 KO sebocytes. Functionally, PARP2 KO sebocytes displayed key hallmarks of sebocyte terminal differentiation, namely lipid accumulation, enlargement, enhanced autophagic flux, and induced cell death, as well as, elevated mitochondrial respiration. Importantly, the more differentiated phenotype of PARP2 KO sebocytes is associated with the induction of androgen hormone production that may be relevant in such sebaceous gland-associated skin diseases as acne or atopic dermatitis.
Collectively, our data identify PARP2 as a novel regulator of sebocyte lipid metabolism.

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A prebiotic-containing emollient counteracts *Staphylococcus aureus* protease-induced barrier damage in a dysbiotic human skin explant model.

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Atopic dermatitis (AD) is a chronic inflammatory skin condition characterized by inflamed, dry and itchy skin. Among the factors that exacerbate this condition, dysbiosis has been particularly highlighted, especially the impact of the bacterial secretome on skin barrier integrity, immune environment and pruritogenic signaling.
The preventive efficacy of a lipidic-replenishing cream anti-iching, containing a prebiotic ingredient, was investigated using an *ex vivo* human skin model replicating *S.aureus* protease-induced damages. Protease exposure induced a significant increase in endothelin-1 and IL-31, concomitant with decreased barrier integrity and reduced expression of key junctional proteins, including desmoglein-1 and claudin-1. Topical application of the emollient significantly counteracted protease-induced barrier impairment and pruritogenic marker expression. In parallel, the prebiotic activity of the formulation was assessed using a co-culture model of *S. epidermidis* and *S. aureus*, demonstrating a selective inhibitory effect on *S. aureus* growth within 8 hours. Overall, this study highlights that a targeted emollient formulation can modulate early AD-associated biomarkers, such as compromised skin barrier integrity, pruritogenic signaling or microbiota balance. Addressing these early pathophysiological

mechanisms may contribute to limiting dysbiosis-driven skin damage and prolonging periods of skin homeostasis between AD flares.

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RANKL-Positive Cells Are Increased in Lesional Skin of Atopic Dermatitis: An Integrative Single-Cell and Spatial transcriptomics Analysis

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Recent studies have suggested that atopic dermatitis (AD) is associated with an increased long-term risk of osteoporosis and fractures. Receptor activator of nuclear factor kappa-B ligand (RANKL) and osteoprotegerin (OPG) are known to be associated with bone mineral density in specific populations. We previously demonstrated that serum RANKL levels and the RANKL/OPG ratio positively correlate with AD severity and may indicate fracture risk in older women with AD. However, the cellular sources contributing to elevated serum RANKL in severe AD remain unclear. In this study, we investigated the cellular origins of RANKL using publicly available single-cell RNA sequencing (scRNA-seq) and spatial transcriptomics datasets derived from AD lesional and non-lesional skin, as well as healthy skin. scRNA-seq data from suction blister-derived samples were reprocessed with quality control, normalization, principal component analysis, clustering, UMAP visualization, and marker-based cell type annotation. RANKL-positive cells were identified, and their distribution across cell populations was quantified. Spatial transcriptomics data from skin biopsy specimens were analyzed to identify RANKL-positive spots and to assess their spatial localization and frequency within tissue sections. In the single-cell analysis, RANKL-positive cells were predominantly observed in three clusters mainly composed of (i) Th, Tc, and Treg cells; (ii) Tc, ILCs, and Th cells; and (iii) differentiated and undifferentiated keratinocytes with Tc cells. The proportion of RANKL-positive cells was significantly higher in AD lesional skin compared to healthy controls. In spatial transcriptomics, RANKL-positive spots were more frequently detected in lesional skin than in non-lesional or healthy skin. These spots were distributed in both the epidermis and dermis, predominantly at the dermoepidermal junction. Previous studies have shown that keratinocytes and T cells can produce RANKL in response to multiple cytokine stimulations. These findings suggest that lesional skin in AD may contribute to increased circulating RANKL levels in patients with severe disease.

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Analyzing the role of CD40L+ helper CD8+ T cells in skin immunity using 3D models and high-plex imaging

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The skin immune system maintains tissue homeostasis through tightly regulated local and systemic interactions. Disruption of this balance contributes to chronic inflammatory diseases such as psoriasis. While the role of CD4+ T cells has been thoroughly studied in psoriasis, CD8+ T cells remain poorly understood despite their abundance and the high prevalence of HLA-C*06:02 among psoriasis patients. Although the “division of labor” concept of adaptive T cell immunity classifies CD8+ T cells as cytotoxic, and CD4+ T cells as CD40L+ helper cells, we have identified a subset of memory CD8+ T cells (Tc2, Tc17, and Tc22) that lack cytotoxic characteristics and instead express CD40L comparable to CD4+ T cells. These Tc cells share cytokine and chemokine receptor profiles with their respective Th counterparts, including Th17 cells which are central to psoriasis pathogenesis. The helper CD8+ T cells display skin-homing signatures and are enriched in the blood of psoriasis patients, suggesting a functional role in inflammatory skin. To further characterize these cells and understand how their priming differs from classical cytotoxic CD8+ T cells, we perform spatial immunoprofiling of T cells and their interaction partners in skin-draining lymph nodes using high-plex imaging (MACSima). To delineate the contributions of cytotoxic and helper T cell subsets to psoriasis induction, we have developed immunized human three-dimensional full-thickness skin equivalent (hFTSE) models containing both CD4+ and CD8+ T cells. Our results demonstrate that both T cell subsets infiltrate the tissue and induce hallmark psoriatic features. We now advance into hFTSE models composed of primary adult cells and integrated with autologous T cells to enable direct comparison of healthy and diseased conditions. Next, we aim to study how cytotoxic and helper T cells respond to triggers such as wounding and infection in autologous hFTSE models. By dissecting the distinct and cooperative roles of cytotoxic and helper T cell subsets, this work aims to deepen our understanding of skin homeostasis and chronic inflammation, ultimately supporting the development of more precise therapeutic strategies.

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Skin-liver crosstalk: Psoriasis-driven IL-17A signaling aggravates metabolic dysfunction-associated liver disease

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Psoriasis patients display cardiometabolic comorbidities, like Metabolic dysfunction-associated steatotic liver disease (MASLD) encompassing metabolic dysfunction-associated steatohepatitis (MASH). This study aims to understand the underlying mechanism of this clinical observation using murine models.
In C57BL/6J mice, psoriasis-like dermatitis was induced using Imiquimod (IMQ) after 16 weeks of high-fat diet (HFD) or high-fat and high-fructose Western diet (WD) to generate MAFLD or MASH, respectively. Skin and liver tissues were analyzed by histology, flow cytometry, single cell RNA-sequencing and metabolomics.
High caloric diets exacerbate IMQ-induced psoriasis-like skin inflammation by increased infiltration of inflammatory cells and IL-17A production. Reciprocally, psoriasis-like dermatitis aggravates liver steatosis and inflammation, shifting the HFD-phenotype

from MASL (steatosis) to MASH (steatosis with inflammation). This is due to increased hepatic IL-17A producing γδ T cells, and impaired very-low-density lipoprotein secretion from hepatocytes under psoriatic MASLD conditions.
This study reveals IL-17A and impaired lipid metabolism as a mechanistic underpinning of the comorbidity of MASLD in psoriasis patients. Psoriatic inflammation acts as a “second hit” in progression from MASL to MASH. Understanding this interorgan crosstalk lay basis for effective therapeutic and preventive approaches for psoriasis patients.

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Impaired JNK signaling disrupts fibroblast–immune crosstalk and skin homeostasis in a cytokine-induced 3D psoriatic skin model

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Psoriasis is associated with impaired c-Jun N-terminal kinase (JNK) signaling in dermal fibroblasts, reducing their capacity to resolve inflammation and promoting pro-inflammatory macrophage activation. We investigated the impact of JNK inhibition on skin homeostasis using a psoriatic-like full-thickness human skin equivalent (FTHSE) and the immunomodulatory role of human dermal fibroblasts (HDF) on monocytes. Psoriatic-like FTHSE were stimulated with TNF-α and IL-22 (10 µg/mL each) in the presence or absence of the JNK inhibitor SP600125 (10 or 50 µM) for 14 days. Skin morphology and barrier proteins were analyzed by immunohistochemistry. Primary HDF were obtained from foreskins of healthy adult donors, and monocytes from buffy coats. Monocytes were treated for 24 h with conditioned media (CM) from HDF previously stimulated with IL-1β (2.5 ng/mL, 24 h) with or without SP600125 (50 µM). Cytokine release (IL-10, TNF-α) was quantified by ELISA. JNK inhibition induced structural alterations in FTHSE under both basal and stimulated conditions, including disrupted keratinocyte organization and changes in epidermal thickness. Psoriatic-like stimulation downregulated barrier proteins (Filaggrin, loricrin, and CK-10) and upregulated involucrin and CK-6. These alterations were exacerbated by JNK inhibition, indicating impaired epidermal integrity. CM from IL-1β-stimulated HDF increased IL-10 and decreased TNF-α release in monocytes, indicating an anti-inflammatory, pro-resolving phenotype. However, CM from SP600125-treated HDF reduced both cytokines, showing this immunomodulatory effect is JNK-dependent. Overall, JNK inhibition disrupts epidermal structure and barrier function in FTHSE and impairs the ability of HDF to promote anti-inflammatory monocyte polarization. These findings suggest reduced JNK signaling may contribute to persistent inflammation in psoriasis.

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$P2Y_2$ is a newly emerging regulator of human sebocyte biology

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We have previously show that A_1, A_{2A} and A_{2B} adenosine receptors are expressed in human sebocytes, where they differentially influence sebaceous lipogenesis and immune phenotype. Within the confines of the current study, we aimed to further explore the role of purinergic signaling using SZ95 sebocytes.
We first challenged the cells by ATP-treatment, and found that ATP increased the $[Ca^{2+}]_{IC}$ of the sebocytes (Fluo-4 AM). Importantly, the effect decreased, but did not disappear in nominally Ca^{2+}-free medium, arguing that it developed in response to the activation of ionotropic P2X and G_q protein-coupled P2Y receptors. Next, we re-analyzed our unpublished RNA-Seq data, and found that multiple P2X and P2Y receptors were expressed in human sebocytes. Among these molecules, the G_q protein-coupled $P2Y_2$ receptor exhibited a remarkably high expression level; hence, we decided to further investigate the expression and role of this receptor. First, we confirmed its expression using independent samples (Fast Universal TaqMan qPCR). Next, we assessed the biological effects of selective $P2Y_2$ modulators. We found that, while the $P2Y_2$ agonist PSB 1114 had no significant effect on the spontaneous, homeostatic sebaceous lipogenesis (Nile Red), it significantly increased the MTT-signal intensity and slightly, but significantly decreased the arachidonic acid-induced, excessive, “acne-mimicking” lipid production, whereas the receptor selective antagonist AR-C 118925XX significantly increased the sebaceous lipogenesis over the course of 48-h treatments.
Our data indicate that $P2Y_2$ is a previously undescribed regulator of sebaceous lipogenesis.

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Insulin-IGF Uncoupling in Vitiligo Patients: Rethinking the Disease from a Systemic Perspective

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Vitiligo is a pigmentary disorder involving progressive melanocyte loss. It is increasingly recognized as a systemic condition rather than a localized skin disease, frequently associated with autoimmune and metabolic comorbidities, such as glucose intolerance and lipid dysregulation. These links underscore the disease's broader systemic nature. However, a significant gap remains in establishing a direct correlation between the clinical phenotype of vitiligo and systemic metabolic dysregulation. This study aims to profile the metabolic state in a well-characterized cohort. 250 vitiligo patients and matched controls were enrolled by four Italian institutes for blood collection and annotation of severity, duration, triggers, comorbidities, family history, and therapies. Blood samples were used for biochemical profiling, antioxidant capacity, quantification of circulating glycated products, cytokine profile and immunoenzymatic quantification of IGF-1, IGF-2, IGFBPs and members of fatty acid binding proteins family (FABPs).
Focusing on glucose metabolism, preliminary results showed a significant positive correlation between fasting glucose and insulin in controls ($r=0.35$, $p<0.0001$), whereas this association was borderline in vitiligo patients ($r=0.13$, $p=0.0588$). ELISA revealed higher IGF-2 levels in vitiligo subjects ($p<0.0001$). Accordingly, a direct correlation between insulin and IGF-2 reached

statistical significance only in the control group (r=0.3968, p=0.0017), indicating a lack of coordinated regulation in patients (r=-0.11, p=0.3862). While IGF-1 was only marginally higher in the vitiligo group, it exhibited a stronger inverse correlation within this cohort (r=-0.5, p=0.0013) compared to controls (r=-0.35, p=0.0273).
A key novelty of this study is the assessment of disrupted insulin-IGF correlations, providing a new perspective on the pathogenesis of vitiligo and its comorbidities.

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IL-13 and IL-4 generate similar Monocyte derived dendritic cells, driving a strong Th2 response in an Atopic Dermatitis like model in vitro

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In atopic dermatitis (AD), skin-resident memory T cells (TRM) reside in the skin even after successful therapy. These TRM may play a critical role during flare-up of skin inflammation due to local reactivation by dendritic cells (DC). IL-4 is believed to play a key role in initiating sensitization to allergens and is also considered to be the main driver of maturation of monocyte DC. However, numerous studies failed to detect IL-4 in AD skin lesions, while IL-13 is readily detectable. This study investigated whether monocyte-derived DC (moDC) generated via IL-13 or IL-4, differed in their capacity to stimulate *in vitro* derived TRM (ivTRM).
Healthy donor monocytes were differentiated into moDCs in presence of either IL-4/GM-CSF, IL-13/GM-CSF or GM-CSF alone. Autologous T cells were isolated and differentiated *in vitro* into ivTRM, expressing CD69+, CD103+, by co-culture with primary human keratinocytes. moDCs were co-cultured with autologous ivTRM and stimulated with Staphylococcus aureus enterotoxin B(SEB). In parallel, memory T cells from circulation of AD patients with house dust mite allergy were also cultured with autologous IL-4/GM-CSF or IL-13/GM-CSF differentiated moDCs and stimulated with mite allergens.
IL-13 matured moDCs were phenotypically indistinguishable from IL-4 matured moDCs, in terms of surface marker expression. Moreover, no difference was observed in the capacity of IL-13 or IL-4 matured moDCs to induce T-cell proliferation and Th2 cytokine release in TRM and CD4+ memory T-cells, or AD allergenic T-cells.
In conclusion, IL-13 and IL-4 have a similar capacity to mature moDC, in terms of being able to promote type 2 responses from both allergen specific memory T-cells and *in vitro* generated ivTRMs. These data strengthen the hypothesis that IL-13 is the driver of DC maturation in AD skin and is not reliant on the presence of IL-4.

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Topical Repurposing of Leflunomide via Methacrylate-Functionalized Hyaluronic Acid-Stabilized Oil-in-Water Emulsions for the Treatment of Atopic Dermatitis

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Topical delivery of immunomodulators represents a promising clinical approach for managing inflammatory skin disorders, such as atopic dermatitis (AD), by localizing therapeutic effects and minimizing systemic adverse events. Leflunomide (LEF), an approved systemic disease-modifying antirheumatic drug, exhibits robust immunomodulatory potential through the inhibition of dihydroorotate dehydrogenase via its active metabolite, teriflunomide (TERIFLU), alongside its ability to activate the aryl hydrocarbon receptor (AHR) pathway. However, LEF's clinical translation for topical administration is hindered by its hydrophobicity and poor skin penetration. To overcome this, we produced an oil-in-water emulsion stabilized by methacrylate-functionalized hyaluronic acid (HA-MA) to encapsulate LEF (EM-LEF), yielding to stable droplets (~282 nm) for skin delivery. In primary human keratinocytes and 3D human epidermal equivalents, topical application of EM-LEF safely and dramatically enhanced AHR signaling and upregulated key epidermal differentiation markers compared to non-encapsulated or systemically administered LEF. Furthermore, therapeutic evaluation in a Th2-mediated AD model revealed that topical EM-LEF successfully limited keratinocyte hyperproliferation and restored essential skin barrier proteins, including filaggrin and hornerin. Crucially, topical EM-LEF facilitated sustained epidermal penetration and enabled the local metabolic conversion of LEF into TERIFLU within the viable epidermis, a biotransformation nonexistent with non-encapsulated LEF.
By enabling the effective topical delivery of LEF, HA-MA-stabilized emulsions offer a powerful repurposing strategy for AD management that achieves targeted anti-inflammatory and barrier-restoring effects while circumventing systemic administration side effects.

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Characterization of Nck's role in the pathogenesis of psoriasis: a potential novel therapeutic target

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The aim of this study was to elucidate the role of the Nck adapter protein in T-cell activation in the pathogenesis of psoriasis. T-cell activation is a cornerstone in the development of plaque psoriasis. Current psoriasis treatments include topical therapies, phototherapy, conventional systemic agents, and biologics. Despite major therapeutic advances, unmet needs remain due to variable treatment responses, adherence challenges, and adverse effects in a subset of patients. Nck has been found to have a modulatory effect on T-cell activation. Thus, we hypothesized that Nck may have a role in T-cell driven inflammation, as seen in psoriasis, and that its inhibition reduces pathogenic cytokine production. First, we investigated the location and gene expression of Nck1 and Nck2 in non-lesional and lesional psoriasis skin, as well as on healthy donor skin, using immunohistochemistry and qPCR. We found, that Nck1 and Nck2 were present in both non-lesional and lesional skin, and in both the epidermal and dermal layer. Interestingly, we found no difference in the gene expression of Nck1 or Nck2 when comparing lesional, non-lesional and healthy skin. Next, we utilized a unique *ex vivo* skin model using 4 mm punch skin biopsies obtained from healthy donors. These were then stimulated with a Th17 driving cocktail and treated with either a vehicle or a specific Nck inhibitor (AX-024) for 48 hours. Following incubation, RNA was extracted for qPCR to identify changes

in the expression of specific psoriasis-associated inflammatory genes. Our preliminary findings demonstrated that pharmacological inhibition of Nck reduced the expression of selected psoriasis-associated inflammatory genes, consistent with modulation of T-cell–driven inflammatory pathways. Taken together, our findings demonstrate that Nck1 and Nck2 are present in both healthy and psoriatic skin, with no differences in expression levels between groups. Furthermore, pharmacological inhibition of Nck modulated psoriasis-associated inflammatory responses in psoriatic skin. These results identify Nck as a potential novel therapeutic target for modulation of T-cell–driven inflammation in psoriasis.

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Beyond lesions: Dupilumab reduces *Staphylococcus aureus* reservoirs in atopic dermatitis

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This study assessed the impact of dupilumab on *Staphylococcus aureus* colonisation in atopic dermatitis (AD), focusing on the anterior nares as a potential reservoir for skin colonisation.
Swabs from anterior nares, lesional and non-lesional skin were collected from adult AD patients (2024–2025). Clinical/demographic data were recorded. *S. aureus* was isolated on selective media, quantified (CFU/mL), and *spa*-typed to infer clonal complexes (CCs). Colonisation prevalence and bacterial load were compared between dupilumab-treated and untreated patients, for each sampling site. Whole-genome sequencing (Illumina) was performed on 19 isolates (dominant CC), and within-patient relatedness assessed by SNP analysis.
Fifty-seven patients (median age 30 years; balanced sex distribution) with variable disease severity (EASI 0–50, median 10; NRS 0–10, median 5.5) were included, of whom 26 were under dupilumab. CC398 predominated overall (47%) and at each site (nares 44%; non-lesional skin 53%; lesional skin 46%). Most patients (84%) carried a single lineage across sites. Dupilumab-treated patients showed lower lesion colonisation (31% vs 71%, p=0.0058) and 2-fold lower bacterial load (p=0.0044). In non-lesional skin and nares, prevalence was unchanged, but CFU/mL was 2-fold lower in treated patients (p<0.01). Within-patient isolates were more closely related (2–46 SNPs) than between patients (162–982 SNPs), supporting intra-host spread.
Dupilumab reduces *S. aureus* burden in AD lesions and is associated with lower bacterial load in non-lesional skin and nares; however, CC398 remains predominant. Genetic relatedness supports the anterior nares as a key reservoir for skin colonisation. Although dupilumab does not alter strain predominance, reduced bacterial load beyond lesions may limit intra-host spread and recolonisation.

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Inflammaskin® enables evaluation of targeted immunomodulatory therapies in a psoriasis-like human skin model

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InflammaSkin® is an *ex vivo* human skin model that replicates a psoriasis-like phenotype. It features *in situ* T-cell activation with Th1/Th17 polarization and remains viable for research use for 7 days. Human skin explants are stimulated with a combination of cytokines and antibodies described in previous scientific papers to induce psoriasis-like inflammation, while non-stimulated samples serve as controls.
Conventional histological markers such as K16 and S100A7 provide limited insight into T cell-driven responses. First, we analyzed the cytokine profile of InflammaSkin® compared with HypoSkin® in 10 donors. We then evaluated inflammation resolution following the administration of betamethasone, and secukinumab to assess the relevance of this model for the evaluation of immunomodulatory therapies.
Initial secretome analysis identified 12 cytokines that were significantly increased in InflammaSkin®: IFNγ, IL-22, IL-17A, IL-10, MCP-1, GM-CSF, IL-15, IL-1α, IL-5, IL-7, TNFα, and VEGF. Flow cytometry analysis revealed that T cells derived from InflammaSkin® models exhibited an activated phenotype, marked by expression of the proliferation marker Ki-67 and the effector molecule Granzyme B. Treatment of InflammaSkin® with clinically relevant molecules induced a statistically significant decrease in several cytokines, including IL-8, IL-17A, MIP-1β, MIP-1α, MIP-3α, and IL-22 after betamethasone treatment, and TNFβ and IL-16 after secukinumab treatment.
These results demonstrate that InflammaSkin® recapitulates key features of T cell-mediated psoriasis-like inflammation. Its translational relevance supports the evaluation of immunomodulatory effects and positions as a valuable solution for assessing inflammation resolution following administration of targeted therapeutics.

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Loss of Regnase-3 triggers cutaneous hypersensitivity via non-canonical activation of interferon-stimulated genes

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Regnase-3, also known as MCPIP3, is an RNase belonging to the MCPIP protein family. Recent studies have demonstrated that it is an important modulator of epidermal proliferation and differentiation under homeostatic conditions. The aim of this study was to elucidate the mechanisms underlying immune cell activation regulated by Regnase-3 in proliferative and inflammatory skin disorders. We employed a 12-O-tetradecanoylphorbol-13-acetate-induced (TPA) skin inflammation model, as well as a 7,12-dimethylbenz[a]anthracene-initiated (DMBA), TPA-promoted skin carcinogenesis model. For that we utilized a mouse model with epidermis-specific deletion of Regnase-3, together with complementary in vitro studies.
In the TPA model, we observed an increased expression of genes associated with interferon response and antigen-presenting cell activation. Furthermore, in the skin carcinogenesis model, mice with Regnase-3 deficiency developed tumors with a less aggressive phenotype, as confirmed by histological analysis. Control animals developed typical features of squamous cell carcinoma tumors, which were completely absent in tumors from keratinocyte-specific Regnase-3-deficient mice. At the molecular level, this phenotype was associated with elevated expression of genes linked to the interferon-independent activation of interferon-stimulated genes, interleukin-33 signaling, chemotaxis, and antigen presentation. We further

demonstrated direct negative regulation of selected factors by Regnase-3 RNase using in vitro approaches. Collectively, these findings indicate that loss of Regnase-3 leads to heightened skin hypersensitivity associated with dysregulated, interferon-independent immune activation. This mechanism may have important implications for inflammatory and hyperproliferative skin disorders, as well as for tumor development and progression.

112 [WITHDRAWN]

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Mild and moderate to severe psoriasis share lesional clinical and transcriptomic profiles during guselkumab induction

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Psoriasis is a chronic inflammatory skin disease with heterogeneous clinical severity and complex immune dysregulation. Mild psoriasis is often underrepresented in molecular studies despite representing the majority of patients. This study characterizes local lesional clinical progression and transcriptomic profiles in mild psoriasis, compares these profiles with lesional features of moderate to severe disease, and evaluates the effects of guselkumab versus placebo on treatment efficacy and biomarker identification. In this randomized, double blind, placebo controlled trial, 20 patients with mild psoriasis (PASI 5 or lower) were randomized 3 to 1 to receive placebo or guselkumab. Clinical outcomes were assessed over 24 weeks, and lesional skin biopsies were collected for RNA sequencing analyses. In addition, a separate cohort of five patients with moderate to severe psoriasis (PASI 10 or higher) received open label guselkumab 100 mg and was included as a reference control for molecular analyses. Guselkumab treated mild and moderate to severe patients showed clinical improvement compared with baseline and placebo. Lesional skin from mild and moderate to severe psoriasis patients exhibited highly similar transcriptomic profiles relative to healthy controls and non lesional skin, with shared psoriasis associated gene expression patterns and minimal differences between severity groups during induction. Guselkumab induction therapy resulted in comparable lesion level clinical improvement and transcriptomic responses in patients with mild and moderate to severe psoriasis, indicating that mild psoriasis can exhibit a local clinical and molecular burden similar to more severe disease. These findings support further evaluation of biologic therapy, including guselkumab, for selected patients with mild psoriasis based on local disease characteristics and clinical impact rather than total body scores.

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Systemic cytokine profiling in lichen sclerosus: insights into disease activity

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Lichen sclerosus (LS) is a chronic inflammatory skin disease affecting mainly the genital and perianal regions, associated with reduced quality of life and potential malignant transformation in long-standing cases. Although immune-mediated mechanisms are recognized, reliable systemic biomarkers of disease activity and treatment response are still lacking. This study aimed to characterize, for the first time, the circulating cytokine profile in LS patients and to evaluate its modulation following regenerative treatment with stromal vascular fraction derived from adipose tissue (AD-SVF), alone or combined with platelet-rich plasma (PRP). A total of 29 LS patients and 17 age- and sex-matched healthy controls were enrolled. Serum levels of CXCL-9, CXCL-10, IFN-γ, IL-1α, IL-1β, IL-6, and TNF-α were measured using the Ella microfluidic platform. A subgroup of 11 patients was reassessed one month after treatment. IL-1α and IL-1β were below the quantification limit in most samples. LS patients showed significantly higher serum levels of CXCL-9, CXCL-10, and IL-6 compared to controls. Sex-stratified analysis revealed higher cytokine levels in females, with a significant difference for CXCL-9. Patients with advanced disease exhibited significantly increased CXCL-9, CXCL-10, and IFN-γ compared to early-stage disease. Pre/post-treatment analyses of the LS cohort showed no significant changes in cytokine levels. Conversely, a high treatment response score tended to be associated with decreased post-treatment cytokine levels. Stratification by treatment suggested lower cytokine levels in patients receiving AD-SVF monotherapy compared with those treated with AD-SVF plus PRP. Overall, these findings suggest that LS, despite being clinically localized, is associated with a systemic modulation of circulating cytokines influenced by sex and disease stage and support a potential role for cytokine profiles as prognostic biomarkers.

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The Potential Skin-Protective Role of Inflammatory ILC2s in Atopic Dermatitis

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The hygiene hypothesis proposes that reduced exposure to microorganisms and parasites alters immune development and regulation, contributing to the rising prevalence of immune-mediated diseases, including atopic dermatitis (AD). Epidemiological and experimental evidence suggest that helminth infection can attenuate allergic diseases. However, the cellular mechanisms remain unclear. Group 2 innate lymphoid cells (ILC2s) are tissue-resident lymphocytes that produce Th2 cytokines and contribute to AD pathology. A distinct subset termed inflammatory ILC2s (iILC2s) is induced during intestinal helminth infection. Unlike tissue-resident ILC2s, iILC2s migrate systemically from the intestine to distal organs. We have previously demonstrated that iILC2s exit the intestinal tissue into afferent lymphatics in an S1PR5-dependent manner and subsequently egress from lymph nodes into efferent lymphatics via S1PR1-dependent mechanisms before entering the circulation. Furthermore, we found that iILC2s promote tissue repair in the helminth-damaged lung. Given their marked expansion during helminth infection, capacity for systemic redistribution, and reparative function, we hypothesized that iILC2s may also reach the skin and modulate inflammation in AD. We performed flow cytometric analysis of the lung, mesenteric lymph nodes, peripheral blood, and skin and observed iILC2 accumulation across these sites, indicating systemic mobilization from the intestine to distal organs during Nb infection. In a combined Nb–AD model, mice were first infected with Nb and subsequently treated with topical calcipotriol (MC903) to induce AD. Infected mice showed a trend toward reduced erythema and ear thickness compared to uninfected AD

controls. These findings may suggest that helminth infection induces systemic iILC2 redistribution, including the skin, potentially contributing to the modulation of AD and providing a mechanistic cellular basis for the hygiene hypothesis.

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Neonatal Langerhans cell dysregulation in dry skin drives atopic dermatitis

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The early-life factors driving atopic dermatitis (AD) remain unclear. We investigated the neonatal pre-disease state using a human birth cohort and mouse models.
Longitudinal skin microbiome analysis in a human birth cohort revealed that infants who developed AD by 1 year of age already showed skin dysbiosis on day 3 after birth. Accordingly, we analyzed host gene expression in neonatal skin. Day 3 skin transcriptome analysis revealed distinct gene expression in infants who later developed AD, including significant upregulation of *GeneX* (name withheld for confidentiality; SNR = 1.41, FDR < 0.05), which enhances Th2 signaling in neonatal skin before AD onset. *GeneX* expression was inversely correlated with stratum corneum water content (Spearman's rank correlation, Rs = –0.678, $p < 0.05$), being higher in drier skin.
In an AD mouse model (*K5-Cre Stat3$^{fl/fl}$*), *GeneX* expression was comparable at E18.5 but significantly increased by postnatal week 1 ($p < 0.01$, Mann–Whitney U test). Single-cell RNA sequencing of an AD mouse model identified Langerhans cells (LCs) as the primary source of *GeneX* (log2FC = 1.45, $p < 0.05$). Notably, the proportion of LCs within the antigen-presenting cell (APC) compartment was significantly reduced in an AD mouse model ($p < 0.001$, Mann–Whitney U test), while the remaining LCs exhibited an activated transcriptional profile. KEGG pathway analysis showed enrichment of inflammatory signaling pathways associated with oxidative stress, supported by the upregulation of *Nfkb1*, *Jak2*, and *Mapk8*.
Finally, neonatal subcutaneous administration (s.c.) of a *GeneX* inhibitor significantly reduced AD incidence in the AD mouse model up to 20 weeks of age ($p < 0.05$, chi-squared test).
These findings suggest that early-life dysregulation of cutaneous LCs, related to skin dryness, contributes to AD development, highlighting a critical window for preventive intervention.

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Spatial and bulk transcriptomic analyses identify dermal inflammatory signatures associated with treatment-resistant pyoderma gangrenosum

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Pyoderma gangrenosum (PG) is a neutrophilic dermatosis characterized by destructive skin ulcers and pathergy, in which minor trauma or surgery triggers lesions. While some cases respond well to treatment with sustained remission, others follow a refractory course. In this study, we aimed to identify factors associated with refractory PG at the tissue, cellular, and molecular levels using bulk and spatial transcriptomic analyses. Bulk RNA sequencing was performed on 12 formalin-fixed paraffin-embedded (FFPE) skin samples from PG patients, which were stratified into refractory PG cases (n = 4; cases with recurrence for more than one year) and resolved PG cases (n = 8). Differentially expressed gene analysis revealed upregulation of *CXCL9* and *CD8A* in refractory PG, whereas the expression of epidermis-derived inflammatory mediators (*CTSG*, *IL36G*, and *DEFB1*) was reduced. Gene Ontology biological process analysis demonstrated enrichment of pathways related to chemokine signaling and dendritic cell differentiation and activation in refractory cases. Spatial transcriptomic analysis using Visium HD was performed on two FFPE samples from refractory PG. The data were output at a nucleus-level transcriptome to achieve single-cell–level resolution. We analyzed chemokine expression cells and infiltrating dendritic cells according to dermal depth. Our findings suggest that distinct cellular and molecular inflammatory signatures within the dermis contribute to the pathogenesis and treatment resistance of PG.

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Non-coding snoRNA Host Genes as novel therapeutic targets in atopic dermatitis and psoriasis

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Atopic dermatitis (AD) and psoriasis are common chronic inflammatory skin diseases characterised by abnormal keratinocyte proliferation and differentiation. Both diseases are associated with increases in different sets of cytokines, resulting in distinct but overlapping clinical features. Current therapies, such as systemic immunosuppressants, biologics and topical corticosteroids, have limitations due to side effects and/or cost, highlighting the need for novel targeted treatments.
Recent findings underscore the importance of non-coding RNAs, particularly microRNAs (miRNAs) and long non-coding RNAs (lncRNAs), in skin biology. MiRNAs are short endogenous RNAs that inhibit the expression of target genes, affecting keratinocyte behaviour and inflammation in both AD and psoriasis. Therapeutic use of miRNAs is hampered by off-target effects, as individual miRNAs can influence hundreds of genes. LncRNAs have the capacity to bind and inhibit multiple miRNAs simultaneously, potentially increasing the therapeutic specificity of miRNA targeting. A group of unusually highly expressed lncRNAs called non-coding snoRNA Host Genes (ncSNHGs) can strongly stimulate keratinocytes to self-renew and inhibit their differentiation by affecting miRNA activity.
We found multiple ncSNHGs to be increased in the lesions of both AD and psoriasis. Several of these lesional ncSNHGs are able to strongly affect keratinocyte proliferation and differentiation and are predicted to bind multiple miRNAs that have been implicated in AD and psoriasis.
Our preliminary results show that a subset of ncSNHGs are promising targets for the treatment of AD and psoriasis lesions. We are engineering models of AD and psoriasis using human skin equivalents challenged by different cytokine cocktails and

generating primary keratinocyte lines that can be induced to downregurate epidermal ncSNHGs to further study their effectiveness and reliance on miRNA interactions.

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Development and validation of a chronic oxazolone-induced mouse model of atopic dermatitis

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Atopic dermatitis is a chronic inflammatory skin disease driven by epidermal barrier dysfunction and type 2 immune activation. While short-term oxazolone-driven mouse models reproduce key disease features, chronicity is required for reliable long-term therapeutic evaluation. The aim of this study was to develop and validate a chronic oxazolone-induced mouse model using interleukin-4 receptor alpha blockade as a clinically relevant benchmark.
Repeated oxazolone challenges were applied to induce sustained skin inflammation. Disease severity was monitored longitudinally by ear thickness, erythema and weight. Immune cell composition was analysed in skin and draining lymph nodes by flow cytometry, while gene expression changes in ear tissue were assessed by quantitative PCR. A murine surrogate of dupilumab was administered therapeutically to evaluate model responsiveness.
Chronic oxazolone exposure induced persistent ear inflammation accompanied by marked epidermal thickening and erythema. Treatment with interleukin-4 receptor alpha blockade consistently reduced clinical disease severity throughout the study. This improvement was associated with downregulation of genes linked to interleukin-4 and interleukin-13 signalling and normalization of markers related to epidermal differentiation and inflammation. Immunophenotyping revealed a pronounced reduction of inflammatory immune infiltrates in the skin, particularly CD4-positive T cells enriched in memory and tissue-resident subsets, while immune cell numbers in draining lymph nodes were largely unaffected.
These findings demonstrate that the chronic oxazolone-induced model recapitulates key immunopathological features of atopic dermatitis and responds effectively to targeted type 2 cytokine inhibition. This model therefore represents a robust and translational platform for preclinical evaluation of novel therapeutic approaches for chronic atopic dermatitis.

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Sex-dependent inflammatory network architecture predicts response to biologic therapy in psoriasis

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Psoriasis is a chronic immune-mediated skin disease driven by the IL-23/IL-17 axis, where immune–keratinocyte interactions and inflammatory network organization contribute to disease persistence and variability in therapeutic response. Although biologics targeting TNF and IL-23 are effective, response heterogeneity remains incompletely understood. We investigated whether sex influences systemic inflammatory architecture and whether baseline cytokine patterns predict biologic response.
We analyzed 34 patients with moderate-to-severe psoriasis (12 females, 22 males) prior to anti-TNF or anti-IL-23 therapy. Plasma inflammatory mediators were quantified, including upstream cytokines (IL-23A, IL-12, IL-27), T-cell effectors (IL-17A, IL-17F, IL-17B, IL-13, IL-25), chemokines and receptor-associated markers (CCL20, CXCL5, CXCL8, CXCL10, CXCL13, CCL16, IL-6R, TNFRI), and tissue remodeling/inflammatory markers (MMP13, PF4, IL-6). Associations with sex, prior biologic exposure, and clinical response were assessed, including decision tree modeling.
Baseline inflammatory mediator levels did not differ significantly between sexes. However, among biologic-exposed patients, females showed higher CCL20 and MMP13, a trend toward lower CXCL10, and reduced soluble IL-6 receptor compared with males. CCL20 is notable for its role in CCR6+ Th17 cell recruitment and epidermal immune amplification. Baseline inflammatory signatures demonstrated sex-dependent predictive value for treatment response. In males, low MMP13 predicted non-response (86%), while in those with high MMP13, low IL-6 identified all responders (100%). In females, soluble IL-6 receptor levels stratified outcomes, with higher levels identifying most responders (85%) and lower levels associated with non-response (80%).
Sex does not primarily affect systemic IL-23/Th17 cytokine abundance in psoriasis but instead reshapes the inflammatory network underlying biologic response. Extracellular matrix remodeling pathways emerge as key predictors in males, whereas IL-6 signaling dynamics dominate in females.

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Mechanical Stress in Pruritus: Effects on Nuclear Morphology, Chromatin, and Gene Expression

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Chronic pruritus is characterized by a self-perpetuating itch-scratch cycle, in which repetitive scratching exerts continuous mechanical stress on skin cells. These forces are transmitted from the cell membrane through the cytoskeleton to the nucleus, altering nuclear structure and chromatin organization. In this context, the study investigates the effects of mechanical stress on keratinocyte nuclei, with a focus on nuclear morphology, chromatin organization, and gene expression.
To assess the molecular impact of mechanical stimulation on the nucleus, primary human keratinocytes were subjected to cyclic strain for six hours using the Cytostretcher platform (Curi Bio). This in vitro model simulates biomechanical forces associated with chronic scratching and allows controlled analysis of mechanotransductive responses. Cells were subsequently examined by immunofluorescence staining, or by quantitative PCR analysis.
We were able to show that mechanical stress induced significant changes in nuclear morphology, characterized by increased nuclear area and irregularity. Chromatin organization shifted toward increased euchromatin presence in stressed cells, whereas heterochromatin predominated in unstressed cells. Gene expression analysis revealed downregulation of genes involved in sensory signal transduction (TRPV1, TRPV3) as well as neurotrophic signaling and neuronal interactions (TAC1, NTRK1). Upregulation was present in genes associated with immune signaling pathways (TSLP, NFKB1) and neuronal plasticity (NRN1), alongside dysregulation of NGF and SEMA3A.
In conclusion, mechanical stress alters the nuclear architecture, chromatin composition, and gene expression in keratinocytes.

These findings highlight the role of mechanotransduction in modulating immune and inflammatory responses as well as neuroepithelial crosstalk, providing a basis for further investigation into mechanically driven mechanisms in chronic pruritus.

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Comparative Neurovascular Signaling in Facial and Abdominal Skin: Implications in Rosacea

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Rosacea is a chronic facial skin disease affecting 5% of the population, combining erythema and telangiectasia. Despite its link to immune, vascular and neuronal dysregulation, rosacea pathogenesis remains poorly understood. Facial skin shows unique adaptation to cold, where vasoconstriction is bypassed by neurogenic inflammation leading to vasodilation. This may result from increased sensitivity of facial endothelial cells to neuropeptides released by sensory innervation upon TRP signaling. Our aim is to study neurovascular interactions through a novel comparative analysis of facial and abdominal cells, targeting their response to neuropeptides and TRP channels. Comparative histological analysis of normal human facial and abdominal skin was performed to characterize vascular and neuronal networks by co-staining of LYVE-1 (lymphatic cells), CD31 (vascular cells) and Neuro-Filament-L fibers. Images were taken by confocal microscopy, then quantified in ImageJ. Expression and distribution of NK-1R (SP receptor) and RAMP1 (CGRP receptor) on endothelial cells, fibroblasts and keratinocytes were analyzed by western blot. Endothelial cells and fibroblasts extracted from facial or abdominal skin (five to seven donors) were treated with the neuropeptides SP or CGRP with or without LL-37. Nitric Oxide (NO) released was assessed as a marker of vasodilatation. Facial skin is more vascularized and more densely innervated than abdominal skin. SP receptor expression was increased in endothelial cells and fibroblasts from facial skin, while CGRP receptor expression was elevated only in fibroblasts. Stimulation with SP, SP with LL-37 or CGRP increased NO release. SP induced stronger NO release in endothelial cells, whereas CGRP had a greater effect in fibroblasts. Furthermore, data suggest that facial skin endothelial cells release increased amounts of NO in response to stimulation by neuropeptides SP, CGRP with or without LL-37. Thus, it appears that vasodilation of skin cells may be more sensitive to neuropeptides which is a well-characterized feature of rosacea. Therefore, preventing neuroinflammation may be a promising therapeutic strategy for treating rosacea.

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Differential effects of type 1 and type 2 cytokines on epidermal barrier function in human *ex vivo* skin

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Atopic dermatitis (AD) is characterised by epidermal barrier dysfunction and immune dysregulation, yet the direct functional contribution of distinct inflammatory axes to human skin barrier impairment remains incompletely defined. This study aimed to compare the effects of type 1, type 2 and IL-22-associated cytokine signalling on epidermal barrier function in a translational AD relevant human *ex vivo* skin model.
Full-thickness healthy human skin explants were cultured *ex vivo* and exposed to representative cytokine conditions reflecting type 1, type 2 or IL-22-driven inflammatory environments. Barrier function was assessed longitudinally by trans-epidermal water loss (TEWL) and at endpoint by passive epidermal penetration of topically applied Lucifer Yellow (LY). Filaggrin (FLG) and loricrin (LOR) expression were evaluated histologically to relate functional changes to alterations in structural barrier proteins, and cytokine blockade was used in selected conditions to assess reversibility of barrier dysfunction.
Type 2 cytokine exposure reduced FLG and LOR expression but did not induce measurable functional barrier disruption, as TEWL was comparable to control skin and LY penetration was limited. In contrast, type 1 and IL-22-associated cytokines both markedly downregulated FLG and LOR and were associated with increased TEWL and enhanced LY penetration into the epidermis, consistent with functional barrier impairment. Blockade of type 1 or IL-22-associated signalling partially reversed water loss.
These findings show that cytokine-driven suppression of barrier markers does not uniformly translate into functional barrier disruption in human skin. Type 1 and IL-22-associated inflammatory signals, but not type 2 cytokine exposure, impaired epidermal barrier function in this *ex vivo* model, highlighting distinct contributions of inflammatory pathways to AD barrier pathology.

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Immune regulation of a prebiotic (GOS/Inulin) supplementation during pregnancy to prevent atopic dermatitis in children

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Atopic dermatitis (AD) affects 10–15% of children in Europe and is associated with a disrupted epithelial barrier, gut microbial imbalance and immune dysfunction leading to loss of tolerance. Prebiotics, with immunomodulatory properties that stimulate the diversity of the gut microbiota, are ideal interventional candidates to improve host's health. Because prenatal life is a crucial period during which different tolerance mechanisms are put in place, we determined whether antenatal prebiotics supplementation prevents AD in high-risk children (the PREGRALL study, NCT03183440). Despite no preventive effect of AD was observed in the children at one year of age from prebiotic-supplemented mothers, their gut microbiota was different from the control children. We aimed to decipher the impact of the antenatal prebiotic supplementation on the immune system of the children.
A blood biocollection was made from children at one year of age (n=41). An exhaustive characterization of the frequency and the functionality of immune cells by spectral cytometry was done. RNA sequencing was performed using the 3'seq-RNA. Gene set enrichment analysis was performed to identify enriched biological pathways. The relationships between spectral cytometry and transcriptomic data were explored in a multiblock analysis with sparse PLS-DA method.
We identified a distinct gene expression profile linked to an immune signature in one-year-old children with AD. Indeed, 20 genes were associated with increased expression of exhaustion or homing biomarkers on pro-inflammatory cells. Interestingly,

antenatal prebiotic supplementation reduced inflammatory biomarkers (at both the gene and protein levels) in children with AD, to levels comparable to healthy children. In contrast, antenatal prebiotic supplementation increased inflammatory markers in non-AD children.
These results suggest that prebiotics may boost the immune system under non-pathological conditions to contribute to the host homeostasis, while dampening inflammation in pathological contexts. This study encourages monitoring the AD children up to the age of five to observe the potential onset of other allergies.

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Visualization of the skin water barrier efficacy as a function of depth
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Confocal Raman Microspectroscopy (CRM) is used to noninvasively generate water concentration through the stratum corneum (SC). This information in combination with the trans epidermal water loss (TEWL) on the same skin site can be used to calculate a profile of the resistance to water diffusion (RWD). Using this method we have demonstrated: a) the immaturity of infant skin barrier compared to adult, b) the compromised barrier in atopic dermatitis according to lesion severity, c) the effects of topical products on enhancing the skin barrier function. Until now the method has been used on average water profiles resulting in a single one-dimensional profile. In this work we generate a 2D map of the RWD profiles projected as a vertical section through the SC.
Raman spectra (~30 um into the skin) and TEWL were measured on the volar forearm of healthy volunteers. Water concentration profiles were generated and transformed to RWD profiles using the equation Ri = (dW/dz)i / TEWL. Finally, the individual RWD profiles were combined into a 2D map corresponding to each volunteer and site.
As we go from the bottom of the SC to the skin surface, the RWD profiles demonstrate an increase in the efficacy of the skin barrier function consistent with the fact that this is the zone of ceramide formation and secretion into the extra corneocyte space as well as the production of natural moisturizing factors, products of the proteolytic activity that begins in this region. The RWD increases towards the skin surface and then decreases close to the surface. This happens as the activity of enzymes that break the corneodesmosomes including kallikreins increases close to the skin surface due to the gradual drop of the skin pH.
2D visualization of the RWD can provide insights into how the skin barrier to water loss is formed within the SC and can be used in better understanding of diseases relating to skin barrier, such as atopic and irritant dermatitis. Moreover, it can be used to demonstrate the effect of topical products aiming to improve conditions of compromised skin barrier.

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Identification of Novel Regulators of the Keratinocyte CBM Complex in Psoriasis
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This study aims to identify novel activators and regulators of the keratinocyte CARD14/BCL10/MALT1 (CBM) complex, a key signalosome in the pathogenesis of psoriasis, to uncover potential therapeutic targets.
Our group previously demonstrated that BCL10 activity correlates with CBM complex function. Using the DepMap database, which enables analysis of the viability effects of knocking out approximately 18,000 genes across 1,070 cancer cell lines, we correlated genetic dependencies with sensitivity to BCL10 knockout. This approach allowed us to identify genes potentially involved in the same signaling pathways as BCL10, and thus candidate regulators of the CBM complex. We further prioritized genes associated with psoriasis based on GWAS studies. ClueGO pathway analysis was performed on significant genes.
Our analysis identified 58 genes whose knockout significantly correlated ($p < 0.05$, FDR < 0.25, $r > 0.2$) with BCL10 dependency. Among the top correlating genes were MALT1 and CARD14, validating the ability of our approach to identify key components of the CBM pathway. In addition, several genes associated with psoriasis in GWAS datasets also showed significant correlation. ClueGO analysis revealed that these genes are involved in more than 100 signaling pathways, including known psoriasis-related pathways such as NF-κB signaling.
Using this approach, we identified several genes that may act as regulators or activators of the CBM complex and could contribute to psoriasis pathogenesis. Future studies will focus on validating these findings through in vitro and in vivo experiments to identify potential therapeutic targets.

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Impact of cell culture medium on epidermal physiology and inflammation parameters in organotypic models
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N/TERT immortalized keratinocyte (KC) cell lines have been widely adopted in investigative dermatology, sharing key characteristics with primary KC and mimicking native epidermis in human epidermal equivalents (HEEs). Their implementation across various laboratories increased variability in culture protocols, complicating cross-study comparisons and reproducibility. To identify best practices, we benchmarked two of the most commonly used cell culture media, EpiLife and CELLnTEC (CnT), for generating HEEs. Longitudinal samples were taken for morphology, transcriptomics, protein expression, and functional barrier properties and compared to *in vivo* epidermis. CnT-HEEs showed higher proliferation resulting in more suprabasal layers compared to Epilife-HEEs. Both media generated a cobblestone-like basal layer and distinct spinous and granular layers, based on morphology and differentiation markers. Keratins 2, 10, 15 and 16, filaggrin, involucrin, transglutaminase-1, cathepsin V staining revealed differences in protein localization and intensity. RNA sequencing revealed 1471 differentially expressed genes (Log2FC≥2, padj≤0.05), with enriched cytokine signaling pathway activation in EpiLife-HEEs, including *TNF* and *IL-17RE* expression. *Stratum corneum* development was distinct between media. CnT-HEEs showed continued cornification throughout culture with more pronounced and stable barrier function. Although *stratum corneum* formation was less apparent in EpiLife-HEEs, barrier function increased progressively between days 8-16 of the air-liquid interface culture. Our in depth head-to-head comparison revealed important physiological differences and time-sensitive skin barrier parameters

determined by culture medium composition. These results should direct researchers in selecting the right organotypic model for the right question.

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Keratinocyte inflammasome activation defines the first wave of ultraviolet-induced inflammation and drives second-wave cytokine release

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Ultraviolet-induced skin inflammation is widely recognized as a cytokine-driven response, yet the early-phase mechanisms that organize subsequent inflammatory amplification remain incompletely defined. In our previous mechanistic studies using a nigericin-stimulated keratinocyte model, we identified inflammasome activation as an early inflammatory response. Here, we investigated whether inflammasome activation functions as a first wave associated with a subsequent second wave of cytokine release under a physiologically relevant ultraviolet stimulus. Keratinocytes were irradiated with ultraviolet light (48 mJ/cm^2), and culture supernatants were analyzed 24 h later. Cells were treated with the caspase-1 inhibitor Z-YVAD-FMK (1–20 μM). To capture the proposed two-wave response, interleukin-1β (IL-1β) release was measured as a readout of early inflammasome activity, whereas interleukin-1α (IL-1α) and tumor necrosis factor-α (TNF-α) release were evaluated as downstream inflammatory outputs using ELISA. Ultraviolet irradiation induced a first-wave inflammasome response. Z-YVAD-FMK suppressed IL-1β release in a dose-dependent manner, confirming effective inhibition of the first wave. Under the same conditions, TNF-α release was also significantly and dose-dependently reduced. Notably, IL-1α release was also significantly reduced across the same concentration range. These findings support a two-wave model in which inflammasome activation in keratinocytes represents a first-wave response associated with downstream cytokine amplification**.** Our data suggest that this early-phase response may be an effective target for attenuating subsequent inflammatory amplification in ultraviolet-induced cutaneous inflammation.

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Dissecting IL4, IL13, and TSLP Signaling Reveals Complementary Roles in Cutaneous Type 2 Inflammation

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The cytokines IL-4 and IL-13 are critical drivers of atopic disease, inducing downstream effectors associated with inflammation, edema, pruritus, airway hyper-responsiveness, and mucus secretion. Engagement of their common receptor, IL4RA, activates STAT6, inducing expression of genes mediating a range of inflammatory responses. TSLP is also elevated in AD, asthma, and COPD and has been implicated in promoting type 2 immune responses at epithelial barrier surfaces. Upon barrier damage, TSLP regulates production of type 2 cytokines and downstream effector responses. Although TSLP is an upstream regulator of IL-4 and IL-13, it also triggers distinct signaling pathways linked to STAT5, the blockade of which may provide additional therapeutic benefit. Antibodies neutralizing IL4RA and IL13 are approved for several atopic indications, and antibodies neutralizing TSLP are approved for use in both allergic and non-allergic asthma.
PF-07275315 is trispecific antibody being developed for the treatment of AD, asthma, and COPD, which simultaneously neutralizes IL-4, IL-13, and TSLP. It is hypothesized that simultaneous blockade of these cytokines will more fully inhibit the effector functions of type 2 immune responses while generating additional efficacy from non-type 2 STAT5-induced pathways. In this study, we stimulated human skin explants with recombinant IL-4, IL-13 and/or TSLP and conducted RNA-sequencing to characterize the unique and overlapping transcriptional signatures. 3749 genes were significantly perturbed by IL-4 and IL-13 and 2066 genes by TSLP. Of these, 838 genes were shared. Shared genes were enriched for markers of type 2 immune activation. Genes unique to IL-4 and IL-13 were enriched in maintenance of keratinocyte barrier function, whereas those unique to TSLP were enriched in lymphocyte survival, stromal and vascular remodeling. These data demonstrate that TSLP orchestrates both shared and unique inflammatory processes compared to IL-4/13 and suggest that combined blockade with PF-07275315 may provide patients with additional efficacy compared to standards of care.

130 [WITHDRAWN]

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PPAR dependent modulation of epidermal lipids by a plant extract

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Ceramides are major lipids of the stratum corneum and play a pivotal role in epidermal barrier integrity, hydration, and resilience to environmental stressors. Alterations in ceramide content, chain length distribution and subclass balance—particularly a reduced NP/NS ratio—are well described in skin diseases and cutaneous aging. The aim of this study was to evaluate the effects of a plant derived extract on key regulatory pathways of epidermal lipid homeostasis and particularly on ceramide synthesis.
The effects of the extract were assessed by lipidomic analyses performed both *in vitro* using differentiated human keratinocytes (dHK) and *in vivo* using tape stripping samples collected from 18 volunteers after 28 days of daily application. Ceramide classes and molecular species were quantified by high resolution Orbitrap mass spectrometry. Transcriptomic analysis was conducted in dHK and PPAR cell reporter assays were performed in presence of the plant extract. Moisturization was assessed on 22 volunteers during the same study.
Transcriptomic profiling revealed activation of sphingolipid metabolism pathways, with *PLIN2* identified as one of the most upregulated genes (+466%). Consistently, reporter assays showed activation of all three PPAR isoforms. Lipidomic analysis confirmed a significant increase in total ceramides in dHK (+137%), a qualitative remodeling characterized by a preferential increase in long chain ceramides (≥C46) and an elevated NP/NS ratio. In vivo, the formulated extract significantly increased long chain NP ceramides (≥C46) (+345%) compared with placebo and raised the NP/NS ratio (+148%). These molecular changes were associated with clinically relevant benefits. Skin hydration increased significantly (+12%) with 86% of subjects showing measurable improvement.

This study demonstrates that a plant derived extract can modulate epidermal lipid metabolism through PPAR dependent pathways, leading to improved barrier related lipid composition and clinically meaningful improvements in hydration and epidermal surface quality.

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Dissecting the *in vitro* effects of individual and combined Th2 cytokines

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The skin of patients with atopic dermatitis (AD) is characterized by impaired keratinocyte differentiation, epidermal barrier disruption, and immune dysregulation driven by Th2-related cytokine signaling. This study aimed to investigate keratinocyte responses to individual and combined Th2 cytokines to better define the specific biosignatures associated with distinct inflammatory cue. To model an atopic dermatitis-like inflammatory condition, human immortalized keratinocytes were exposed to IL-4, IL-13, and IL-31, either alone or in combination and analyzed for expression of genes and proteins involved in proliferation/differentiation, lipid barrier-associated enzymes and inflammatory lipid mediators. By applying targeted lipidomic analysis, we investigated the formation of bioactive lipids, including prostanoids, leukotrienes, and lypoxins, in the promotion of inflammatory response in AD. As expected, the expression of key early and late keratinocyte differentiation markers was globally reduced. Furthermore, we observed decreased levels of corneodesmosin, an adhesion molecule key in the maintenance of epidermal cohesion and barrier integrity. Consistently, several enzymes involved in lipid barrier metabolism were downregulated in response to Th2 cytokines. We also detected a significant upregulation of carbonic anhydrase II, known to be specifically expressed in lesional AD skin. Th2 cytokine treatment modulated linoleic and arachidonic acid metabolism pathway. Notably, for some parameters, IL-31 elicited effects distinct from those of IL-4 and IL-13, suggesting cytokine-specific alterations of keratinocyte response that do not always overlap with those induced by combined stimulation. These findings mirror the complexity of cytokine interplay within the inflammatory skin microenvironment in atopic dermatitis.

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KIOM-A Attenuates Acne-Like Inflammation and Sebum Dysregulation via Suppression of NF-κB and AKT/SREBP Signaling Pathways

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Acne vulgaris is a multifactorial inflammatory skin disorder characterized by excessive sebum production and *Cutibacterium acnes*-induced inflammation. Given the limitations of current therapies, natural products with multi-target activities are of growing interest. This study investigated the anti-acne potential of KIOM-A, a standardized herbal extract, by evaluating its effects on lipogenesis and inflammatory signaling *in vitro* and *in vivo*.
In sebocytes, KIOM-A significantly reduced oleic acid/palmitic acid-induced lipid accumulation and downregulated lipogenic genes such as ACC, SREBP1, FAS and inflammatory cytokines. Protein analysis revealed suppression of FASN, PPARγ, and phosphorylated AKT, indicating inhibition of the AKT-mediated lipogenic pathway. In a *C. acnes*-induced mouse ear model, KIOM-A alleviated acne-like lesions, including ear swelling, epidermal hyperplasia, and inflammatory cell infiltration. Consistently, pro-inflammatory cytokines and TLR2 expression were significantly reduced in ear tissues.
Furthermore, KIOM-A improved lipid dysregulation by decreasing triglyceride, cholesterol, and free fatty acid levels and modulating SREBP1, FAS, and PPARγ expression. Mechanistically, it suppressed NF-κB/MAPK-mediated inflammation and AKT/mTOR/SREBP-driven lipogenesis *in vivo*.
These findings demonstrate that KIOM-A exerts anti-acne effects by simultaneously targeting inflammatory and lipogenic pathways, highlighting its potential as a multi-functional therapeutic agent.

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Molecular Determinants of Type-2 CD4+ Tissue Resident Memory T Cells In Atopic Dermatitis

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Atopic dermatitis (AD) is a chronic inflammatory skin disease driven by type-2 immune responses and epidermal barrier dysfunction. In contrast to CD8+ tissue-resident memory T cells (TRMs), the mechanisms governing the differentiation and maintenance of IL-4/IL-13-producing CD4+ TRMs (TRM2) remain poorly understood. Single-cell transcriptomic studies in human AD have revealed disease-associated stromal and immune programs, suggesting that tissue context and skin microanatomy may influence the establishment of a TRM2 residency program.
To address this question, we leveraged preclinical models of skin inflammation representing distinct immune endotypes: type 2 inflammation induced by repeated exposure to calcipotriol (MC903), type 1 inflammation induced by the hapten DNFB, and type 17 inflammation induced by the TLR7 agonist imiquimod. Using these models, combined with single-cell RNA sequencing, spectral cytometry and lineage-tracing approaches, we performed comparative phenotypic and functional analyses of skin CD4+ TRM subsets associated with each inflammatory context and assessed their persistence over time.
Our preliminary data reveal that CD4+ TRMs arising in these three inflammatory settings display distinct transcriptional programs and that the persistence of CD4+ TRM2 in the skin is limited, not exceeding three months in total ear tissue. Ongoing studies aim to determine the fate of CD4+ TRM2 during the resolution of AD-like inflammation. In parallel, we are testing the contribution of several candidate regulators that may control the acquisition of the TRM2 program in AD skin. Together, this work will delineate the molecular circuits that govern the formation and persistence of CD4+ TRM2 cells in the skin. These insights may pave the way for the development of novel therapeutic strategies aimed at modulating pathogenic TRM populations in allergic skin diseases.

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Expression of dopamine receptor 2 in allergic contact dermatitis. A kinetic study

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The dopamine receptor 2 (DRD2) has been appointed an antiinflammatory role in both innate and adaptive immunity. It has been reported to be able to potentiate the activity of T regs. The results with expression of DRD2 on inflammatory cells have, so far, been contradictory. The expression of DRD2 in allergic contact dermatitis (ACD) was studied using biopsies, collected at 0, 48 and 72 h, from evolving positive patch reactions to nickel sulphate, from 10 nickel-allergic female subjects, using immunohistochemistry. The sections were stained using polyclonal antibodies to DRD2, IFN-γ, FOXP3 and IL-17, in order to investigate a possible co-expression of DRD2 with Tregs, and pro/inflammatory cytokines. DRD2 was expressed by keratinocytes in the basal epidermal layer, the number of cells and their signal strength decreasing with time after application of the antigen. DRD2 expression in inflammatory cells in the papillary dermis was hardly detectable, and only at a high degree of tissue inflammation. In comparison, IFN-γ was expressed by inflammatory cells in the papillary dermis and also, and in a diffuse pattern, in disintegrating basally located keratinocytes, adjacent to spongiosis. FOXP3 was expressed by inflammatory cells in the papillary dermis, in biopsies with increasing inflammation, but also in basally located keratinocytes. IL-17 was expressed by basally located keratinocytes, the number increasing with time. In addition, in inflammatory cells of the papillary dermis in biopsies with the heaviest inflammation. In conclusion we could not detect an evident DRD2 expression in inflammatory cells in evolving ACD biopsies, but in basally located keratinocytes. FOXP3 and IL-17 were, like DRD2, expressed in keratinocytes in a similar fashion.

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Oleocanthal attenuates IL-1β secretion by suppressing NLRP1 inflammasome activation in human epidermal keratinocytes

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Keratinocytes play critical roles in maintaining epidermal barrier function and serving as a first line of innate immune defense. Among the innate immune sensors in human epidermal keratinocytes, the nucleotide-binding oligomerization domain-like receptor family pyrin domain-containing protein 1 (NLRP1) has been identified as the principal inflammasome sensors. Oleocanthal, a secoiridoid phenolic compound in extra-virgin olive oil, exerts anti-inflammatory effects by inhibiting cyclooxygenase-1 (COX-1) and COX-2. However, the effects of oleocanthal on inflammasome activation in epidermal keratinocytes remain unknown. Therefore, we investigated whether oleocanthal modulates NLRP1 inflammasome activation in normal human epidermal keratinocytes (NHEKs). NHEKs were stimulated with the bacterial toxin nigericin to induce NLRP1 inflammasome activation. IL-1β levels in culture supernatants were quantified using ELISA, caspase-1 activity was assessed using Z-YVAD-APC, intracellular ROS levels were quantified using the fluorescent probe CM-H2DCFDA, and intracellular potassium levels were measured using ICP-OES. Oleocanthal suppressed nigericin-induced IL-1β secretion. In contrast, oleocanthal did not directly inhibit caspase-1 activity. In addition, oleocanthal did not inhibit either ROS production or potassium efflux, both of which are involved in nigericin-induced NLRP1 inflammasome activation. These results suggest that oleocanthal may inhibit NLRP1 inflammasome assembly. Talabostat induced NLRP1 inflammasome assembly and oleocanthal inhibited talabostat-induced IL-1β secretion. Collectively, these findings suggest that oleocanthal is a potential modulator of NLRP1 inflammasome signaling in keratinocytes.

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A Clinicopathological Scoring System Enabling Individualized Therapeutic Decision-Making in Cutaneous Sarcoidosis

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Sarcoidosis is a multisystem inflammatory disease most commonly affecting the lungs and skin, with limited effective treatment options. Recent clinical and translational studies suggest that targeted therapies, including Janus kinase (JAK) inhibitors and mechanistic target of rapamycin (mTOR) inhibitors, can induce clinical responses; however, outcomes are heterogeneous, raising the possibility of distinct molecular subtypes characterized by differential pathway activation.
To investigate this, we performed immunohistochemical analyses of lesional skin from 11 patients with histologically confirmed cutaneous sarcoidosis, assessing activation of the JAK/STAT pathway (STAT1, STAT3) and mTOR signaling (phosphorylated S6, pS6). Based on these data, we developed a semi-quantitative clinicopathological scoring system integrating expert dermatopathological assessment with quantitative image-based analysis to classify lesions according to dominant signaling profiles.
We identified distinct and variable patterns of JAK/STAT and mTOR pathway activation across patients, allowing stratification into pathway-defined subgroups. Importantly, baseline signaling profiles were significantly associated with in vivo treatment responses, with differential outcomes observed for JAK/STAT- versus mTOR-targeted therapy. These findings support the biological and clinical relevance of pathway-based stratification.
In conclusion, we present a clinicopathological scoring system that predicts therapeutic response and enables individualized treatment selection in cutaneous sarcoidosis, representing a step toward precision medicine in granulomatous skin diseases.

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Fate Diversification of Human CD8$^+$Tissue-Resident Memory T Cells in Psoriasis

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CD8$^+$tissue-resident memory T cells (TRM) are key mediators of persistent local immune memory in psoriasis, yet it remains unclear when heterogeneity first emerges during human TRM development. Here, we identify phenotypic and molecular diversity within early CD69$^+$CD103$^+$CD8$^+$ T-cell states in human skin using single-cell RNA sequencing of healthy and psoriasis-derived tissues.

Primary human skin–derived T cells from healthy individuals and patients with psoriasis were differentiated in vitro, where they acquired heterogeneous patterns of residency-associated features. These findings support a model in which heterogeneity is established early during TRM differentiation, prior to full acquisition of canonical residency markers and suggest that early tissue-adaptive $CD8^+$ T-cell states may serve as a checkpoint for the emergence of pathogenic TRM programs in psoriasis.

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Resident macrophage–derived C1q restrains DNA sensing and shapes treatment response in psoriasis

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Psoriasis is widely considered a Th17-driven disease; however, incomplete responses to Th17-targeted therapies suggest additional pathogenic mechanisms. Here, we identify persistent activation of DNA-sensing pathways as a common inflammatory program in established psoriasis that drives dominant type I interferon (IFN) responses associated with therapeutic resistance. Type I IFNs are produced predominantly by plasmacytoid dendritic cells (pDCs) through C1q-binding protein (C1qBP)–mediated uptake of LL37–DNA complexes, enabling endosomal DNA sensing. This pathway is normally restrained by the complement component C1q, produced by tissue-resident macrophages, which limits LL37–DNA engagement with C1qBP. In therapy-resistant lesions, selective loss of C1q enhances DNA sensing and amplifies IFN-driven inflammation. Targeting DNA sensing or restoring macrophage-derived C1q-mediated regulation suppresses IFN responses, identifying innate DNA sensing as a key determinant of therapeutic response in psoriasis.

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Systemic Th17 Activation in Viral Warts: Elevated Serum IL-17 Levels in a Cross-Sectional Comparative Study

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Viral warts (verrucae), caused by human papillomavirus, are common benign skin lesions with variable clinical behavior and inconsistent treatment response. The contribution of host immune dysregulation, particularly the Th17 axis, remains incompletely defined, with limited data from South Asia. This study aimed to evaluate serum interleukin-17 (IL-17) levels in patients with viral warts and healthy controls.
This cross-sectional comparative study was conducted at Bangladesh Medical University (former Bangabandhu Sheikh Mujib Medical University), Dhaka. Thirty clinically diagnosed viral wart patients and thirty age- and sex-matched healthy controls were included. Serum IL-17 levels were measured using enzyme-linked immunosorbent assay. Statistical analysis was performed using IBM SPSS Statistics version 26.0, with results expressed as mean ± standard deviation and group comparisons conducted using independent samples t-test.
Patients with viral warts demonstrated significantly elevated serum IL-17 levels compared to controls (37.8 ± 12.6 vs 20.7 ± 8.2 pg/mL, $p < 0.001$), indicating activation of Th17-mediated immune pathways.
This study provides novel evidence from Bangladesh demonstrating systemic upregulation of IL-17 in viral wart patients. These findings support a role for Th17-driven inflammation in disease pathogenesis and highlight the IL-17 pathway as a potential target for future immunomodulatory therapies.

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Oleic acid induces distinct transcriptional programs in proliferating and differentiated keratinocytes

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Sebum-derived lipids are increasingly recognized as active regulators of epidermal homeostasis. However, the effects of these lipids on keratinocytes at different stages of differentiation are not yet fully understood. In this study, we investigated the transcriptional response of human keratinocytes to oleic acid, a major sebum component, in proliferating and differentiated states.
HaCaT keratinocytes were cultured under low-calcium (representing the proliferating keratinocytes' layer of the epidermis) or high-calcium (representing the more differentiated keratinocytes' layers) conditions. The differentiation state of the cells was validated by assessing the expression of established proliferation and differentiation markers using quantitative PCR (qPCR), after which the cells were treated with oleic acid. Global gene expression changes were analyzed using RNA sequencing, followed by differential expression and pathway enrichment analyses.
Oleic acid induced distinct and robust transcriptional responses in the two cellular states, with keratinocytes' layers showing a markedly higher number of differentially expressed genes observed in differentiated keratinocytes than in proliferating cells. Importantly, there was minimal overlap between the regulated gene sets, indicating a strong differentiation-dependent effect. In proliferating keratinocytes, oleic acid primarily modulated genes associated with lipid metabolism and early differentiation. By contrast, in differentiated keratinocytes, oleic acid significantly impacted pathways related to cell cycle regulation, including G1/S transition and S phase progression, as well as immune signaling pathways such as mTOR, IL-17, and TGF-β signaling. These findings demonstrate that oleic acid elicits context-dependent transcriptional programmes in keratinocytes, with more pronounced effects in differentiated cells. Overall, our results emphasize the importance of the cellular differentiation state in shaping keratinocyte responses to sebum lipids, providing insight into how alterations in lipid composition may contribute to epidermal dysregulation in skin disorders.

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Toxin-Triggered Epithelial Systems Failure in Buruli Ulcer: A Mechanistic Model of Epidermal Barrier Breakdown

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This study aimed to determine whether Buruli ulcer exhibits a reproducible cascade of epidermal barrier failure driven by toxin-mediated disruption of skin structure and function. A structured mechanistic synthesis was conducted using PubMed free-text and MeSH strategies, identifying 459 records, of which over 120 were screened using predefined criteria. Studies reporting histological, molecular or cellular findings relevant to epidermal pathology were included and analysed across structural disruption, keratinocyte dysfunction, inflammatory signalling and repair dynamics. Consistent structural features included epidermal necrosis, ulceration and basement membrane disruption across lesion-based studies. Experimental and in vivo data demonstrated mycolactone-mediated keratinocyte apoptosis, cytoskeletal disruption and sustained toxin presence within affected tissue. Immune profiling revealed dysregulated cytokine responses, including IL-1β, IFN-γ and IL-10, alongside vascular dysfunction and fibrin deposition. Evidence of impaired re-epithelialisation and delayed repair was also observed. These findings demonstrate coordinated disruption across structural, cellular and immunological levels. Buruli ulcer therefore represents a reproducible model of toxin-triggered epithelial systems failure, providing a unifying mechanistic framework for epidermal barrier breakdown in inflammatory skin disease and informing future studies of barrier dysfunction in inflammatory skin disease.

144 [WITHDRAWN]

AUTOIMMUNITY, AUTO-INFLAMMATION

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Monocyte-derived dendritic cells may contribute to lung fibrosis in systemic sclerosis through reduced IRF8 expression

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The pathogenesis of systemic sclerosis (SSc) is closely associated with the type I interferon signature and IRF family genes have been identified as susceptibility loci. Our previous studies found that altered IRF8 expression contributed to the fibrotic process in SSc skin, directing monocytes toward profibrotic M2 macrophages. However, the characteristics of monocyte-derived dendritic cells (moDCs) emerging from the same IRF8-low precursors are yet to be defined. Therefore, we aimed to evaluate the functional role of moDCs in SSc, specifically from the perspective of IRF8 regulation.
IRF8 mRNA levels were determined in moDCs generated from SSc patients (n=21) and healthy controls (HC, n=8) using qRT-PCR. For functional analyses, we used myeloid-specific IRF8-knockout (LysM-Cre-IRF8$^{flox/flox}$: IRF8 cKO) mice. Protein membrane arrays and bead-based immunoassays quantified secreted factors, while flow cytometry was performed to evaluate surface markers on moDCs. Furthermore, moDC infiltration was examined in lungs of mice treated with intratracheal bleomycin (BLM) or saline.
Our results revealed that IRF8 mRNA expression level was significantly reduced in moDCs from diffuse cutaneous SSc patients compared to that in HC. In murine models, moDCs from IRF8 cKO mice showed upregulated CD11c and H-2kb, suggesting enhanced maturation and antigen-presenting capacity. Protein membrane arrays and bead-based immunoassays confirmed elevated secretion of CCL3, CCL5, and CCL17 in IRF8 cKO moDCs. Interestingly, these chemokine levels in moDCs from SSc patients correlated with respiratory functional parameters (%VC, %DLCO) and interstitial lung area obtained by HRCT. Finally, increased moDC infiltration was identified in the lungs of BLM-treated mice. These findings suggest that moDCs may contribute to lung fibrosis in SSc through enhanced maturation, chemokine secretion, and lung infiltration. Reduced IRF8 expression appears to play a key role in this process.

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A novel humanized mouse model closely mimics human vitiligo, its complex pathogenesis, and its response to therapy

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Vitiligo pathogenesis remains incompletely understood, in part because existing animal models in sufficiently recapitulate human vitiligo. To overcome this limitation, we grafted darkly pigmented human skin onto SCID/beige mice and applied a protocol that includes coordinated application of oxidative stress, HSP70-mediated innate immune activation, transfer of autologous CD8/NKG2D T-cell primed against melanocyte-associated autoantigens, and patient-derived IgG4 autoantibodies. Only with this complete protocol, 80% of the human skin xenotransplants developed characteristic vitiligo lesions, showing melanocyte loss, interferon-g- and -a-, IL-15- and IL-18 dominated inflammation, dendritic-cell expansion, MICA induction, and accumulation of epidermal resident memory T cells (TRM). Omission of any of the above induction elements, including IgG4 autoantibodies or HSP70, prevented vitiligo induction. Prior to developing visible vitiligo lesions, human epidermal melanocytes and keratinocytes showed oxidative stress–associated senescence (p16^INK4A, p-S6, Lamin B1), mitochondrial dysfunction (PGC-1α, MTCO1, VDAC), and impaired antioxidant defense (NRF2, HO-1), documented by qIHC/IF**.** Transcriptomic profiling revealed selective suppression of MITF-dependent melanocyte programs, alongside dominant IFN-γ/JAK–STAT1 network activation in infiltrating CD8 T cells. Tacrolimus induced partial repigmentation, whereas ruxolitinib achieved substantial repigmentation and normalized the above stress signatures. This novel, clinically predictive humanized vitiligo mouse model demonstrates that coordinated oxidative stress–induced, senescence-promoting keratinocyte and melanocyte damage, HSP70-dependent innate immune activation, IgG4-mediated melanocyte cytotoxicity, and antigen-specific CD8 activation are all required to induce vitiligo in healthy human skin in vivo. Thus, optimal future vitiligo therapy should target as many of these pathogenesis elements as possible.

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From Genetic Risk to Tissue Circuitry: IL-23–Driven Remodeling of Stromal–Immune Niches in Psoriasis

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Psoriasis is a chronic immune-mediated skin disease with a strong genetic basis, in which multiple susceptibility loci converge on the IL-23 signaling pathway, including variants in *IL23R*. The remarkable clinical efficacy of IL-23-targeted therapies emphasize the central role of this pathway in psoriasis pathogenesis. IL-23 signaling through IL23R activates STAT3-dependent programs in T cells, driving IL-17 production and downstream epidermal inflammation. However, despite the clear genetic and therapeutic importance of the IL-23/IL-17 axis in psoriasis, cellular and molecular mechanisms by which IL-23 blockade induces durable disease remission remain incompletely understood.

To address this, single-cell RNA sequencing, TCR sequencing, and spatial transcriptomics were performed on matched pre- and post-treatment psoriatic skin samples from patients (n=16) treated with the anti–IL-23 monoclonal antibody risankizumab (300 or 600 mg at Weeks 0, 4, and 16), followed without further dosing until Week 52. IL-23 blockade resulted in a durable reduction of IL-17–producing $CD8^+$ tissue-resident memory T cells ($CD8^+$ TRM17) and a reprogramming of the local immune microenvironment, including decreased interactions between TRM17 cells and conventional dendritic cells (cDCs), consistent with long-term disease resolution.

Mechanistically, integrative *in silico* and *in vivo* analyses identified suppression of the c-Maf–IL-7 axis, a key survival pathway for TRM17 cells, as a defining feature of the post-treatment state. In parallel, IL-34 produced by $APOE^+$/$IL34^+$ fibroblasts was identified as a central signal promoting monocyte-to-dendritic cell differentiation, while spatially restricted vascular expression of CCL19 and CCL21 coordinated immune cell recruitment. Notably, both fibroblast-derived and vascular niche signals were attenuated following IL-23 blockade.

Together, these findings demonstrate how genetic risk converging on IL-23 signaling is translated into pathogenic cellular circuits, and how targeted blockade of this pathway disrupts stromal–immune interactions and sustains long-term therapeutic responses.

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Selective JAK Inhibition Ameliorates Disease Severity in Experimental Mucous Membrane Pemphigoid and highlights a pathogenetic role of fibroblasts

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Mucous membrane pemphigoid (MMP) is an autoimmune blistering disease characterized by progressive mucosal inflammation and scarring. Current therapeutic approaches show limited efficacy and considerable toxicity, highlighting the need for targeted treatment strategies. Here, we evaluated the therapeutic potential of Janus kinase inhibition (JAKi) in a mouse model of MMP. Disease was induced in C57BL/6J mice by injections of anti-murine laminin-α3 (mLAMA3) IgG. Animals were treated with selective inhibitors targeting JAK1, JAK2, JAK3, TYK2, or pan-JAK using upadacitinib, fedratinib, decernotinib, deucravacitinib and peficitinib, respectively. All inhibitors significantly reduced skin lesions extent compared to vehicle-treated controls. In contrast, TYK2 inhibition showed no significant effects in oral lesions, whereas other inhibitors demonstrated amelioration, with JAK2 being most effective. To mechanistically contextualize these findings, we investigated fibroblast responses upon anti-mLAMA3 IgG treatment. After confirming laminin α3 expression and specific binding of anti-mLAMA3 IgG to murine fibroblasts and in experimental MMP skin, treatment of cultured murine fibroblasts with anti-mLAMA3 IgG induced the secretion of CXCL2. Proteomic cytokine profiling of the cell lysates further revealed upregulation of mediators including M-CSF and PAI-1, while transcriptomic analyses identified activation of inflammatory, oxidative stress, and extracellular matrix remodeling pathways. Importantly, anti-mLAMA3 IgG-driven responses were attenuated by JAKi without compromising cell viability. Collectively, our data demonstrate strong in vivo efficacy of JAKi in experimental murine MMP and identify JAK signaling as a central regulatory axis. Furthermore, fibroblasts were highlighted as active contributors to disease pathogenesis, providing a potential link between stromal activation and therapeutic response in experimental models of MMP.

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Extracellular Granzyme B Promotes Vitiligo-Associated Depigmentation with Melanocyte Detachment and Is Attenuated by Topical Inhibition in Mice

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Vitiligo is an acquired depigmenting disorder characterised by melanocyte loss. While autoimmune-driven apoptosis is a major pathogenic mechanism, protease-mediated melanocyte detachment has also been implicated. However, the responsible proteases remain incompletely defined. Granzyme B (GzmB), a serine protease, has been reported to accumulate in vitiligo lesions. Beyond its classical intracellular apoptotic role, GzmB has recently been implicated in extracellular functions in inflammatory skin diseases, where its inhibition shows therapeutic benefit. However, its extracellular role in vitiligo remains unclear. Here, we demonstrate a 17-fold increase in GzmB-positive cells in the upper dermis of vitiligo skin, predominantly mast cell-derived and perforin-negative, suggesting a non-canonical extracellular function. In vivo, subcutaneous administration of GzmB in mice induced marked depigmentation, characterised by reduced basal melanocyte numbers and increased melanocyte detachment. In cell culture assays, GzmB exerted no cytotoxic effects but induced melanocyte senescence and promoted differentiation-related changes in keratinocytes, as shown by RNA-seq and validation analyses. Consistently, $p16^{INK4a}$-positive detached melanocytes and aberrant perpendicular division of basal keratinocytes were spatially associated in GzmB-treated mice, with a similar pattern observed in lesional vitiligo skin. Furthermore, topical treatment with the GzmB-specific inhibitor VTI-1002 significantly attenuated depigmentation and aberrant perpendicular keratinocyte division in a rhododendrol-induced vitiligo mouse model. Collectively, our findings identify extracellular GzmB as a driver of melanocyte

senescence and perpendicular keratinocyte division, promoting melanocyte detachment in vitiligo. Targeting GzmB may represent a potential therapeutic strategy for vitiligo.

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Schnitzler Syndrome as an Autoinflammatory Disease Driven by B-Cell-Specific Somatic MYD88 Mutation

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Schnitzler syndrome (SchS) is an acquired autoinflammatory disease that typically develops after the age of 50 and is characterized by chronic urticaria and monoclonal gammopathy, most commonly of the IgM class. To elucidate the genetic basis underlying this phenotype, we performed cell type-specific mutational analysis in 10 patients clinically diagnosed with SchS. First, in three patients from whom fresh blood samples were available, peripheral blood was fractionated into purified B cells, T cells, monocytes, and neutrophils. Each fraction underwent deep whole-exome sequencing to identify somatic mosaic variants. The MYD88 p.L265P mutation was detected in bulk PBMCs in two of the three patients, whereas it was identified in purified B cells in all three. No mutation was detected in T cells, monocytes, or neutrophils. Based on these findings, seven additional patients were analyzed by separating PBMCs into CD19-positive and CD19-negative fractions and performing an allele-specific quantitative PCR assay targeting MYD88 p.L265P. In six of the seven patients, the mutation was undetectable in bulk PBMCs and in the CD19-negative fraction but was consistently detected in the CD19-positive fraction, with variant allele fractions ranging from 1.24% to 26.69%. The single patient without detectable mutation had previously received rituximab. Among the patients with detectable mutations in B cells, one had an IgA-type monoclonal gammopathy. No IgG-type monoclonal gammopathy cases were included in this cohort. These findings indicate that SchS is an acquired autoinflammatory disease driven by somatic mosaic mutations arising in the B-cell lineage and may explain why a subset of patients subsequently develop B-cell-lineage hematologic malignancies. In addition to adult-onset autoinflammatory syndromes such as CAPS and VEXAS syndrome, our results suggest that some adult-onset febrile disorders may similarly be driven by acquired somatic mosaic mutations in genes involved in inflammation.

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Inflammatory Infiltration as a Precursor to Immune Privilege Collapse in Alopecia Areata

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Alopecia areata (AA) is a common immune-mediated, non-scarring hair loss disorder with a highly variable clinical course and heterogeneous treatment responses. Although CD8+NKG2D+ T-cell–mediated cytotoxicity targeting the lower part of the anagen hair follicle (HF) and collapse of HF immune privilege (IP) are widely regarded as central pathogenic mechanisms, the upstream drivers of AA remain poorly understood. For extensive cases, high-dose systemic corticosteroids or Janus-Kinase (JAK) inhibitors represent the first-line treatment to which only ~ 40% of patients respond. Moreover, discontinuation of treatment frequently leads to relapses.
Here, we performed single-cell RNA sequencing of lesional, perilesional, and control skin biopsies from patients with localized and generalized AA, totalling in 42 samples and more than 180,000 analysed cells. Our data reveal that perilesional skin samples, 2–3 cm from active lesions, exhibit a cytotoxic T cell infiltrate indistinguishable from active lesions. This observation enables a precise dissection of the temporal dynamics underlying human AA progression. Notably, we demonstrate that CD8+ T cell infiltration precedes the collapse of the IP. In samples obtained 4-6 weeks after therapy initiation - coinciding with clinical response - HF IP persists beyond the resolution of inflammation. Furthermore, our findings identify disease-specific inflammatory hair follicle fibroblast populations and dendritic cells, which may collaboratively drive the breakdown of HF IP. For the first time, our data resolves a long-standing immunological paradox in human AA, demonstrating that the immune infiltrate precedes and likely drives the collapse of the HF IP. While therapeutic suppression of inflammation successfully restores hair growth, we show that HF IP is not reconstituted - a critical insight that mechanistically explains the high recurrence rates observed in AA.

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Self-peptides presented by HLA-C*06:02 in psoriatic lesions share sequence homology with Streptococcal peptides

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HLA-C*06:02, carried by ~10% of the global population and ~50% of psoriasis patients, is the strongest genetic risk factor for the disease. Notably, these individuals also show a higher prevalence of pharyngeal Group A Streptococcus (GAS) infections at disease onset. Firstly, to identify peptide self-antigens presented by HLA-C*06:02 in psoriatic skin we profiled the immunopeptidome of lesional and non-lesional skin biopsies from patients with psoriasis (n=20) and from healthy individuals (n=16). Of the 99,204 peptides identified in these studies, 27,456 HLA I- and 15,324 HLA II-bound peptides were detected exclusively in lesional skin. Notably, 196 of these peptides were detected in at least four of seven HLA-C*06:02$^+$ lesional samples and mapped back to motifs of HLA-C*06:02 or the often co-inherited HLA-B*57:01.
To identify streptococcal antigens capable of triggering autoimmunity in genetically predisposed individuals, we exposed HLA-C*06:02$^+$ cells to live or heat-inactivated GAS. This yielded 63 GAS-derived predicted peptides of which 39 showed high sequence similarity to human peptides (78–89%), supporting molecular mimicry in psoriasis pathogenesis. We next tested CD8+ T cell reactivity to these lesional self- and GAS-derived peptides using PBMCs from psoriasis patients and healthy donors. Preliminary responses were observed to peptide pools, and single-peptide deconvolution is ongoing. Overall, our study defines the human- and GAS-derived HLA-C*06:02 immunopeptidome, revealing disease-relevant streptococcal antigens and providing a molecular framework for understanding how microbial exposure may trigger or amplify HLA-C*06:02-associated psoriasis.

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Role of B Cells, Plasma Cells and Autoantibodies in Hidradenitis Suppurativa

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Hidradenitis suppurativa (HS) is a chronic inflammatory skin disease characterized by painful nodules, abscesses, and tunnel formation, leading to profound impairment in quality of life and reduced life expectancy. HS immunopathogenesis remains poorly understood, but recent association to HLA suggests an autoimmune component. Concordant with this, HS lesions display marked infiltration of B cells and plasma cells with local antibody production, although, the contribution of B cell responses to disease remains unclear.
To define antigen-driven B cell responses and establish a systematic pipeline for autoantibody and target discovery, lesional HS skin was analyzed using single-cell transcriptome and immune receptor sequencing. Our analyses revealed extensive clonal expansion and diversification of B cells and plasma cells in HS lesional skin, as well as the presence of public B cell clones across HS patients. Seventy plasma cell-derived antibody sequences were expressed as recombinant monoclonal antibodies. Immunohistochemical profiling on HS and healthy skin demonstrated heterogeneous reactivity patterns, including epithelial- and tunnel-associated reactivity, indicating antibodies recognizing self-antigens in human skin. Selected antibodies were further integrated into downstream antigen discovery workflows, including pulldown-based proteomics and serologic analyses across multiple donors.
Together, our findings identify a distinct panel of self-reactive antibodies targeting diverse human skin structures and provide additional evidence for an autoimmune component in HS. Importantly, this integrated approach demonstrates the feasibility of deriving tissue specificity and antigen identity by leveraging single-cell antibody sequence data at scale, establishing a functional framework for autoantibody and target discovery in inflammatory disease more broadly.

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Precision medicine in psoriasis: predicting treatment response to biologic therapies through the analysis of disease-predisposing allelic variants

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Emerging evidence indicates that SNP-based genetic variability influences treatment outcomes, but its clinical translation remains limited, highlighting the need for precision tools to improve patient stratification and therapeutic decision-making. This study aimed to identify genetic biomarkers predictive of optimal response to biologic therapies, in order to support personalized treatment strategies in psoriasis management.
A customized panel of 583 SNPs located within psoriasis susceptibility loci was analyzed by Next-generation sequencing in three cohorts of moderate-to-severe psoriasis patients (n=626) undergoing long-term treatment with anti-IL-12/IL-23, -IL-17A, and -IL-23. Associations between genetic variants, treatment response, and clinical parameters were evaluated. Functional relevance was investigated through GTEx-based eQTL analysis.
Unsupervised clustering and PCA analyses identified distinct genetic signatures defining specific profiles within the psoriatic population. The identified SNP clusters were predominantly enriched within the *PSORS1* locus, including genes involved in skin barrier function (*CDSN, CCHCR1*), antigen presentation (*HLA-C, HLA-B, ERAP1*), and T-cell activation pathways (*IL-23R, TNFA*), underscoring the pivotal role of this region in psoriasis heterogeneity. Integrating clinical data demonstrated that specific genetic signatures were associated not only with key disease characteristics but also with treatment-specific predictors of response to biologics. Specifically, baseline disease severity and prior exposure to biologics negatively impacted response to anti-IL-12/IL-23 therapy, while a BMI >30 and generalized or palmoplantar psoriasis were associated with a reduced response to anti-IL-17 and anti-IL-23 agents.
These findings suggest that the genetic landscape of psoriasis significantly contributes to disease heterogeneity and may influence long-term response to biologic therapies, paving the way for more personalized treatments.

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Pemphigus vulgaris B-cell receptor sequencing for a targeted systemic antibody-drug conjugate delivery strategy

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There have been many advancements in skin gene therapy research for genodermatoses such as recessive dystrophic epidermolysis bullosa (RDEB), however effective systemic delivery continues to be a major hurdle. To address this, we aim to develop an antibody-drug conjugation strategy for targeted systemic delivery of oligonucleotides. In this study, we captured the genetic sequence of the B-cell receptor (BCR) of desmoglein 3 (dsg3)-targeting B-cells of pemphigus vulgaris (PV) patients. Peripheral blood mononuclear cells were isolated from PV patients and stained for B cell markers and their BCR using AF647-labelled recombinant dsg3. Afterwards, positive cells were single cell sorted by FACS, cultured and stimulated for antibody production. A dsg3 ELISA was performed for IgG producing colonies, where positives were selected for B cell receptor sequencing. Subsequently, we recombinantly expressed multiple IgG sequences and produced several monoclonal hu-a-dsg3 antibodies in HEK293T cells. With this method, we isolated and expressed four unique clones. Interestingly, we found three different anti-dsg3 lambda B cell sequences within one patient and another anti-dsg3 kappa sequence from another patient. Subsequent characterization followed by immunofluorescence staining on cryosections of 3D human skin equivalents stained with these clones. All clones showed the distinct ‘chicken wire’ DSG3 pattern. When tested for pathogenicity in a keratinocyte dissociation assay, all clones turned out to be pathogenic. Taken together, we have successfully developed a method for specific BCR-sequencing and isolated and expressed monoclonal hu-a-dsg3 antibodies which lays the foundation for the

development of a universal delivery platform for gene therapies as oligonucleotide-based therapies. Currently, we are generating antibody-drug conjugates that we will test in cell and model systems.

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Mitochondrial Stress–Driven Interferon Signaling in Dermatomyositis Skin Revealed by Spatial Transcriptomics

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Dermatomyositis (DM) is a rare autoimmune disease characterized by muscle weakness, atrophy, and inflammatory skin involvement, with elevated type I interferon (IFN) activity across tissues. However, the exact mechanisms sustaining chronic inflammation remain unclear. Here, we performed spatial profiling of lesional DM skin using high-resolution transcriptomics (XENIUM).
The spatial abundance of IFNA2, -B1, and -K was quantified across the epidermis, the dermo-epidermal interface, and the upper and lower dermis. We observed a pronounced upregulation of IFNB1 specifically within the interface and upper dermis regions. IFNK was strongly upregulated within epidermis. Ligand–receptor analysis using LIANA identified fibroblasts (FB) and vascular cells (VASC) as key signaling hubs driving IFN-associated communication. XENIUM demonstrated close proximity of FBs and VASC, with highest IFN stimulated gene (ISG) expression in dermal regions surrounding VASC niches.
Consistent with prior RNA sequencing, mitochondrial (MITO) pathways were downregulated in DM skin. MITO dysfunction was pronounced in FBs and overlapped spatially with ISG-high areas. Functional assays (Seahorse and MitoSOX) in patient-derived FBs confirmed impaired respiration and increased oxidative stress.
Analysis of the DNA sensor cyclic GMP-AMP synthase (cGAS) revealed enrichment of cGAS-high cells near MITO damage regions in DM.
These findings suggest that MITO stress and the release of MITO DNA may activate cGAS-dependent pathways, thereby driving sustained IFN responses in situ. This study identifies a previously underappreciated link between MITO dysfunction and chronic inflammation in DM skin, providing new insights into disease pathogenesis and potential therapeutic targets.

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Integrative Modeling Reveals that the Th1/Th2–Treg Axis Orchestrates Mechanistic Endotypes and Therapeutic Responses in Alopecia Areata

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Alopecia areata (AA) is an inflammatory hair loss disorder traditionally regarded as a Th1-mediated disease driven by IFN-γ–dependent immune responses. However, substantial heterogeneity in disease progression and therapeutic efficacy suggests that this paradigm alone is insufficient. Moreover, emerging clinical responses to dupilumab in AA patients with concomitant atopic dermatitis implicate a potential role for Th2-associated pathways. Despite these observations, how the Th1/Th2 balance and other underlying mechanisms shape AA pathogenesis and treatment responsiveness remains poorly understood.
To address this gap, we developed an integrative methodological framework that combines mechanistic mathematical modeling with spatial transcriptomic data and immunohistochemistry, and extends to virtual patient analysis and validation against clinical trial outcomes to redefine AA endotypes and therapeutic determinants.
Using this framework, we identified four distinct AA endotypes defined by key biomarkers, including the Th1/Th2 balance, IFN-γ–MHC-I signaling, and Treg activity. We then characterized therapeutic responses to the JAK inhibitors baricitinib and ritlecitinib, as well as dupilumab. We found that JAK inhibitor efficacy is governed by restoration of the Th1/Th2 balance or modulation of the IFN-γ–MHC-I axis, whereas dupilumab responsiveness depends critically on Treg-mediated regulation. Collectively, this study identifies four immunologically distinct AA endotypes and establishes the Th1/Th2-Treg balance as a key organizing axis underlying AA pathophysiology, providing a foundation for endotype-guided precision medicine.

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Basophils exacerbate bullous pemphigoid by promoting natural killer cell-mediated skin inflammation and by regulating dendritic cell accumulation and T cell proliferation in secondary lymphoid organs

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Bullous pemphigoid (BP) is an autoimmune blistering disorder driven mainly by autoantibodies against type XVII collagen (COL17). Although basophils comprise only 0–2% of circulating leukocytes, these innate immune cells infiltrate BP lesions. Previous single-cell analyses demonstrated a lesional accumulation of basophils and suggested interactions with neutrophils and macrophages; however, their exact pathogenic contribution remains unclarified. To elucidate their role in BP inflammation, we treated active BP model mice with basophil-depleting anti-CD200R3 monoclonal antibody. Basophil depletion ameliorated clinical skin scores in male mice without altering systemic anti-COL17 autoantibody titers. Subsequent evaluation using scRNA-seq with Cellular Indexing of Transcriptomes and epitopes by sequencing (CITE-seq) on lesional skin, inguinal lymph nodes and spleen was performed on day 11 and week 5. Transcriptomic profiling of the affected skin revealed reductions in natural killer (NK) cells and plasmacytoid dendritic cells (pDCs) across both timepoints, alongside a decline in inflammatory monocytes by week 5. Conversely, lymph node analyses demonstrated an early (day 11) decrease in migratory type-2 conventional dendritic cells (cDC2s) and proliferating T cells, followed by a reduction in interferon-activated B cells at week 5. Finally, splenic cDC1s and neutrophils declined on day 11. These findings indicate that basophils drive local tissue inflammation in active BP independently of autoantibody generation. This pro-inflammatory function is likely mediated through immunomodulatory effects on NK cells and pDCs within the skin and cDC1s, cDC2s and T cells within secondary lymphoid organs.

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When skin tolerance falters: Decoding the collapse of dendritic tolerance and activation of resident memory T cells in vitiligo

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Skin immune homeostasis relies on tightly regulated interactions between dendritic cells (DCs) and resident memory T cells (T_{RM}). In vitiligo, autoreactive $CD8^+$ T_{RM} already infiltrate the clinically non-lesional (NL) skin, raising the question of how local tolerance is maintained and ultimately lost. However, the specific tolerance mechanisms preserving this fragile balance, and how a stressed microenvironment disrupts it, remain largely unknown. Flow cytometry analysis revealed a high expression of immune checkpoint molecules, specifically PD-1/PD-L1, respectively on $CD8^+$ T cells and DCs in the NL skin of vitiligo patients, suggesting a role of these regulatory mechanisms in restricting T cell activation. Single-cell RNA sequencing (scRNA-seq) analyses of NL and peri-lesional (PL) skin identified a distinct regulatory DC subset enriched in patient NL skin, whereas gene ontology analysis revealed an enrichment of T cell activation pathways in PL skin.
To assess the impact of environmental triggers on this delicate balance and understand the mechanisms driving lesion appearance, we modeled a stressed microenvironment using a high but non-toxic dose of UVB irradiation to mimic a sunburn. scRNA-seq of NL skin treated with or without high-dose UVB revealed a major « transcriptional downregulation » of immune checkpoint ligands across the myeloid compartment. Specifically, PD-L1 gene expression significantly decreased in DC subpopulations. Concurrently, mature DCs and Langerhans cells (LCs) significantly upregulated the expression of genes related to antigen presentation and pro-inflammatory cytokines (IL-1β, TNF-α). Furthermore, we observed an upregulation of genes associated with T cell activation (e.g., IL2RA, CD28, and CD44), pointing to the reactivation of skin T_{RM}. In addition, flow cytometry showed a significant decrease in PD-L1 and PD-L2 expression on CD14+ myeloid cells and cDC2s, alongside a significant decrease in PD-1 on $CD8^+$ TRM.
In summary, these findings demonstrate that UVB stress triggers vitiligo progression by collapsing DC-mediated immune checkpoint expression, thereby licensing the activation of pathogenic skin T_{RM}.

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Impaired Mitophagy and Pol γ-Driven mtDNA Synthesis Fuel Sterile Inflammation in Vitiligo Keratinocytes

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Vitiligo is an autoimmune skin disorder driven by progressive melanocyte loss. While adaptive immunity is well-characterized, the role of keratinocytes in triggering upstream innate responses remains less understood. This study demonstrated that vitiligo keratinocytes (VHK) exhibit a profound metabolic crisis involving ATP depletion, low mitochondrial DNA (mtDNA) copy number, and antioxidant imbalance.
Impaired mitochondrial ATP synthesis mimics hypoglycemia, enhancing Glut-4 expression, glucose uptake, and autophagy to meet energetic demands. However, consequent cytoplasmatic hyperglycemia causes insulin resistance and intracellular accumulation of glucose-related toxic by-products. Importantly, their release into the microenvironment suggests these molecules act as messengers within surrounding tissue.
Crucially, reduced mtDNA copy numbers correlate with mitophagic failure and the active release of mtDNA into the extracellular space. Analysis detected 8-OHdG damage in VHK supernatants, reflecting mtDNA vulnerability to oxidative stress. This displacement is fueled by Pol γ-driven compensatory mtDNA synthesis, particularly under insulin and IGF-1 metabolic stimulation, which paradoxically amplifies cellular stress. Ultimately, this triggers the cGAS-STING pathway, driving a robust release of type I interferons and inflammatory mediators that activate innate immunity, as evidenced by THP-1 monocyte differentiation and network amplification.
Further evidence for a central role of metabolism in VHK dysfunction emerged from transcriptomic analysis which indicated “ATP hydrolysis activity”, “ADP binding”, “negative regulator of D-glucose import”, “negative regulator of lipid storage” among the significantly upregulated pathways.
These findings identify mitochondrial DNA "leakage" as a critical DAMP triggering the innate immune cascade in vitiligo. Consequently, stabilizing mitochondrial turnover and restoring mitophagy represent essential targets to arrest the onset of localized autoimmunity.

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Endothelial propagates inflammation through STING vesicle-mediated intercellular signaling in psoriasis

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The cGAS-STING signaling pathway has been implicated in various inflammatory diseases, but its role in psoriasis remains incomplete This study aimed to determine whether endothelial STING activation propagates inflammation in psoriasis through vesicle-mediated intercellular signaling. Transcriptomic and single-cell analyses were performed to define cell-specific activation patterns, followed by validation in patient skin samples. Endothelial cells were stimulated to activate STING, and released vesicles were isolated to evaluate their role in intercellular communication. STING signaling was enriched in endothelial cells, whereas downstream response signatures were predominantly observed in immune and mesenchymal populations. Patient samples confirmed endothelial STING activation in psoriatic lesions. Activated endothelial cells released vesicles containing phosphorylated STING, which induced inflammatory responses in recipient fibroblasts and dendritic cells. Genetic inhibition of STING in endothelial cells markedly attenuated these effects. Notably, blockade of interferon receptor signaling only partially suppressed downstream activation, indicating that vesicle-mediated STING transfer contributes

independently of canonical interferon signaling. These findings demonstrate that endothelial STING activation propagates inflammation through a vesicle-mediated mechanism. This noncanonical mode of intercellular signaling provides insight into endothelial-driven inflammatory amplification and suggests STING as a potential therapeutic target in psoriasis.

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Stage-dependent spatial niches surrounding CD3$^+$CD69$^+$ T cells in alopecia areata

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Alopecia areata (AA) is a T cell-mediated autoimmune disease, but the spatial mechanisms underlying disease chronicity remain incompletely understood. We performed Xenium spatial transcriptomic analysis of perifollicular cells from three acute and three chronic AA lesions to investigate T-cell-associated microenvironments. Because CD69 may reflect both tissue residency and T-cell activation, CD3$^+$CD69$^+$ T cells within the T-cell cluster were used as a proxy for resident memory T (T_{RM})-like cells and were not considered definitive T_{RM} cells. Acute and chronic perifollicular cells were integrated and annotated using a shared clustering framework to enable direct stage comparison. We then identified non-T cells located within 50 μm of CD3$^+$CD69$^+$ T cells and evaluated their cellular composition and gene expression profiles. Integrated analysis identified major perifollicular populations, including keratinocytes, fibroblasts, endothelial cells, T cells, and myeloid and antigen-presenting cell (APC) populations. Although the overall proportion of CD3$^+$CD69$^+$ T cells was comparable between acute and chronic lesions, their surrounding non-T-cell niches showed clear stage-dependent differences. In acute lesions, cells proximal to CD3$^+$CD69$^+$ T cells showed enrichment of keratinocyte-associated genes, including *KRT15*, *COL17A1*, *TP63*, and *KRT5*, consistent with an epithelial-associated microenvironment. In contrast, chronic lesions demonstrated enrichment of myeloid/APC-related populations and genes, including *LYZ*, *CLEC10A*, *FCER1A*, and *CD68*, indicating a shift toward an immune-dominant niche. These findings suggest that CD3$^+$CD69$^+$ T cells in AA are associated with stage-dependent perifollicular niches, transitioning from an epithelial-associated microenvironment in acute lesions to a myeloid/APC-enriched immune niche in chronic lesions. This spatial reorganization may contribute to persistent inflammation in chronic AA.

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Transfer of BP230-primed CD4+ T cells trigger Basement Membrane-specific Antibodies in Nude mice

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Bullous pemphigoid (BP) is an autoimmune blistering disease characterized by autoantibodies directed against BP180 and BP230, key structural components of the hemidesmosomes. Although BP230-specific antibodies are found in BP patient sera, their pathogenic relevance, especially with reactivity to the N-terminus, remains unclear, likely due to the limited accessibility of this intracellular antigen.
To assess the immunogenicity of N-terminal BP230 epitopes in vivo, K5-Cre:BP230 knock-out mice were immunized with BP230 fragments spanning the N-terminal domain. Repeated administration induced BP230-specific antibody responses in K5-Cre+:BP230fl/fl mice. Antibody specificity was validated by IIF on salt-split palate tissue, demonstrating linear staining restricted to samples expressing BP230, and absent signal upon keratinocyte-specific antigen deletion. CD4+ T cells were FACS-isolated from spleen of immunized mice and adoptively transferred into nude mice. To investigate the potential of BP230-primed CD4+ T cells as BP initiators, recipient mice were monitored for disease induction. BP230-primed CD4+ T cells elicited production of IgG antibodies targeting the dermal-epidermal junction, detectable as early as two weeks post transfer in some animals. In contrast, adjuvant-only primed CD4+ T cells failed to induce skin-specific antibody responses. Despite serological evidence of autoreactivity, recipient mice did not develop clinical skin manifestations or exhibit weight loss, irrespective of the immunizing BP230 epitope. Cell analysis revealed an elevated frequency of regulatory T cells in skin-draining lymph nodes of mice receiving BP230-primed CD4+ T cells.
These findings indicate that BP230-primed CD4+ T cells can provide help to inherent B cells, promoting the generation of basement membrane zone-specific antibodies. However, this response was insufficient to recapitulate the full clinical and cellular BP phenotype. The Treg expansion may have limited pathogenic autoimmunity and prevented disease onset. Further studies are required to establish a BP230-driven disease model and to clarify the pathogenic relevance of BP230-specific antibodies.

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Notch Inhibition by Nirogacestat Attenuates Fibrotic Processes in Fibroblasts from Patients with Systemic Sclerosis and Localized Scleroderma

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Systemic sclerosis (SSc) and localized scleroderma (LS) are chronic disorders characterized by autoimmunity, vasculopathy, and fibrosis. While LS fibrotic processes primarily affect the skin, SSc can present multisystem involvement and severe complications. Regardless of aetiology, acquired and inherited fibrotic diseases, such as the skin fragility disorder recessive dystrophic epidermolysis bullosa (RDEB), are known to share some molecular signatures and pro-fibrotic pathways, including Notch. Based on our previous evidence demonstrating the effective repurposing of nirogacestat (NIR), a Notch inhibitor and anti-tumor drug (Ogsiveo), as a novel anti-fibrotic agent in RDEB preclinical models, this study aims to test its activity in SSc and LS. Using patient fibroblasts (SSc-FBs and LS-FBs), we showed that NIR markedly attenuates several pro-fibrotic traits, including cell contractility, TGF-β1 secretion, fibrogenic marker expression (e.g. α-SMA, TNC), and collagen synthesis and deposition. In addition, RNA-seq on NIR-treated SSc-FBs revealed the modulation of both known and previously uncharacterized molecules involved in ECM stiffening (e.g. LOXL4), immune response and ROS scavenging (e.g. CYGB). Selected dysregulated molecules were validated by molecular analyses and cytokine arrays. Of note, the latter showed that NIR-treated SSc-FBs exhibit an up-regulation of the intracellular form of IL-1RA, relevant in light of the pivotal role of IL-1 pathway in SSc. These findings unveil novel aspects in fibrosis pathogenesis and confirm NIR ability to counteract fibrotic processes in different diseases sharing Notch hyperactivity. Ongoing studies on an animal model of SSc will hopefully lead to

the demonstration of NIR effectiveness *in vivo* and thus pave the way for its translation into clinical practice as cost-effective and easy-administrable therapeutic option for SSc.

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GE8820: a selective IgG4 degrader for the treatment of pemphigus vulgaris (PV)

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Pemphigus vulgaris (PV) is an autoimmune skin blistering disease driven by autoantibodies (autoAb) against the desmosomal protein DSG3. Circulating autoAbs, primarily of the IgG4 isotype, cause damage by disrupting cell adhesion and signaling pathways and autoab titers correlate with disease severity.
GE8820 is a biologic degrader developed for the treatment of PV that exhibits selective binding to IgG4 and hepatic ASGPR thereby inducing rapid and deep degradation of circulating IgG4.
GE8820 was characterized using *in vitro* assays with a cohort of 28 patient sera and *in vivo* rodent models for its ability to reduce PV pathology. GE8820 selectively and completely (>95%) immunodepleted total IgG4 and decreased IgG4 specific DSG3 specific autoab in PV sera by mean of 99% (range 95-100%). Consistent with previous reports, IgG4 was the most enriched anti-DSG3 autoAb isotype in PV sera (range 2-95%) in a direct autoAb isotyping assay. Treatment with GE8820 eliminated the pathogenicity in 80% of PV sera to that of healthy donor controls in a cell-based keratinocyte fragmentation assay. Furthermore, IgG4 was the dominant contributor of pathogenicity (~ 70% of PV sera) with residual contribution from IgG1. In a pharmacodynamic mouse model, treatment of GE8820 rapidly (< 1hr) and completely removed (98%) circulating autoAbs demonstrating an IgG4 degrader MoA. Finally, in a neonate mouse passive transfer model of PV, sub-Q administered GE8820 prevented disease.
These data show that IgG4 autoAbs are key drivers of pathogenicity and support the therapeutic potential of an IgG4 degrader in PV treatment. GE8820 is a novel first-in-class IgG4 selective degrader, with potential non-immunosuppressive and steroid sparing activity due to its selective, rapid and deep IgG4 degradation.

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EGL-003: a novel Treg biased IL-2 mutein conserving full agonism mobilizes Tissue Regulatory T Cells and Resolves Inflammation

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Defective regulatory T-cell (Treg) function is implicated in various autoimmune and inflammatory diseases. A major therapeutic challenge is not only to expand Tregs, but also to efficiently mobilize their function within inflamed tissues to restore homeostasis. Low-dose interleukin-2 (IL-2) has emerged as a promising strategy to preferentially expand Tregs; however, its clinical application remains limited by a narrow selectivity window between Tregs, which express the high-affinity IL-2 receptor (IL-2Rαβγ), and other T cells expressing the intermediate-affinity receptor (IL-2Rβγ).
EGL-003 is a novel Fc-fused IL-2 mutein engineered to enhance Treg selectivity through mutations that reduce binding to IL-2Rβγ, while incorporating stabilizing mutations that preserve signaling potency through IL-2Rαβγ.
In human PBMC stimulation assays, EGL-003 induced pSTAT5 signaling with high selectivity for Tregs while maintaining potency comparable to native IL-2. This translated into robust Treg activation, including acquisition of tissue effector functions, both in vitro and following administration in mice.
In an acute DSS-induced mouse colitis model, EGL-003 promoted resolution of intestinal inflammation, associated with marked expansion of colonic Tregs displaying a mucosal healing phenotype. Single-cell transcriptomic analyses further showed that EGL-003 increased Treg dominance in both lymphoid and peripheral tissues and induced colon-specific activation of Treg functional programs.
In lesional biopsies from patients with dermatological disease, single-cell transcriptomic analysis demonstrated that ex vivo incubation with EGL-003 selectively enhanced Treg functional pathways, highlighting its ability to mobilize tissue-resident Tregs even within a chronic inflammatory environment.
Taken together, these data identify EGL-003 as a potent and selective IL-2 mutein capable of mobilizing tissue Tregs effector functions and promoting inflammation resolution. EGL-003 is currently tested in a first-in-human study (EU CT n° 2025-521658-40-00) that comprises a SAD part in healthy volunteers and a MAD part in patients with moderate to severe atopic dermatitis.

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A bead aggregation assay reveals functional autoantibody activity as a key determinant of disease severity in pemphigus

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Pemphigus autoantibodies induce acantholysis through direct steric inhibition of desmoglein (Dsg)-desmocollin (Dsc) trans-interaction and signaling-driven desmosomal disassembly. Anti-Dsg ELISA quantifies total IgG regardless of pathogenicity, and titers do not always reflect disease severity. The aim is to determine whether functional autoantibody activity measured by a bead aggregation assay correlates with disease activity more reliably than ELISA across pemphigus subtypes, and whether stratified analyses provide additional insights. Steric inhibitory activity of autoantibodies was measured by previously developed bead aggregation assays using Protein G beads coated with Fc-tagged Dsg and Dsc. Sera from 37 patients (16 pemphigus foliaceus [PF], 14 mucosal-dominant PV [PV-MD], and 7 mucocutaneous PV [PV-MC]) were analyzed in relation to the Pemphigus Disease Area Index (PDAI), including longitudinal samples from 8 cases. Additional analyses were performed under ELISA-matched conditions to assess antibody quality independent of quantity. Inhibitory activity correlated with PDAI across disease subtypes, whereas ELISA did not correlate consistently. In PV-MD, Dsg3 inhibitory activity correlated strongly with PDAI ($\rho=0.81$, $p<0.01$), while Dsg3 ELISA did not. In PV-MC, stratified analyses revealed that Dsg3 inhibitory activity correlated with mucosal PDAI, whereas Dsg1 inhibitory activity correlated with skin PDAI; in contrast, ELISA showed site-dependent and less consistent associations. In PF, PDAI correlated with Dsg1/Dsc1 inhibitory activity ($\rho=0.75$, $p<0.01$) but not

with Dsg1 ELISA. Notably, sera with comparable ELISA titers exhibited markedly different inhibitory activities, and a patient with low titers but severe disease showed disproportionately high activity. Longitudinally, inhibitory activity paralleled clinical fluctuations in most cases. Bead aggregation assays more consistently reflect disease severity than ELISA across pemphigus subtypes. These findings indicate that antibody pathogenicity, rather than titer alone, is a key determinant of disease activity and support the use of functional assays for improved disease monitoring.

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Dynamics of TFH subsets in bullous pemphigoid during active disease and remission

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In this study, we aimed to characterize activated circulating T follicular (TFH) helper cells and their subsets, together with T follicular regulatory (TFREG) cells in bullous pemphigoid (BP) and to evaluate their association with disease severity. We collected peripheral blood mononuclear cells from patients with BP in flare (n=41; mean ± SD age 75.8 ± 13.9), in remission (n=14; mean ± SD age 72.2 ± 11.4), and from 25 age- and sex-matched healthy controls (HC).
Frequencies of TFH (CD4+CXCR5+CD25$^{neg\text{-}low}$CD127$^{low\text{-}high}$ ICOS+) were markedly higher during BP flare than in HC ($p<0.0001$) and in remission ($p=0.0015$), with no difference between remission and HC ($p=0.47$) and correlated with body surface area (BSA) involvement ($\rho=0.332$, $p=0.034$). Subset analysis revealed an expansion of TFH2 cells (CXCR3−CCR6−) within the TFH fraction in flare vs remission vs HC (median 67.1 vs 30.8% vs HC 37.6%, respectively; $p<0.0001$) that correlated with BSA ($\rho=0.327$, $p=0.037$). TFH17 cells (CXCR3−CCR6+) were reduced during flare compared with remission and HC ($p=0.034$). TFREG cells (CD4+CXCR5+CD25^{high} CD127^{low}ICOS+) differed significantly among groups ($p<0.0001$), with the highest values observed during flare, intermediate values in remission and the lowest values in HC. Notably, TFREG and TFH2 frequencies correlated strongly ($\rho=0.746$, $p<0.0001$). The TFH2+TFH17/TFH1 ratio (TFH1 defined as CXCR3+CCR6−), also differed significantly among groups ($p=0.0081$), being highest in flare, intermediate in HC, and the lowest in remission.
Compared with prior work showing expansion of memory TFH cells with an increased TFH2+TFH17/TFH1 ratio in active BP—largely driven by TFH17 polarization—our study, focusing on activated ICOS+TFH subsets, identifies a distinct TFH2-skewed activation profile. These findings suggest that TFH17 cells may reflect long-term autoimmune imprinting, whereas activated TFH2 cells represent dynamic effector responses. The increase in TFREG cells during flare supports a compensatory but insufficient regulatory response. Collectively, these findings highlight dysregulation of the TFH–TFREG axis in BP and support activated TFH2 cells as biomarkers of disease activity and potential therapeutic targets.

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Chronic discoid lupus erythematosus shows a specific hair follicle signature.

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It is not well understood why the most common cutaneous lupus subtype - chronic discoid LE (CDLE) – presents with scarring outcome. We investigated the expression of LE-associated parameters in different body compartments in CDLE, SLE and healthy donors.
PBMCs and plucked hair follicles (HF) were investigated for the expression of IFN-stimulated genes (ISGs) by qPCR in CDLE, SLE patients (n=40) and healthy individuals (n=8). 5 Hair follicles(HF) were plucked from an uninvolved scalp area. Serum was analysed for parameters linked to B-cell activation.
SLE PBMCs showed a 20-fold increase, and CDLE ones a 10-fold increase in ISG expression compared with healthy controls. By contrast, the expression of ISG in HF was markedly higher in CDLE compared to SLE and healthy controls. Scarring CDLE is believed to start at the HF. We observed a high expression of the MHC class I-related beta 2-microglobulin in LE samples with high “hair follicle ISG” expression. Serum levels of CD40L and APRIL were increased in SLE compared to healthy, but in CDLE, the mean expression was only 16% below SLE for sCD40L and APRIL.
B-cell activation is detectable in CDLE serum, pointing to “systemic” involvement of the disease. It remains to be shown if activated B cells functionally contribute to the scarring skin inflammatory response. Our results show that non-lesional HF are “poised” for a CDLE response. Our data suggest that a collapse of the HF immune privilege occurs even at non-lesional sites in CDLE. Plucked HF are a suitable source to determine increased IFN response, and high HF ISG signatures could serve as an “early” marker of CDLE inflammation, allowing treatment before irreversible scarring damage.

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The Interrelationship between Periodontitis, Bullous Pemphigoid, and Mucous Membrane Pemphigoid.

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Patients with bullous pemphigoid (BP) and mucous membrane pemphigoid (MMP) often present with poor oral conditions and may ultimately require multiple tooth extractions. We hypothesized that oral lesions may worsen periodontitis by impairing oral hygiene, while chronic periodontal inflammation may in turn enhance autoimmune responses. Therefore, we investigated the bidirectional association between periodontitis and BP/MMP. We first evaluated oral lesions in active BP model mice. The model was generated by transferring splenocytes from wild-type mice immunized with skin from type XVII collagen (COL17) humanized mice into Rag2-/-COL17-humanized recipient mice. This model enables continuous production of anti-human COL17 antibodies and reproduces BP-like skin lesions. Compared with negative control mice, active BP model mice showed tongue epithelial detachment and linear deposition of IgG along the basement membrane zone (BMZ) in the tongue and buccal mucosa by direct immunofluorescence, indicating that this model reproduces oral manifestations relevant to pemphigoid. We then combined a periodontal model with the active BP model mice to investigate their interaction. Experimental periodontitis was induced by oral administration of two periodontal bacterial species and evaluated by micro-CT and H&E staining. After administering periodontal bacteria, splenocytes were transferred. Mice exposed to periodontal bacteria showed slightly higher skin scores than controls, suggesting that periodontal inflammation may aggravate systemic disease activity. These findings

suggest that the active BP model mice combined with experimental periodontitis may be a useful tool for studying the interaction between periodontitis and BP and MMP. Further studies are needed to evaluate alveolar bone loss, oral and skin lesions, and immunological changes.

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Investigational, orthosteric STAT6 inhibitor, REX-8756, demonstrates potent, selective and reversible inhibition and achieves preclinical efficacy and safety profiles comparable to combined anti-IL-4/IL-13 biologics

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The IL-4/IL-13 signaling pathway, mediated by STAT6 via its SH2 domain, drives Th2 immune responses in inflammatory and pruritic diseases. Anti-IL-4Ra/IL-13 biologics are approved in multiple diseases driven by Th2 inflammation, including atopic dermatitis. However, there is a significant need for oral therapeutic options targeting this biology for patient convenience and preference. While oral JAK inhibitors impact the pathway downstream of the receptor, their use can lead to broad immunosuppression and hematologic side effects. Selective, oral STAT6 inhibition therefore represents an opportunity to phenocopy the efficacy of anti-IL-4 Ra /IL-13 monoclonal antibodies while avoiding known JAK-mediated adverse events.
Here we describe REX-8756, an oral, reversible, non-degrading, SH2 domain-targeting STAT6 inhibitor that demonstrates on-target sub-nanomolar potency with an exquisite off-target selectivity and safety profile.
REX-8756 was well tolerated in 28-day GLP tox studies with no adverse findings. Toxicokinetic analysis revealed broad distribution in key type-2 inflammatory target tissues. In a preclinical model of atopic dermatitis, oral treatment with REX-8756 attenuated skin inflammation with efficacy comparable to antibody-mediated blockade of IL-4/IL-13 pathways and reductions of Th2 cytokines (IL-13, IL-5) within the immune compartment.
REX-8756 is currently in a first-in-human, randomized, double-blind, placebo-controlled phase 1 trial assessing safety, pharmacokinetics and pharmacodynamics of single and multiple day ascending doses in healthy volunteers.
REX-8756 inhibition of STAT6 brings the promise of combining the efficacy of clinically validated biologics with the convenience of oral administration, while potentially avoiding known JAK inhibitor safety concerns.

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Efficacy of Dupilumab for Cutaneous Immune-Related Adverse Events: A Multi-Institutional Cohort Study

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Although immune checkpoint inhibitors (ICIs) have shown promise in treating a growing number of malignancies, they often lead to cutaneous immune-related adverse events (cirAEs). Data on the use of biologics to treat various cirAE morphologies remain limited. This study investigates the efficacy of dupilumab on cirAEs in ICI patients.
Retrospective cohort study of patients treated with ICIs and dupilumab at Massachusetts General Hospital and Dana-Farber Cancer Institute was performed. ICIs included anti-PD-1 (pembrolizumab, nivolumab, cemiplimab), anti-PD-L1 (atezolizumab, avelumab, durvalumab), anti-CTLA-4 (ipilimumab).
Among 31 patients, 61% of received anti-PD-1 monotherapy and 35% received ICI combination therapy. The most common malignancies were genitourinary (29%), followed by skin (19%), thoracic (19%), and head and neck (16%). 87% of patients started dupilumab for cirAEs, while 13% of patients were treated for flare of their pre-existing dermatoses. Median time from ICI initiation to cirAEs or flare was 15 weeks, while median time from cirAEs or flare to dupilumab initiation was 31 weeks. Median duration of dupilumab treatment was 23 weeks.
Eczematous (42%) and bullous (29%) morphologies predominated, followed by lichenoid (10%), pruritus (6%), morbilliform (3%), and morphea (3%). Most cirAEs were grade 2 (39%) or 3 (32%). 74% of patients had significant improvement, while 10% had partial improvement. All (13/13) patients with eczematous eruptions and 66% (6/9) of patients with bullous eruptions improved significantly.
Our multi-institutional cohort demonstrates that dupilumab is effective in treating cirAEs, especially for eczematous eruptions. Dupilumab is often initiated after multiple unsuccessful trials of conventional therapy, such as topical and systemic steroids, and steroid-sparing immunomodulators and delayed by the insurance approval process. Toxicity-directed biologics have not been associated with worsened survival, suggesting preservation of ICI antitumor efficacy. This underscores the potential of dupilumab in optimizing both quality of life and survival outcomes for patients on ICIs.

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PDE4 Inhibition Targets CX3CL1/CX3CR1-Dependent Myeloid Cell Recruitment and Ameliorates Oral Lesions in Two Mouse Models of Mucous Membrane Pemphigoid

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Mucous membrane pemphigoid (MMP) is a chronic autoimmune blistering disease characterized by tissue-specific inflammation and scarring. Progress in therapy development has been hampered by the lack of preclinical models that recapitulate both the disease induction and its effector mechanisms. Here, we establish an antigen-driven mouse model of MMP based on immunization with murine laminin-α3, which reproducibly induces a chronic disease closely recapitulating key clinical, histopathological, and immunological features of human MMP. Using this model alongside an antibody-transfer approach, we demonstrate that phosphodiesterase-4 inhibition (PDE4i) significantly reduces disease severity across mucosal and cutaneous sites. Therapeutic efficacy was associated with reduced local inflammation, while autoantibody levels and tissue IgG/C3-deposition remained unchanged. Transcriptomic profiling across models revealed conserved signatures of myeloid cell activation and chemokine signaling, particularly involving the CX3CL1/CX3CR1 axis, independent of systemic autoantibody levels. Mechanistically, PDE4i reduced the anti-laminin 332 IgG-mediated CXCL2 and IL-6 release from cultured keratinocytes and impaired CX3CL1-mediated macrophage chemotaxis without altering CX3CR1 expression. Consequently, CX3CR1-deficient mice exhibited significantly attenuated disease, highlighting the role of myeloid cell trafficking in tissue-specific

pathology. Translational relevance was supported by elevated CX3CL1 serum levels in two independent MMP patient cohorts and co-localization of CX3CR1 with macrophages in lesional mucosal and skin tissues. Collectively, these findings propose PDE4i as a promising therapeutic approach for MMP and highlight CX3CL1/CX3CR1, CXCL2, and IL-6 as drivers and keratinocytes and macrophages as key elements of PDE4i effects in this disease.

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Transcriptomic associations with ultraviolet-B phototherapy and clinical response in psoriatic skin and blood

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Psoriasis is a systemic autoimmune skin disease associated with elevated inflammatory cytokine levels in blood. Ultraviolet-B radiation (UVB) induces diverse effects in human skin, including apoptosis, and full-body narrowband UVB phototherapy (NbUVB PT) is an effective treatment for psoriasis, although clinical response varies. Our aim was to identify transcriptome biomarkers of clinical response to NbUVB PT in skin and investigate the effects of UVB in blood. Prior to starting NbUVB PT, 36 psoriasis subjects provided untreated lesional (LS) and nonlesional (NLS) skin biopsies; LS and NLS samples treated with one (1MED) or three (3MED) minimal erythemal doses of UVB were also taken. Blood samples were collected at baseline and week (wk) 2, 4 and 8 during NbUVB PT. All samples underwent bulk RNASeq. Enrichment scores for functional pathways were obtained with gene set variation analysis. Clinical response was defined by percentage reduction in psoriasis area and severity index at the end of therapy compared to baseline (ΔPASI). Differential expression analysis was used to identify genes and pathways (i) modulated by 1MED or 3MEDs in skin, (ii) associated with ΔPASI in skin, and (iii) modulated by NbUVB PT in blood. Irradiation with 3MEDs in LS led to upregulation of apoptosis pathway expression (log fold change (logFC) 0.16, q-value (q) 0.02). Upregulation of this gene set in untreated LS was associated with ΔPASI (logFC 0.05, q 0.0003). In blood, 4047 genes were differentially expressed at wk2, wk4 or wk8 compared to baseline (F-test $q < 0.05$), including genes involved in TNF-NFkB signalling and neutrophil degranulation, which exhibited transient downregulation at wk2 and stronger downregulation at wk8. These findings underscore the role of UVB in apoptosis induction and suggest that higher baseline apoptosis expression is a biomarker of NbUVB PT response. They also suggest that the therapeutic effects of NbUVB PT extend beyond the skin and include downregulation of inflammatory signatures in blood.

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GenSci161, a novel IL-1α and IL-1β bispecific antibody for moderate-to-severe hidradenitis suppurativa

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Hidradenitis suppurativa (HS) is a chronic inflammatory disorder, which affects the intertriginous skin and is associated with numerous systemic comorbidities. Emerging evidence identifies that IL-1α and IL-1β play critical roles in HS pathogenesis, with both cytokines significantly upregulated in lesional tissue and correlating directly with disease severity. Therefore, dual inhibition of the IL-1 signaling cascade represents a promising strategy to ameliorate the inflammatory burden of HS. GenSci161 is a novel, fully human IgG1 bispecific antibody developed by GeneScience Pharmaceutical Co., Ltd. Utilizing the proprietary EvoMab™ platform, GenSci161 is engineered to simultaneously neutralize both IL-1α and IL-1β while sparing endogenous IL-1 receptor antagonist (IL-1RA), thereby preserving natural inflammatory regulation. The molecule features an engineered Fc region designed to extend half-life, facilitating convenient subcutaneous administration every 8 to 12 weeks.
In LPS-stimulated PBMCs, GenSci161 significantly suppressed the secretion of IL-6, IL-8, TNF-α, and IFN-γ, with maximum inhibition rates of 47.32%, 44.84%, 65.09%, and 100%, respectively. In the murine air pouch model, treatment significantly reduced monosodium urate (MSU) crystal-induced IL-6 production in pouch exudates and attenuated neutrophil recruitment. In the 3D skin co-culture model, GenSci161 effectively reversed the profound dysregulation of HS-associated mediators. Furthermore, GenSci161 dose-dependently reduced levels of key cytokines and biomarkers linked to neutrophil biology, fibroblast activation, and tissue remodeling.
GenSci161 effectively suppressed the broad inflammatory cascade and its unique ability to spare IL-1RA, combined with an extended half-life, positions GenSci161 as a promising candidate for the treatment of moderate-to-severe HS, addressing a critical unmet medical need.

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Metabolic kinetics in cutaneous Graft versus Host Disease

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Inflammation driven tissue remodeling and fibrosis characterize chronic cutaneous Graft versus Host Disease (GvHD). Recent data have spotlighted causal links between cell metabolism shifts and development of skin fibrosis, but the temporal relationship between fibroblast dynamics and mitochondrial activity remains incompletely defined. We therefore aimed to characterize structural, cellular and metabolic changes in chronic GvHD in murine skin. We induced systemic GvHD in female recipient mice by chemotherapeutic conditioning and intravenous transplantation of bone marrow and splenic T cells from donors. We collected consecutive lesional skin samples at days 0, 3, 7, 14, 21. Results of bulk RNA sequencing are currently pending. Analyses included epidermal and dermal thickness, proliferation (Ki67), a smooth muscle actin (αSMA) and mitochondrial activity by Complex IV (MTCO1). Epidermal and dermal thickness remain unaffected in the early phases and then both increase, peaking at day 14 and subsequently decreasing, corresponding to macroscopically progressive alopecia and eczema plaques around this time point. Proliferative activity in dermal fibroblasts, as assessed by Ki67, increased markedly after GvHD induction and reached a maximum at day 7 before gradually decreasing, indicating a pronounced early increase in proliferation that precedes and overlaps with maximal tissue thickening. Myofibroblast and total nuclei counts increased from day 3 to a peak at day 14 and decreased at later time points, suggesting that fibrotic remodeling is associated with expansion of the myofibroblast population in cutaneous GvHD development. Along these lines, mitochondrial activity

increased from day 3 to a maximum at day 14 and then declined, consistent with the cellular pattern. Myofibroblasts again showed the strongest mitochondrial signal during highest myofibroblast density and dermal thickening, indicating a temporal coupling between mitochondrial activity and myofibroblast driven fibrotic remodeling. Whether this is a causal correlation, needs further validation in vivo. Taken together, these findings suggest mitochondrial pathways as promising stage specific therapeutic targets in GvHD associated skin fibrosis.

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Single-Cell Analysis Reveals Cytotoxic CD4 T Cell Remodeling in Sitosterolemia-Related Necrobiotic Xanthogranuloma

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Sitosterolemia-related necrobiotic xanthogranuloma (SNXG) is a rare cutaneous manifestation of inherited sterol transporter dysfunction, but its immune alterations remain unclear. We aimed to define peripheral immune remodeling and disease-enriched CD4 T cell states in SNXG.
Peripheral blood mononuclear cells from four patients with SNXG and four healthy controls were analyzed by single-cell RNA sequencing. We integrated miloR, starCAT/custom module scoring, pseudobulk analysis, VIPER transcription factor (TF) inference, monocle3/slingshot, condiments, tradeSeq, and CellOracle.
Patients showed CD4 T cell contraction and a shift toward effector memory (EM) states. Functional analysis showed attenuated homing, translation and metabolic programs in patients, with pre-activation in naïve and central memory cells and sustained activation in EM cells. Program stratification identified a patient-enriched cytotoxic EM CD4 T cell state, termed C2, with inflammatory trafficking, multi-cytokine and cytotoxic features. Exact permutation confirmed C2 enrichment ($P=0.0286$). Pseudobulk analysis of C2 versus other EM CD4 T cells identified 93 robustly upregulated and 179 robustly downregulated genes. VIPER inferred increased activity of nine TFs in C2, including TBX21. Trajectory analysis placed C2 along a late lineage with significant patient-control pseudotime differences ($q<0.001$). tradeSeq identified 117 branch-associated genes, including TBX21, potentially involved in C2-lineage differentiation. Among 19 TFs nominated from VIPER and cytotoxic T cell literature, CellOracle predicted that TBX21 knockout would selectively and markedly suppress transition toward this program; top predicted TBX21 network genes included NKG7, GNLY and FGFBP2.
These findings suggest that SNXG involves systemic CD4 T cell remodeling toward a TBX21-linked cytotoxic EM state, potentially linking cutaneous xanthogranulomatous disease to sterol-driven vascular pathology.

181 [WITHDRAWN]

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Regulatory Science Manoeuvred Fabrication and Pre-clinical Evaluation of Novel Budesonide Embedded Nanoemulgel for the Effective Management of Psoriasis

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Psoriasis is a persistent inflammatory dermatosis characterised by redness, flaking, and pruritus. Because existing therapies often struggle with inconsistent results or significant side effects, this research explores the potential of repurposing Budesonide (BUD), a corticosteroid typically used for respiratory conditions, as a topical therapy for psoriasis. Due to its poor aqueous solubility, a nanoemulgel is aimed to be developed using natural anti-psoriatic oils.
Initially, nanoemulsion was optimised by Design Expert software's D-mixture design. The optimised nanoemulsion consists of 7.9% rose oil, 49.3% water, and 42.8% Smix (a mixture of Transcutol and Tween 80 in a 1:1 ratio). A globule size of 152.4 ± 13.99 nm particle size and 0.26 ± 0.02 PDI was observed for the blank nanoemulsion, whereas BUD-nanoemulsions 0.025, 0.0188, & 0.0125 % showed a globule size of 162 ± 13.89 nm, 159.2 ± 5.89, 151.7 ± 12.67, and a PDI of 0.23 ± 0.02, 0.26 ± 0.03, 0.27 ± 0.02. Incorporating the formulation into a Seppic gel base produced a nanoemulgel (NEMG) characterized by favourable spreadability, sterility, and shear-thinning properties. Biological assays using RAW 264.7 and HaCaT cell lines confirmed the nanoemulgel's safety profile, showing low cytotoxicity alongside potent anti-inflammatory effects against LPS-induced stress. Furthermore, in an imiquimod-induced mouse model, histopathological analysis revealed that the nanoemulgel-treated groups experienced significantly greater recovery from psoriatic symptoms than those receiving untreated, plain gel, or commercial formulations. ELISA results further indicated a significant reduction in cytokine levels in the nanoformulation-treated groups.
BUD nanoemulgels demonstrated superior efficacy in ameliorating psoriatic symptoms in the mice and could be an alluring approach in comparison to other topical formulations. As the first reported formulation of its kind, this delivery system represents a promising translational strategy for clinical disease management.

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Type 17 memory T cells with tissue-resident features are enriched in tumor necrosis factor inhibitor non-response in axial spondyloarthritis

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Axial spondyloarthritis (axSpA) is an immune-mediated skeletal disease involving the interleukin-17 (IL-17) axis, a pathway also central to psoriasis and psoriatic arthritis. Tumor necrosis factor inhibitors (TNFi) are first-line biologics, but response is heterogeneous and stratification markers are limited. In psoriatic arthritis, TNFi non-response has been linked to rising

circulating T helper 17 (Th17) cells and IL-17F-related gene activity. As axSpA target tissues are difficult to sample, we assessed whether circulating type 17-polarized T cells expressing tissue-resident memory (TRM)-associated markers are persistently enriched in TNFi non-responders.
Peripheral blood mononuclear cells from six non-radiographic axSpA patients with active disease (ASAS criteria, 4/6 HLA-B27 positive) were analyzed at baseline and after 6 months of TNFi therapy. Responders (n=3) reached an Axial Spondyloarthritis Disease Activity Score <1.3; non-responders (n=3) did not. Flow cytometry quantified chemokine-receptor-defined Th1, Th2, Th17, Th17.1 and Th22 polarizations and regulatory/$CD8^+$ counterparts in memory T cells, with co-staining for CD103 and CXCR6.
Responders showed decreased $CD3^+$ frequencies among live lymphocytes, whereas non-responders remained stable or increased. Circulating Th17 cells increased only in non-responders, consistent with observations in psoriatic arthritis. Non-responders displayed approximately twofold enrichment of $CD103^+$ T helper cells at both timepoints, driven mainly by persistently elevated $CD103^+$ Th17.1 cells, with elevations of $CD103^+$ Th17, Th22 and Tc2 fractions. CXCR6 was elevated within Tregs and Tr22 cells from non-responders.
These data suggest that TNFi non-response in axSpA is associated with sustained enrichment of circulating type 17-polarized T cells expressing TRM-associated markers. This supports further evaluation of tissue-imprinted barrier-tissue programs as candidate markers of biologic therapy response.

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Endosomal Toll-Like Receptor3 (TLR) imbalance and PTPN22 polymorphism in vitiligo

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Vitiligo is a chronic autoimmune disorder characterized by selective melanocyte destruction driven by dysregulated interactions between innate and adaptive immunity. Evidences support a key role of nucleic acid-sensing Toll-like receptors (TLRs) in initiating inflammatory cascades that promote autoimmunity. This study investigated endosomal TLR3 expression and PTPN22 gene polymorphism in vitiligo patients. TLR3 expression was measured using ELISA assay, while PTPN22 polymorphism was analyzed by PCR-based methods in peripheral blood samples. A significant downregulation of TLR3 was observed in patients compared to controls ($p = 0.032$), indicating impaired interferon-mediated immune regulation and a shift toward persistent innate immune activation. PTPN22 genotyping revealed that 50% of patients carried the heterozygous CT variant, while 12.5% were homozygous TT. PTPN22 is a gene that encodes a p*rotein tyrosine phosphatase non-receptor type 22* acting as a negative regulator of T-cell activation, helping to maintain immune tolerance. Polymorphism variants may reduce this inhibitory function, resulting in easier activation of autoreactive T cells. Altered endosomal TLR signaling may amplify antigen presentation and pro-inflammatory cytokine release, while PTPN22 polymorphism may facilitate excessive T-cell activation.The convergence of these pathways may disrupt immune tolerance and may promote sustained melanocyte-specific autoimmunity. Observed findings support a model in which imbalance of nucleic acid-sensing innate immunity, combined with genetic predisposition affecting T-cell regulation, drives vitiligo pathogenesis.

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Therapeutic relevance of soluble Fas Ligand in skin and systemic disorders

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The Fas/Fas Ligand (FasL) system is implicated in several physiological and pathological processes, yet no therapies currently target this pathway. This is largely due to the complexity of the Fas/FasL signalling and the risk of interfering with membrane-bound FasL, which is involved in immune surveillance and tumor control. PC111 is a human monoclonal antibody that selectively binds soluble FasL (sFasL) but not its membrane-bound form. This selectivity may allow therapeutic modulation of pathogenic sFasL activity without affecting the protective FasL functions. We previously demonstrated the efficacy of sFasL blockade in preclinical models of Pemphigus vulgaris and Stevens-Johnson Syndrome/Toxic Epidermal Necrolysis. Here, we identify additional diseases associated with elevated sFasL, including drug reaction with eosinophilia and systemic symptoms (DRESS), drug-induced hyper-sensitivity syndrome (DIHS), systemic lupus erythematosus, rheumatoid arthritis, Sjögren's syndrome, and acute respiratory distress syndrome. In these settings, sFasL may contribute to tissue damage and inflammation and is worth additional validation testing, so as to support its selective inhibition as a promising therapeutic strategy.

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Tissue-Resident Memory T Cells as Autonomous Drivers of Disease Persistence in Inflammatory Skin Disorders: A Systematic Review and Semi-Quantitative Synthesis

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Chronic inflammatory skin diseases, including psoriasis, vitiligo, and atopic dermatitis, frequently relapse at previously affected sites despite effective systemic therapy, suggesting persistence of local pathogenic immune populations. Tissue-resident memory T cells (TRM) have emerged as key candidates, yet their role in disease maintenance remains incompletely defined. We conducted a systematic review and semi-quantitative synthesis of human and translational studies investigating TRM in inflammatory skin disease. MEDLINE, Cochrane and Embase were searched from inception to 28 April 2026 using predefined terms. Studies were included if they assessed TRM abundance, phenotype, clonality, cytokine activity, persistence, or functional pathogenicity. Evidence was synthesised using a predefined framework for TRM pathogenicity. Of 247 records identified, 38 met inclusion criteria, comprising single-cell transcriptomic (n=14), spatial/imaging (n=10), functional (n=8), and clinical observational studies (n=6). TRM populations were enriched in lesional and previously affected skin in 34/36 studies, with clonal expansion reported in 29/32. Sustained expression of cytotoxic mediators (granzyme B, perforin) and effector

cytokines (IFN-γ, IL-17) was observed in 31/34 studies. Persistence of disease-associated TRM signatures in clinically resolved skin was reported in 21/24 studies. Functional studies demonstrated maintenance of TRM activity via IL-15 and IL-7 signalling, independent of ongoing antigen presentation. Directional consistency across study types was high, although the magnitude of clonality and cytokine dependence varied between diseases. We propose a model in which TRM act as semi-autonomous effectors sustaining local inflammation independently of systemic immune activation. This provides a mechanistic basis for site-specific relapse and incomplete disease resolution with current therapies. Targeting TRM survival and retention pathways may therefore be required to achieve durable remission in inflammatory skin disease.

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Comparison of Serum and Blister Fluid Anti-Desmoglein 1 and 3 Antibodies in Pemphigus Vulgaris

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Pemphigus vulgaris (PV) is an autoimmune blistering disease mediated by pathogenic autoantibodies against desmoglein (Dsg) 1 and 3, detectable both in the skin and circulation. Serum antibody evaluation is crucial in PV diagnosis and monitoring. Similarly, blister fluid (BF) is considered a readily accessible local disease compartment, but its diagnostic utility remains insufficiently defined, particularly given the fragility of PV blisters and potential differences in antibody concentration. This study aimed to compare anti-Dsg1 and Dsg3 antibody levels in paired serum and BF samples from 35 newly diagnosed PV cases. All patients were treatment-naïve at sampling and analyzed by ELISA (Euroimmun, Germany). Agreement in antibody positivity was assessed using Cohen's κ, correlation of titres by Spearman's ρ, and paired differences by the Wilcoxon signed-rank test (z). Anti-Dsg1 antibodies were detected in 29 serum and 23 BF samples, with moderate agreement ($\kappa = 0.568$, $p < 0.001$). Anti-Dsg3 antibodies were detected in 30 serum and 25 BF samples ($\kappa = 0.588$, $p < 0.001$). Full agreement was observed in 29/35 (82.9%) for anti-Dsg1 (23 both positive, 6 both negative) and in 30/35 (85.7%) for anti-Dsg3 (25 both positive, 5 both negative), with discordance only in cases with positive serum and negative BF results. Strong correlations were observed between serum and BF titres for anti-Dsg1 ($\rho = 0.845$, $p < 0.001$) and anti-Dsg3 ($\rho = 0.858$, $p < 0.001$). However, BF titres were significantly lower than serum titres ($z = –5.035$ and $–4.984$, respectively; $p < 0.001$), indicating consistent underestimation in BF. These findings show that although BF antibody levels closely reflect relative serum variation, they are consistently reduced, suggesting compartmental attenuation of antibody titres in PV. Consequently, while BF may support qualitative antibody detection, it is not directly interchangeable with serum for quantitative assessment or disease monitoring.

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Genetic determinats and immune pathways in Psoriatic Arthritis: Implications for biomarker discovery

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Psoriatic arthritis (PsA) is a chronic immune-mediated disease affecting skin and joints, frequently developing in patients with cutaneous psoriasis (PsC). While both conditions share genetic risk factors, their divergent clinical manifestations suggest distinct pathogenic mechanisms. Here, we aimed to identify susceptibility variants associated with PsA and PsC and to define the immune pathways differentially involved.
We analyzed curated genome-wide association study (GWAS) datasets to compile PsA- and PsC-associated single nucleotide polymorphisms (SNPs). Pathway enrichment analysis showed a significant overrepresentation of PsA-associated variants in Tumour Necrosis Factor (TNF) and NF-κB signaling, as well as Interleukin (IL)-12B and IL-23 pathways. These findings support a distinct pro-inflammatory signature in PsA and point to key pathways driving disease progression.
Despite the availability of targeted therapies, including TNF inhibitors, IL-17A antagonists and JAK/STAT modulators, early diagnosis of PsA remains limited. As cutaneous disease typically precedes joint involvement, identifying predictive biomarkers in PsC patients is critical for early intervention.
Overall, this study provides mechanistic insight into the differential immunopathogenesis of psoriatic disease and highlights candidate genetic pathways for biomarker development and precision medicine.

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Therapeutic Challenges in Pemphigus Foliaceus: Balancing Immunosuppression and Infection Risk

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Pemphigus foliaceus (PF) is an autoimmune blistering disorder limited to the skin, characterized by superficial acantholysis. Systemic corticosteroids and immunosuppressive agents represent the mainstay of treatment but are associated with an increased risk of infections.
We report the case of a 53-year-old male presenting with a 3-year history of generalized skin lesions, initially managed as seborrheic dermatitis, with partial response to topical and systemic therapies. The patient received repeated courses of systemic corticosteroids, followed by persistence and progression of lesions. Clinical examination revealed flaccid bullae, erosions, and poorly demarcated erythematous plaques with greasy scales, predominantly involving seborrheic areas and the scalp. Mycological examination identified Malassezia spp. and dermatophyte infection consistent with tinea capitis, likely favored by prior corticosteroid exposure.
Histopathological examination demonstrated superficial acantholysis within the granular layer, confirming PF. The presence of active fungal infection posed a therapeutic challenge, as initiation of systemic immunosuppressive therapy was deferred. Antifungal treatment led to clinical improvement, allowing subsequent initiation and escalation of azathioprine.
This case highlights the complexity of managing PF in the context of treatment-related infections. Recognition and control of active infections are essential prior to immunosuppression in order to minimize complications and optimize outcomes.

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From plaques to blisters: pemphigus vulgaris complicating chronic psoriasis vulgaris

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Pemphigus vulgaris (PV) is a rare, chronic autoimmune muco-cutaneous disease causing severe blistering and erosions on the skin and mucous membranes. It is characterized by the presence of autoantibodies targeting desmogleins 1 and 3. Drug-induced pemphigus vulgaris is an autoimmune blistering disease with multifactorial, incompletely understood pathogenesis. Characteristic lesions appear weeks to months (up to 1 year) after drug exposure.
Psoriasis vulgaris is among the most prevalent chronic inflammatory skin conditions. It is frequently associated with other autoimmune and autoinflammatory diseases, amplifying the complexity of its clinical management.
In this regard, we present the case of a 50-year-old female with a history of psoriasis vulgaris and breast cancer (ongoing hormone therapy with goserelin and tamoxifen), who develops bullae and erosions on the face, scalp, trunk and extremities. Other comorbidities include hypertension, prior cholecystectomy, tinnitus. Current medications: betahistine, ramipril, amlodipine, irbesartan. Prior psoriasis treatments included methotrexate, secukinumab (discontinued after breast cancer diagnosis), and tildrakizumab (stopped after bullous eruption). Skin biopsy confirmed the diagnosis of pemphigus vulgaris. ELISA showed elevated anti-DSG 1 with normal anti-DSG 3. Additional workup ruled out malignancy. Methotrexate treatment was initiated, with favorable response for both psoriasis vulgaris and PV lesions.
PV may be drug-induced, associated with psoriasis vulgaris, or coincidental. Potential mechanisms include psoriatic inflammation inducing epidermal changes that expose hidden desmoglein antigens (triggering PV via epitope spreading), shared genetic predisposition, and overlapping immune dysregulation. Both require different management, and treatments for one can sometimes aggravate the other.

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Clinical Application and Efficacy of Zhi Ju Qing Re Wash in the Treatment of Pediatric Eczema

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To evaluate the clinical efficacy of Zhi Ju Qing Re Wash combined with conventional treatments in pediatric eczema, focusing on symptom relief, inflammation reduction, and skin barrier function.
80 children with moderate-to-severe eczema were randomized into treatment (n=40) and control (n=40) groups. Both received topical corticosteroids for 4 weeks, while the treatment group additionally applied Zhi Ju Qing Re Wash externally. Outcomes included SCORAD index, clinical symptoms (erythema, edema, pruritus), inflammatory markers (IL-4, IL-6, TNF-α), skin hydration, and transepidermal water loss (TEWL), assessed post-treatment and at one-month follow-up.
The treatment group demonstrated significant improvements in SCORAD scores ($P<0.05$), faster symptom relief, enhanced skin hydration, reduced TEWL, and lower inflammatory markers. No serious adverse events occurred, and patient satisfaction was higher in the treatment group.
Zhi Ju Qing Re Wash enhances the efficacy of corticosteroids in pediatric eczema by improving symptom control, skin barrier function, and reducing inflammation, offering a safe and effective complementary therapy.

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Lichen Planus Pigmentosus Inversus Mimicking Acanthosis Nigricans: A Diagnostic Pitfall

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This study aimed to highlight a clinically relevant diagnostic pitfall in the evaluation of intertriginous hyperpigmented lesions, in the context of a rare and likely underrecognized entity.
We report the case of a 44-year-old female presenting with diffuse hyperpigmented macules and plaques involving the axillary, submammary, inguinal and nuchal folds. The lesions were brown, poorly demarcated and mildly lichenified, raising initial clinical suspicion of acanthosis nigricans. However, the absence of metabolic abnormalities, including normal glycemic and endocrine parameters, created a clinicopathological discrepancy, prompting further investigation.
Dermoscopy revealed a light-brown background with irregularly distributed granular brown-gray dots, consistent with pigment incontinence and atypical for acanthosis nigricans, thereby redirecting the diagnostic hypothesis toward a lichenoid dermatosis. Histopathological examination demonstrated epidermal atrophy with focal basal vacuolar degeneration and occasional colloid bodies, alongside a superficial perivascular lymphocytic infiltrate with numerous melanophages and marked pigment incontinence. These findings supported the diagnosis of lichen planus pigmentosus inversus.
The patient was initiated on topical corticosteroids and calcineurin inhibitors, with partial clinical improvement observed during follow-up.
This case underscores that lichen planus pigmentosus inversus may closely mimic acanthosis nigricans in intertriginous areas, creating potential for misdiagnosis, particularly in the absence of metabolic abnormalities and highlights the pivotal role of dermoscopy and histopathological assessment, as well as the importance of thorough clinicopathological correlation in atypical presentations, to ensure accurate diagnosis and appropriate management.

SKIN CANCERS: PATHOMECHANISMS, INNOVATIVE THERAPIES

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ROR1 drives melanoma development identifying a potential therapeutic target

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Despite the successful use of immune-checkpoint inhibitors and first targeted therapies, melanoma remains an aggressive and challenging tumor to treat. Consequently, there is a continued clinical need for additional, potentially synergistic treatment strategies. One emerging novel target for the treatment of various cancer types is ROR1.
We thus aimed to identify the role of ROR1 in melanoma development and metastasis formation to assess its potential as a therapeutic target. Therefore, we compared primary and metastatic tumor formation of ROR1-deficient and wildtype B16 melanoma cells in preclinical models, and investigated the effects of ROR1 inhibition in vivo and in vitro.
We showed that ROR1-deficiency prevents metastasis formation after intravenous injection of B16 melanoma cells in C57BL/6 mice. Furthermore, intradermally inocculated ROR1-deficient B16 cells resulted not only in reduced tumor formation and restricted proliferation, but also led to a significantly increased infiltration of $CD4^+$ and $CD8^+$ T cells, as well as natural killer (NK) cells. Similarly, peritumoral ROR1 inhibitor treatment of melanoma-bearing mice resulted in a significantly decreased tumor growth and induced a favorable immune infiltrate with pronounced numbers of $CD4^+$, $CD8^+$ T cells, and NK cells. Finally, we could demonstrate that ROR1 knockdown and inhibitor treatment also affects key features of malignancy in human melanoma cell lines as evidenced by reduced proliferative and migratory capacity, impaired spheroid formation and enhanced arrest in G0/G1 cell cycle phase.
Taken together, our findings underscore that ROR1 is a promising target for melanoma treatment through both direct antitumoral and immune-mediated anti-tumor effects.

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Integrated Molecular Profiling Reveals the Origins and Clinical Heterogeneity of Basal Cell Carcinoma

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Basal cell carcinoma (BCC), the most common human malignancy, remains incompletely understood at the molecular level. We performed integrated genomic, methylomic, transcriptomic and single-cell transcriptomic profiling of 443 BCCs to define the determinants of tumor development and heterogeneity.
Tumor mutational burden varied significantly with clinical and geographical factors. Replication timing and *TP53* status jointly shaped UV mutagenesis: TP53-mutant tumors accumulated substantially more UV-induced mutations, particularly in early-replicating regions, consistent with impaired DNA-damage repair.
Evolutionary analyses suggested that *TP53* inactivation often precedes *PTCH1* loss during BCC development. We also identified *PAK2* gene as a putative novel oncogenic driver, with recurrent missense mutations affecting its autoinhibitory and kinase domains, implicating this pathway as a potential therapeutic vulnerability in a subset of BCCs.
Histological subtypes did not form separate clusters based on RNA-seq but showed marked differences in the tumor microenvironment (TME), despite similar driver mutation landscapes: nodular BCC displayed an immune-desert phenotype, whereas aggressive tumors were enriched in cancer-associated fibroblasts. In contrast to localized BCCs, rare metastatic tumors exhibited pronounced aneuploidy and frequent whole-genome duplication, indicating that genomic instability rather than specific driver events underlies metastatic potential.
Single-cell RNA sequencing identified *LGR5+* hair follicle stem cells as the most likely cell of origin, with low *MHC-I* and high *TGF-β* expression suggesting an intrinsic immunosuppressive state. Integrative multi-omic clustering defined four molecular BCC subtypes distinguished by histology, immune infiltration, Hedgehog pathway activity and mutational burden. Together, these findings establish a molecular framework for BCC pathogenesis, clarify the role of *TP53* in UV-driven mutagenesis, and identify biologically distinct subtypes with potential therapeutic relevance.

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Combined TCR-T therapy and ferroptosis induction overcome melanoma heterogeneity including escape variants

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Tumor heterogeneity, including the emergence of antigen-loss or antigen-presentation-deficient escape variants, remains a major obstacle to effective cancer immunotherapy. Ferroptosis is a form of regulated cell death characterized by iron-dependent lipid peroxidation, and ferroptosis inducers have shown antitumor activity across multiple cancer types. Interferon-γ (IFN-γ), a key cytokine produced by activated T cells, has been reported to promote ferroptosis through suppression of system Xc^-.
Based on these observations, we hypothesized that combining T cell–based immunotherapy with ferroptosis induction could enhance antitumor efficacy against both antigen-positive and antigen-negative tumor populations. To test this, we established a heterogeneous tumor model composed of human melanoma cells and β2-microglobulin knockout (B2M KO) variants lacking antigen presentation.
We found that treatment with the ferroptosis inducer RSL3 in combination with MART-1-specific TCR-engineered T cells (TCR-T cells) effectively eliminated mixed tumor populations in vitro. Furthermore, in a NOG mouse model, the combination therapy demonstrated significant antitumor effects compared with either treatment alone.
These findings suggest that integrating ferroptosis induction with TCR-T therapy may overcome tumor heterogeneity by targeting both immunogenic and non-immunogenic tumor clones, thereby providing a promising strategy to improve the efficacy of cancer immunotherapy.

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Non-Invasive Molecular Diagnosis and Early Cancer Detection in Epidermolysis Bullosa *via* AI-Enhanced Raman Spectroscopy ('READ-EB' Platform)

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Epidermolysis bullosa (EB) is a severe inherited skin disorder characterised by tissue fragility, chronic wounds and a high risk of aggressive squamous cell carcinoma (SCC). Current diagnostic and monitoring approaches rely on invasive biopsy and delayed analysis, limiting early detection of malignant transformation and posing significant challenges in fragile skin.
We present a *non-invasive, point-of-care* molecular profiling platform for *real-time* characterisation of EB and associated carcinogenesis. The approach integrates confocal Raman spectroscopy with a purpose-built, self-optimising neural network (SKiN-D) for automated spectral feature extraction and classification.
Ex-vivo patient-derived samples from recessive dystrophic EB, EB-associated SCC, sporadic SCC and healthy skin were analysed alongside extracellular matrix biomarkers, including collagen-VII and laminins. Distinct molecular fingerprints enabled robust stratification of disease states. EB was differentiated from healthy skin with accuracies exceeding 98% (≥99.3%, AUC=0.96, sensitivity/specificity >96%), while EB-associated SCC was identified with 100% classification accuracy.
A collagen VII-specific spectral signature enabled direct, label-free quantification, demonstrating reduced expression consistent with disease pathology. Tumour-associated spectra revealed reproducible biochemical alterations in protein conformation, lipid organisation and nucleic acid content, enabling discrimination between EB-associated and sporadic SCC.
This approach enables simultaneous diagnosis, risk stratification and molecular characterisation without tissue excision. The **READ-EB** platform (**R**apid **E**valuation, **A**ssessment and **D**etection for **EB**) provides rapid, clinically interpretable outputs and is designed for *point-of-care* deployment. Ongoing work is translating this technology towards *in-vivo* handheld use for bedside assessment and longitudinal monitoring.
These findings establish a clinically translatable, *non-invasive* diagnostic paradigm addressing a critical unmet need in EB and skin cancer surveillance.

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Tumor-margin immune niches associated with immunotherapy response in cutaneous squamous cell carcinoma

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Clinical response to programmed cell death protein 1 (PD-1) blockade in cutaneous squamous cell carcinoma (cSCC) varies substantially between patients, yet the baseline tissue programs associated with response remain insufficiently defined. We analyzed clinically annotated responder and non-responder samples using spatial transcriptomic profiling of formalin-fixed paraffin-embedded (FFPE) biopsies with Visium HD, Xenium, and MERSCOPE, complemented by peripheral blood single-cell RNA sequencing and serum proteomic profiling with Olink Reveal and Immuno-Oncology panels. Samples from patients who responded showed a distinct organization of immune and stromal cells at the tumor margin, marked by chemokine-enriched transcriptional programs and activated myeloid or dendritic-cell features, including LAMP3 and FCER1A. Spatial neighborhood and proximity analyses further showed close positioning of T cells with myeloid and dendritic-cell populations within these interface niches, consistent with coordinated local immune activation. In parallel, patients who responded showed increased serum levels of immune-recruiting chemokines, including CXCL9, CXCL10, and CCL19. By contrast, samples from non-responders showed less structured tumor-stromal borders and more diffuse immune infiltration within repair-associated stromal regions. These findings suggest that sensitivity to PD-1 blockade in cSCC is linked to a chemokine-rich myeloid-lymphoid interface niche at the tumor margin and support baseline spatial immune organization as a candidate framework for patient stratification.

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1-methylnicotinamide is an immunosuppressive metabolite in human basal cell carcinoma

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Human basal cell carcinoma (BCC) is the most common form of skin cancer. Growing evidence indicates that tumor-derived metabolites actively shape immune evasion, yet the metabolic microenvironment of human BCC remains incompletely understood. We investigated the interstitial metabolic milieu of BCC and its impact on T cells. We isolated interstitial fluids from BCC and adjacent healthy skin and performed mass spectrometry-based metabolic profiling, revealing a accumulation of 1-methylnicotinamide (1-MNA) in BCC. 1-MNA is generated from nicotinamide by nicotinamide N-methyltransferase (NNMT. Immunohistochemical staining demonstrated elevated NNMT expression in BCC tumor cells, and re-analysis of single-cell RNA-sequencing data confirmed high NNMT expression in BCC-associated fibroblasts, suggesting stromal NNMT contributes to increased 1-MNA levels. To investigate the impact of 1-MNA on T cells, we studied human blood-derived T cell lines and skin-derived tumor-infiltrating T cells (T-TIL) from BCC. We analyzed BCC T-TILs for expression of organic cation transporters SLC22A1, SLC22A2, and SLC22A3, which presumably mediate uptake of 1-MNA. Proteomics analyses of 1-MNA-treated T cells revealed a decrease in OXPHOS-associated proteins, indicating metabolic rewiring. IFN-γ-pathway proteins were repressed, while exhaustion markers PD-1, PD-2, and LAG3 were increased. Flow-cytometry analyses of 1-MNA-treated CD8+ T cells confirmed increased PD-1 and TIM-3 expression and reduced IFN-γ production, while IL-10 expression and proliferation were sustained. Overall, these findings demonstrate that 1-MNA induces metabolic and functional rewiring, promoting an immune-regulatory, exhausted CD8+ T cell phenotype. Our data suggest that 1-MNA contributes to an immunosuppressive environment in human BCC and identify NNMT as a potential therapeutic target.

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Modelling Innate Immune Dysregulation in Recessive Dystrophic Epidermolysis Bullosa-cSCC using a Multicellular Spheroid

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Recessive Dystrophic Epidermolysis Bullosa (RDEB) is a severe blistering skin disorder, characterised by chronic inflammation and fibrosis, predisposing patients to aggressive squamous cell carcinoma (cSCC). However, the immunopathogenic mechanisms driving tumour progression remain poorly understood, and relevant preclinical models that recapitulate immune dysregulation are lacking. Here, we present an integrated analysis combining peripheral blood immunophenotyping with bulk RNA sequencing of tumour biopsies to define systemic and local innate alterations associated with precancerous inflammation and RDEB-cSCC disease severity. These features were modelled in an immunocompetent three-dimensional multicellular spheroid, incorporating tumour, epithelial, stromal and immune components. Peripheral immune profiling identified an early systemic shift towards a pre-educated, alternatively activated monocyte phenotype, prior to tumour formation, that worsens with RDEB-cSCC progression. Transcriptomic and immunofluorescence analysis revealed a myeloid-rich tumour microenvironment with spatial immune compartmentalisation, restricting antigen-presentation. These features were recapitulated in the spheroid model, which captured fibroblast-driven monocyte recruitment and monocyte differentiation towards a tumour-promoting phenotype. In addition, patient plasma, tumour tissue and spheroid supernatant exhibited concurrent upregulation of both pro- and anti-inflammatory soluble mediators, consistent with constitutive JAK/STAT3-driven signalling. Pharmaceutical inhibition of JAK signalling resulted in a dose-dependent growth suppression, increased necrosis, and a reprogramming of monocytes towards an anti-tumoral homeostatic state, within the spheroid model. Collectively, these findings identify innate immune dysregulation as a central driver of RDEB-cSCC and establish the multicellular spheroid model as physiologically relevant platform to interrogate monocyte plasticity and evaluate therapeutic strategies.

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A POSTN fibroblast niche sustains keratinocyte Carcinoma and is therapeutically targetable

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Basal cell carcinoma (BCC) and squamous cell carcinoma (SCC), collectively termed keratinocyte carcinomas (KC), represent the most common malignancies worldwide. Although most lesions are surgically curable, a subset of high-risk patients, including those with immunosuppression, extensive ultraviolet exposure, or genetic susceptibility can develop multiple recurrent tumours requiring repeated and often disfiguring procedures. Effective preventive therapeutic strategies for this population remain limited.

Using single-cell RNA sequencing and spatial transcriptomics, we previously identified periostin-expressing (POSTN) cancer-associated fibroblasts (CAFs) in BCC that correspond to a population associated with hair follicles in healthy skin. In ongoing work, we demonstrate expansion of POSTN CAFs in squamous cell carcinoma, supporting a conserved stromal programme across KC subtypes. Transcriptomic analyses identified key signalling pathways mediating epithelial-stromal crosstalk between POSTN fibroblasts and KC keratinocytes; however, specific pathway components are not disclosed due to a pending patent. Gene set enrichment analysis comparing tumour-derived BCC keratinocytes with healthy keratinocytes further identified upregulation of key signalling pathways.

To investigate the functional role of these pathways, we established a 3D keratinocyte-fibroblast spheroid co-culture model replicating early tumour-stromal architecture. KC derived spheroids displayed distinct morphological and organisational differences compared with healthy spheroid controls, driven by pathway-specific signalling interactions. Pharmacological inhibition of these pathways altered spheroid structure and tumour-associated phenotypes, supporting a functional role for POSTN CAF-mediated signalling in early KC development.

This platform provides a functional model for dissecting stromal contributions to keratinocyte carcinoma initiation and progression and has enabled identification of candidate targeted therapies for basal cell carcinoma. Targeting the associated signalling pathways represents a promising strategy for preventing tumour development in patients with high KC burden.

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Efficacy and Pitfalls of RAS Inhibition in NRAS-Mutant Melanoma

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Nearly 25% of cutaneous melanomas are driven by NRAS mutations, which typically affect older patients and are associated with a poorer prognosis than BRAF V600-mutant melanoma. Currently, the only effective systemic treatment is immunotherapy with immune checkpoint inhibitors. Targeted therapies have shown limited clinical benefit, highlighting the need for new strategies for patients who do not respond or cannot tolerate immunotherapy.

The recent development of RAS inhibitors has transformed the therapeutic landscape of RAS-mutant cancers. Daraxonrasib (RMC-6236) and the related compound RMC-7977 are multiselective inhibitors targeting the active state of RAS, enabling inhibition of all isoforms (KRAS, NRAS, HRAS) regardless of the specific mutation. These agents have demonstrated preclinical efficacy, and daraxonrasib is in phase III trials in KRAS-mutant lung and pancreatic cancers.

Here, we evaluated the antitumor activity of RMC-7977 in NRAS-mutant melanoma, focusing on mutations at codon 61 (NRAS Q61X). We show that this compound potently inhibits tumor growth and induces apoptosis, both *in vitro* and *in vivo*. We

characterize its effects on oncogenic signaling, revealing that Q61X mutations functionally decouple NRAS from both receptor tyrosine kinase activation and PI3K/AKT/mTOR signaling. Accordingly, combined inhibition of NRAS and receptor tyrosine kinases does not enhance the antitumor efficacy of RMC-7977, whereas co-inhibition of NRAS and PI3K results in significant synergistic effects.
We also developed preclinical models of resistance, revealing a combination of genetic and non-genetic mechanisms. These range from activating MEK mutations to global reprogramming of melanocyte identity, characterized by loss of SOX10 expression, reduced pigmentation, and acquisition of a fibroblast-like phenotype. These findings support preliminary evidence indicating that daraxonrasib induces rapid but short-lived responses in patients with NRAS-mutant metastatic melanoma. Overall, multiselective inhibition of active RAS is a promising therapeutic strategy in NRAS-mutant melanoma, but durable responses will require addressing context-specific signaling dependencies and adaptive resistance mechanisms.

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Telocytes as key marker of cutaneous carcinogenesis fate

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Skin cancer ranks as one of the most prevalent globally. Tumor invasion depends on interactions between cancer cells and Cancer-Associated Fibroblasts (CAFs). Recently a new cell type: telocyte (TC), often misidentified as fibroblast has been discovered. TC, characterized by long dendrites enabling them to communicate with a wide variety of cell types, are progressively lost during tissue disruption. However they reappear after treatment, highlighting their role in maintaining tissue homeostasis.
Our aim was to decipher the role of telocytes in squamous cell carcinoma (cSCC). Using immunohistochemistry on cSCC patients samples at different stages, we observed a progressive decrease of telocytes, revealed by loss of CD34+ stromal cells, consistent with data in other cancer types. More interestingly, loss of CD34+ was not observed in all actinic keratosis samples, suggesting a protective role of TC.
However, in a 3D spheroid model, TC isolated from healthy skin donors enhanced tumor cells growth while tumor cells did not affect TC proliferation rate.
On the other hand, in a 3D-based hydrogel model, tumor cells are able to decrease TC dendricity partly via TGF-β1 paracrine secretions. This phenomenon is reversible. This morphological change is associated by a loss of TC markers and gain of CAF markers, leading to the hypothesis that (1) CD34+ cells did not disappear but instead underwent differentiation and lost the CD34+ marker (2) TC may be a novel identified source of CAFs.
This was confirmed by our proteomics profiling showing that some identified TC biomarkers such as CD10, known for being expressed by a CAF subtype responsible for sustaining cancer stemness properties.
In conclusion, this work aims to highlight the potential role of telocytes in the progression of squamous cell carcinoma and raises the question of whether targeting TCs at early stages of cSCC development may offer therapeutic benefit.

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Melanotan II Augments the Therapeutic Efficacy of c-Met Inhibitor in Suppressing B16-F10 Melanoma.

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Melanoma is refractory to conventional cancer therapies and associated with a high risk of mortality. Cabozantinib acts as a potent multi-tyrosine kinase inhibitor (TKI) in melanoma by targeting VEGFR/cMET/AXL/RET pathways. However, the response rate of advanced melanoma patients to TKI Cabozantinib remains poor, underscoring the demand of novel therapeutic strategies. Melanotan II (MT II), a synthetic analog of alpha-melanocyte stimulating hormone (α-MSH), has been applied in humans for tanning purposes. Our previous study indicates topical MT II application effectively attenuated the oncogenic behaviors *in vitro* and progression of B16-F10 melanoma *in vivo*. RNA sequence analysis reveals that MT II upregulates the expression of c-Met, one of the targeting pathways by carbozantinib, in B16-F10 melanoma cells. Thus, we investigated whether MT II supply enhanced the anti-neoplastic potential of Cabozantinib in B16-F10 melanoma. It was found that that MT II significantly enhanced the tumor-suppressive effects of cabozantinib by inhibiting the proliferation and colonies formation of B16-F10 cells. Moreover, MT II diminished the pro-invasive and epithelial mesenchymal transition (EMT)-promoting functions of cabozantinib in B16-F10 cells. In mice bearing established B16-F10 melanoma, combination therapy using both MT II and cabozantinib significantly reduced the tumor burden. Notably, histological analysis showed that MT II reversed the cabozantinib-mediated EMT whereas cabozantinib abrogated the MT II–induced c-Met upregulation. These findings advocate the potential of MT II and carbozantinib combinatory therapy for melanoma suppression in the future.

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HyBryte - A Promising Photodynamic Therapy for Cutaneous T-Cell Lymphoma

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The management of cutaneous T-cell lymphoma (CTCL) remains a clinical challenge, with treatment primarily focused on skin-directed therapies to control disease progression while minimizing cumulative toxicity. Current standards of care are limited by local irritation and long-term safety concerns, necessitating the development of novel therapies. The potential use of topical hypericin (HyBryte) for CTCL has advanced significantly in recent years, with the completion of multiple Phase 2 and 3 clinical studies evaluating safety and efficacy. Hypericin is activated by visible light, generating reactive oxygen species (ROS) that induce apoptosis. With topical delivery and potentially enhanced absorption into malignant T cells, this approach offers an improved safety profile. In the double-blind, placebo-controlled Phase 3 FLASH study (n = 166), the primary endpoint was the overall response rate (ORR) after one 6-week cycle, assessed using the modified Composite Assessment of Index Lesion Severity (mCAILS) score. The ORR was statistically significant after one cycle and further improved to 40% and 48% after one and two additional treatment cycles, respectively. An open-label study directly compared HyBryte with the gold-standard topical mechlorethamine (Ledaga) in patients with stage IB mycosis fungoides (MF). Over 12 weeks, the objective response rate was

60% (3/5) in the HyBryte group compared to 20% (1/5) in the Ledaga group. SCORD scoring demonstrated no adverse effects with HyBryte but significant contact dermatitis with Ledaga. Another open-label study evaluated long-term HyBryte therapy for up to one year. After 18 weeks, the overall response rate was 75% (6/8) in early-stage MF. Based on these results, an additional Phase 3 FLASH2 study is ongoing, enrolling up to 80 patients. Hypericin is minimally detectable systemically, contributing to a favorable safety profile. The rate of application-site adverse events has been consistently low compared with standard therapies. Consequently, HyBryte represents a major step forward in the skin-directed treatment of early-stage CTCL.

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Intratumoural B cell frequency predicts coordinated immune organisation at the tumour-stroma boundary in melanoma

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Immune checkpoint inhibitors have revolutionized treatment of melanoma, however, over 50% of patients fail to achieve durable responses. We and others previously showed that B cells play a major role in anti-tumour immunity and are predictive of response to immunotherapy in melanoma.
To characterise B cells in melanoma tumour microenvironment (TME), we used single-cell spatial transcriptomics on 275 tissue microarrays from 142 patients with primary or metastatic disease with high or low B cell infiltration. Overall, metastatic patients showed loss of antigen-presenting cells, transition of fibroblasts from inflammatory to myofibroblasts, and expansion of SPP1+ and interferon-gamma+ tumour-associated macrophages (TAMs). Sex-stratified analyses showed female primary tumours had higher proportions of exhausted CD8 T cells and inflammatory macrophages than males, suggesting a more inflamed primary TME. This difference in the TME was reversed in metastases, with males showing enrichment of SPP1+ immunosuppressive TAMs associated with T cell exclusion and poor prognosis. Spatial analyses showed SPP1+ TAMs enriched at the direct tumour-stroma boundary, potentially shielding the tumour from cytotoxic T cells. Additionally, tumours with high B cell infiltration showed enrichment of plasma cells at tumour-stroma boundary as expected, but also CD8 T cells co-expressing cytotoxic and exhaustion genes. This T cell population is consistent with a progenitor-derived transitional exhausted state (TPEX) - the primary responder to PD-1 blockade in melanoma. B cells may therefore shape functional immune organisation at the tumour-stroma boundary and, by extension, immunotherapy response.
Our results show that the tumour-stroma boundary in melanoma acts as a microniche where immunosuppressive players, such as SPP1+ macrophages, and anti-tumour effectors, such as TPEX, coexist and collectively shape the local immunity.

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Oncogenic *HRAS*-driven immune escape in cutaneous squamous cell carcinoma

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Oncogenic HRAS is frequently mutated in cutaneous squamous cell carcinoma (cSCC), the second most common non-melanoma skin cancer, yet its role in shaping the tumor microenvironment remains poorly understood. We investigate how oncogenic *HRAS* drives immune evasion and establishes a tumor-promoting microenvironment in cSCCs.
To address this, we modeled acute HRAS activation by transiently introducing $HRAS^{G12V}$ into immortalized primary human keratinocytes and examined chronic HRAS activation in vivo using mouse models bearing $Hras^{Q61}$- and $Hras^{G12V}$-mutated skin tumors.
We demonstrate that oncogenic *HRAS* exerts context-dependent effects on the tumor microenvironment, inducing a strong pro-inflammatory response alongside the upregulation of immunosuppressive genes and metabolic pathways. In particular, HRAS activation increased IDO1 abundance, promoting tryptophan depletion and kynurenine accumulation, consistent with an immunosuppressive metabolic shift. Additionally, *Hras*-mutated tumors displayed distinct immune infiltration patterns, with prominent neutrophil recruitment in both models and macrophage infiltration restricted to G12V-mutated tumors, suggesting mutation-specific immune modulation. Notably, oncogenic HRAS induced neutrophil extracellular trap (NET) formation, or NETosis, contributing to further inflammation and immune evasion within the TME of *Hras*-mutated cSCCs.
In conclusion, oncogenic *HRAS* drives both inflammatory and immunosuppressive features within the tumor microenvironment, mediating cSCC progression. Targeting HRAS-associated pathways, such as IDO1 signaling and NETosis, may represent promising strategies to enhance anti-tumor immunity and improve outcomes in *HRAS*-mutated cSCCs.

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Beyond cell death: XIAP drives melanoma vascular mimicry

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The X-linked inhibitor of apoptosis protein (XIAP) belongs to a group of proteins that regulate programmed cell death, and beyond this, it exerts additional apoptosis-unrelated cellular activities. Its expression correlates and contributes to tumor progression and therapy resistance across several tumor entities, including melanoma. In melanoma, in addition to conferring resistance to apoptosis, XIAP drives cell migration and neutrophil infiltration. Further, in advanced human melanoma specimens, we detected a correlation between XIAP expression and the number of vascular mimicry (VM) structures. These tumor cell-derived blood vessels enable tumor perfusion independent of classical angiogenesis. Extensive *in vitro* studies with murine and human melanoma cells demonstrated that both pharmacological inhibition and genetic deletion of XIAP impaired VM formation without affecting cell viability and proliferation, thus establishing a direct role for XIAP in that process. Domain-specific analyses and genetic deletion identified the XIAP-BIR2 domain, acting through a RIPK2-TAB1 signaling axis. Additional pharmacological studies pointed NF-κB as the downstream effector of the identified axis leading to VM. Transcriptomic profiling

of XIAP-deficient melanoma cells undergoing VM revealed dysregulation of genes linked to angiogenesis and cellular plasticity. Among these, IL-1B and TG2 emerged as critical intracellular effectors, as their intracellular reconstitution by forced expression, but not extracellular supplementation, rescued VM capacity in the absence of XIAP. Collectively, our findings establish XIAP as a central regulator of non-apoptotic signaling pathways that drive melanoma progression, and highlight the XIAP-RIPK2-TAB1-NF-κB axis as a potential therapeutic target for vascular mimicry in melanoma.

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Human Multicellular 3D In Vitro Melanoma Models for Studying Tumour-Microenvironment Interactions and Therapy Response

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Malignant melanoma is the most aggressive form of skin cancer, characterised by high metastatic potential and limited long-term therapeutic success. Progress in targeted therapies remains constrained by the lack of predictive preclinical models, as conventional 2D cultures and animal systems fail to recapitulate the architecture and complexity of human skin and its tumour microenvironment (TME). Here, we present two complementary human-derived 3D in vitro models of increasing complexity: full-thickness skin equivalents (FTSEs) and hiPSC-derived skin organoids (SOs).
In FTSEs, melanoma cells carrying distinct oncogenic mutations are introduced in a controlled manner, enabling modelling of disease progression from early to advanced stages. This system reveals mutation-specific differences in tumour growth and invasion, emphasizing the role of genetic background in melanoma behaviour. It further allows precise manipulation of tumour distribution and microenvironmental composition. Incorporation of cancer-associated fibroblasts (CAFs) reconstructs tumour stroma and significantly increases melanoma invasion depth, highlighting their role in promoting tissue remodelling and progression. Additionally, fluorescent labelling enables spatial and temporal tracking of tumour dynamics.
To enhance physiological relevance, melanoma cells are co-cultured with hiPSC-derived skin organoids, which recapitulate key features of native human skin architecture. This model provides a more complex and integrated TME for studying tumour-tissue interactions. Analysis of molecular and cellular responses using PCR and immunofluorescence reveals distinct marker expression patterns that vary depending on both the mutational status of the tumour cells and the surrounding microenvironment.
Together, these complementary 3D human skin models enable the investigation of melanoma progression and tumour-stroma interactions in controlled and physiologically relevant settings. They provide a promising platform for mechanistic studies and for improving the translational value of preclinical therapeutic testing in melanoma.

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Donor–recipient sex–mismatch tracing uncovers immune tolerance of donor cells in relapsed cutaneous T-cell lymphoma after stem cell transplantation

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Hematopoietic stem cell transplantation (HSCT) is the reference treatment for many hematologic malignancies including cutaneous T-cell lymphoma (CTCL). Tumor escape mechanisms after HSCT remains incompletely understood.
We investigated immune exhaustion in CTCL relapse after sex-mismatched HSCT with scRNA-seq of skin lesions of one Sézary syndrome patient, and immunofluorescence staining of immune checkpoint molecules of longitudinal skin samples of 9 allografted CTCL.
Using a Y-score to discriminate donor cells, we highlighted immune exhaustion in donor-derived immune compartments, and immune tolerance interactions in scRNA-seq data. We confirmed the overexpression of PD1, CTLA4, ICOS, and LAG3 by $CD8^+$ T-cells in skin at baseline and at relapse after HSCT.
This score-based method allows for the study of tumor escape mechanisms at single-cell-resolution in sex-mismatched HSCT in relevant models.

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Spatial remodeling of tertiary lymphoid structures at the invasive front of extramammary Paget's disease

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Extramammary Paget's disease (EMPD) is a rare cutaneous adenocarcinoma with an incompletely characterized tumor immune microenvironment. Tertiary lymphoid structures (TLSs) are important mediators of local antitumor immunity. We recently reported reduced antitumor immunity, including fewer TLSs, at infiltrating sites of EMPD, but the underlying mechanism remains unclear. We investigated regional differences in TLS architecture across tumor-associated compartments in EMPD. A total of 32 TLSs from four EMPD cases were analyzed after each tissue section was divided into three spatial regions according to the distance from the tumor: far from tumor, near tumor, and invasive area. Spatial proteomic profiling was performed using PhenoCycler (Akoya Biosciences). By combining 17 oligonucleotide-barcoded antibodies with machine learning-based analysis, we identified approximately 40 cellular phenotypes within a single tissue section. TLSs in each region were evaluated for cell density, cell number, and spatial organization, and cellular neighborhood analysis was performed to assess regional changes in TLS composition and immune cell arrangement. TLS architecture showed progressive remodeling across the three regions. In areas far from the tumor, TLSs exhibited a more organized structure, consistent with preserved lymphoid compartmentalization. By contrast, TLSs closer to the tumor, particularly in the invasive area, became increasingly disorganized. This change was accompanied by greater intermixing of B and T cells, suggesting loss of canonical TLS segregation and structural maturity. Quantitative analyses of cell density and cell number further supported regional differences

in TLS composition. Overall, TLSs adjacent to the invasive front displayed a more disordered immune architecture than those farther from the tumor. These findings suggest that TLSs in EMPD undergo spatial remodeling according to proximity to the tumor invasive area. Progressive disruption of TLS organization and increased B/T-cell intermixing near the invasive front may reflect chronic tumor-immune interactions and broader remodeling of the local microenvironment at the invasive front.

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DHODH Inhibition Triggers Redox-Driven Differentiation in Cutaneous Squamous Cell Carcinoma

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Alteration in metabolic activities is a critical step in cancer progression, and several metabolic based therapy options are increasingly being proposed for human tumors. Dihydroorotate dehydrogenase (DHODH), a key enzyme in *de novo* pyrimidine biosynthesis, has recently emerged as a therapeutic target in various cancers. We have previously identified a pivotal role of DHODH in initiation of cutaneous squamous cell carcinoma (cSCC), the second most common type of non-melanoma skin cancer. We also showed that pharmacological inhibition of this enzyme suppresses ultraviolet (UV)-induced tumor formation. However, the key mechanisms driving the anticancer activity of DHODH inhibition remain unexplored in cSCC.
We investigated the biological consequences of pharmacological and genetic DHODH inhibition in cSCC using xenograft models derived from two human cell lines, A431 and SCC13, implanted in immunodeficient NSG mice. DHODH activity was suppressed pharmacologically with leflunomide (LFN) and the potent DHODH inhibitor PTC299, or genetically via lentiviral shRNA-mediated DHODH silencing (shDHODH). Proteomic and metabolomic analyses were integrated with histopathological, immunohistochemical, and immunoblotting evaluations to delineate the downstream effects of DHODH blockade.
Comprehensive proteomic and metabolomic profiling revealed that DHODH inhibition induces a coordinated adaptive program involving keratinization, differentiation, redox homeostasis, and metabolic stress responses. Histological and immunostaining analyses demonstrated marked reductions in Ki67-positive proliferating cells and a corresponding increase in pan-cytokeratin (PanCK) and keratin 10 (Krt10) expression, indicative of enhanced epithelial differentiation. These changes were most pronounced in PTC299-treated and shDHODH xenografts, whereas LFN displayed minimal or no efficacy in SCC13 tumors.
DHODH inhibition drives tumor differentiation and suppresses proliferation in cSCC, highlighting metabolic dependency as a potential therapeutic vulnerability. These findings position DHODH as a promising target for bioenergetic vulnerability-based cancer therapy in advanced or treatment-resistant cSCC.

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Integrated Spatial and Circulating Chemokine Profiling in Merkel Cell Carcinoma

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Merkel cell carcinoma (MCC) is a rare, aggressive neuroendocrine skin cancer with poor prognosis. Although immune checkpoint inhibitors (ICIs) have improved outcomes, reliable biomarkers for disease activity and treatment response are lacking. Chemokines regulate immune cell trafficking and tumor–immune interactions, but their role in MCC remains unclear. We analyzed circulating and spatial chemokine profiles in MCC. 30 plasma samples from 11 patients treated with surgery, ICIs, radiotherapy, or combinations were analyzed. 42 chemokines were quantified by Bio-Plex multiplex immunoassay and correlated with clinical course and treatment response. Post-treatment samples showed increased CXCL10 ($p = 0.033$) and CCL26 ($p = 0.046$) compared with pre-treatment. IL-16 levels were higher during tumor progression than complete response (CR) ($p = 0.0012$), whereas CCL24 was elevated during CR ($p = 0.019$). Similar trends were observed for metastasis: IL-16 increased in metastatic cases ($p = 0.0038$), while CCL24 was higher in non-metastatic cases ($p = 0.00058$). Among ICI-treated patients, responders showed higher levels of CCL24 ($p = 0.0000045$) and CCL19 ($p = 0.0035$), whereas non-responders showed increased TNF-α ($p = 0.0099$) and CXCL9 ($p = 0.022$). MCPyV-positive cases showed upregulation of CCL13 ($p = 0.000032$), CCL4 ($p = 0.0010$), CXCL6 ($p = 0.0086$), and CXCL8 ($p = 0.02$), whereas no chemokines were significantly upregulated in MCPyV-negative cases. Spatial transcriptomic analysis using Visium HD was performed in 10 MCC tumors. MCPyV-positive tumors showed intratumoral upregulation of 22 chemokines, including CCL2, CCL8, CXCL10, CCL4, CCL24, CCL13, and CXCL8, whereas MCPyV-negative tumors showed increased CXCL16, CCL27, CCL1, CCL26, and CCL21. ICI-responsive tumors showed upregulation of 17 chemokines, including CCL19, CXCL14, CCL2, CXCL10, and CCL24, whereas non-responsive tumors showed increased CCL4L2, XCL1, XCL2, CCL23, and CCL4. These findings indicate that circulating and spatial chemokine profiles are associated with disease status, viral subtype, and treatment response in MCC. Both plasma and spatial analyses suggest a positive association between CCL24 expression and favorable prognosis.

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YAP engages RIF1 to dampen replication stress-induced DNA damage in squamous cell carcinoma.

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Squamous cell carcinomas (SCCs) are among the most common solid tumours, with cutaneous SCC (cSCC) being the most prevalent type, imposing a major healthcare burden due to its high incidence. The paralogous transcriptional co-regulators YAP

and TAZ have emerged as essential drivers of SCC development and progression by executing transcriptional programmes that promote cell proliferation, survival, and epithelial-to-mesenchymal transition.
SCC cells experience high levels of replication stress. How such cells sustain rapid proliferation despite replication stress is still not fully understood. Here, we show that oncogenic YAP activation induces replication stress in human cSCC cells. By performing RIME (Rapid Immunoprecipitation Mass Spectrometry of Endogenous Proteins) analysis, we have characterised the nuclear interactome of chromatin-associated YAP in a cSCC cell line that is transcriptionally addicted to YAP, and discovered that YAP engages with RIF1, a key regulator of DNA replication and DNA damage repair under replication stress. RIF1 is highly expressed in human SCC cell lines and tissues and upregulated during tumour progression. RIF1 protein levels are modulated by YAP together with its transcriptional binding partner TEAD. Depletion of RIF1 in cSCC increases DNA replication fork speed and thereby exacerbates endogenous replication stress by preventing robust activation of the ATR/CHK1-mediated replication stress checkpoint. We further show that under severe replication stress, YAP interacts with RIF1 near sites of DNA damage caused by replication fork collapse, and that YAP depletion impairs DNA damage repair.
Our results thus demonstrate that YAP's oncogenic functions in squamous cancers involve both transcriptional and non-transcriptional mechanisms.

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XPC dependent epigenetic deregulation promotes keratinocyte carcinoma aggressiveness in xeroderma pigmentosum
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Xeroderma pigmentosum (XP) is a rare DNA repair disorder caused by defects in the nucleotide excision repair pathway or in the translesion synthesis polymerase η, resulting in extreme UVR sensitivity and a dramatically increased risk of keratinocyte carcinomas (KCs). XP patients frequently develop aggressive, early onset tumors, yet the epigenetic mechanisms underlying this susceptibility remain poorly understood. DNA methylation regulates transcription, shaping cell identity and guiding responses to environmental cues, and is dynamically altered by aging and chronic UVR exposure. However, XP specific methylation changes and their functional impact are still undefined. Gaining insight into these alterations could open new avenues for therapeutic strategies aimed at XP patients and potentially benefit the broader population at risk for KCs.
Here, we provide the first genome wide DNA methylation analysis of XP epidermis and XP associated KCs. XP epidermis displays a profoundly disrupted methylome that closely resembles premalignant and malignant lesions, indicating an intrinsic predisposition to tumor development. We further show that XPC loss enhances cSCC aggressiveness through a distinct methylation landscape and the hypomethylation of repetitive elements. Finally, integrated methylation and transcriptomic profiling of CRISPR/Cas9 engineered XPC deficient cSCC models exposed to chronic UVR demonstrates that XPC deficiency establishes the epigenetic basis for tumor aggressiveness in XP, which UVR subsequently amplifies into an invasive phenotype.

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ROBO-Slit signaling links lineage programs to migration and therapy resistance in melanoma
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Given the neural crest origin of melanocytes, this study aims to identify lineage-dependent transcriptional programs and signaling pathways reactivated during melanoma formation and progression.
Public and in-house single-cell RNA sequencing (scRNA-seq) datasets were analyzed using the SCENIC algorithm to identify lineage-associated gene regulatory networks. Candidate pathways were functionally validated in melanoma cell lines and cancer-associated fibroblasts (CAFs) by assessing receptor and ligand expression via qPCR. Pathway components were manipulated using siRNA-mediated knockdown and stimulation with recombinant ligands. Downstream signaling activity was evaluated by western blot analysis of phosphorylated target proteins. Functional effects on cell migration were assessed using an Incucyte® chemotaxis assay. Gene expression changes following pathway stimulation were analyzed by qPCR and bulk RNA sequencing.
Screening of melanoma and CAF cell lines revealed ubiquitous ROBO1 expression and variable ROBO2 levels in tumor cells. Integration of qPCR and public RNA-seq data showed that Slit ligands are primarily expressed by CAFs and pericytes within the tumor microenvironment, rather than by melanoma cells. This expression pattern recapitulates the ROBO-Slit distribution observed during neural crest cell migration. Slit stimulation induced ROBO-dependent phosphorylation of Akt and Erk and altered expression of Erk target genes, indicating Erk-dependent transcriptional changes. In the presence of MAPK inhibitors, Slit treatment restored Erk phosphorylation, suggesting a potential role in therapy resistance. Functionally, Slit ligands reduced melanoma cell migration by approximately 50% in chemotaxis assays, mirroring its chemorepulsive role during NC development
These findings identify ROBO-Slit signaling as a lineage-dependent pathway reactivated in melanoma that contributes to interactions between tumor and its microenvironment, limits melanoma cell migration and may be a potential target to overcome resistance to targeted therapy.

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T Cell Immune responses to Human Papillomavirus among Asian Organ Transplant Recipients and Immunocompetent Individuals with Cutaneous Squamous Cell Carcinoma
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To date, there is a knowledge gap about cell mediaated immunity responses to cutaneous Human Papillomavirus (HPV) in patients with cutaneous squamous cell carcinoma (SCC), especially in organ transplant recipients (OTR) who are heavily burdened with multiple viral warts and SCC. This study aims to characterize the HPV E6/E7-specific T-cell responses in OTR

with SCC, compared to immunocompetent individuals with SCC.
This multi-center study recruited participants from 4 tertiary hosipitals in Singapore. Cases were organ transplant recipients who had a history of histologically confirmed post organ transplant primary SCC. The comparator group for the study was non-transplant participants who had developed SCC. Fifteen-mer peptides that overlapped by 10 amino acids and covering the E6/E7 region of HPV 8, 17, 49, and 98 were synthesized. To measure the frequency of HPV-specific T cell epitopes, an Enzyme-linked immune absorbent spot (ELISpot) assay was deployed.
The majority of participants in each group mounted robust T-cell responses, with non response rates of 18% among OTRs, 30% among non-OTRs, and 25% among healthy controls. In the OTR cohort, βHPV17 elicited the highest proportion of responders, followed by βHPV98, whereas responses to βHPV8 and βHPV49 were low. In the non-OTR cohort, overall responses were low across all four βHPV genotypes; however, two non-OTR participants exhibited strong responses to βHPV49. Participants with positive ELISpot responses have higher wart body surface area and more SCC events.
Our study demonstrated that HPV E6/E7-specific T-cell responses are preserved in OTR with persistent multiple cutaneous warts and cutaneous squamous cell carcinoma. Advanced age, long duration of organ transplant, and multiple immunosuppression medications did not dampen their HPV E6/E7-specific adaptive immunity. Future work can build on studies to focus on protecting high-risk individuals with the prospects of T cell-centered vaccines or immunotherapy against commonly occurring cutaneous βHPVs and prognostication with βHPV-specific T cells.

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Shared p63 and p73 enhancer landscapes sustain mitogenic signaling in skin squamous cell carcinoma

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Aberrant transcriptional regulation is a defining feature of squamous cell carcinoma, yet how lineage transcription factors coordinate shared and factor-specific oncogenic programs remains unclear. This study investigated the cooperative contribution of p63 and p73 to skin squamous cell carcinoma tumorigenesis. We combined expression analyses in human tumors with loss-of-function assays, chromatin profiling, transcriptomics and functional validation in cellular and tumor models. p73 was upregulated together with p63 in skin squamous cell carcinoma and was required for efficient tumor growth. p63 and p73 formed heteromeric complexes and co-occupied distal enhancer elements, establishing a shared chromatin regulatory framework. Integrated chromatin and transcriptomic analyses showed that this enhancer landscape supports both convergent and divergent transcriptional outputs. Both factors sustained proliferation programs, while p63 preferentially reinforced epithelial lineage circuits and p73 contributed more prominently to DNA replication and stress-associated pathways. Among shared targets, p63 and p73 co-regulated multiple epidermal growth factor receptor ligands, establishing a feed-forward signaling module that amplifies mitogenic signaling. Amphiregulin emerged as a dominant functional mediator, and its depletion phenocopied key effects of p63 and p73 loss, including impaired proliferation and reduced tumor formation. These findings support a model in which shared enhancer occupancy by p63 and p73 drives cooperative and factor-biased transcriptional programs linking chromatin regulation to growth factor signaling and tumor maintenance.

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Spatial Differences in Immune Microenvironments across Actinic Keratosis, Bowen's Disease and Cutaneous Squamous Cell Carcinoma

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Actinic keratosis (AK), Bowen's disease (BD) and cutaneous squamous cell carcinoma (cSCC) are keratinocyte tumors with distinct clinicopathological features, yet how their immune microenvironments differ spatially remains insufficiently characterized. We investigated disease-associated differences using PhenoCycler spatial multiplex imaging (Akoya Biosciences) with 33 oligonucleotide-barcoded antibodies targeting immune cell markers and immune checkpoint proteins. A total of 5 AK, 7 BD and 8 cSCC samples were analyzed. For each sample, 5–12 immune hot zones of 40,000 square micrometers were selected, followed by cell segmentation, phenotype annotation and quantitative spatial analysis. For cellular composition, a significant enrichment of CD3e+CD4+ICOS+ T cells in AK compared with BD (P=0.0369) and cSCC (P=0.046) was observed, whereas CD20+ B-cell density was significantly higher in cSCC than in AK (P=0.0479). For pairwise immune cell organization, Pearson cell-cell correlation coefficients within 60 μm diameter spots followed by Welch's t-test showed that CD3e+CD4+ T cells were spatially closer to CD3e+CD4+PD1+TOX+ cells in AK than in BD (P=0.0110), and closer to CD3e+CD8+TOX cells in AK than in cSCC (P=0.0089). In contrast, CD3e+CD4+ T cells were closer to CD3e+CD8+CD107a+ cells in BD than in cSCC (P=0.0074). These findings suggest that keratinocyte tumors exhibit disease-specific characteristics in both cellular composition and spatial organization. AK was characterized by enrichment of ICOS+ CD4+ T cells and close spatial association between CD4 T cells and TOX-expressing T-cell subsets, whereas cSCC showed increased B-cell accumulation and reduced proximity among selected T-cell subsets. This immune profiling approach may help define lesion-specific immune microenvironments associated with keratinocyte tumor biology.

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Spatial and Bulk Transcriptomics Defines the Molecular Evolution of Cutaneous Squamous Cell Carcinoma and Reveals Stage-Specific Biomarkers of Clinical Relevance

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Cutaneous squamous cell carcinoma (cSCC) is a common skin cancer associated with substantial morbidity and mortality in advanced stages. Despite its well-described stepwise progression from actinic keratosis to invasive disease, robust molecular markers for stage discrimination and clinical decision-making remain limited. We sought to define the transcriptional continuum underlying cSCC progression, identify stage-associated biomarkers, and assess the broader relevance of these programs across human malignancies.
Bulk RNA sequencing (HTG EdgeSeq) and spatial transcriptomics (GeoMx) were performed on biopsies from eight patients, each presenting multiple disease stages (healthy skin, premalignant lesion, tumor core, and invasive front) within the same lesion field, enabling within-patient analysis of progression.
Spatial transcriptomic analyses identified more than 2,000 differentially expressed genes whose expression varied across disease stages. These genes were organized into 18 coordinated expression programs reflecting progressive biological rewiring during tumor evolution. Proliferation, extracellular matrix remodeling, inflammation, and stress-response pathways were progressively upregulated, whereas epithelial differentiation and metabolic processes, including lipid and amino acid metabolism, were downregulated. Macrophages exhibited distinct metabolic reprogramming, with increased purine metabolism, glycolysis, and pyruvate metabolism across progression. A cSCC-derived gene signature was associated with poor overall survival in multiple TCGA cancer types. We further identified 12 stage-informative biomarkers, whose spatial expression patterns were validated using Visium HD.
This study provides a spatially resolved and stage-aware transcriptomic map of cSCC progression, identifies coordinated gene programs underlying disease evolution, and defines progression-associated signatures with prognostic relevance across multiple cancers, highlighting their potential translational value.

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A dissolvable microneedle platform for intradermal chemoimmunotherapy in full-thickness human skin cancer models

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Vulvar squamous cell carcinoma (VSCC) is an invasive squamous malignancy of the vulvar epithelium characterized by pronounced tumor-stroma interactions. Surgery remains the mainstay treatment for localized VSCC; however, preserving local tissue integrity and function underscores the demand for less invasive, locally targeted strategies. Dissolvable microneedle arrays (dMNAs) offer a promising approach by enabling minimally invasive intradermal delivery into tumor-bearing skin.
Our objective was to develop and evaluate a dMNA platform for intradermal co-delivery of a chemotherapeutic and a stromal-modulating agent in human full-thickness skin models (FTMs).
We developed dMNAs co-loaded with doxorubicin (DOX) and pirfenidone (PFD) for delivery in HT117 VSCC FTMs. DOX was employed as a cytotoxic model chemotherapeutic, while PFD was included to modulate fibroblast activity and extracellular matrix (ECM) remodeling. Microneedles were characterized for morphology, mechanical integrity, insertion, dissolution, and delivery depth using fluorescence-based confocal z-stack imaging. In fibroblast-containing VSCC FTMs, responses were assessed by tumor viability, histology, proliferation, DNA damage, apoptosis markers, and stromal readouts including α-SMA, fibronectin, collagen I, and TGF-β/SMAD signaling.
dMNAs inserted reliably and delivered DOX/PFD to target depths. Local treatment induced antitumor effects, with reduced tumor viability, decreased proliferation, and increased DNA damage and apoptosis. PFD co-delivery further modulated stromal responses, reflected by altered fibroblast activation and ECM-related readouts.
This study establishes a human-relevant workflow for evaluating localized chemo-stromal therapy in VSCC. By combining spatially controlled intradermal delivery with stromal modulation, DOX-PFD co-loaded dMNAs represent a promising platform for investigating local combination therapies targeting tumor cells and the fibroblast-rich tumor microenvironment, with potential to enhance local efficacy while minimizing systemic exposure.

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Highly-targeted personalised siRNAs for *BRAF*-fusions trigger melanocytic apoptosis

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Congenital melanocytic naevus (CMN) syndrome is a mosaic disorder caused by classic melanoma oncogenic variants developing in utero, representing the highest melanoma risk in children globally. Recently, BRAF-fusions have been identified as a cause, presenting with a particularly proliferative and symptomatic phenotype. While oral trametinib (MEK inhibitor) can decrease cutaneous proliferation, the naevi remain, and the melanoma risk persists. We previously demonstrated that allele-specific siRNA can induce apoptosis in NRAS-variant naevus cells in vitro and successfully deliver it into mouse skin. Given the sequence specificity of gene fusions and the targeted design of siRNA against the fusion breakpoint, together with the skin's accessibility, we aimed to investigate the potential of BRAF-fusion siRNA therapy in primary cell cultures.
Primary melanocytic cells were obtained from skin biopsies of four patients with BRAF-fusion-driven CMN syndrome. Three samples were from benign naevus tissue and one from primary melanoma. Each patient had a different 5' partner gene fused to the BRAF kinase domain: *PHIP:BRAF*, *ZNF213:BRAF*, *QKI:BRAF*, and *EVI5:BRAF*. siRNAs designed to target the fusion transcripts were transfected either alone or in combination with trametinib, and compared with non-targeting siRNA and vehicle controls.
Fusion-specific siRNAs effectively lowered fusion transcript levels compared to non-targeting controls. A single siRNA dose markedly decreased cell proliferation, outperforming the current standard, trametinib. Combining siRNA with trametinib did not improve efficacy beyond that of siRNA alone. All patient cell lines showed signs of apoptosis. Mechanistically, siRNA treatment notably decreased MAPK activation across all lines.
Taken together, these findings suggest that siRNA therapy should serve as a more effective treatment for CMN patients with *BRAF* fusions than trametinib, and induction of apoptosis supports potential resolution of lesions rather than only stabilisation. The accessibility of skin for siRNA delivery and the specificity of fusion-targeted siRNA would be expected to minimise off-target effects, but formal toxicity studies would be required.

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In-depth proteomic analysis of extramammary Paget's disease subtypes and associated adenocarcinomas

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Extramammary Paget's disease (EMPD) is a rare epidermal adenocarcinoma, presenting as slow-growing lesions in genital areas. It can be primary or secondary to other adenocarcinomas, such as breast or prostate cancers. The molecular mechanisms distinguishing EMPD subtypes and their association with concurrent tumors remains poorly understood. To identify pathophysiological features and potential therapeutic targets, we analysed the proteomes of microdissected tumor cells and cell islands from formalin-fixed paraffin-embedded tissue of 19 EMPD patients and nine associated adenocarcinomas by liquid chromatography tandem mass spectrometry. We detected 7977 proteins across all samples, with 20 unique to EMPD and 288 unique to the associated cancers. EMPD-specific proteins implicated melanin synthesis and kinase binding pathways. Differential expression (DE) analysis revealed 1165 significantly DE proteins between EMPDs and other tumors (FDR<0.05, $|\log_2 FC|>1$). Top EMPD-enriched proteins included CALML3, LGALS7B and TRIM29, implicating oncoproteins, tumor suppressors, calcium signaling, cell differentiation, trafficking, and adhesion. Site-specific EMPD pathways were identified, including RAGE signaling and ion sequestration in anal EMPD, providing insights to support future diagnostic efforts. This study characterises EMPD-specific pathways distinct from concurrent adenocarcinomas and provides an in-depth proteomic analysis of anatomical EMPD subtypes, contributing towards improved diagnosis and, with continued functional exploration, improved treatments, for this poorly-understood cancer.

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Therapeutic approaches to *NRAS*-driven leptomeningeal melanoma in congenital melanocytic naevus syndrome

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Primary *NRAS*-driven leptomeningeal melanoma is a universally fatal childhood cancer, with trametinib the only therapy that confers temporary benefit. We sought to identify novel therapies using two complementary approaches.
Four patients with *NRAS*-mosaic congenital melanocytic naevus syndrome underwent biopsy of progressive diffuse leptomeningeal proliferations, three subsequently diagnosed as melanocytic dysplasia with malignant potential and one as melanoma. Primary cell lines were established and *NRAS* genotype confirmed. An in vitro screen of 2529 FDA-approved drugs was performed in one dysplastic line and wild-type immortalised control melanocytes. In parallel, two siRNAs were designed to target oncogenic alleles *NRAS*: c.181C>A, p.(Q61K) and *NRAS*: c.182A>G, p.(Q61R). IncuCyte® phase-contrast imaging and a DNA-binding fluorescent caspase 3/7 dye measured confluency and apoptosis, respectively. RNA sequencing was performed post-treatment with either $siNRAS^{Q61K}$ or $siNRAS^{Q61R}$.
While trametinib lacked preferential activity against *NRAS*-mutant cells, epirubicin was the most effective in selectively suppressing mutant cells' proliferation, but it did not induce apoptosis. Moreover, epirubicin does not cross the blood-brain barrier and cannot be given intrathecally. In contrast, treatment with siRNAs $siNRAS^{Q61K}$ or $siNRAS^{Q61R}$ triggered apoptosis in all mutant lines. Mechanistically, oncogenic NRAS knockdown downregulated *HIF1A*, antiapoptotic gene *ARL6IP1* and melanoma progression genes *DTL* and *CDC6*. Separate knockdown of *HIF1A* and *ARL6IP1* induced apoptosis across all lines. Precision siRNA against oncogenic *NRAS* is a new candidate therapy for untreatable leptomeningeal dysplasia and melanoma. Intrathecal administration is a potential delivery route, subject to safety studies.

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Mutational landscape of *FOXA1* and *ERBB2* in extramammary Paget's disease: A retrospective multicenter study of 99 cases across Japan and Taiwan

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Extramammary Paget's disease (EMPD), a cutaneous malignancy typically arising in apocrine-rich areas, is known to harbor specific somatic hotspot mutations, notably g.chr14:38064406G>A in the *FOXA1* promoter and S310F in *ERBB2*. However, the prevalence of *FOXA1* promoter mutations outside Japan and their clinicopathological significance across different Asian populations remain poorly defined. This study aimed to evaluate the frequency and clinical relevance of these hotspot mutations in EMPD cohorts from Japan and Taiwan.
We conducted a multicenter retrospective analysis of 99 surgically treated EMPD patients from five institutions in Japan and Taiwan between January 2013 and March 2024. Genetic profiling was performed on surgical specimens using amplicon sequencing for *FOXA1* and droplet digital PCR for *ERBB2*. Additionally, immunohistochemistry was utilized to evaluate FOXA1 protein expression in a subset of 38 cases.
Among the 93 non-fresh-frozen samples, the *FOXA1* (g.chr14:38064406G>A) mutation and the *ERBB2* S310F mutation were identified in 8.6% (8/93) and 40.0% (37/93) of the cases, respectively. Notably, patients with the *FOXA1* mutation were detected across all five participating centers. IHC analysis revealed robust nuclear FOXA1 expression in Paget cells across all evaluated samples, independent of their genetic mutational status. Neither *FOXA1* nor *ERBB2* mutations demonstrated

statistically significant associations with the assessed clinical parameters.
Our findings confirm the presence of *FOXA1* hotspot mutations in both Japanese and Taiwanese EMPD patients and highlight their potential biological significance.

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TGF-β-mediated loss of endothelial AMBRA1 as a potential marker of melanoma progression
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Loss of the autophagy regulatory protein AMBRA1 in combination with the terminal epidermal differentiation protein Loricrin in the epidermis overlying primary melanoma is a validated prognostic biomarker for nonulcerated stage I/II disease. Mediated by tumoural secretion of isoform specific transforming growth factor-beta 2 (TGF-β2), our recent data shows epidermal AMBRA1 downregulation is also associated with loss of AMBRA1 in the tumour endothelial microenvironment, prompting the current study aimed at determining the contribution of TGF-β ligands to endothelial AMBRA1 regulation and the consequences of its loss.
Primary human endothelial cells (HUVECs and HDBECs) were cultured in vitro with TGF-β1, 2 or 3 alone or in combination, followed by assessment of AMBRA1 expression by Western blot densitometry. To investigate the consequences of AMBRA1 loss, siRNA-mediated depletion of AMBRA1 in HUVECs was performed prior to bulk RNA sequencing and pathway enrichment analyses.
Individually TGF-β isoforms induced downregulation of endothelial AMBRA1 expression, whereas pooled TGF-β1/2/3 induced the most robust effect. Transcriptomic profiling of AMBRA1-depleted endothelial cells demonstrated suppression of endothelial proliferation, adhesion and extracellular matrix remodelling genes. Gene ontology analysis identified reduced cell-cell adhesion signalling, while enrichment using a bespoke endothelial-to-mesenchymal transition (endoMT) gene set revealed loss of endothelial identity markers, consistent with a partial endoMT phenotype.
Collectively these data suggest TGF-β-mediated suppression of endothelial AMBRA1 modulates endothelial dysfunction and plasticity, which may facilitate tumour cell intravasation and dissemination. In the context of epidermal and endothelial AMBRA1 loss, this pathway may contribute to metastatic progression in early-stage melanoma and represent a potential source of prognostic biomarkers and therapeutic targets, providing a foundation for future translational studies.

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MITF-driven regulation of cellular ROS homeostasis in cutaneous melanoma
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Microphthalmia-associated transcription factor (MITF) is crucial in melanocyte development, function, and melanoma pathogenesis. MITF amplification is common in melanoma and is associated with resistance to targeted therapies. The production of eumelanin is thought to shield the melanocytes and keratinocytes from UV damage by buffering the accumulation of UV-induced reactive oxygen species (ROS) during pigment production. We hypothesized that, in addition to controlling the synthesis of the antioxidant eumelanin, MITF might promote ROS clearance by regulating cellular ROS homeostasis. We aim to demonstrate that MITF regulates a global antioxidant program that increases the survival of melanoma cell lines by protecting the cells from reactive oxygen species (ROS)-induced damage. These studies build on our published work demonstrating that MITF regulates a transcriptional antioxidant program in melanoma. Pathway analyses were performed using publicly available datasets in DAVID and Cytoscape. Cells were transfected with a siRNA delivery system. Cytosolic ROS levels were measured using the DCFDA fluorescent dye by flow cytometry. Cell viability was measured with trypan blue. Zebrafish were injected with MiniCoopR MITF overexpression plasmid. Zebrafish older than 15 days were euthanized by rapid chilling via submersion in ice water for 30 minutes. 8-oxoG was detected by mass spectrometry.
Pathway analyses indicate that MITF might regulate a transcriptional redox program in melanoma. Functional studies demonstrate the role of MITF and its target genes in reducing cytosolic and mitochondrial ROS. In vivo experiments using a zebrafish melanoma model showed that MITF decreases ROS-mediated DNA damage. Some MITF target genes, such as IDH1 and NNT, are regulated through direct MITF binding to canonical E-BOX sequences proximal to their promoters. Our data suggests that MITF is a major driver of the cellular antioxidant state, possibly explaining earlier observations that MITF may protect melanoma cells against ferroptosis. These findings further provide a foundation for exploring how lineage-specific redox programs may differentially influence distinct oxidative stress-related cell death pathways.

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Environmentally stressed drug-naive melanoma cells share a molecular signature with drug-tolerant and drug-resistant cells
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Melanoma drug resistance, driven predominantly by dynamic heterogeneity, prevails in slow-cycling rather than proliferating tumour cells. We analyzed the transcriptome of 3D melanoma spheroids, where the inner cell-layers are slow-cycling while the outer cell-layers remain proliferating. The differentially expressed genes (DEGs) and pathways affecting the spheroid cell cycle (inner vs. outer cells, IVO) were correlated with drug-sensitive vs. drug-tolerant (TVS) and drug-resistant (RVS) melanoma. IVO shared 14.7% (192 genes) of their upregulated and 36.6% (357 genes) of their downregulated DEGs with the TVS group, and 9.2% (120 genes) and 15.8% (154 genes) with the RVS group. The number of shared enriched pathways in IVO vs. TVS was higher than IVO vs. RVS. In addition, hsa-miR-4497 is the only miRNA that is dysregulated significantly in all three experimental conditions. Our comparative analysis of hsa-miR-4497 target genes revealed it plays a key role in all three different experimental groups through distinct but overlapping mechanisms. We speculate that hsa-miR-4497 drives both inner and drug-tolerant cells to exhibit early adaptive responses to stress. Although through regulation of different genes in inner and resistant cells, the genes affected by hsa-miR-4497 exhibit long-term adaptive processes that involve a strategic reallocation of resources to enhance survival under prolonged adverse conditions. Also, adaptation of inner cells to hypoxia mirrors stable resistance (RVS), which focuses on maintaining critical survival pathways and reducing cell migration and invasion. In conclusion, environmentally stressed inner cells revealed gene expression patterns more similar to drug-induced early persister than irreversibly drug-resistant cells.

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Immune landscape remodeling in cSCC progression: unveiling the phenotypic diversity of the cSCC immune microenvironment

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The transition from actinic keratosis (AK) to cutaneous squamous cell carcinoma (cSCC) is driven by a profound remodeling of the tumor microenvironment (TME).This study aims to characterize the immune drivers of this progression and define distinct TME phenotypes.We profiled 119 matching samples (42 field of cancerization [FC], 35 AK, and 42 cSCC) using the OIRRA transcriptomic panel, validating immune profiles via IHC in 10 FFPE cases.Comparison between AK and FC revealed a significant upregulation of S100A8(FDR=2.3e-4) and S100A9(FDR=1.7e-4) in AK, associated with increased infiltration of neutrophils(p=1.9e-5).The progression from AK to cSCC was defined by a signature of 53 DEGs showing 83.5% concordance with existing literature (GSE32628), characterized by the downregulation of DGAT2(FDR=9.4e-9) and EGR3(FDR=5.2e-7), and the upregulation of GBP1(FDR=2.0e-6) and CXCL8(FDR=2.0e-5).cSCC exhibited a significant shift towards M1 macrophage polarization(p=3.2e-7), while CD4+ T-cells(p=0.021), Th2(p=9.7e-8),Th17(p=1.8e-5), and M2 macrophages(p=9.3e-4) were reduced compared to AK. Functional analysis via ssGSEA showed a marked increase in IFNg response(p=1.4e-5), Type I IFN(p=5.3e-6), tertiary lymphoid structures (p=0.001) and keratinocyte-driven proliferation(p=0.034), alongside a depletion of basal epidermal identity signatures(p=7e-4), reflecting the loss of structured epidermal programs during invasion.Within cSCC, a Thorsson-based stratification identified 4 phenotypes (Immune-Activated, Immune-Stromal High, Immune-Desert, and Myeloid-Suppressed) that significantly correlated with tumor thickness and the Ayers GEP score (p=0.004), the gold standard for predicting immunotherapy response.These subgroups were characterized by distinct marker expression: DGAT2(Activated), IL18(Desert), CD27(Stromal High), and CCL4(Myeloid-Suppressed). Decoding this TME complexity is pivotal for developing next-generation personalized strategies in cSCC progression.

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Epigenetic reprogramming of cancer-associated fibroblasts by HDAC inhibition induces senescence-driven tumor-supportive signaling in melanoma

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Targeting the tumor microenvironment (TME), particularly cancer-associated fibroblasts (CAFs), by reversing their activation or disrupting tumor-supportive functions is an emerging therapeutic strategy in cancers including melanoma. While histone deacetylase inhibitors (HDACis) demonstrate potent pro-apoptotic effects in melanoma cells, their impact on stromal compartments remains underexplored.
Our objective was to investigate the effects of HDACis on CAF function and tumor-CAF crosstalk in melanoma.
In 2D cultures, CAFs were treated with valproic acid (VPA) or vorinostat (SAHA). Proliferation, contractility, and migration were assessed using CCK-8, gel contraction, and scratch assays, respectively. CAF phenotypes were characterized by bulk RNA sequencing and validated experimentally. Tumor-stroma interactions were evaluated using co-culture systems. 3D melanoma-like human skin equivalents (HSEs) were generated to assess drug responses in in a physiologically relevant context.
HDAC inhibition suppressed myofibroblast-like CAF (myCAF) signatures and tumorigenic extracellular matrix production, while promoting a shift towards a quiescent, reticular fibroblast-like state. However, HDACis concurrently induced CAF senescence, characterized by cell cycle arrest and a senescence-associated secretory phenotype which enhanced NFκB-dependent compensatory tumor cell survival and monocyte chemotaxis. In 3D melanoma HSEs, HDACi application reduced tumor burden and myCAF activation but increased melanoma epithelial-to-mesenchymal transition-like plasticity. Notably, co-targeting NFκB signaling mitigated CAF senescence-associated tumor-promoting effects and enhanced the anti-tumor efficacy of HDAC inhibition.
HDAC inhibition reshapes the melanoma microenvironment by suppressing CAF activation while inducing senescence-driven, NFκB-dependent tumor-supportive signaling. These findings provide a mechanistic explanation for the limited clinical efficacy of HDACis in melanoma and support combined HDAC and NFκB targeting as a rational therapeutic strategy.

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Loss of deubiquitinase function drives tumor-like transformation in Schwann cells

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Tumors arising from Schwann cells affect peripheral nerves and may present as subcutaneous lesions encountered in dermatological practice. Current care is predominantly surgical, and targeted pharmacological strategies are lacking due to

incomplete understanding of the underlying molecular mechanisms. This study investigates how loss of a deubiquitinating protein contributes to Schwann cell transformation and tumor-associated phenotypes.
CRISPR/Cas9-mediated knockout was established in human Schwann cells and validated by genotyping and immunoblotting. Phenotypic characterization included proliferation assays to assess contact inhibition, immunofluorescence to evaluate growth patterns, and anchorage-independent growth assays. Rescue experiments were performed via re-expression to confirm specificity of the observed phenotype. Molecular analyses were conducted by immunoblotting and qPCR. In addition, whole-genome sequencing of patient-derived schwannoma samples was performed, revealing alterations in ubiquitin pathway-associated regulatory components.
Loss of deubiquitinase function resulted in reduced contact inhibition, multilayer growth, and anchorage-independent proliferation, consistent with tumor-like behavior across all assays. Molecular analyses confirmed dysregulation of ubiquitin pathway activity. Re-expression partially restored the phenotype.
These findings demonstrate that disruption of ubiquitin-dependent protein homeostasis drives Schwann cell transformation and identify ubiquitin-mediated regulation as a critical pathway in peripheral nerve tumor pathogenesis.

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Cancer Associated Fibroblasts Switch from Immune Activating to Immune Suppressive States During Cutaneous Squamous Cell Carcinoma Progression

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Cancer associated fibroblasts (CAFs) of the tumour microenvironment contribute significantly to tumour progression and immune regulation. While tumour cell evolution in cutaneous squamous cell carcinoma (cSCC) is well characterised, how CAF epigenetic and transcriptional programs evolve from primary tumours through recurrence to metastasis remains unclear.
Here, we developed a screening pipeline to profile primary CAFs and patient matched normal fibroblasts across cSCC stages. Using this approach, we generated a unique isogenic CAF progression series from a severe (T3) cSCC spanning primary tumour, recurrence and metastasis, and performed RNAseq and ATACseq. Functional heterogeneity was explored through chemical modulation of chromatin state.
Analysis of multiple cellular parameters across 17 primary CAF cultures from early and late stage cSCC (T1 to T3) confirmed marked fibroblast heterogeneity. Unexpectedly, nuclear features most strongly correlated with disease severity, implicating chromatin remodelling as a key driver of progression. Integrated ATACseq and RNAseq analysis of the isogenic CAF series revealed clustering by disease stage, acquisition of epithelial mesenchymal transition associated gene signatures and progressive global chromatin compaction. During progression, CAFs shifted from immune activating towards stress adapted, immune suppressive states characterised by AP1 and NFκB activity, resulting in distinct IFNγ responses upon inflammatory stimulation and chromatin modulation. Whereas early stage CAFs exhibited both immune activating (CD40high) and immune suppressive (PDL1high) features, advanced disease was associated with epigenetic remodelling, loss of immune activating programs, and dominance of immune suppressive states.
These findings identify CAF chromatin state as a stage specific regulator of immune function in cSCC, highlighting epigenetic targeting of CAFs as a potential strategy to restore anti tumour immunity and improve therapeutic responses in advanced disease.

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Modulating phenotypic melanoma heterogeneity and immune cell infiltration

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Microphthalmia-associated transcription factor (MITF) is a key player in melanoma phenotype switching. We showed previously that MITF regulates phenotypic melanoma heterogeneity through changing extracellular matrix (ECM) composition and tumour microarchitecture. ROCK (Rho kinase) inhibition mimics the MITF-high phenotype by generating a loose spheroid architecture and a wider distribution of proliferating cells and thus a smaller G1-arrested core.
We hypothesise that MITF-upregulation or ROCK inhibition regulate immune cell infiltration through changed ECM structure. We show that MITF-upregulation or ROCK inhibition affect immune cell infiltration in a cell line-dependent manner. The spatial distribution pattern of PBMCs infiltrating into spheroids matched the pattern of ECM fiber organization, suggesting that ECM structure, regulated by MITF expression and ROCK activity, plays a critical role in PBMC penetration.
Notably, PBMCs preferentially targeted proliferating cells. ROCK inhibition boosted PBMC activity regarding eliminating proliferating melanoma cells in the spheroid periphery. Natural Killer (NK) cells are likely responsible for the melanoma cell death, given the short duration of the experiment, which precludes T cell activation. Indeed, NK cell cytotoxicity in 2D culture resulted in an increase in cell death, indicating that NK cells were responsible for the melanoma cell elimination.
Given the melanoma cell death in PBMC-infiltrated groups primarily emerged in the peripheral zone, which was occupied by proliferating cells, we further sought to understand why NK cells specifically targeted the proliferating peripheral cell population. By investigating NK cell activation and inhibitory ligands in both melanoma subpopulations, we found that proliferative melanoma cells produced more MICA/B, a critical activation ligand for NK cells.
In summary, dormant cells are often responsible for melanoma relapse, which is a challenge for current clinical treatments. We propose that ROCKi will increase the total number of immune-sensitive proliferating cells and allow for a better innate immune response, suggesting a potential combination with immune checkpoint inhibitors.

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Integrative molecular profiling reveals determinants of cutaneous squamous cell carcinoma metastasis

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Although most cutaneous squamous cell carcinomas (cSCCs) are curable, the minority that metastasise account for substantial disease-related mortality and remain difficult to predict. We aimed to define genomic and transcriptomic features of metastatic progression and integrate these data to identify linked programmes. Formalin-fixed paraffin-embedded perilesional normal skin, primary tumours and metastases from immunocompetent patients with cSCC underwent whole-exome sequencing and TempO-Seq expression profiling. Primary tumours were classified as non-metastasising after three years of follow-up. Mutational burden, signatures, driver genes, clonality and copy-number alterations were assessed alongside differential expression and cell-type deconvolution. Copy-number calls were integrated with matched expression data, and multi-omics factor analysis was used to examine shared molecular structure. Metastasising primaries and metastases showed higher mutational burden, increased subclonal complexity and greater copy-number disruption than non-metastasising primaries. Metastasising primaries were transcriptionally distinct from non-metastasising primaries but resembled matched metastases, with modest expression change after accounting for tissue context. Samples became progressively more progenitor-like across disease progression. Copy-number and expression integration identified gain-associated genes with concordant increased expression and enrichment for proliferative programmes, including cell-cycle and MYC/E2F-related biology. Exploratory multi-omics factor analysis supported coordinated RNA and copy-number variation across disease progression. These findings suggest that metastatic potential in cSCC may be established within the primary tumour through genomic instability, copy-number dosage effects and transcriptional reprogramming.

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Inhibition of endothelial and melanoma cell migration by a vascular endothelial growth factor receptor (VEGFR)-1-derived peptide blocking neuropilin-1/VEGFR-1 interaction

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Neuropilin-1 (NRP-1) is a semaphorin receptor involved in neuronal guidance and functions as a co-receptor for specific isoforms of the vascular endothelial growth factor (VEGF) family. Binding of NRP-1 to VEGF-A isoforms enhances their interaction with VEGF receptor-2 (VEGFR-2), promoting receptor phosphorylation and downstream signaling. Although NRP-1 can also interact directly with VEGFR-1, this interaction competes with VEGF-A binding and does not enhance VEGFR-1 activation.

In a previous work, we investigated the interaction between NRP-1 and the soluble isoform of VEGFR-1 (sVEGFR-1), which acts both as a decoy receptor for VEGFs and as an extracellular matrix protein capable of binding α5β1 integrin. Our findings demonstrated that the interaction between sVEGFR-1 and NRP-1 is essential for α5β1 integrin binding to sVEGFR-1, as well as for endothelial cell adhesion to a matrix containing sVEGFR-1. We also showed that the anti-angiogenic peptide Flt2-11, derived from the second Ig-like domain of VEGFR-1, specifically binds NRP-1 and inhibits the NRP-1/sVEGFR-1 interaction, likely contributing to its anti-angiogenic effects.

In the present study, we characterized a shorter peptide derived from Flt2-11, consisting of five amino acids (Flt2-5). This peptide binds to NRP-1 and disrupts its interaction with sVEGFR-1. Functionally, Flt2-5 inhibits endothelial cell adhesion to sVEGFR-1-containing matrices, as well as endothelial and melanoma cell migration toward sVEGFR-1. Alanine scanning identified key residues required for its activity.

Overall, Flt2-5 emerges as a promising anti-angiogenic and anti-melanoma candidate, as well as a valuable tool for investigating the role of sVEGFR-1/NRP-1/α5β1 integrin complex in angiogenesis and melanoma progression.

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Comparative spatial transcriptomic profiling of nevi-associated melanomas and their corresponding nevi

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Malignant melanoma is an aggressive skin cancer frequently arising from preexisting nevi, making molecular characterization of this complex transformation of high clinical interest. Here, we applied a spatially-resolved, multi-omic approach to identify transcriptomic and proteomic alterations associated with malignant transition. RNA expression profiling was performed on eight paired formalin-fixed, paraffin-embedded nevi-associated melanomas (NAM) and corresponding nevus samples using the GeoMx Digital Spatial Profiler. Regions of interest were selected based on MART1 immunostaining for melanocytic enrichment of both compartments, enabling spatially resolved whole-transcriptome analysis. Gene profiles were compared to published proteomes from the same tumors. We quantified 10,877 genes, including 109 significantly differentially expressed genes in NAM versus corresponding nevi (FDR<0.01,|FC|>0.5). Pathway analysis of NAM and corresponding nevi revealed an upregulation of several cancer hallmarks in melanoma, including cell migration, immune modulation, metabolic reprogramming and angiogenesis. Comparative analyses of gene and protein fold changes identified 21 concordantly regulated genes/proteins in melanoma, including downregulation of DCN and CCL19, and upregulation of SND1, consistent with tumour progression and immune evasion. This spatially-resolved, multi-omic study reveals important differences associated with nevus-to-melanoma

transformation, suggesting distinct molecular pathways involved in its progression with partly convergent transcritomic and proteomic evidence.

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Non-invasive profiling of epidermal methylomes for keratinocyte cancer risk stratification

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The epidermis is continuously exposed to environmental stressors such as ultraviolet radiation, which drive progressive genetic and epigenetic alterations linked to aging and the emergence of keratinocyte carcinomas. Because DNA methylation patterns reflect these cumulative changes, they offer a powerful readout of epidermal health and carcinogenic potential. Yet, the application of methylation-based assays to the epidermis has been constrained by the low quantity and compromised quality of genomic DNA obtained through conventional non-invasive sampling.
To address this limitation, we developed TapeLift, a workflow that enables the generation of high-quality epidermal methylomes from tape-stripped material using Infinium microarray technology. The method combines efficient keratinocyte capture, an optimized extraction protocol for low-input DNA, and a curated probe set tailored to the characteristics of superficial epidermal samples. Analysis of ~100 TapeLift derived methylomes demonstrates that this approach supports accurate modeling of epidermal aging and the detection of methylation features associated with keratinocyte cancer development, including signatures linked to aberrant proliferative history and tumor suppressor gene silencing. These results highlight TapeLift as a practical and minimally invasive platform for large scale epidermal methylation profiling, biomarker discovery, and improved assessment of skin health and cancer risk.

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Comparative Assessment of Hybryte™ and Ledaga® in Early-Stage Mycosis Fungoides

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This phase 2 trial provided preliminary comparative assessment of safety and efficacy of chlormethine (Ledaga) gel versus 0.25% synthetic hypericin (HyBryte) ointment. HyBryte is a novel non-mutagenic photodynamic therapy activated with visible light that previously demonstrated safety and efficacy in a multicenter randomized placebo-controlled phase 3 trial (FLASH) for early-stage mycosis fungoides/cutaneous T-cell lymphoma (MF/CTCL). This was an open-label trial enrolling patients with early-stage CTCL randomized to receive HyBryte or Ledaga for 12 weeks. Patients randomized to HyBryte applied the drug to all accessible lesions twice weekly followed by visible light treatment 21 (±3) hours later (starting dose 6 J/cm^2, max 50). Index lesions were assessed every 3 weeks using the modified Composite Assessment of Index Lesion Severity (mCAILS) score. Efficacy endpoints included mCAILS, the modified Severity-Weighted Assessment Tool, and Physician Global Assessment scores. Ten patients were enrolled (median age 62 years); 9 completed the study, as 1 patient in the Ledaga group discontinued due to an adverse event (AE). HyBryte treatment resulted in 60% of patients achieving a ≥50% improvement in their mCAILS score versus 20% of patients treated with Ledaga. No HyBryte patients had AEs related to therapy, whereas 60% of the Ledaga patients had at least 1 AE related to therapy. SCORD scoring noted contact dermatitis with Ledaga and none with HyBryte. Patients receiving HyBryte had higher treatment response rates compared to those receiving Ledaga (60% vs 20%) over 12 weeks. By the end of the study (Week 16), significantly more plaque lesions had responded to HyBryte, whereas both therapies showed a similar response in patches. The rapid benefit of HyBryte is clinically important as it provides motivation for patients to continue therapy due to observable early benefit. These findings reinforce the potential of HyBryte as a well-tolerated and effective treatment for CTCL.

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Population- and haplotype-dependent variation around *TYR* rs1126809: an *in silico* study suggesting new directions for melanoma risk research

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The common missense variant rs1126809 in the *TYR* gene, encoding a key enzyme in melanin synthesis, is linked to both albinism and melanoma susceptibility. While its role in hypopigmentation as part of a risk haplotype is established, its function in melanoma is less understood. To investigate population- and haplotype-dependent associations surrounding *TYR* rs1126809 that may suggest directions for future studies on melanoma risk and melanocyte biology. We analysed melanoma GWAS summary statistics using FUMA to identify lead SNPs in linkage disequilibrium (LD) with rs1126809 and assessed their regulatory potential using chromatin state data from melanocytes, keratinocytes, and fibroblasts. Haplotype structures were examined in European, American, and South Asian populations using LDlink. rs1126809 emerged as lead SNP in the *TYR* locus. Thirty SNPs were in high LD, six of which reside in melanocyte-specific regulatory regions. These variants form population-specific haplotypes that may modulate TYR expression. In Europeans and Americans, two distinct haplotypes carrying the risk allele showed different linked SNP profiles, suggesting haplotype-dependent effects. The findings suggest that variations surrounding *TYR* rs1126809 may exhibit population- and haplotype-dependent patterns relevant to melanoma risk, pointing to new directions for future research and refinement of polygenic risk modelling.

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Proteomic Response to Cryotherapy and Topical Therapy in Actinic Keratosis

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Actinic keratoses (AKs) are common premalignant keratinocytic lesions managed with lesion-directed cryotherapy or field-directed topical 5-fluorouracil (5-FU). Comparative molecular responses within treated epidermis are incompletely characterised. We compared pre and post-treatment tape-striped proteomic changes following cryotherapy and topical 5-FU.
In a comparative observational study, paired tape-striped samples were collected from AKs before and after treatment with cryotherapy (n=20) or topical 5-FU (n=13). Proteins were identified and quantified using liquid chromatography–mass spectrometry-based proteomics. Paired differential protein abundance was analysed using limma with Benjamini–Hochberg false discovery rate (FDR). Pathway enrichment was assessed using pre-ranked GSEA across GO Biological Process, KEGG, Reactome and Hallmark gene sets; significance was defined as FDR q<0.25.
Cryotherapy produced limited single-protein change, with 5/3492 proteins significantly altered (FDR<0.05; 4 upregulated, 1 downregulated), including increased ITIH3, LRG1 and TMPRSS11E, and decreased CYTH1. Despite this, GSEA identified 450 significant gene sets, including enrichment of Lysosome (NES 2.71) and ECM degradation (NES 2.10) with depletion of translation programs (cap-dependent translation initiation, NES −2.53). Topical 5-FU induced broader disruption, with 150/3556 proteins significantly altered (130 upregulated, 20 downregulated), including complement/coagulation and inflammatory proteins (e.g., C1R, CFI, C4BPA, PLG, MPO, ELANE). GSEA identified 683 significant gene sets, including enrichment of complement and coagulation cascades (NES 2.66) and depletion of translation initiation pathways (translation initiation complex formation, NES −3.07).
Both therapies shift the AK surface proteome toward innate immune activation and tissue remodelling with concurrent suppression of ribosome/translation programs. The molecular response is substantially stronger following topical 5-FU, consistent with a pronounced inflammatory field reaction, supporting tape-strip proteomics for mechanistic assessment and biomarker discovery in AK management.

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The urinary microbiome as a potential biomarker of response to immune checkpoint inhibitors in advanced melanoma

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We aimed to characterize the urinary microbiome (UMB) in malignant melanoma (MM) and its association with immune checkpoint inhibitor (ICI) response in a prospective study.
Urine samples from 26 MM patients (female: n=12; age: 66.5) and 24 healthy controls (female: n=11; age: 63.0) were analyzed using 16S rRNA gene sequencing. Samples were collected prior to initiation of ipilimumab and nivolumab. Best overall response (BOR) and overall survival (OS) were evaluated after a median follow-up (FU) of 216 days.
No significant differences in microbial community structure were observed between MM patients and controls (Shannon: p=.946; Bray-Curtis (BC): R^2=.018, p=.727). However, within the MM cohort (n=26), reduced alpha diversity was associated with unfavorable BOR (progressive disease: n=11/26; Shannon: p=.036) and mortality during FU (death: n=8/26; Shannon: p=.026). Beta diversity analysis suggested modest group differences when stratified by mortality (BC: R^2=.052, p=.028). ANCOM and LEfSe identified multiple clinically relevant taxa, with Bacteroidota (LDA: 4.9), Fusobacteriota (LDA: 3.9), and *Prevotellaceae* (LDA: 4.9) of particular interest, all associated with favorable outcomes.
UMB-derived parameters (Shannon, presence/absence of Fusobacteriota, relative abundance of Bacteroidota) better predicted survival after ICI therapy (p=.002, p=.018, p<.001) than established biomarkers (BRAF status: p=.700, LDH: p=.634, S100: p=.286).
While comparable between MM patients and matched controls, the UMB is associated with BOR and OS within the MM cohort, with reduced baseline diversity linked to unfavorable outcomes and specific taxa associated with favorable outcomes.

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Tyrisonase activity increases sensitivity to ferroptosis in melanoma

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The limited efficacy of current treatments in overcoming resistance to targeted and immunotherapies in melanoma highlights the need to identify novel therapeutic targets and to better understand the regulation of cell death pathways. Reactive oxygen species (ROS) play a key role in both pigmentation and melanoma pathogenesis. However, the regulation of iron-dependent, lipid peroxidation-mediated ferroptotic cell death in melanoma remains poorly understood. Therefore, we aimed to investigate the role of pigment production in modulating ferroptosis sensitivity in melanoma.
We used glutathione peroxidase 4 (GPX4) inhibitors (ML210, RSL3) to induce ferroptosis in pigmented mouse (B16F0) and human (MEL202) melanoma cell lines. Tyrosinase (TYR) knockout clones were generated in both models using CRISPR-Cas9. Interestingly, TYR-expressing wild-type cells exhibited increased sensitivity to ferroptotic cell death compared to TYR knockout cells in both mouse and human melanoma lines. Consistently, overexpression of tyrosinase further increased sensitivity to ferroptosis and pharmacological inhibition of tyrosinase activity reduced sensitivity to ferroptotic cell death. In line with these findings, increased lipid peroxidation following GPX4 inhibition was observed in cells with active tyrosinase. Importantly, we examined multiple cell death pathways and confirmed that pigmented cells treated with ML210 and RSL3 underwent ferroptosis rather than apoptosis, necroptosis, parthanatos or pyroptosis.
Transcriptomic profiling indicated decreased NRF2 activity in tyrosinase-expressing melanoma cells. Consistent with this, NRF2 target genes, including GPX4, as well as its cofactor glutathione, were present at lower levels in TYR-active cells, correlating

with increased ferroptosis sensitivity. Reducing glutathione levels in unpigmented cell mimicked the TYR active cells, highlighting the functional role of glutathione.
In conclusion, our results suggest that pigment production increases ferroptosis sensitivity in melanoma cells. However, further studies are required to precisely elucidate the mechanisms underlying the increased susceptibility of pigmented cells to ferroptosis.

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Blocking Two-Pore channel 2 suppresses BRAF V600-mutant melanoma aggressiveness

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The endo-lysosomal ion channel Two-Pore channel 2 (TPC2) regulates Ca^{2+} and Na^{+} signaling and has been linked to tumor progression, angiogenesis, and immune modulation. In melanoma, TPC2, primarily expressed on the membranes of lysosomes, late endosomes, and melanosomes, appears to play a context-dependent role influenced by BRAF status, genetic background, and tumour stage.
To investigate the role of TPC2 in human melanoma, patient biopsies from different clinical stages and paired cell lines derived from early and late tumour stages harboring BRAF mutations were analyzed. TPC2 was silenced using siRNA and pharmacologically inhibited using naringenin (flavonoid) and the synthetic inhibitor SG-094.
The analysis of patient biopsies revealed a correlation between TPC2 expression and melanoma aggressiveness. In vitro 3D migration and invasion assays showed that TPC2 inhibition markedly reduced cell viability and spheroid growth. Moreover, TPC2 blockade limited migration and invasion both *in vitro* and *in vivo* in a zebrafish model, while modulating integrin-mediated adhesion, melanosome dynamics, and cancer-associated fibroblast transformation. Importantly, TPC2 inhibition also enhanced the efficacy of chemotherapy and BRAF-targeted therapies. In conclusion, targeting TPC2 represents a promising therapeutic strategy in melanoma, as its inhibition not only impairs tumour progression but may also enhance the efficacy of existing therapies. By potentially overcoming acquired resistance to BRAF inhibitors, TPC2 emerges as a clinically relevant target, supporting the development of pharmacological approaches aimed at improving outcomes for patients with BRAF-mutant melanoma.

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A combined *NRAS* and *BRAF* inhibitor to treat naevi and melanoma

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A substantial fraction of melanomas arise from melanocytic naevi, or moles, and the total number of naevi remains a key predictor of melanoma risk. Current approaches to monitoring and excising naevi cause scarring, and are poorly tolerated in older adults due to impaired wound healing. A medical therapy to regress naevi completely would therefore have major clinical value. Here we design a combined short interfering RNA therapy to target the three commonest driver variants in naevi, termed siCOMBO$^{NRAS/BRAF}$. siCOMBO$^{NRAS/BRAF}$ treatment triggers pronounced apoptosis in both melanoma and primary naevus cultures, while treatment with MEK- and BRAF-small molecule inhibitors does not. Mechanistically we show that apoptosis is mediated via *ARL6IP1,* acting downstream of oncogenic *BRAF* or *NRAS*, but not via the MAPK pathway. In an intradermal xenograft model, intra-tumoural siCOMBO$^{NRAS/BRAF}$ treatment induces regression of melanoma. Our findings establish siCOMBO$^{NRAS/BRAF}$ as a triple-target combination siRNA therapy that induces apoptosis in both naevus cells and melanoma, supporting further exploration as a non-surgical strategy to eliminate naevi.

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Simultaneous silencing of multiple oncogenic drivers induces apoptosis across cancer types

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Short interfering RNA (siRNA) therapies are now a reality for genetic disorders. Their precision and safety led us to study the biological effects of approaching cancers from a genetic therapy angle.
SK-MEL-28 (melanoma), SK-OV-3 (ovarian carcinoma), and HCT-116 (colorectal carcinoma) lines were genotyped using deep Ampliseq for Illumina Cancer HotSpot v2 and Focus Panel sequencing. Based on the genotyping results, two different siRNAs were designed for each cell line, targeting known oncogenic alleles. IncuCyteâ high-throughput phase contrast imaging combined with fluorescent caspase 3/7 dye monitored confluency and apoptosis. Transcriptomic effects of treatment were interrogated by RNA sequencing. Intradermal xenograft models of SK-OV-3, SK-MEL-28 and HCT-116 were established in NSG mice, and siRNA therapies delivered by intratumoural injection for initial proof of concept.
We show that treatment with single siRNAs targeted to cell-line-specific oncogenic variants in genes *BRAF*, *EGFR*, *FBXW7*, *KRAS* or *PIK3CA* triggered significant apoptosis across cancer types *in vitro*. Strikingly, combining two siRNAs further increased the potency of apoptotic induction in melanoma and colorectal lines. Treatment with these combined siRNAs *in vivo* induced significant reduction in tumour growth in all three XG models. Mechanistically, combined silencing of oncogenic *PIK3CA* and *KRAS* led to notable *TP53* upregulation in HCT116, confirmed by *TP53* knockdown rescue of apoptosis.
Simultaneous silencing of multiple oncogenic drivers induces apoptosis across cancer types including melanoma. The simplicity of this genetic therapy approach renders it agnostic to cancer type or stage and lends itself to an automated platform approach.

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UVB exposure drives a tumour-permissive epithelial–immune microenvironment that promotes early cSCC development

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Chronic ultraviolet B (UVB) exposure is one of the major risk factors for developing cutaneous squamous cell carcinoma (cSCC). However, the early cellular and molecular events that govern tumour initiation remain incompletely understood. Here, we performed an integrated single-cell transcriptomic analysis of human UV-exposed and UV-protected skin, actinic keratoses (AK), and cSCC lesions, complemented by spatial transcriptomics and a mouse model of chronic low-dose UVB exposure.
UV-exposed skin showed a shift in epidermal composition, with an increased basal-to-differentiated keratinocyte ratio and expansion of a highly proliferative basal population. These cells were enriched for cell cycle programs (YBX1, RUNX1, STAT3) and conserved across UV-exposed skin, AK, and cSCC, indicating a persistent proliferative state. However, they retained differentiation signatures and lacked canonical cancer stem cell markers, suggesting they are not fully tumour-initiating.
UV exposure broadly reprogrammed keratinocyte transcriptional profiles, with reduced differentiation, adhesion, and immune regulatory pathways. These findings suggest that most keratinocytes act as modulators of the UV-exposed microenvironment rather than direct cells of origin for cSCC. Consistently, ligand–receptor analysis revealed extensive remodelling of epithelial–immune crosstalk, including impaired IL34–CSF1R signalling linked to reduced Langerhans cell maturation, enrichment of immunoregulatory T cells (Tregs, NKT), and increased immune checkpoint and cytokine interactions.
Together, our findings define a UV-induced tissue ecosystem in which proliferative keratinocyte states and coordinated immune remodelling act in concert to create a tumour-permissive microenvironment. This work provides a framework for distinguishing tumour-initiating processes from adaptive field effects and highlight actionable epithelial-immune signalling pathways, including IL34-CSF1R and immunoregulatory networks, as potential targets for early intervention to prevent or limit cSCC development.

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Cytokines derived from cutaneous T cell lymphoma T cell lines disrupt epidermal differentiation and barrier formation in 2D and 3D human skin models

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Cutaneous T-cell lymphomas (CTCL) are a group of rare malignancies characterized by the presence of malignant T cells in lesional skin. In early stage disease, CTCL lesions often mimic benign inflammatory dermatoses, which may progress in tumors and/or generalized erythroderma. The pathogenesis and pathophysiology of CTCL remains incompletely understood. In particular, the impact of the altered cytokine milieu produced by malignant T cells on epidermal homeostasis is poorly defined, hampering the development of effective, targeted therapies and limiting biologically driven disease stratification. This study aims to investigate the effect of cytokines secreted by malignant CTCL T cell lines on epidermal structure and function, to elucidate their contribution to CTCL associated skin pathology.
Monolayer (2D) cell cultures and human skin equivalents (HSEs) (3D) were generated and exposed to conditioned media containing cytokines from malignant CTCL T cell lines (HH, MyLa, and SeAx) during the final 72 hours before harvesting. Epidermal morphogenesis, structure and function were evaluated based on histology and the expression of differentiation, barrier and inflammatory markers.
Histological analysis revealed that exposure to CTCL-derived cytokines impaired epidermal structure. The expression of differentiation and barrier-associated markers were altered, indicating that CTCL-derived cytokines negatively affected epidermal stratification and barrier formation. Inflammatory markers interleukin (IL)-1β was upregulated in response to MyLa derived cytokines, whereas IL-8/CXCL8 was induced by cytokines from SeAx and HH.
Cytokines derived from malignant CTCL T cell lines profoundly affect epidermal morphogenesis, structure and function in both 2D and 3D cell cultures. Ongoing analyses aim to define the functional consequences of these cytokine induced epidermal alterations and to clarify their relevance for CTCL pathology.

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Cancerous inhibitor of PP2A (CIP2A) as an Oncogenic Driver and Prognostic Biomarker in Cutaneous Squamous Cell Carcinoma

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Incidence of cutaneous squamous cell carcinoma (cSCC) is rising worldwide, underscoring the need for new therapies for recurrent and metastatic disease. Protein phosphatase 2A (PP2A) is a key tumor suppressor, and its inhibition promotes tumorigenesis. We investigated the role of the cancerous inhibitor of PP2A (CIP2A) in cSCC progression. RNA-seq and qRT-PCR showed significant CIP2A mRNA overexpression in cSCC cells. Western blotting confirmed CIP2A protein overproduction in cSCC cell lines compared with NHEKs. Analysis of single-cell RNA-sequencing data from cSCC revealed CIP2A mRNA expression in epithelial cells and fibroblasts. CIP2A knockdown with specific siRNA revealed decreased cSCC cell growth and viability by inhibiting MEK1 and ERK1/2 phosphorylation. *In vivo*, strong CIP2A immunostaining was noted in 51% of primary cSCCs (n=162) and 81% of cSCC metastases (n=63), while staining was absent or weak in all actinic keratoses (n=73) and 98% of cSCC in situ (n=59). Kaplan-Meier analysis revealed that all patients with high CIP2A expression had significantly worse disease-specific survival than those with low expression (p=0.04). These results identify tumor cell-derived CIP2A as a clinically relevant oncogene in the progression of cSCC, with abundant overexpression in the invasive primary tumors and metastases. CIP2A may serve as a biomarker of progression and metastasis of cSCC and as a potential therapeutic target in metastatic cSCC.

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Deregulation of intercellular ERK MAPK signal network in epidermal cancer cells

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Extracellular signal-regulated kinase (ERK) regulates multiple cellular functions and its precise regulation is essential in physiological tissues. We previously found physiological epidermal cells show dynamic spatiotemporal ERK activation patterns such as pulsatile activities and local propagations. However, their physiological significance and potential alterations in pathologies such as cancer remain unstudied.
Here we performed single-cell live imaging of ERK activity in normal epidermis (HaCaT) and epidermoid carcinoma (A431) cell lines using a FRET (Förster Resonance Energy Transfer)-based biosensor. We found reduced ERK activity pulse frequency and impaired cell-cell ERK activity propagation in A431 cells, suggesting the intercellular signal network is impaired in cancer cells. Supportively, co-cultured HaCaT and A431 cells revealed that A431 cells block ERK activity propagation between HaCaT cells. Furthermore, pharmacological analyses indicated that Raf activity is critical for the regulation of the spatiotemporal ERK activity patterns.
Our live imaging approach underpins the significance of cell-cell signal network in oncogenesis. Controlling the ERK-mediated intercellular communications may provide novel therapeutic targets for the prevention of early-stage cancer development.

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Thermal conductivity measurement for noninvasive assessment of melanoma and dermal invasion

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Early detection of melanoma and dermal invasion is essential, but current diagnosis relies on dermoscopy and biopsy, and may lead to unnecessary invasive procedures. We conducted a prospective exploratory single-centre feasibility study to evaluate a novel double-pulse device that noninvasively measures effective thermal conductivity of skin lesions. Adults with lesions clinically suspected of melanoma and requiring biopsy were recruited at Tohoku University Hospital between January and December 2025. Thermal conductivity was measured at the lesion and adjacent healthy skin before biopsy, and histopathology served as the reference standard. Twenty patients were enrolled, 19 were included in the full analysis set, and 10 in the per-protocol set after excluding one basal cell carcinoma and nine cases with abnormal device signals related to low temperature. In the per-protocol analysis, sensitivity and specificity for distinguishing benign from malignant lesions were 77.8% and 85.7%, respectively, with an area under the curve of 0.8695. For detecting dermal invasion, sensitivity and specificity were 83.3% and 66.7%, respectively, with an area under the curve of 0.7284. No serious device-related adverse events occurred. These findings suggest that thermal conductivity measurement is a safe, rapid and quantitative noninvasive approach that may support melanoma diagnosis and assessment of dermal invasion. Larger multicentre studies with improved temperature control are warranted.

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Autophagy dysregulation in cutaneous Squamous Cell Carcinoma (cSCC): tumorigenesis and therapeutic potential

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Cutaneous Squamous Cell Carcinoma (cSCC) is a is a keratinocyte-derived skin cancer which can develop from premalignant lesions known as actinic keratosis (AK). While most cSCC are effectively treated by surgical excision, a high-risk subset progresses to invasive or metastatic disease. Investigating alterations in cellular signalling pathways may reveal what drives cSCC progression and development. Autophagy is an essential process in which cellular components are sequestered into autophagosomes and degraded. Analysis of single-cell RNA sequencing databases revealed high mutational frequency in autophagy-related genes between AK and cSCC, suggesting that autophagy deregulation may drive progression of cSCC and could provide a novel therapeutic avenue.
Preliminary *in vitro* autophagic flux assays revealed autophagy was impaired in premalignant cSCC cell lines, while active in metastatic cell lines. To investigate this further, expression of key autophagic markers were examined in AK and cSCC tissue, where protein accumulation indicates regions of autophagic dysfunction. A cell DIVE multiplex immunofluorescence platform was used to spatially map multiple autophagy markers across epidermal, tumour and stromal compartments using lineage-specific markers.
In AK, autophagy marker accumulation was higher in regions of severe dysplasia than normal epidermis. In primary cSCC, autophagy marker expression was heterogenous across samples, so tumours were classed into phenotypes; autophagy-intact tumours which were typically well-differentiated, and autophagy-deficient tumours which tended to be poorly differentiated. Autophagy appeared intact in stromal fibroblasts, implicating a potential role for autophagy in the cSCC stroma.
In conclusion, impaired autophagy may contribute to transition from normal epidermis to AK. cSCC tumours appear to segregate into autophagy-intact or impaired subtypes, suggesting autophagy status may influence tumour differentiation and behaviour. Future work aims to elucidate how these autophagy phenotypes shape tumour progression and evaluate whether targeted autophagy modulation represents a viable therapeutic strategy in cSCC management.

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Anatomical location influences clinicopathologic features and tumor stage in acral melanoma: a single-center analysis

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Acral melanoma represents a distinct subset of cutaneous melanoma arising on glabrous skin and within the nail unit, yet the extent to which different acral sites are associated with specific clinicopathologic patterns remains unclear. The aim of this study was to evaluate the impact of anatomical location on the clinical and pathological features of melanoma arising on acral sites.
We conducted a retrospective analysis of 183 patients with primary melanoma arising on acral sites diagnosed at a tertiary referral center, stratified into lesions of the soles and palms (n=56), dorsum of the hands and feet (n=47), and the nail unit (n=80). Continuous and categorical variables were compared using non-parametric and chi-square tests with appropriate corrections.
Significant differences were observed across anatomical sites. Melanomas arising on the dorsum were diagnosed at a younger age and were significantly thinner than those located on soles/palms and in the nail unit (median Breslow thickness 0.4 mm vs 1.2 mm and 0.7 mm, respectively; $p<0.001$). This group was predominantly composed of superficial spreading melanoma (83.3%) and radial growth phase lesions (80.0%), and was largely confined to early-stage disease (73.9% T1). In contrast, soles/palms melanomas showed a shift toward more advanced disease, with greater thickness, predominance of vertical growth phase (58.3%), and a higher proportion of T3–T4 stages (35.2%). Melanoma of the nail unit displayed a heterogeneous pattern, combining the highest proportion of in situ lesions (38.3%) with a substantial representation of nodular melanoma (30.2%) and advanced stages.
Anatomical location identifies distinct clinicopathologic phenotypes within melanoma arising on acral sites. Dorsal lesions appear less aggressive at presentation, palmoplantar melanomas are associated with more advanced disease, and nail unit melanoma shows marked heterogeneity. These findings suggest that anatomical site may reflect both biological differences and variability in diagnostic timing, with potential implications for risk stratification and early detection strategies.

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CD276 and PRAME Expression in Malignant Melanoma and Dysplastic Nevi: Clinicopathological and Dermoscopic Correlations

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Distinguishing malignant melanoma from dysplastic nevi remains diagnostically challenging because of overlapping clinical, dermoscopic, and histopathological features. This study evaluated CD276 (B7-H3) and PRAME immunohistochemical expression in melanocytic lesions and investigated their associations with clinicopathological and dermoscopic characteristics. Sixty-two archived melanocytic lesions (34 malignant melanoma, 28 dysplastic nevi) were retrospectively analyzed. Immunohistochemical staining was performed on formalin-fixed paraffin-embedded tissue sections, and expression levels were assessed using immunoreactive score. Both CD276 and PRAME expression were significantly higher in malignant melanoma than in dysplastic nevi ($p=0.007$ and $p=0.001$, respectively). High PRAME expression was significantly associated with advanced tumor stage ($p=0.005$). Dermoscopic features including gray-blue structures, angulated lines/polygons, and blue-white veil were more frequent in lesions with high PRAME and CD276 expression, while malignant melanoma demonstrated significantly higher frequencies of gray-blue structures, atypical pigment network, white lines, angulated lines/polygons, irregular lines/pseudopods, regression structures, blue-white veil, and irregular blotches compared with dysplastic nevi. Although CD276 was not independently associated with prognostic clinicopathological variables, it may reflect tumor biology. These findings suggest that CD276 and PRAME may enhance diagnostic discrimination between malignant melanoma and dysplastic nevi, and that integrating immunohistochemical biomarkers with dermoscopic evaluation may improve the diagnostic and biological characterization of melanocytic lesions.

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SKIN AGEING, SENESCENCE, PROTECTIVE STRATEGIES

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The role of cellular senescence in facial senile lentigo

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Skin aging is accelerated by exposure to environmental factors such as ultraviolet (UV) radiation and pollution. Senescent cells, characterized by an irreversible growth arrest, accumulate in aging tissues, including skin, and are implicated in pre-neoplastic lesions and various age-related pathologies. A significant challenge in the senescence field has been the unequivocal identification of senescent cells through reliable biomarkers, both *in vitro* and *in vivo*. Over the past decade, we have characterized the downregulation of lamin B1, a nuclear intermediate filament protein, as a robust biomarker for detecting and quantifying senescent cells *in vitro* and *in vivo*. Our findings demonstrate that lamin B1 protein is downregulated in cells undergoing replicative or oncogene ($BRAF^{V600E}$)-induced senescence. Furthermore, senescent (lamin B1-negative) cells accumulate in chronologically aged human skin, following UVB exposure, and within precancerous actinic keratosis lesions. Here, we extended these findings to quantifying senescent cells in the epidermis of lesional versus adjacent non-lesional facial senile lentigo (SL) - a common skin condition linked to prolonged sun exposure and age. To investigate the physiological relevance of these observations, we conducted co-culture experiments to quantify the impact of senescent cells on melanin production and transport. Collectively, these results underscore the contribution of senescent cells to the development of age-

related skin conditions, and lay the foundation for targeted treatment strategies to mitigate skin aging and age-related pigmentation disorders.

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Topical reverse transcriptase inhibitors reduce retrotransposon-associated skin inflammaging

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Aging promotes chronic low-grade inflammation and drives dysregulation of innate immune signaling. This phenotype is linked to type I interferon signaling and a senescence-associated secretory phenotype (SASP). Mechanisms underlying this phenotype remain to be explored. Using RNA *in situ* hybridization, we have found that retrotransposons, including the human endogenous retrovirus K (HERV-K) and the long interspersed element-1 (LINE-1), which are usually silenced in healthy cells, were derepressed in the epidermis of old compared to young skin. These retrotransposons retain partial reverse transcriptase activity, enabling them to produce cytosolic DNA that activates the cyclic GMP-AMP synthase-stimulator of interferon genes (cGAS-STING) pathway. This triggers a type I interferon-regulated immune response and amplifies senescence-associated inflammatory signaling. HERV-K expression positively correlated with M1 pro-inflammatory macrophages ($p<0.002$) in old skin, suggesting that retrotransposon activation may be an upstream factor influencing the macrophage phenotype associated with skin inflammaging. We treated human skin explants with the reverse transcriptase inhibitors zidovudine (AZT) and lamivudine (3TC) to test whether retrotransposon-associated inflammatory signaling can be blocked. Treatment significantly reduced CXCL10 ($p<0.001$) and TNF-α ($p<0.03$) expression. A topical formulation showed similar effects. These findings identify retrotransposon derepression as a contributor to skin inflammaging and establish topical reverse transcriptase inhibitors as a potential therapeutic strategy.

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miR-383 promotes senescence in human dermal fibroblasts by targeting PCLAF and suppressing DNA repair

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Cellular senescence is a fundamental driver of skin aging, characterized by persistent DNA damage signalling and proliferative arrest. While the transcriptional landscape of senescent fibroblasts is well-defined, the post-transcriptional mechanisms enforcing the senescence program remain incompletely understood. Here, we identify miR-383-5p (miR-383), a skin ageing-associated miRNA, as a potent pro-senescence inducer in human dermal fibroblasts. We show that miR-383 is induced by replicative exhaustion and in response to genotoxic stress. Functionally, ectopic expression of miR-383 is sufficient to trigger a senescence-associated phenotype, including cell cycle arrest, increased senescence-associated β-galactosidase activity, p16/INK4a-induction, increased expression of inflammatory factors, decreased level of SIRT1, as well as transcriptomic changes overlapping with those seen in replicative senescence. Conversely, CRISPR-Cas9-mediated knockout of the endogenous MIR383 locus extends the replicative lifespan of fibroblasts and delays the onset of senescence. Transcriptome profiling reveals that miR-383 suppresses a broad network of DNA damage response genes. Mechanistically, within this network, we identify the PCNA clamp-associated factor (PCLAF, KIAA0101) as a direct target of miR-383. We demonstrate that PCLAF is essential for efficient DNA repair and that its loss recapitulates the DNA repair defects and damage accumulation driven by miR-383. Collectively our data establish the miR-383/PCLAF-regulatory circuit as a critical regulator of genomic stability, offering a novel molecular target to mitigate fibroblast senescence.

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Aging-Associated Mitochondrial Decline Accelerates Skin Aging and Obesity

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Age-related decline in epidermal function is considered a contributing factor to hair thinning and delayed wound healing. In contrast, epidermal keratinocytes possess high proliferative capacity and rapid turnover, suggesting that the impact of cellular senescence in these cells is limited. Therefore, this study focused on mitochondria as a potential cause of age-related epidermal dysfunction and aimed to investigate whether mitochondrial impairment in epidermal cells contributes to skin aging and systemic metabolism.
First, young wild-type mice (2 months old) and aged wild-type mice (24 months old) were compared to evaluate age-associated changes in the epidermis. Next, epidermis-specific TFAM (mitochondrial transcription factor A) knockout mice, designated *Tfam*^{EKO} mice, were generated to induce mitochondrial dysfunction in epidermal cells. Phenotypic analyses of aged wild-type mice and *Tfam*^{EKO} mice were then performed to determine the effects of epidermal mitochondrial dysfunction on aging.
Aged wild-type mice showed reduced dorsal hair follicle number, delayed hair regrowth, and impaired wound healing compared with young mice. In the epidermis of aged mice, mitochondrial DNA copy number was decreased, and mitochondrial respiratory capacity was reduced, whereas no significant differences were observed in the expression of senescence-associated genes. *Tfam*^{EKO} mice also exhibited reduced epidermal mitochondrial DNA copy number and impaired respiratory function, recapitulating the age-related decline in epidermal mitochondrial function. At 12–14 months of age, *Tfam*^{EKO} mice demonstrated delayed hair regrowth and impaired wound healing, resembling the skin aging phenotypes observed in aged wild-type mice. Furthermore, *Tfam*^{EKO} mice showed increased body weight and accumulation of subcutaneous fat on CT analysis.
These findings indicate that mitochondrial dysfunction in epidermal cells contributes to skin aging and may also promote age-associated obesity through disruption of systemic metabolic homeostasis.

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Melatonin Suppresses Dermal Fibroblast Senescence by Targeting the CXCL9 Axis and Preserving Mitochondrial Function

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Skin aging is driven by interactions between intrinsic and extrinsic factors, including oxidative stress and mitochondrial dysfunction, that promote fibroblast senescence and dermal extracellular matrix (ECM) degradation. Melatonin, an indoleamine neurohormone whose endogenous production declines with age, has potent antioxidant and anti-inflammatory activities. We investigated the anti-aging effects of melatonin in human dermal fibroblasts and aged mouse skin, focusing on the pro-inflammatory chemokine CXCL9, an emerging biomarker of inflammaging. Replicative-, irradiation-, and doxorubicin-induced senescence models of human dermal fibroblasts were used to assess melatonin's effects. Melatonin significantly reduced senescence-associated β-galactosidase–positive cells (38.5–41.6%) and suppressed senescence markers such as p16^INK4a, γH2AX, and the senescence-associated secretory phenotype (SASP) factors *IL1A* and *IL6*. Additionally, melatonin lowered intracellular ROS levels (50.8–87.7%) and restored mitochondrial membrane potential. CXCL9 expression was markedly upregulated in senescent fibroblasts, whereas melatonin treatment effectively inhibited CXCL9 expression. siRNA-mediated CXCL9 knockdown mimicked melatonin's anti-senescent effects, while the melatonin receptor antagonist luzindole abolished these actions, indicating MT1/MT2 receptor-dependent suppression of CXCL9 signaling. Furthermore, melatonin pretreatment protected young fibroblasts against irradiation-induced senescence, preserving mitochondrial integrity and attenuating inflammatory cytokine expression. In aged mice, intradermal melatonin administration enhanced dermal thickness (152–253%) and collagen density while reducing SASP factor expression in vivo. Collectively, these findings show that melatonin exerts potent anti-aging effects on dermal fibroblasts by mitigating ROS accumulation and inhibiting CXCL9-driven inflammatory pathways, highlighting its potential as a therapeutic strategy for skin rejuvenation.

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Decay of the *Lef1*-*Srsf3*-microRNA axis underlies stem cell aging

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Stem cell (SC) aging is implicated in tissue dysfunction and organismal aging. However, the molecular basis of aging shared across diverse adult SC types remains unclear. Here, we identify the transcription factor *Lef1*, the splicing regulator *Srsf3*, and a subset of DNA damage response microRNAs (DDR-miRs) as a common molecular axis underlying SC aging. *Lef1* was the most consistently downregulated transcription factor during aging, common to mesenchymal stem/stromal cells (MSCs) and hematopoietic stem/progenitor cells (HSCs), and this reduction was also observed in other tissue SC types in both mice and humans based on reanalysis of public RNA-seq datasets. In young MSCs, *Lef1* knockdown reproduced a miRNA expression profile similar to that of aged MSCs. Using *in vitro* reporter assays for mirtron biogenesis, a non-canonical miRNA biogenesis process, we identified age-associated cytoplasmic translocation and functional attenuation of Srsf3 as the causal molecular alteration responsible for impaired miRNA biogenesis under reduced Wnt signaling. Consistent with these *in vitro* findings, DDR capacity was reduced in aged SCs *in vivo*. Stem cell-specific *Lef1* deletion in either intestinal or epidermal stem cells in young mice induced premature DDR impairment and elicited inflammatory and senescence-like phenotypes in remote organs including the brain, skin, and kidney. These findings define the decay of the *Lef1*-*Srsf3*-miRNA axis as a core molecular alteration underlying SC aging and reveal that local SC aging may contribute to organismal aging.

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Pharmacological Targeting of the DPP4–GLP-1–NLRP3 Signaling Network Attenuates HDM-Driven Skin Inflammaging

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Chronic exposure of the skin to environmental allergens may accelerate inflammaging through persistent inflammation and cellular senescence. Here, we examined the role of the DPP4–GLP-1–NLRP3 inflammasome axis in house dust mite-induced skin inflammaging and assessed the protective activity of DCM5, a small-molecule sugar alcohol. HDM stimulation induced senescence-like changes in human dermal fibroblasts, including increased p21, p16, SA-β-gal activity, and SASP factors such as IL6, IL1β, and MMP1. HDM also reduced NQO1 and FLG expression, suggesting impaired antioxidant defense and barrier-associated responses. DCM5 post-treatment reversed these HDM-induced effects and restored NQO1 and FLG expression. Mechanistically, docking analysis and enzymatic assays demonstrated that DCM5 interacts with the S2 pocket of DPP4 and inhibits DPP4 activity. HDM reduced active GLP-1 levels and decreased GLP-1/GLP-1R expression, whereas DCM5 restored GLP-1 signaling. In addition, HDM activated the NLRP3 inflammasome, as shown by increased NLRP3, ASC, cleaved caspase-1, IL-1β, and IL-18, while DCM5 markedly suppressed this inflammatory cascade. Single-cell RNA-seq analysis of human skin further showed that aged fibroblast subsets display increased DPP4 and NLRP3 expression with reduced GLP-1/GLP-1R expression. These results identify the DPP4–GLP-1–NLRP3 inflammasome pathway as an age-associated and druggable regulatory axis in HDM-induced skin inflammaging. DCM5 may serve as a pharmacological candidate for mitigating allergen-driven inflammatory skin aging.

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N-acetylcysteine Exerts Senescence-selective Antifibrotic Effects in Fibroblasts from Chronic Wounds in Recessive Dystrophic Epidermolysis Bullosa

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Chronic non-healing wounds in recessive dystrophic epidermolysis bullosa (RDEB) are characterized by progressive fibrosis and impaired tissue repair. We hypothesized that senescent fibroblasts may accumulate within these wounds and drive a pro-fibrotic, pro-inflammatory microenvironment through their senescence-associated secretory phenotype (SASP), and that their selective elimination could restore a regenerative environment conducive to wound closure. Primary fibroblasts from healthy skin, non-lesional RDEB skin, and chronic RDEB wound dressings (RDEB-CW) were studied as a non-invasive, clinically relevant model of active wound biology. Transcriptomic profiling showed that RDEB-CW fibroblasts form a distinct cluster enriched in senescence and fibrosis pathways. Functionally, in RDEB-CW fibroblasts multiple senescence markers were elevated, including SA-beta-galactosidase activity, nuclear enlargement, and increased p21 expression. Treatment with N-acetylcysteine (NAC, 1 mM) reduced fibrotic activity, evidenced by decreased collagen gel contraction, reduced alpha-smooth muscle actin, and lower tenascin-C and periostin expression. Dose-response viability assays revealed that NAC-induced cell loss correlated with baseline senescence burden, consistent with senolytic rather than nonspecific cytotoxic activity. Their selective elimination preserved proliferative and migratory capacity in the remaining non-senescent population, supporting repopulation of a regeneration-competent environment. These findings propose NAC-mediated senolysis as a strategy to clear dysfunctional cells and restore wound healing potential in RDEB. Given its established safety profile, topical repurposing of NAC represents an accessible therapeutic avenue warranting direct evaluation in patients.

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Marein alleviates cutaneous photodamage through AHCY-dependent restoration of PINK1/Parkin-mediated mitophagy

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While ultraviolet A (UVA) radiation is a primary driver of extrinsic skin aging, the molecular targets that link metabolic enzymes to mitochondrial homeostatic regulation remain insufficiently defined. This study investigated the protective efficacy and underlying mechanisms of marein, a secondary metabolite from *Coreopsis tinctoria* Nutt., using HaCaT cells and a unique adenosylhomocysteine hydrolase (AHCY) overexpressed mouse model. UVA radiation was employed to establish photodamage models, and the effects of marein were evaluated through antioxidant enzyme assays and transmission electron microscopy. To identify the direct cellular target of marein, a label-free drug affinity responsive target stability (DARTS) strategy coupled with liquid chromatography-tandem mass spectrometry (LC–MS/MS) was utilized. The physical interaction was validated through cellular thermal shift assays, surface plasmon resonance, and molecular dynamics simulations. Furthermore, bisulfite sequencing and methylation-specific PCR were conducted to investigate the epigenetic regulation of the PINK1 promoter.
The results identified AHCY as the direct molecular target of marein. Mechanistically, UVA irradiation induced AHCY-mediated hypermethylation of the PINK1 promoter, which suppressed its expression and impaired mitophagic flux. Marein treatment effectively inhibited this hypermethylation, thereby restoring transcriptional expression of PINK1 and activating the PINK1/Parkin-mediated mitophagy pathway. This restoration preserved mitochondrial ultrastructure, as evidenced by recovered cristae and reduced vacuolization. In vivo, the use of AHCY-overexpressed mice demonstrated that the loss of marein efficacy in the presence of excess target protein, confirming the relevance of the AHCY-PINK1 axis. Marein significantly attenuated epidermal hyperplasia and collagen fragmentation in mice model compared to UVA photodamaged groups. These findings establish marein as a promising lead compound for targeted photoprotective therapies that maintain mitochondrial integrity by modulating the AHCY-mediated epigenetic landscape of the skin.

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Endothelial Ageing Drives Matrix Breakdown and Blood-Lymphatic Disorganisation in a Human Eyelid Skin Equivalent

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Periocular skin ageing is characterised by microvascular dysfunction and dermal matrix decline, but the specific contribution of endothelial ageing remains poorly modelled. We engineered a fully human, scaffold-free eyelid skin equivalent from primary eyelid-derived cells, preserving key anatomical features including a thin stratified epithelium, a dermal matrix, and combined CD31-positive blood and podoplanin-positive lymphatic networks.
To isolate endothelial ageing, two variants were reconstructed within the same aged stromal environment, incorporating either young or aged microvascular endothelial cells. Endothelial ageing alone induced tissue alterations: aged-endothelial constructs showed reduced epidermal organisation, impaired terminal differentiation, marked capillary dilation, disrupted VE-cadherin junctional continuity and decreased lymphatic network organisation. Dermal alterations included fragmented collagen architecture and reduced elastin distribution.
Treatment with escin, used as a vasoprotective reference compound, improved the aged-endothelial phenotype by restoring CD31-positive capillary morphology, reinforcing VE-cadherin junctional continuity and enhancing podoplanin-positive lymphatic organisation. Dermal matrix compactness and elastin homogeneity were also improved, supporting the responsiveness of the model to vascular-targeted intervention.
This human eyelid skin equivalent integrates blood and lymphatic microvascular networks and demonstrates that endothelial ageing is sufficient to drive dermal degradation and microvascular decline. It provides a mechanistic and translational platform to investigate periocular skin ageing and evaluate strategies aimed at preserving or restoring microvascular integrity.

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Skin Ageing-Related Loss of Basement Membrane Collagens Disrupts Nuclear Mechanics and Epidermal Homeostasis.

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Skin ageing is a universal problem that leads to the dysregulation of epidermal differentiation and increased mechanical stress. Basement membrane collagens (BMCs) decline with age, but their mechanistic consequences remain unclear. BMCs transmit mechanical forces via F-actin and the LINC complex to the nuclear lamina and chromatin. We hypothesised that the loss of BMCs impairs nuclear mechanotransduction and epidermal differentiation in keratinocytes.
To investigate whether the loss of BMCs disrupts nuclear integrity, we generated stable *COL7A1* and *COL17A1* shRNA knockdowns (shCol7, shCol17) and a non-targeting control (shC) in immortalised N/TERT-1 keratinocytes. Protein levels were assessed by Western blotting and immunofluorescence, with and without mechanical stretch to evaluate nuclear mechanics. Multi-omics profiling (RNA-seq, ATAC-seq, CUT&Tag-seq, and ChIC-seq) characterised gene expression, chromatin accessibility, histone marks, and lamina- and nucleolus-associated domains (LADs and NADs).
Type VII collagen loss reduced proliferation and SUN2 ($p<0.05$), elevated Lamin B1 puncta ($p<0.01$), and strengthened nuclear–nucleolar shape coupling ($R=0.25$, $p<2.2\times10^{-16}$). Type XVII collagen loss increased proliferation, nuclear area ($p<0.01$) and nucleolar size ($p<0.05$), and reduced SUN2 ($p<0.001$). Transcriptomic analysis revealed chromatin and cell cycle suppression in shCol7 cells and ribosomal gene upregulation in shCol17 cells. Epidermal Differentiation Complex (EDC)-related genes were dysregulated with either knockdown. shCol7 increased chromatin opening, and H4K16ac and LINE-1 transposable element levels; shCol17 increased H3K9me3. Transcription factor footprinting identified differential regulators of the EDC, including p63, GRHL, KLF, Sp, C/EBP and AP-1 family proteins. Chromosome 1 LAD–NAD uncoupling ($R < 0$) was observed in shCol7 cells.
To conclude, the loss of BMCs perturbs nuclear architecture, mechanotransduction and epigenomic regulation of epidermal differentiation. This work implicates epigenetic regulation as a potential therapeutic anti-ageing target in keratinocytes, with possible applications in epidermolysis bullosa and skin cancer.

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Interplay between the microbiome and skin aging

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With age, the skin microbiome undergoes significant shifts in diversity and species composition. Emerging evidence links these changes to hallmarks of skin aging, yet the link/impact between/of commensals bacteria and/on skin aging features such as barrier function alteration or pigmentation defect remains poorly understood.
The aim is to investigate the impact of *C. acnes, S. epidermidis* and *C. kroppenstedtii* on skin aging features by using young and aged human skin equivalents (HSEs) integrating those commensals.
HSEs containing keratinocytes, melanocytes, papillary (Pf) or reticular (Rf) fibroblasts, representing young or aged skin, were generated and inoculated with the combined three commensals. HSEs were assessed for epidermal morphogenesis, skin barrier structure and function, and pigmentation.
Pf-HSEs exhibited a fully-differentiated epidermis with an organized basal layer, and granular layers while Rf-HSE showed altered cellular proliferation and barrier markers expression. After microbial inoculation, Pf-HSE models did not reveal morphological or functional changes while Rf-HSE showed distinct modifications in barrier markers expression, such as Loricrin and Involucrin as well as increased number of melanocytes, elevated melanin and distinct expression of melanogenesis-related genes compared to non-inoculated models. *S. epidermidis* alone appeared to increase melanocyte number in, Rf-HSEs, though its exact role in melanocyte survival and skin pigmentation warrants further investigation.
These findings reveal a role for age-associated skin commensals in modulating barrier integrity and skin pigmentation, processes central to skin aging, and position HSEs as a platform to study microbiome-skin interactions.

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REDD1 Deficiency Converts Glucocorticoid-Induced Atrophy into Hyperplasia in Aged Skin

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Glucocorticoids (GCs) are the drugs of choice for treating inflammatory skin diseases in the elderly despite their well-known side effects, including skin atrophy. We previously identified mTOR inhibitor REDD1/DDIT4 as a causative factor in steroid-induced atrophy in young mice and showed that REDD1 knockout (KO) or pharmacological inhibition protected them against GC-induced skin thinning. Here we examined the role of REDD1 in GCs-induced skin atrophy in aged (12-month old) mice. Epidermal thickness, a marker of age-related skin atrophy, was similarly reduced in untreated aged REDD1 KO and isogenic WT mice. However, chronic treatment with fluocinolone acetonide (FA; 2 µg every 72 h for 2 weeks) produced opposing effects in skin. As expected, it caused epidermal hypoplasia and reduced basal keratinocyte proliferation in WT animals but surprisingly induced hyperplasia and increased basal keratinocyte proliferation in REDD1 KO mice. Transcriptomic analysis following acute FA treatment (2 µg, 24 h) revealed a comparable number of differentially expressed genes (DEGs) between genotypes, with similar induction of classical glucocorticoid receptor (GR) metabolic targets. Notably, REDD1 KO mice exhibited a shift toward transrepression, with 76% of DEGs downregulated and with greater magnitude of expression changes than in WT mice. Among these DEGs, ~20% were proliferation-related genes. While 296 DEGs were unique to REDD1 KO mice (including *p19INK4d*), 165 were shared between genotypes with many shared anti-proliferative genes (e.g., *Ptch2*) stronger repressed in REDD1 KO mice. Gene set enrichment analysis further identified enrichment of histone genes, including targets of Polycomb Repressive Complex 2 (PRC2), as down-regulated DEGs specifically in REDD1 KO epidermis. Overall, these findings suggest that loss of REDD1 mitigates glucocorticoid-induced skin atrophy in aged mice by shifting GR signaling toward repression of anti-proliferative pathways and modulating epigenetic regulators of epidermal homeostasis.

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Ageing impairs wound closure in a human 3D skin model dynamically monitored by line-field confocal optical coherence tomography

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Skin ageing delays wound repair, yet human-relevant systems capturing age-dependent defects in re-epithelialisation remain limited. We developed a bilayered scaffold-free human 3D wound healing skin model to compare repair kinetics in young and aged tissues and their responsiveness to a pro-healing treatment. After standardised wounding, young and aged tissues were untreated or exposed to a wound repair activator and monitored at D3, D5 and D7. Repair was assessed by MTT wound area measurement, histological analysis and line-field confocal optical coherence tomography (LC-OCT), enabling label-free, non-invasive visualisation of wound morphology, epidermal regeneration and tissue architecture. Untreated aged equivalents showed delayed wound area reduction and histological closure *versus* young tissues across D3-D7. LC-OCT provided dynamic, tissue-scale evidence of this defect, revealing persistent wound discontinuity, delayed epidermal resurfacing and slower restoration of wound-site architecture. This phenotype was associated with altered Ki67-positive activity at wound margins, delayed reconstruction of a CK10- and loricrin-positive differentiated neoepidermis, postponed restoration of dermal-epidermal junction markers including collagen VII, laminin-332 and collagen XVII, and altered provisional matrix remodelling involving fibronectin and hyaluronic acid. The pro-healing treatment accelerated closure and improved remodelling in both phenotypes, confirming model responsiveness. However, aged tissues responded less efficiently, with incomplete rescue of closure kinetics and wound-site architecture, mirroring aged skin *in vivo*. LC-OCT captured these phenotype-dependent treatment responses without sample processing. This age-stratified human model recapitulates key features of impaired wound healing in aged skin, while remaining responsive to pro-regenerative stimulation. It provides a translational platform to investigate age-impaired wound healing and evaluate repair-enhancing strategies.

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Distinct spatial transcriptomic signatures of photoageing in light and dark skin

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Photoageing differs clinically between light and dark skin, with darker skin showing relative resistance to ultraviolet radiation (UVR)-induced damage. We hypothesised that increased pigmentation confers protection by attenuating UVR-driven transcriptional and cellular responses. To test this, we profiled spatial gene expression in photoexposed and photoprotected skin from individuals with light and dark skin across age groups.
Paired skin biopsies from photoexposed forearm and photoprotected buttock were collected from younger (22–29 years) and older (>69 years) adults (n=3 per group per skin type). Sections were analysed using the Xenium spatial transcriptomics platform with a 475-gene panel. Differential expression (|log2FC| ≥1, adjusted q<0.1) and cell annotation were performed in R using Seurat.
Whole-tissue analysis comparing photoexposed and photoprotected sites identified upregulation of extracellular matrix remodelling and immune-related genes, including *MMP9, CD3E, LYZ, IDO1, IL4I1,* and *CCL19,* in photoexposed light skin from older individuals, whereas these changes were not observed in dark skin. Consistent with this, photoexposed light skin exhibited increased immune cell proportions in both age groups, a response absent in dark skin.
At the fibroblast level, *LEPR* was downregulated in photoexposed skin from both age groups with light skin and in older individuals with dark skin, but not in younger individuals with dark skin, suggesting age- and pigmentation-dependent sensitivity to UVR.
In contrast, fibroblast subpopulation analysis revealed an age-associated increase in reticular-like fibroblast signatures in both photoexposed and photoprotected skin from older individuals with light skin, a shift not observed in dark skin, indicating differential ageing trajectories independent of UVR exposure.
Together, these findings demonstrate enhanced immune activation and stromal remodelling in photoaged light skin, with comparatively attenuated responses in dark skin. This supports a protective role for pigmentation against UVR-induced damage and highlights the importance of pigmentation-informed approaches in skin ageing research.

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Using an immune-responsive human skin equivalent ageing model to dissect cutaneous immune-ageing

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Skin ageing is associated with visible phenotypical changes such as wrinkling. On a cellular level, the number of senescent cells accumulates with age, which can drive skin ageing phenotype. During ageing, there are also profound changes in cutaneous immunity which render older adults more susceptible to skin cancers and infections.
We have previously shown that skin from older adults (≥65yrs) has impaired antigen-recall response against pathogens. This age-associated impairment is due to recruitment of proinflammatory monocytes by activated senescent fibroblasts, these monocytes blunt antigen-specific immunity by prostaglandin E2 secretion. However, the mechanisms of senescent fibroblast activation are currently unknown. Due to ethical limitations of performing repeated biopsies on humans, a novel ageing skin model needs to be developed.
The aim of this project is to build immune responsive ‘young’ and ‘old’ *in vitro* 3D human skin equivalent (HSE) model to identify the cellular source of this age-associated skin inflammation.
We have built ‘young’ (100% proliferating fibroblasts) and ‘old’ (10% senescent; 90% proliferating) HSEs. Furthermore, we established immune-responsive HSEs with the addition of primary human monocytes into the dermal layer.
The “old” HSEs exhibited epidermal thinning and ECM disorganisation. Additionally, senescent fibroblast within the dermis is associated with epidermal development impairment defined by epidermal growth marker expressions. The monocytes within the HSEs differentiated into dermal macrophages as defined by CD68 expression.
We have developed HSEs that recapitulate human skin ageing. Research is ongoing to determine the influence of the old skin environment on mononuclear phagocyte differentiation. This project will aid our understanding of age-related cutaneous immune decline, and ultimately, the identification of druggable pathways to help improve skin health in older adults.

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How ageing reshapes cutaneous autophagy and skin health

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Epidermal and dermal homeostasis are essential for maintaining skin structure and function. Ageing progressively disrupts this balance through intrinsic and extrinsic factors, promoting a chronic low-grade inflammation (inflammageing) and functional decline. Autophagy, a lysosome-mediated degradation pathway responsible for turnover of damaged proteins and organelles, is a key regulator of cellular homeostasis in the skin. However, how intrinsic ageing alters autophagic activity across distinct skin compartments remains unclear. Here, we investigate age-associated changes in autophagy-lysosome pathways in human skin.
Quantitative expression of autophagy related proteins (ARPs) across distinct skin layers, ARP expression, and spatial distribution were assessed by multiplex immunofluorescent imagery of formalin fixed, paraffin embedded human skin sections (n=24) from young and aged donors. Comparisons across compartment and aged were complemented by western blot densitometry of epidermal and dermal lysates (n=12), and human dermal fibroblasts (n=13).
Expression of lysosomal-associated membrane protein (LAMP) 1 and 2 showed a similar age related decline, most prominently within the basal epidermis. Western blot analysis from epidermal and dermal compartments corroborated these findings, demonstrating age-associated declines in LAMP1 and LAMP2. Dermal lysates from older donors also showed reduced LAMP1 expression. Notably, decreases in lysosomal markers were accompanied by reduced levels of degradation markers - adaptor proteins p62 and NDP52.
Collectively, our findings reveal a coordinated, age related decline in autophagy-lysosome pathway components with age across human skin. The parallel reduction in lysosomal and cargo adaptors proteins challenges the prevailing model of autophagic impairment driven by defective clearance, instead suggests a broader contraction in overall autophagic capacity and initiation. These findings provide potential new insight into the role of autophagy in skin ageing and support targeting autophagy-lysosome function as a potential strategy for restoring cutaneous homeostasis in ageing.

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A BrdU-induced hair follicle aging model demonstrates protective effects of rapamycin

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Hair aging involves prolonged catagen and telogen phases that drive hair follicle (HF) miniaturization, reduced density, and thinner fibers. Influenced by genetic and environmental factors, this process is linked to DNA damage and cellular senescence. Our BrdU-induced HF senescence ex vivo model captures aspects of these mechanisms and allows the testing of interventions. To benchmark the model, we examined whether thymidine could counteract BrdU effects by competing for DNA incorporation, and whether rapamycin, an mTORC1 inhibitor, could suppress BrdU-induced senescence in HFs *ex vivo*.
Using human HF organ culture, microdissected temporal/occipital follicles from healthy donors were co-treated with 250 µM BrdU plus either thymidine (250 or 500 µM) or rapamycin (5 or 21.9 µM) every other day for 5-7 days. HFs were assessed macroscopically, by quantitative (immuno-)histomorphometry and LegendPlex.
Consistent with earlier findings, BrdU treatment induced a clear senescence-associated phenotype in human HFs, including increased $p21^+$ cell numbers, elevated SASP factor release, premature catagen entry, and reduced hair matrix keratinocyte proliferation and hair shaft production. Co-treatment with either thymidine or rapamycin mitigated several of these effects, notably reducing the release of IL-6, MCP-1, and IL-8, as well as CXCL10 expression in the HF epithelium. Thymidine additionally maintained hair matrix proliferation, decreased apoptosis, and reduced $p21^+$ cell accumulation, while rapamycin dose-dependently improved hair shaft production and limited the increase in $p21^+$ cells.
Overall, these data demonstrate that BrdU-induced HF senescence is pharmacologically modifiable, reinforcing the value of this ex vivo model as a screening platform for compounds designed to preserve hair follicle function and support hair longevity.

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Skin Commensal *Moraxella osloensis* 4-210 Drives Skin Aging by Regulating Keratinocyte Homeostasis and Lipid Peroxidation

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Skin aging is accompanied by marked cutaneous microbiome remodeling, yet mechanistic evidence linking defined microbes to aging remains scarce. In our previous cohort analyses, *Moraxella osloensis* was positively associated with age and aging-related skin features. Herein, we isolated *M. osloensis* from the facial skin of individuals across different age groups, characterized microbial phenotypes, and investigated microbe–host interactions using an *in vitro* keratinocyte model. Our results demonstrated that *M. osloensis* was preferentially isolated from prematurely aged middle-aged individuals and elderly donor, whereas it was barely detectable in young healthy skin. All isolates alkalinized the culture environment, suggesting a shared capacity to modify local pH. Strain 4-210 from premature-aged skin exhibited the strongest biofilm formation and unique floc-like aggregation, reflecting potent colonization and adhesive properties. In HaCaT keratinocytes, 4-210 supernatant disrupted extracellular matrix and collagen programs, while lipid-conditioned 4-210 supernatant uniquely showed malondialdehyde-reactive signals and further activated oxidative stress and cellular senescence pathways. Functionally, 4-210 supernatant suppressed keratinocyte proliferation and migration, independently of the aryl hydrocarbon receptor. Collectively, strain 4-210 possesses strong colonization capacity and secretes lipid-regulated soluble factors to dysregulate keratinocyte matrix homeostasis. This study highlights the critical role of specific skin commensals in driving skin aging, and provides a valuable model strain for mechanistic aging research and the development of targeted skincare strategies.

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Targeting Dermal Fibroblast Senescence in Skin Aging via Lipid Nanoparticle-Mediated mRNA Delivery

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Cellular senescence is a key driver of skin aging, contributing to progressive dermal tissue dysfunction through extracellular matrix (ECM) degradation and impaired fibroblast activity. In the skin, these alterations compromise structural integrity and reduce the capacity for tissue maintenance and repair. This complex process involves multiple molecular pathways and transcriptional regulators, including HES1, whose modulation may influence senescence-associated phenotypes and matrix remodeling in dermal fibroblasts. However, RNA-based approaches remain limited by poor stability, low cellular uptake, and rapid degradation of naked nucleic acids. To overcome these challenges, lipid nanoparticles (LNPs) have emerged as effective carriers to improve RNA stability and delivery efficiency in target cells relevant to skin biology.
In this study, we explored the impact of HES1 overexpression using RNA-loaded LNPs in senescent human dermal fibroblasts, a key cellular model of skin aging. HES1 expression was first validated in HEK293T and U2OS cells via plasmid vectors. Subsequently, HES1-encoding RNA was encapsulated into LNPs and delivered to senescent fibroblasts. Transcriptomic analysis revealed modulation of key pathways involved in ECM organization, including genes associated with collagen synthesis, matrix remodeling, and fibroblast function. Functionally, HES1 overexpression was associated with partial restoration of ECM-related gene expression and attenuation of senescence-associated markers in dermal fibroblasts.
These findings demonstrate that LNP-mediated mRNA delivery enables targeted modulation of transcriptional programs linked to ECM homeostasis in skin-relevant fibroblast senescence, providing insight into mechanisms underlying dermal aging and matrix remodeling in the skin.

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Histone deacetylase inhibition impairs epidermal homeostasis and barrier function in human skin models

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The regulation of gene expression involves numerous mechanisms, including the deposition of epigenetic marks that can adjust the chromatin compaction level. Among these marks, histone modifications play a crucial role in skin development and function. These include histone tail deacetylation which is mediated by the activity of specialized enzymes named histone deacetylases (HDACs). The role of HDACs in maintaining epidermal homeostasis remains poorly understood. To clarify this point, we investigated how increasing histone acetylation through HDAC inhibition affects keratinocyte function and overall epidermal homeostasis. To do so, we used a specific HDAC inhibitor, LMK235, on N/TERT1 and primary keratinocytes in monolayer and 3D cultures. As expected, LMK235 increased histone H3 acetylation in 2D-cultured keratinocytes, but also strongly impacted both cell proliferation and differentiation. The generation of reconstructed human epidermis (RHE) models treated with LMK235, using either N/TERT1 cells or primary keratinocytes, confirmed these results. RHE models treated with LMK235 revealed a thinner epidermis, an abnormal organization of the stratum corneum and an impaired barrier function, as well as the expression of typical aging-associated epidermal markers. Lipidomic analysis revealed an altered representation of the epidermal lipids in LMK treated skin models, which may explain the compromised barrier function. Finally, using RNA-seq analysis, we confirmed a strong deregulation of the epidermal transcriptome after HDAC inhibition. Altogether, we confirm that HDAC plays a key role in keratinocyte gene regulation and in epidermal function. We show that repression of HDAC impairs epidermal barrier function, in particular because of its harmful effect on lipid metabolism. This work paves the way for the development of innovative models of skin aging based on the modulation of histone acetylation.

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Wavelength-Dependent Skin Responses to Visible Light and Light-Harnessing Strategies

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Contemporary photoprotection targets ultraviolet (UV) radiation, leaving visible light—approximately 40% of solar irradiance—largely unaddressed. Visible light penetrates skin more deeply than UV and may contribute to cutaneous ageing, including barrier deterioration, pigmentation, and extracellular matrix remodelling; however, wavelength-specific effects remain incompletely understood. This study examined the biological impact of four visible light wavelengths—blue, green, amber, and red—on keratinocyte and melanocyte cultures, reconstructed three-dimensional skin equivalents, and ex vivo skin explants. Markers of barrier integrity, melanogenesis, and dermal matrix homeostasis were assessed to construct a wavelength-resolved map of cutaneous responses. Blue and amber light consistently induced barrier impairment and elevated melanin synthesis, while green light exerted minimal effects. Conversely, red light reinforced epidermal barrier integrity, demonstrating that visible light exerts divergent, wavelength-dependent effects on the skin. These findings informed a dual strategy: deploying photoreactive materials that selectively shield skin from harmful wavelengths while harnessing beneficial ones. Chlorophyllin sodium copper salt, formulated to absorb and attenuate blue light, and C-phycocyanin, which absorbs red light to activate pro-collagen and antioxidant signalling pathways, were each applied prior to irradiation. C-phycocyanin drove a 1.68-fold increase in collagen synthesis and a 4.47-fold suppression of matrix metalloproteinase activity, while chlorophyllin sodium copper salt conferred a 1.61-fold improvement in epidermal barrier function. This study maps wavelength-specific skin responses to visible light and validates photoreactive materials tailored to distinct spectral ranges, supporting a transition from uniform light blockade to wavelength-selective utilisation for skin health.

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Elucidation of Factors Associated with Easily Improved Senile Lentigines

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Senile lentigines (SLs) are an age-related skin concern resulting from accumulated ultraviolet damage that impairs facial aesthetics. Although numerous studies have been conducted and a wide variety of active ingredients have been developed to improve SLs, their efficacy varies. For example, even when the same active ingredient is used, some SLs show rapid improvement, whereas others exhibit little improvement. Such variability is often attributed to individual differences in responsiveness or differences in underlying causes; however, the possibility that SLs themselves intrinsically differ in their ease of improvement has not been fully explored. In this study, we investigated gene expression differences between easily improved and poorly improved SLs using a minimally invasive microbiopsy technique that enables gene expression analysis of SL sites prior to intervention and longitudinal tracking of the same sites over time. Using this approach, we analyzed characteristic genes associated with differences in improvement outcomes following treatment with two active agents, hydroquinone and 4-n-butylresorcinol. SLs showing early improvement consistently exhibited significantly higher expression of the *RPTN* gene, irrespective of the active ingredient used. To explore the mechanism underlying the contribution of *RPTN* to SL improvement, gene expression changes were examined in keratinocytes overexpressing *RPTN*. This revealed increased expression of genes related to inflammation suppression, antioxidant activity, and epidermal barrier function, including *ATF3*, *PON3*, *EPHB6*, and *FLG*. In addition, screening of plant-derived extracts identified Paeonia and Codonopsis species extracts as enhancers of *RPTN* expression, accompanied by upregulation of these associated genes. This study demonstrates that, beyond individual differences in responsiveness to active ingredients, intrinsic skin characteristics of SLs themselves regulate their ease of improvement. Modulation of *RPTN* expression in SL sites may therefore provide a promising strategy for achieving more consistent efficacy without reliance on trial-and-error ingredient selection.

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Biomimetic hydrogels restore fibroblast quiescence and enhance the predictive value of in vitro models

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Conventional plastic substrates commonly used for cell culture are up to a million times stiffer than human skin, resulting in a major mechanical mismatch that limits their physiological relevance and reduces the predictive value of the cellular responses they elicit. To address this limitation, we rely on biomimetic hydrogels engineered to match the stiffness of human skin (~5 kPa), thereby improving the physiological relevance of the biomechanical environment. Normal human dermal fibroblasts cultured on plastic substrates exhibited a proliferative phenotype (54.7% of Ki67-positive cells), consistent with elevated metabolic activity. By comparison, cells grown on hydrogels displayed absent or minimal Ki67 expression and a 1.67-fold decrease in metabolic activity (Alamar Blue), while maintaining an unchanged mtDNA/nDNA ratio. Cells maintained on hydrogels did not express αSMA, whereas re-seeding onto plastic substrates induced its expression, albeit at levels 1.37-fold lower than in cells continuously cultured on plastic. Consistently, phalloidin staining revealed a poorly developed actin cytoskeleton in hydrogel-cultured cells, whereas re-seeding onto plastic substrates restored a dense and contractile actin fiber network, comparable to that observed in cells continuously cultured on plastic. Taken together, these results indicate that conventional plastic substrates promote baseline biomechanical activation of fibroblasts, thereby limiting their responsiveness to additional stress or perturbation. By contrast, biomimetic hydrogels maintain dermal fibroblasts in a quiescent, skin-like state, providing a model that more closely recapitulates native physiology and enhances predictive power for mechanistic investigations and efficacy assessments. This platform is therefore relevant for elucidating the contribution of mechanobiological cues to the regulation of skin aging and cellular senescence, and for evaluating strategies aimed at preventing or reversing age-associated dermal alterations.

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Modular deconstruction of DNA methylation clocks identifies drivers of human skin aging

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Aging and skin aging are major risk factors for the development of skin cancer. DNA methylation clocks are widely used tools to estimate biological age. However, their underlying cellular mechanisms are not well understood, although they could offer promising approaches for rejuvenation. Here, we demonstrate that modularization of epigenetic clocks for different human skin layers allows the identification of age-tracking substructures. We used a multi-step clustering approach to assign CpGs of epigenetic clocks to co-varying and comparable modules. Characterization of these modules revealed distinct features on the level of genomic localization and chromatin modification. Notably, only three out of 12 modules tracked the majority of age-related changes. Additional analysis of these modules confirmed their association with various known and novel pathways relevant for skin aging. Finally, we validated our computational predictions by knocking down certain module genes, which resulted in a pronounced deceleration of epigenetic age in human dermal fibroblasts. Our findings thus contribute to our knowledge of skin aging and facilitate the identification of novel targets for its rejuvenation.

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A Human Hair-bearing Organoid Model for Premature Skin Ageing

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Human skin undergoes morphological, biochemical, and functional alterations during ageing. However, current *in vitro* ageing models lack sufficient cellular and tissue complexity to capture these changes at the tissue level. Stem cell-derived skin organoids, despite being more complex and closely resembling in vivo skin, remain developmentally immature and embryonic

in nature. Here, we aim to establish an aged, hair-bearing human skin organoid model by expressing progerin, a mutant form of lamin A, that causes the premature ageing syndrome Hutchinson-Gilford progeria (HGPS), combined with repeated ultraviolet (UV) irradiation. Induced pluripotent stem cells expressing progerin in a doxycycline-inducible manner were generated and differentiated into skin organoids under optimised doxycycline and UV treatment conditions. Exposure to UV irradiation in conjunction with progerin expression induced key hallmarks of cellular senescence and age–associated skin histology, including increased melanin deposition within the epidermis and decreased dermal collagen content beneath the epidermal-dermal junction. Furthermore, single-cell transcriptome profiling identified senescence-enriched cell clusters and altered intercellular communication networks within the tissue. These changes were associated with increased expression of ageing-associated factors including GDF15 and IGFBP2. The concomitant alterations across epidermal and dermal compartments suggest coordinated tissue-level changes associated with skin ageing. Under optimised experimental conditions, these histological phenotypes were partially reversed by treatment with selected agents targeting pathways implicated in skin ageing, supporting the functional responsiveness of the model. Collectively, our human skin organoid model captures key histological and cellular aspects of skin ageing in vitro and provides a robust platform for mechanistic analysis and evaluation of candidate anti-ageing interventions.

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MicroRNA-focused CRISPR-Cas9 screen identifies key regulators of keratinocyte aging

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Cellular senescence is a major barrier to effective skin wound healing, as it compromises keratinocyte regenerative capacity and contributes to delayed tissue repair. MicroRNAs (miRNAs) are versatile regulators of gene expression that modulate complex regulatory networks and diverse cellular activities through coordinated post-transcriptional control of multiple targets. Here, we aim to explore the role of miRNA in regulating keratinocyte senescence and human skin wound healing.
We firstly established the replicative senescence model of keratinocyte through serial passaging and observed that late-passage keratinocyte displayed pronounced senescence-associated phenotype, including increased proportion of SA-β-gal-positive cells, elevated p16 and p21 expression, alongside with the reduced levels of cell proliferation, migration, and colony formation capacity. Using the CRISPR-Cas9-based miRNA screen, we identified miR-X as a potential regulator for keratinocyte senescence.
Functional studies demonstrated that miR-X knockdown and inhibitor treatment enhanced keratinocyte proliferation and migration, whereas miR-X mimic treatment produced the opposite effects, indicating miR-X acts as a negative regulator of keratinocyte regenerative capacity. Transcriptomic analysis further suggested that miR-X modulates signalling pathways associated with cellular metabolism, proliferation, and senescence-related programs, supporting the role of miR-X in regulating keratinocyte regeneration.
Together, this study identifies miR-X as a key regulator of keratinocyte senescence and highlights its potential role as a therapeutic target for improving human skin wound healing.

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Spatial Transcriptomic Profiling Reveals Extracellular Matrix Remodelling Differences Between Intrinsically Aged Light and Dark Skin

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Intrinsic skin ageing is characterised by progressive structural and functional decline driven by time-dependent biological processes. However, differences in transcriptional profiles between skin of varying pigmentation remain poorly understood. We used spatial transcriptomics to compare gene expression in intrinsically aged skin from light and dark pigmented individuals. Photoprotected buttock biopsies were obtained from adults aged >69 years with dark (n=3) and light (n=3) skin, classified by individual typology angle. Participants with dark skin were of African American ancestry, while those with light skin were of Northern European ancestry. Cryosections were analysed using the 10x Genomics Xenium In Situ platform with a custom 475-gene panel. Differentially expressed genes (DEGs) were identified using a thresholds of >1 log-2 fold-change and FDR < 0.1. Comparison of intrinsically aged light and dark skin identified 23 candidate DEGs, with 19 upregulated and 4 downregulated in light skin. Upregulated genes were enriched in extracellular matrix (ECM) remodelling pathways, including *FMOD*, *LUM*, *MFAP5*, and *TFPI2*, alongside the stem cell-associated marker *PROM1*. Downregulated genes included cell cycle-associated genes (*CCNB1*, *UBE2C*) and structural/adhesion-related genes (*COL23A1*, *PCDH7*). Spatial mapping localised ECM-related gene expression predominantly to fibroblasts, whereas downregulated cell cycle genes primarily associated with T cells. Intrinsically aged light skin demonstrates a more transcriptionally active profile than dark skin, particularly in ECM remodelling pathways. These findings identify candidate mechanisms that may contribute to known structural differences between skin types and highlight the importance of considering pigmentation in studies of intrinsic ageing.

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Heat Shock Protein 47-Mediated Restoration of Extracellular Matrix Homeostasis Attenuates UVB-Induced Proteotoxic Stress and Senescence in Human Dermal Fibroblasts

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UVB-driven dermal photoaging is typically defined by oxidative stress and extracellular matrix (ECM) degradation, yet whether proteostasis failure actively drives ECM disorganization and premature senescence remains unresolved. Heat shock protein 47(HSP47) is a collagen-specific molecular chaperone that supports procollagen folding and maturation; however, whether this collagen proteostasis machinery can be leveraged to counteract UVB-induced proteotoxic stress and matrix dysfunction has not been established. We therefore investigated the ability of HSP47 to restore ECM homeostasis and suppress senescence in UVB-exposed HS68 human dermal fibroblasts. UVB-irradiated cells (25 mJ/cm^2) were treated with HSP47, followed by assessment of viability, intracellular ROS, senescence, protein aggregation, collagen processing and secretion, and ECM-related gene expression. UVB induced a broad proteotoxic photoaging phenotype characterized by reduced viability, marked

ROS accumulation, extensive intracellular protein aggregation, collagen disorganization, and widespread senescence. HSP47 significantly reduced intracellular ROS levels ($p<0.01$) and improved cell viability ($p<0.001$). Importantly, HSP47 reduced intracellular protein aggregation, decreased misfolded collagen III, improved ER-associated collagen processing, and increased soluble collagen secretion. At the transcriptional level, HSP47 increased collagen type I alpha 1 chain(COL1A1), collagen type III alpha 1 chain(COL3A1), elastin(ELN), and fibrillin 1(FBN1) expression while suppressing matrix metallo peptidase-1(MMP-1). These coordinated effects were accompanied by a marked reduction in SA-β-gal-positive senescent cells. Together, these findings identify proteostasis collapse as a key pathological axis of UVB-induced dermal aging and highlight HSP47 as a compelling chaperone-based strategy to preserve ECM integrity and suppress premature senescence.

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Melatonin rescues epigenetic dysregulation associated with human skin aging

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Chronological aging is driven by progressive epigenetic dysregulation which impairs cellular homeostasis. DNA methylation patterns are preserved during cell division by DNMT1 (methylation maintenance), while DNMT3a/3b establish new methylation patterns (*de novo* methylation). These marks can be enzymatically removed by TET3 protein. Here, we asked if accelerated skin aging involves disruption of DNA methylation dynamics and if this is modulated by the potent, but safe anti-aging neurohormone, melatonin (MT). Full-thickness scalp skin samples (6 healthy donors, 43–75 yrs) were organ-cultured in our serum-free “speed-aging” assay and treated topically with MT (200μM) or vehicle. Quantitative immunohistomorphometry showed reduced DNMT1 and DNMT3a expression, indicating impaired methylation maintenance and *de novo* methylation. UHRF1, which supports DNMT1 function, was upregulated in control skin, while DNMT3b remained unchanged, however TET3 levels, global levels of 5-methylcytosine (5-mC), and 5-hydroxymethylcytosine (5-hmC) decreased. This suggests disrupted methylation balance driven by passive hypomethylation and reduced active demethylation. These epigenetic changes were associated with significantly reduced protein expression of PGC1α and TFAM (mitochondrial impairment), collagen 17a1 (stem cell niche degeneration), gp100 (melanocyte dysfunction), and g-H2Ax (DNA repair imbalance), and with significantly increased p16ink4 (cellular senescence), suggesting that these genes may be epigenetically regulated. Importantly, MT treatment partially restored epigenetic balance by increasing DNMT1, TET3, and global 5-mC levels, suggesting improved maintenance methylation and demethylation capacity, while DNMT3a/3b remained unchanged. Thus, human skin aging is linked to a disrupted DNA methylation machinery and suggests that topical MT can partially restore epigenetic stability.

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Beyond Erythema: Molecular Prevention of UV-Induced Photoageing in 3D Human Skin Models

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Sunscreen efficacy is conventionally determined using minimal erythemal dose (MED)-based endpoints that quantify visible redness. However, visible photoageing reflects cumulative molecular damage including extracellular matrix degradation, DNA damage responses, cellular senescence and inflammatory activation. Evaluating photoprotection using biologically relevant ageing biomarkers may provide a more meaningful assessment of long-term skin ageing prevention. We therefore aimed to determine whether photoprotection prevents UV-induced activation of key transcriptomic drivers of skin photoageing in a human 3D skin model.
Phenion® 3D full-thickness human skin equivalents were irradiated with 5 Standard Erythemal Doses (SED) of solar-simulated UV (≈50–75 minutes Mediterranean midday sun) with or without topical application (2 mg/cm2) of a daily photoprotective formulation combining encapsulated SPF filters, liposomally delivered DNA repair enzymes and antioxidants. RNA was isolated 24 hours post-irradiation and gene expression quantified by RT-qPCR. Biomarkers included MMP1 (collagenase induction), CDKN1A/p21 and CDKN2A/p16 (senescence), PTGES (prostaglandin E2 synthesis) and GLB1 (β-galactosidase).
UV exposure significantly induced MMP1 (13.7-fold), p21 (2.3-fold) and PTGES (3.9-fold), confirming activation of extracellular matrix degradation, early senescence and inflammatory pathways. Topical photoprotection abrogated UV-induced MMP1, p21 and PTGES expression to levels not significantly different from non-irradiated controls ($p<0.01$–0.001). GLB1 showed a modest UV-induced increase that was directionally reduced but did not reach statistical significance. As anticipated within a 24-hour window, p16 expression was unchanged.
Photoprotection prevented activation of key molecular pathways underpinning skin photoageing beyond erythema, including collagenase induction, early senescence and prostaglandin-mediated inflammation. Mechanism-based evaluation using transcriptomic biomarkers offers a biologically relevant framework for assessing photoprotective efficacy in the context of skin ageing prevention.

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Investigating the Mechanisms Underlying Mitochondrial Dysfunction in Skin Ageing

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This study aims to investigate Laminin 332 as a target of age-related mitochondrial dysfunction leading to altered epidermal homeostasis.
To investigate mitochondrial dysfunction associated with age-related Laminin332 reductions, laminin α3, β3, and γ2 chain knockdowns (shLAMA3, B3 and C2) were generated in keratinocytes. Live and fixed immunofluorescence was used to assess mitochondrial morphology and function, while long term culturing of shLAMA3 cells on human recombinant Laminin 332 ascertained whether these morphological and functional changes were reversible. Generation of 3D skin organotypic models with shLAMA3 keratinocytes will further support changes at the tissue level.
Laminin332 deficient keratinocytes exhibit significantly increased mitochondrial size, perinuclear mitochondrial clustering, and

distinct dysfunction of mitochondrial clearing pathways. Mitochondrial dynamics regulating proteins, including Drp1, Opa1 and MFN2, were significantly altered in shLAMA3 cells. Calcium-induced differentiation as well as CCCP-induced mitophagy of shLAMA3 highlighted the impairment of mitochondrial clearing as assessed by the maintained mitochondrial perinuclear clustering. Culturing of shLAMA3 on human recombinant Laminin 332 for 3 days restored mitochondrial morphology and distribution.
Our findings indicate that Laminin 332 loss profoundly impacts mitochondrial morphology, organisation and function. Laminin 332 restoration in shLAMA3 keratinocytes reverses altered mitochondrial morphology, distribution. Collectively, these data suggest laminin 332 structural or downstream signalling functions impacts mitochondrial network balance and may be a target to restore mitochondrial function in epidermal homeostasis.

297 [WITHDRAWN]

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Supporting hair follicle recovery in stress-related *telogen effluvium* through autophagy induction and adenosine receptor activation
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Telogen effluvium is a frequent stress-related and reversible hair loss marked by premature transition of follicles from anagen to telogen. Psychological and environmental stresses disrupt follicle homeostasis by altering dermal papilla activity, growth factor secretion and scalp balance.
Hair follicle dermal papilla cells (HFDPC) regulate hair cycling through paracrine secretion of keratinocyte growth factor (KGF) and vascular endothelial growth factor (VEGF), essential for anagen induction. Excess sebum and scalp dysregulation may further impair follicular function under stress. Autophagy has emerged as a key mechanism in follicle integrity, hair cycle control and oil management.
Pharmacological stimulants such as minoxidil are commonly used as a treatment for *Telogen effluvium*. However, they may induce an intolerance, thus supporting the need for dermocosmetic alternatives. SiAd, a complex combining a silicon-derivative (extracted from rice husks) and adenosine, was evaluated for its ability to restore follicular homeostasis through autophagy induction and adenosine receptor activation.
In vitro, SiAd reduced lipid production in sebocytes under pro-seborrheic conditions. In isolated human hair follicles, SiAd stimulated hair shaft elongation comparable to minoxidil. SiAd induced autophagy and stimulated both VEGF and KGF secretion in HFDPCs, while also upregulating K15 expression in outer root sheath cells. Two independent clinical studies in women with stress-related *telogen effluvium* and oily scalp showed improved hair density and thickness, reduced shedding and normalized sebum levels without side effects. These results indicate that targeting autophagy and adenosine signaling is a relevant dermocosmetic strategy to support hair follicle recovery in *telogen effluvium* by acting on dermal papilla function and scalp environment.

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Deciphering the impact of cortisol-mediated stress on epidermal homeostasis: A multi-scale *in vitro* platform for mechanistic decoding and evaluation of protective strategies.
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Chronic psychological stress is a significant factor in skin barrier dysfunction and accelerated ageing. While clinical manifestations such as increased transepidermal water loss, surface roughness and delayed wound healing are well-documented, the specific molecular and cellular mechanisms remain to be fully elucidated. This study established a mechanistic framework using a multi-scale *in vitro* platform to decipher cortisol-driven epidermal defects. We established a controlled hormonally standardized environment using human epidermal keratinocytes and 3D reconstructed human skin models. Chronic stress was induced by persistent exposure to hydrocortisone to mimic systemic cortisol elevation. Evaluations included transcriptomic profiling, colony-forming efficiency (CFE) of highly proliferative progenitor cells, and histological analysis. Transcriptomic results identified a specific biological signature characterized by a downregulation of genes essential for barrier integrity (filaggrin, loricrin), while local cortisol amplification (11β-HSD2) and neurosignalling markers (NTF4) were upregulated. At the cellular level, chronic exposure induced stem cell exhaustion, resulting in a significant reduction in colony number and total area, alongside the formation of smaller colonies. In 3D models, stress provoked a significant decrease in epidermal thickness and reduced filaggrin expression. Crucially, treatment with the glucocorticoid receptor antagonist mifepristone demonstrated that these alterations are reversible physiological disturbances rather than permanent defects. Furthermore, evaluation of targeted active ingredients such as ectoin or *Tephrosia purpurea* extract showed a restoration of stress-impacted gene expression and a significant recovery of tissue thickness. These findings provide a biological basis for the "fatigue look" and barrier failure reported in stressed skin. This methodology offers a robust tool for identifying interventions aimed at restoring skin resilience and preserving longevity in the face of modern lifestyle-induced stress.

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New Insights into the Role of Brain-Derived Neurotrophic Factor in Skin Cell Senescence
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Brain-Derived Neurotrophic Factor (BDNF) is a critical protein in maintaining brain health, with well-established roles in mitigating neurodegenerative diseases. Despite extensive research into its neuroprotective effects, the involvement of BDNF in skin aging process remains largely unexplored. In this study, we investigated the impact of aged skin cells on BDNF-TrkB signaling, shedding light on its novel role in skin cell senescence.
By employing advanced AI-driven screening technology, we identified a species of Mimosa flower as the primary source of the active agent (MFE). Human primary fibroblasts (NHDFs) and keratinocytes (NHEKs) were obtained from a mature donor. NHDFs at low-passage (young NHDFs) and at high-passage (aged NHDFs) were used. After a 24-hour incubation in the

absence or presence of MFE, BDNF and elastin protein levels were measured in NHDF lysates. Following the incubation of low-passage NHEKs in the absence or presence of NHDF media, TrkB and SASP factor levels were measured in NHEK lysates.
A significant decrease in elastin and an increase in BDNF levels was observed in high-passage NHDFs compared to low-passage NHDFs. Treatment with MFE effectively attenuated this increase in high-passage cells. No significant difference in TrkB levels was detected between NHEKs cultured without NHDF medium and those cultured with low-passage NHDF medium. A significant upregulation of TrkB and SASP was observed in NHEKs exposed to high-passage NHDF medium, a trend that was markedly diminished upon MFE treatment of high-passage NHDFs. A similar pattern was observed for senescence markers such as beta-galactosidase, CXCL10, Ki67 and collagen XVII in explants.
The BDNF-TrkB autocrine signaling loop has been shown to enhance the viability of senescent cells, thereby promoting their accumulation. In this study, we demonstrate for the first time that the paracrine signaling network of BDNF-TrkB derived from aged fibroblasts may influence keratinocyte senescence, contributing to epidermal aging. This novel finding suggests that the BDNF-TrkB axis plays a key role in skin senescence and may offer new perspectives for developing strategies to prevent or mitigate skin aging.

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Rejuvenating pre-senescent 'mid-old' fibroblasts rather than senolysis
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'Mid-old' cells, a pre-senescent intermediate cellular state, have recently emerged as promising anti-aging targets because they are more prevalent in tissues and retain reversibility, unlike fully senescent cells. A single-cell transcriptomic study identified a distinct subset of dermal fibroblasts exhibiting a mid-old state in human skin. These fibroblasts were characterized by upregulation of pro-inflammatory genes, including IL1B and SAA1, and downregulation of anti-inflammatory genes, including SLIT2 and CXCL12. Analysis of human skin from young, middle-aged, and elderly individuals revealed progressive expansion of this population with chronological aging, with a marked increase in middle-aged skin. Dermal-targeted therapy using energy-based devices effectively shifted the mid-old fibroblast population toward a more youthful fibroblast profile. Taken together, these findings identify mid-old fibroblasts as a reversible therapeutic target for skin rejuvenation.

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Longitudinal Multi-omics Reveals Host–Microbiome–Metabolome Remodeling During Topical Anti-ageing Intervention
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The skin microbiome contributes to skin health and ageing, yet longitudinal evidence on how skin microbes and host metabolism co-remodel during human skin ageing remains limited. We conducted a 60-day prospective study in 100 healthy Chinese women, including 50 receiving a topical active ingredient and 50 placebo controls. Skin phenotypes, metagenomes and pseudotargeted metabolomes were profiled at baseline, Day 30 and Day 60.
The intervention induced biphasic improvement in ageing-related phenotypes. Early treatment increased stratum corneum hydration, elasticity and firmness and reduced fine lines, whereas later treatment sustained these benefits and further reduced fine-line area. Late-phase metagenomics showed reduced microbial alpha diversity and inter-individual heterogeneity, with significant community remodeling. *Deinococcus cavernae*, *Staphylococcus hominis* and *Micrococcus luteus* were enriched, while *Acinetobacter johnsonii* decreased.
Pseudotargeted metabolomics revealed marked temporal remodeling. Early intervention upregulated flavonoids, amino acids and derivatives, glycerophospholipids, fatty acyls and organic acids, while suppressing sphingolipids and 4-tert-octylphenol. Later intervention maintained amino acid-related increases and decreased glycerolipids, suggesting remodeling of the skin surface metabolic microenvironment and barrier-related lipid metabolism. Integrated multi-omics analyses further underscored the tightly interconnected regulation of host-lipid metabolism, antioxidant defense and microbial nutrient substrate supply during topical perturbation.
These findings reveal temporal host-microbiome-metabolome remodeling associated with improved skin ageing phenotypes and identify candidate biomarkers for personalized skincare interventions.

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Impact of senescent keratinocytes on skin cancer progression
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Several studies highlighted that senescent cells are involved in the development of age-related diseases including cancer. To further investigate the link between the senescent microenvironment and cancer progression in a skin context, our study focuses on the impact of senescent keratinocytes on several hallmarks of skin cancer cells. Human epidermal keratinocytes were exposed to UVB to induce their senescence. Then, their conditioned medium (CM) was collected 3 or 7 days after UVB exposure. Melanoma (A375) or squamous cell carcinoma (A431) cells were then incubated with the CM to evaluate its potential effect on their proliferation and migration.
We demonstrated that CM from senescent keratinocytes had a pro-migratory effect on both A431 and A375 cells. In order to identify the senescence-associated secreted proteins involved, we undertook an analysis of the secretome composition by mass spectrometry. As expected, we found that the secretome composition is modified after UVB exposure but also over time. We identified several candidates that could potentially take part in the pro-migratory effect of CM, including calpains. We are

currently validating their putative role by depleting these proteins from the keratinocyte CM.
In parallel, to study the interactions between senescent keratinocytes and cancer cells in more complex models, we are developing 3D models of reconstructed epidermis including cancer cells and senescent keratinocytes. We showed that according to the cancer cell line, the distribution of the cancer cells with the reconstructed epidermis was different.
In conclusion, the results evidenced that UVB-induced senescent keratinocytes favor the migration of squamous cell carcinoma and melanoma cells by secreting proteins that are currently being identified.

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Proteomic insights into the dermatological potential of rooibos and its phytosome formulation to enhance skin barrier integrity

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Rooibos (*Aspalathus linearis*) is rich in polyphenols with antioxidant and anti-inflammatory properties relevant to skin health. However, clinical translation of plant polyphenols is often limited by poor bioavailability, low cellular uptake, and reduced stability. Phytosome technology, which complexes polyphenols with phospholipids, has emerged as an effective strategy to improve bioavailability and biological efficacy in epithelial systems. This study investigated proteomic effects of unfermented and fermented rooibos extracts in human keratinocytes and integrated these findings with the development and biological evaluation of a rooibos-phytosome to enhance polyphenol bioavailability and epithelial barrier protection under inflammatory conditions. HaCaT keratinocytes were treated with unfermented and fermented rooibos extracts followed by label-free quantitative proteomic analysis using LC–MS/MS to identify differentially abundant proteins. Pathway enrichment and validation were performed to confirm key molecular targets. Based on these findings, a rooibos-phytosome complex was formulated and tested in epithelial cells exposed to lipopolysaccharide (LPS)-induced inflammation to evaluate its effects on cellular bioavailability and barrier integrity. Proteomic analysis revealed significant modulation of proteins involved in extracellular matrix organisation, hemidesmosome assembly, and oxidative stress defence. The rooibos-phytosome demonstrated improved cellular bioavailability and enhanced epithelial barrier integrity under inflammatory conditions compared to dexamethasone, supporting its functional advantage as a delivery system. This study provides novel proteomic evidence that rooibos modulates key pathways involved in skin barrier integrity and stress defence. Furthermore, phytosome encapsulation enhances polyphenol bioavailability and improves epithelial barrier protection during inflammation. The findings support potential application of rooibos-phytosome formulations in the development of therapeutic and cosmeceutical products targeting inflammatory and barrier-related skin disorders.

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Minoxidil positively impacts aging-associated pathways in human skin and murine intestine *in vivo*

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We recently demonstrated anti-aging effects of minoxidil (MXL) in male androgenetic alopecia scalp hair follicles. As low-dose systemic MXL is increasingly used for hair loss, including in women, we dissected its impact on aging-associated pathways in aged human skin and internal organs in vivo. Forehead and scalp skin from older women (n=8, 71 ± 3.1 years) was xenografted onto aged female SCID mice to eliminate host-induced skin rejuvenation (Keren et al. Sci Adv 2022). In some mice (n=18), only xenotransplants were treated for 8 weeks with topical MXL 5%, while others received oral MXL (0.025 mg/mouse, daily gavage; n=18). Xenografts and selected internal organs were compared to topical/systemic vehicle controls by quantitative immunohistomorphometry (qIHM) and RNA-seq.
qIHM showed that topical and systemic MXL improved aging-associated biomarkers in aged human female scalp and facial skin xenografts: MXL reduced p16INK4a and p-S6, indicating reduced mTORC1 activity, and increased lamin B1, PGC1α, NRF2, fibrillin-1, and VEGF-A, a driver of human skin rejuvenation. At the tested dose, only murine intestine — but not brain, heart, skeletal muscle, or eyes — showed improved aging-associated biomarkers after systemic MXL. RNA-seq revealed coordinated suppression of senescence, inflammatory/SASP, and oxidative stress programs in aged human skin. In contrast, murine intestine did not show similar suppression of aging-associated programs, but selectively activated circadian, PPAR/metabolic, immune, and tissue-remodelling programs. These results reveal that aging is highly context-dependent: human skin adopts a suppressive, damage-limiting program, whereas murine intestine engages active metabolic and remodelling pathways. This divergence cautions against direct cross-species extrapolation.
Thus, MXL improves multiple aging-associated markers in aged human female skin, while its selective effects in aged murine intestine suggest tissue-specific benefits beyond hair growth.

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Skin Aging Across Fitzpatrick Phototypes I–VI: Distinct Clinical Signatures and the Impact of Lifestyle

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Skin aging affects all humans, but the onset, severity and pattern of visible signs differ across skin phototypes and populations. Data from 7,593 women aged between 20 and 70 years, representing phototypes I to VI across multiple populations, were analyzed. High resolution frontal and profile facial photographs were acquired under rigorously standardized conditions. Skin aging features were assessed using a set of validated photographic grading scales by trained dermatologists. Lifestyle data were collected through structured questionnaires. Composite scores were developed to summarize wrinkles, sagging, lentigines, and overall skin aging. Associations with chronological age were modeled using polynomial regression, and multivariate analyses were used to assess the influence of lifestyle factors.
Marked differences in skin aging patterns were observed across phototypes and geographic regions. Women with phototypes I to IIIa exhibited the highest overall severity of skin aging, predominantly driven by wrinkles. Phototypes IIIb to IV showed a higher propensity for aging spots, particularly in Asian populations. Despite presenting fewer wrinkles and lentigines, women with dark phototypes (V and VI) displayed sagging severity comparable to other phototypes. Geographical location played a significant role, with increased wrinkle severity observed across all phototypes in regions of high UV exposure. Several lifestyle

factors, including sun protection practices, cosmetic habits, and diet, were significantly associated with skin aging severity. All phototypes exhibit the same major skin aging features, but their frequency and severity vary substantially between populations. Skin color and sun exposure emerged as key drivers of these differences. Understanding population specific aging profiles may help identify targeted risk factors and support the development of more personalized skin aging prevention and care strategies.

307 [WITHDRAWN]

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Spatial transcriptomics reveals spatially organised basal cell heterogeneity in the human interfollicular epidermis

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The interfollicular epidermis is maintained by transcriptionally and spatially heterogeneous basal stem cells; however, their organisation in healthy human skin remains incompletely characterised. This study aimed to design and apply a targeted spatial transcriptomic panel to define interfollicular epidermal keratinocyte states in situ.
A custom 10x Genomics Xenium panel was created by combining the 260-gene human skin base panel with 100 additional genes selected from published single-cell datasets, prioritising markers of basal stem cells, lineage identity, and differentiation. Photoprotected buttock skin biopsies from nine young adult donors (aged 18 to 28) were profiled. Cells were segmented, normalised, and clustered in R using Seurat. Interfollicular epidermal keratinocytes were subsequently extracted and re-clustered, with cell states assigned using established undifferentiated and differentiated marker signatures. Spatial localisation was assessed using cell-boundary polygons.
A total of 36,761 interfollicular epidermal keratinocytes were analysed. The panel resolved a differentiation continuum encompassing basal, transitional, spinous, and granular states, as well as a proliferative population. Within the undifferentiated keratinocyte cluster, three transcriptionally distinct subpopulations were identified. One co-expressed POSTN, KRT15, DLL1, LFNG, and JAG2 and was enriched at the base of the rete ridges. A second, defined by APSN, ASS1, CSPG4, and DLL1, localised predominantly to inter-ridge regions. The third population expressed NOTCH1, HES1, CCND1, and MYC and was positioned superficial to the basal sub-populations.
These findings demonstrate that targeted spatial transcriptomics can resolve previously uncharacterised heterogeneity within the human interfollicular epidermal stem cell compartment and reveal its spatial organisation in situ.

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Revisiting Photoaging: Melatonin Controls Mitochondrial Dysfunction–Associated Senescence

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Cellular senescence is an irreversible growth arrest that occurs as a result of different damaging intrinsic and extrinsic stimuli, including DNA damage, telomere shortening and dysfunction or oncogenic stress. Human skin, the largest organ of the body, provides a physical barrier against harmful microbes, toxins, and protects from ultraviolet radiation (UVR). Increasing evidence suggests that senescent cells accumulate in chronologically aged and photoaged skin; and may contribute to age-related skin changes and pathologies. Skin health is considered one of the principal factors representing overall “well-being” in humans. Thus, there is an imperative to consider melatonin’s regulatory activity on cellular senescence of the skin.
Melatonin, an evolutionarily ancient derivative of serotonin with hormonal properties, is the main neuroendocrine secretory product of the pineal gland. It regulates circadian rhythmicity and exerts anti-oxidative, anti-inflammatory, immunomodulatory, and anti-tumor capacities.
Herein, in vitro studies using human epidermal keratinocytes exposed to UVB (50 mJ/cm^2), we noticed that melatonin attenuates altered cell survival ratio, and affects expression of senescence markers (p53, p16, γH2AX, IL-6). Moreover, melatonin ameliorates UVB-induced oxidative stress and depolarization of mitochondrial transmembrane potential (mtΔΨ) indicating the mitochondrial dysfunction-associated senescence (MiDAS). In vivo studies have been performed using C57BL/6 mice treated subcutaneously biennially with melatonin (10 mg/kg) and changes in skin aging has been substantially ameliorated followed by melatonin-treated mice compared to control ones. These data enclose changes within skin aging and the impact of the melatoninergic anti-oxidative system controlled by melatonin, targeting the prevention or reversal of skin senescence.

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Isatis tinctoria extract – enriched in sinapic acid – as a novel active to counteract neuro-cutaneous decline for skin neuro-longevity

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Aging is associated with a decline in neuro-cutaneous communication, including reduced neurite density, impaired mechano-transduction, and disrupted neuron–keratinocyte crosstalk. This leads to diminished skin trophic support and well-being sensory signaling, a phenomenon referred to as *neuro-aging*. While conventional skincare targets mainly structural changes, our researchs overlook neuro-epidermal dysfunction. In this study, we used precision indoor farming and elicitation in *Isatis tinctoria* L. to boost the production of sinapic acid, known to be antioxidant and neuroprotective. A neuro-aging *in vitro* co-culture model combining human sensory neurons and keratinocytes from young and aged donors were used to measure neurite outgrowth, mechanosensitive ion channels expression (PIEZO1/2, TRPα1, TRPV4, ASIC2/3, TRAAK, TREK1/2), and glutamate release. Skin explants were then used to assess collagen properties. A 2% formulation was tested in a randomized, double-blind, placebo-controlled clinical study to assess neurocutaneous responses, wrinkles, skin resilience, and hydration. Aged neurocutaneous co-culture showed reduced neurite outgrowth (−23%), deficits of all mechanosensitive channel expressions,

and diminished glutamate release. Treatment with *Isatis tinctoria* extract reversed these deficits, increasing neurite length (+32%) and restoring mechanosensitive ion channel expressions. Glutamate release was concomitantly restored (+23.8%). *Ex vivo*, it restored collagen fiber diameter (−13.4%) and orientation (−8.6%). Clinically, it enhanced EEG responses, reduced wrinkles count and volume (−16.2%/−8.6%), improved resilience, and increased hydration (+8%). Taken together, these findings advance our understanding of skin aging mechanisms and support the use of a sinapic acid-enriched *Isatis tinctoria* extract as an effective strategy in preserving skin innervation and mechanosensory signaling to counteract age decline and drive neuro-longevity–based innovation.

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Targeting Early Photoageing: Histological and Spatial Transcriptomic Responses to a Novel Cosmetic Formulation

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Adults aged 35–40 typically show minimal visible photoageing, although early structural alterations such as reduced elasticity, thinning of the epidermis and degradation of fibrillin-rich microfibrils at the dermal–epidermal junction (DEJ) are already evident. This study evaluated a novel cosmetic formulation containing peptides and antioxidants using the Manchester Patch-Test to assess early repair responses. Five healthy volunteers (4 female, 1 male) with mild photoageing underwent a 12-day occluded patch-test on extensor forearm skin with vehicle, vehicle plus actives, full product and untreated control. Post-treatment, 3 mm skin biopsies were analysed histologically for epidermal thickness, DEJ convolution and fibrillin organisation. Spatial transcriptomic analysis (Xenium) was additionally performed. All treatments were well tolerated with minimal irritancy reported. Epidermal thickness and DEJ convolution were not significantly altered by any treatment. In contrast, fibrillin deposition was significantly increased following treatment with vehicle plus actives (2.9 ± 0.3; $p = 0.03$) and full product (3.3 ± 0.4; $p = 0.01$) compared with control (1.4 ± 0.3), with no significant effect of vehicle alone (1.7 ± 0.3). Xenium analysis demonstrated reduced expression of SOD3, MMP2 and MMP19 in both active-containing formulations relative to control, suggesting modulation of oxidative stress and matrix remodelling pathways. These findings indicate that targeted cosmetic intervention can stimulate early repair processes in mildly photoaged skin, particularly through enhancement of fibrillin-rich microfibrils, representing a potential strategy to support dermal integrity prior to the development of more advanced clinical features.

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Age-related Alterations in Subcutaneous Support Structures of the Lower Eyelid and Putative Regulation by the Muscle-derived Secretory Factor Irisin

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The lower eyelid is particularly susceptible to age-related morphological changes, including skin laxity and sagging. The retinacula cutis (RC)—connective tissue structures that anchor the skin to the underlying subcutaneous tissue and contribute to mechanical support—exhibit reduced density and thinning of fiber bundles with age in the facial cheek skin. However, quantitative analyses focusing on the lower eyelid are limited.
Owing to its proximity to the orbicularis oculi muscle and constant exposure to mechanical stress, preservation of the subcutaneous supporting architecture of the lower eyelid is crucial. Therefore, we hypothesize that muscle-derived factors regulate RC integrity. Among such factors, irisin, a myokine, has been implicated in structural homeostasis. In this study, we investigated age-related alterations in the RC of the lower eyelid.
Histological analyses were performed on lower eyelid tissue sections obtained from women in their 20s to 90s using Elastica van Gieson staining. RC density, fiber bundle thickness, and fiber orientation were assessed. In addition, tendon cells were cultured under irisin-supplemented conditions, and collagen type I and microfibril-associated protein 4 (MFAP4) expression was evaluated.
Both RC density and fiber bundle thickness decreased with age. Moreover, in older subjects, RC fiber bundle orientation shifted toward the direction of gravity, suggesting that age-related weakening of the RC increases susceptibility to gravitational forces and contributes to structural instability within the lower eyelid. Irisin supplementation significantly increased both collagen type I and MFAP4 expression in cultured tendon cells.
Altogether, these findings suggest that age-associated deterioration of the RC underlies degeneration of subcutaneous support structures in the lower eyelid and that muscle-derived secretory factors, such as irisin, may regulate this process. This study provides a scientific basis for developing preventive and therapeutic strategies to preserve subcutaneous supporting structures and mitigate age-related cosmetic changes, including lower eyelid sagging.

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Dual Role of Sorbic Derivatives in Melanin Photoprotection: Probing and Quenching Triplet-State Photoreactivity

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The purpose of this study was to define the role of melanin triplet excited states in aerobic photochemistry and to determine whether sorbic acid derivatives can act simultaneously as triplet-state probes and photoprotective quenchers.
Synthetic eumelanin and pheomelanin were photoexcited under aerobic conditions in the presence of selected sorbic derivatives used as diene quenchers of melanin triplet states. Singlet oxygen generation was assessed as a readout of triplet-state energy transfer to molecular oxygen, whereas extended measurements of photodegradation and photoreactivity were performed in a synthetic eumelanin model.
Both synthetic melanins generated singlet oxygen upon photoexcitation, supporting energy transfer from melanin triplet excited states to dioxygen. Sorbic derivatives efficiently quenched the triplet states responsible for singlet oxygen sensitization and revealed at least two functionally distinct triplet-state pools differing in apparent energy, accessibility, and contribution to photochemical reactivity. In the eumelanin model, the same derivatives reduced pigment photodegradation, indicating a dual role as mechanistic probes of triplet-state populations and protective quenchers of triplet-mediated photoreactivity. The results also shown a correlation between photobleachning, triplet state pools and incresed photosensitization of singlet oxygen in eumelanin model.
These results support a heterogeneous model of melanin photochemistry in which distinct triplet-state populations drive

different aerobic outcomes. Sorbic derivatives therefore provide a practical approach for mapping melanin triplet-state photoreactivity and may help suppress oxidative photodegradation of eumelanin by intercepting reactive triplet states before singlet oxygen formation and pigment bleaching occur.

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From molecular biomarkers to clinical outcomes after microneedling: a patented multi-target bioactive complex promoting skin longevity

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1-LGVTY, a patented complex of baicalin, chlorogenic acid, sodium carboxymethyl beta-glucan and oligosaccharides, was assessed for translation of its molecular anti-aging activity through ex-vivo experimental model into clinical benefit when delivered as a 7.5% serum in post-procedure care associated with collagen precursor peptides.
Ex vivo, human skin explants were pre-treated with 1-LGVTY for 3 days, then co-exposed to urban particulate matter (PM10, 0.375 µg/cm2) and ultraviolet-A (6 J/cm2); biomarkers of oxidative damage, inflammation, pigmentation and dermal matrix were quantified by epifluorescence microscopy. A 28-day randomized split-face clinical study (n=31 women, 30-65 years) evaluated the 7.5% serum applied immediately pre- and post-microneedling on one randomized hemiface, contralateral as untreated control. Erythema (VISIA), transepidermal water loss, firmness, elasticity (Cutometer), crow's feet severity (Bazin scale) and hydration were assessed at baseline, post-procedure, 5h post-procedure and Day 28.
Ex vivo, 1-LGVTY reduced stress-induced protein carbonylation, normalized interleukin-1α and interleukin-6, decreased melanin by 80% and fully suppressed tyrosinase upregulation ($p<0.01$ vs stressed control). Clinically, post-procedure barrier disruption was attenuated by 40% on the treated side versus control ($p=0.001$) and inter-side erythema reduced by 22.5% at 5h ($p=0.020$). At Day 28, firmness improved by 26% ($p<0.0001$), crow's feet depth decreased by 16% ($p=0.001$), skin tone homogeneity improved by 78% ($p=0.001$) and hydration increased by 10% ($p=0.002$). Tolerance was excellent.
This bench-to-bedside translation shows that a multi-target bioactive complex modulating key hallmarks of skin aging like chronic inflammation delivers clinical benefit, supporting adjunctive use after aesthetic procedures.

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Reinforcing Skin Barrier Function through pH and Ca^{2+} Modulation: Clinical Evidence from a Novel Topical Formulation

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Stratum Corneum (SC) barrier integrity is determined by its protein architecture — predominantly keratins, comprising 80–90% of its dry mass. The cornified envelope and corneodesmosomes form a regulated network, influenced biologically and physicochemically by multivalent ions and pH. The cornified envelope is stabilized via Ca^{2+}-dependent transglutaminase crosslinking; corneodesmosomal cohesion relies on Ca^{2+}-mediated adhesion and pH-dependent proteolytic regulation. Beyond their biological roles, pH and Ca^{2+} modulate protein charge and conformation. Keratin studies in hair showed that Ca^{2+} promotes electrostatic repulsion, while rising pH (beyond the hair isoelectric point ~3.7) drives negative charge accumulation, enhancing water uptake and swelling. Despite the complexity of the SC, such effects may extend to its proteins, where optimal pH and Ca^{2+} could mitigate these changes and strengthen barrier function.
On this basis, this study examined pH and Ca^{2+} modulation of SC as a strategy to reinforce skin barrier function. Two oil-in-water formulations were tested: a vehicle containing linoleic acid and glycerin, and a verum based on this vehicle containing Ca^{2+} hypophosphite and adjusted to pH 4.5. In a randomized, placebo-controlled trial in participants with very dry, atopic-prone skin, penetration of two model molecules was measured at baseline and after two weeks using confocal Raman spectroscopy. Both formulations improved hydration comparably, but the verum produced significantly greater barrier reinforcement, with reduced penetration versus both baseline and vehicle. In a further study, the verum improved core symptoms — dryness, scaling, roughness and pruritus — based on subjective and dermatologist assessments, with excellent tolerability.
Collectively, these data indicate that the new formulation enhances epidermal barrier function beyond standard lipid and moisture replenishment and provides clinical symptom relief in very dry, atopic-prone skin. As SC proteins are active barrier determinants, targeting their organization through pH and Ca^{2+} may represent a promising and underexplored strategy in topical dermatological formulation.

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The Skin Effects of Feeding and Fasting at the Single Cell Level

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As a homeostatic organ, the skin regulates itself in response to a variety of stimuli, including food intake. Caloric restriction has been shown to modify hair growth and skin aging whereas fasting decreases inflammation and stimulates wound healing. Long term studies have shown how intermittent feeding can induce collagen production whereas extended fasting can alleviate psoriasis and atopic dermatitis symptoms. Although caloric restriction and fasting have demonstrated beneficial effects in skin, the core transcriptomic drivers for these differences are not well described. Using single cell transcriptomics, we describe crucial skin regulatory networks that highlight differences in ketone body metabolism, autophagy, and immune response upon feeding. Furthermore, we highlight the impact of specific genes in each compartment which govern autophagy and keratinocyte stem cell regulation, thereby identifying potential targets for skin disease and regenerative medicine.

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The melatonin metabolite, AFMK, inhibits human eyelid skin aging *ex vivo* independently of melanin production

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We previously showed that Melatonin (MT), a safe indoleamine neurohormone, reduces epidermal aging and stimulates melanogenesis in human eyelid skin *ex vivo*. Since MT's stimulatory effect on melanin production limits its use for facial skin rejuvenation, we asked if MT's major metabolite, N^1-acetyl-N^2-formyl-5-methoxykynuramine (AFMK), exerts similar anti-aging effects without stimulating melanogenesis (currently unknown). Full-thickness human eyelid skin of aged female and male donors (49-74 y-o) was organ-cultured for 6 days with AFMK (50-200μM, added to the medium), and skin aging biomarkers (incl. collagen I/III, SIRT1, LaminB1), pigmentation, proliferation, and apoptosis were analyzed by quantitative immunohistomorphometry (qIHM). 200μM AFMK significantly increased both proliferation (Ki-67) and the number of Lamin B1+ cells in eyelid epidermis. Moreover, 50μM and 100μM AFMK significantly increased intraepidermal protein expression of VEGF-A, the recognized key driver of human skin rejuvenation. 100μM AFMK also reduced dermal aging *ex vivo*, as indicated by a lower collagen I/III ratio, due to increased collagen III protein production. Notably, the mitochondrial marker MTCOI remained unaltered, which differed from what the observed increased expression with MT treatment. Interestingly, in contrast to MT, 200μM AFMK significantly reduced epidermal melanogenesis, as indicated by decreased histochemical melanin content and tyrosinase activity *in situ*, and reduced the total number of gp100+ melanocytes. The latter was not due to increased melanocyte aging, as the % of LaminB1+/MITF+ cells remained unchanged; neither did AMFK promote intraepidermal apoptosis (Caspase 3). Collectively, our study shows that AFMK exerts potent anti-aging effects in human eyelid epidermis and dermis without enhancing the risk of stimulating (cosmetically undesired) periorbital hyperpigmentation.

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Melatonin inhibits melanocyte senescence in human epidermis *ex vivo*, in part via VEGFA stimulation

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The role of senescent epidermal melanocytes (MCs), which secrete inflammatory mediators and proteases that promote the senescence of adjacent cells, in human epidermal aging remains insufficiently understood. Since we recently showed that the safe indoleamine neurohormone, melatonin (MT), retards human skin aging and, unexpectedly, stimulates epidermal melanogenesis ex vivo, we asked in the current study if MT inhibits MC aging/senescence in aged human skin.
100 or 200 μM MT were administered for 6 days to the medium of organ-cultured full-thickness human eyelid skin of aged female donors (47-72y-o), mimicking "systemic" MT application. Double-immunostains for gp100 as an MC marker, with key aging/senescence biomarkers, were performed and assessed by quantitative immunohistomorphometry (qIHM). qIHM showed that both MT concentrations significantly reduced the % of gp100+ MCs double-positive for $p16^{INK4}$, CXCR3, or IL-6. Instead, MT increased the % of gp100+ MCs co-expressing perinuclear Lamin B1, SIRT1, and VEGF-A, the key driver of human skin rejuvenation. The % of MCs expressing p21, p-S6 (=mTORC1 activity marker), and IP-10/CXCL10 remained unchanged compared to vehicle-treated control skin. Interestingly, adding VEGF-A-neutralizing antibody to the medium significantly antagonized the effect of MT on the % of MCs expressing SIRT-1, $p16^{INK4a}$, IL-6, or VEGF-A. Thus, these anti-aging and anti-senescence effects of MT were VEGF-A-dependent (as expected, the other markers remained unchanged).
Therefore, "systemic" high-dose MT can reduce human epidermal MC senescence/aging in aged human eyelid skin, at least *ex vivo,* in part by directly stimulating VEGF-A production in MCs, thereby recruiting the key growth factor that drives human skin rejuvenation. This may slow overall human skin aging driven by senescent intraepidermal MCs and identifies the latter as a novel target for MT therapy.

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Non-Invasive LED Therapy Drives Epidermal Renewal and Functional Improvement in Photoaged Skin

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Photoageing, driven by cumulative sunlight exposure and intrinsic ageing, is characterised by epidermal thinning, dyspigmentation, and progressive loss of structural integrity. Light-emitting diode (LED) therapy using red and near-infrared (NIR) wavelengths represents a promising non-invasive approach for improving visible features of photoaged skin; however, its effects on photoaged human skin at the histological level remain incompletely characterised. This *in vivo* human study evaluated functional and histological effects of a commercially available LED face mask in women with moderate photoageing. Healthy female participants aged 35–55 years were recruited (n = 20). Participants used an LED face mask and a matched forearm device incorporating Veritace®-tested LEDs emitting 633 nm (red), 830 nm (NIR) and 1072 nm (deep NIR) wavelengths simultaneously, for 10 minutes, five times per week, over 12 weeks. Full-thickness forearm skin biopsies were obtained at baseline and week 12, and skin biomechanics were assessed using cutometry. Cryosections were stained with haematoxylin and eosin to determine epidermal thickness, and Ki67 immunostaining was used to assess keratinocyte proliferation.
Mean epidermal thickness significantly increased from 40.1 ± 3.9 μm at baseline to 43.7 ± 6.3 μm at week 12 (paired t-test, $p = 0.031$). This was accompanied by significant changes in cutometry parameters, including a reduction in R9 ($p = 0.044$), indicating increased resistance to mechanical deformation. Consistent with these findings, the number of Ki67-positive basal keratinocytes significantly increased from 2.41 ± 0.57 to 3.25 ± 0.92 per 100 μm ($p = 0.006$), suggesting enhanced epidermal proliferative activity.
Overall, 12 weeks of combined red/NIR LED therapy induced measurable improvements in epidermal structure and mechanical function in photoaged skin. These findings support the potential of LED-based interventions to enhance epidermal renewal and

contribute to improved skin resilience. Future analyses will further characterise additional epidermal changes and interrogate potential remodelling within the dermis, including effects on structural protein organisation.

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Investigating the combined effects of solar light and particulate material on dermal fibroblast viability

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Environmental factors such as sunlight and pollution exacerbate skin aging. Whilst solar radiation is well-studied, most photoaging research focuses on ultraviolet (UV) light alone and fails to consider other solar components e.g. visible (VIS) and infrared (IR) light. Moreover, the combined effects of sunlight and pollution on skin aging remain uncharacterised. Since skin is often exposed to these stressors simultaneously, this study aimed to investigate their collective toxicity in human neonatal dermal fibroblasts (HDFn).
A tetrazolium-based assay was used to quantify live and metabolically active cells post-stressor exposure. To assess whether VIS and IR potentiate UV-induced cell death, HDFn were exposed to full solar light or filtered UV at UV-equivalent doses (0, 1.08, 2.16, 4.32 and 8.64 SED). Likewise, to determine whether particulate material (PM) enhances solar-induced cell death, HDFn were exposed to full solar light (2.16 or 4.32 SED) with PM at 0, 5, 25 or 50µg/mL.
Full solar light induces dose-dependent cell death at 24 hours post-irradiation (up to 27% with 8.64 SED; $p<0.01$). UV alone results in less cell death compared to full solar light counterparts at all doses, with minor increases in metabolic activity at lower doses (0.54 and 1.08 SED). This demonstrates that UV alone causes low level damage relative to full solar light, highlighting the synergistic toxicity of UV, VIS and IR. Co-exposure with PM further reduces viability in irradiated cells, with a dose-dependent effect related to both irradiation and PM. At 50µg/mL PM, a 3- to 7-fold increase in cell death was observed compared to irradiation alone ($p<0.0001$) suggesting that PM enhances solar-induced damage.
By mimicking real-world exposure to solar light and pollution, this study provides valuable insight into how they may interact in skin. Ultimately, elucidating their individual and combined damage mechanisms further advances our understanding of their role in skin aging.

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Anti-aging protein expression is induced by TGFβ2 in dermal sheath fibroblasts isolated from normal human scalp hair follicles

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Terminal-haired skin heals better than non-haired or vellus-haired skin, and it is thought that hair follicles (HF)-associated dermal sheath (DS) fibroblasts exhibit enhanced regenerative potential. Wound healing capacity declines with age and while TGFβ2 is a recognized wound healing growth factor, it is unclear how TGFβ2 influences collagen levels and subtype in skin aging.
DS fibroblasts were isolated from scalp HF of healthy adult donors. Proteomic data from DS fibroblasts treated with TGFβ2 [5 ng/ml @24h] or [10 ng/ml @30mins] versus untreated DS fibroblasts were obtained with LC/MS.
TGFβ2 stimulation (24h) resulted in a statistically-significant upregulation of the following: upregulated - COL1A1 (2.93-fold), COL3A1 (5.46-fold), PCOLCE (2.69-fold), PLOD3 (1.97-fold), TGFB1 (3.58-fold), LMNA (3.29-fold), MXRA7 (20.53-fold), ITGA4 (2.97-fold), ITGA2 (5.20-fold), ITGA8 (2.03-fold) while MMP14 (2.08-fold), while S100A4 (-25.99-fold) was reduced. IPA canonical pathways that were increased included *Integrin cell surface interactions* (Z-score=1, p-value=5.24E-04) and *Cell junction organization* (Z-score=1.41, p-value=9.34E-04), while the *Collagen degradation pathway* (Z-score=-1.41, p-value=3.44E-04) decreased.
Separately, short-term TGFβ2 stimulation (30min) increased: COL1A1 (2.36-fold change), COL3A1 (6.59-fold), PCOLCE (4.00-fold), PLOD3 (2.16-fold), TGFB1 (3.86-fold), HMOX1 (3.34-fold), LMNA (5.20-fold), MXRA7 (12.55-fold), ITGA4 (3.60-fold), ITGA2 (3.92-fold), while both S100A4 (-38.05-fold) and MMP14 (-3.16-fold) were downregulated. IPA canonical pathways that were increased included: *Telomere maintenance* (Z-score=2.449, p-value=2.76E-02) and *Cytoprotection by HMOX1* (Z-score=1.89, p-value=2.87E-03).
In summary, our results suggest that TGFβ2 stimulates a collagen-promoting and maintenance of skin integrity protein profile in healthy scalp HF DS fibroblasts.

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Cutaneous ageing in Asian skin: Loss of biomechanical resilience with preservation of epidermal architecture

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Ageing is characterized by a progressive decline in physiological function and is influenced by both intrinsic genetic and extrinsic lifestyle and environmental factors. Our current understanding of cutaneous ageing has been derived from studies of predominantly Caucasian populations, with little to no studies performed on Asian skin. Our previous research has demonstrated that the structural organization of healthy young skin is ethnicity-dependent, and that alterations in epidermal morphology in Caucasian subjects are closely associated with biomechanical decline. Here we explore the consequences of ageing on biomechanical function (cutometry of both protoprotected and chronically photoexposed skin; N=203) and epidermal morphometry of biopsy tissues (N=30; 21-76 years) from healthy participants of Chinese heritage. Our data suggests that for photoprotected skin, a biomechanical transition occurs around 50 years of age, with significantly reduced elasticity (R2, R7; $p<0.001$) and an increase in tissue "fatigue" (R4, $p<0.001$; R6, $p<0.0001$; R9, $p<0.01$). By 70 years of age, the skins' biomechanical profile has fundamentally shifted from elastic to fatigue-prone (R10; $p<0.05$). Notably, histologic interrogation of intrinsically aged skin revealed no significant age-related differences in epidermal thickness, dermal-epidermal junction (DEJ) convolution or *stratum corneum* thickness. At photoexposed sites, biomechanical decline was accelerated and measurable around 40 years of age. Histologically, we observe a flattened DEJ ($p<0.05$) but with no alteration to epidermal thickness or to the *stratum corneum*. This contrasts sharply to observations of Caucasian skin, where DEJ flattening and epidermal atrophy

are well-documented features of ageing at both photoprotected and photoexposed body sites. These findings suggest that the trajectory of intrinsic cutaneous ageing is ethnicity-dependent, highlighting the importance of including diverse ethnic populations in cutaneous ageing research. It further suggests that a precision medicine approach may be successfully deployed to address the impact of cutaneous ageing across heritage groups.

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UV-induced 3D senescent epidermal model

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Senescent cells are cells that have reached an irreversible cell cycle arrest due to internal or external stresses. These cells, often termed "zombie" cells can cause very detrimental effects to the surrounding tissue by the secretion of pro inflammatory cytokines, MMPs and growth factors.
Senescent cells have been detected in the epidermis and dermis of human skin in older people as well as actinic keratosis. Although we are still missing valuable information on the clinical consequences of senescent cells in skin, studies have described a decrease of wrinkles and an increase in epidermal thickness or decrease of TEWL (Transepidermal Water Loss) after senolytic or senomorphic treatment, suggesting that elimination or silencing of senescent cells leads to an improvement of clinical signs linked to aging.
As mentioned, senescence can be induced by external stressors (such as UV exposure) or internal stresses (such as oxidative stress or telomere shortening).
To address these different types of senescence, we have developed an *in vitro* 3D senescent RHE (Reconstructed Human Epidermis) model that mimics premature photoaging. Senescence was induced in RHE (reconstructed Human Epidermis) by chronic exposure to low doses of UVSSR. We observed an increase of P21 and γH2Ax at day 17, 3 days after the final exposure, showing early senescence, whereas P16 increase, or laminB1 decrease are observed at day 19 and day 21, showing deeper, late-stage senescence.
Increase of senescence markers is coupled with a decrease of Ki67 and Filaggrin, showing the impact of senescence on other skin parameters.
Addition of Honokiol at 5 µg/ml, from day 17 to day 19 of tissue reconstruction restores expression of LaminB1 and decreases expression of P16, P21 and γH2Ax showing a possible senolytic activity and providing a robust 3D senescent model for cosmetic actives screening.

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Topical cosmetic formulations provide protection and repair of the skin proteome against UV-A and pollution-induced oxidative stress in human skin explants

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Chronic UV-A and particulate matter (PM10) exposure induces irreversible protein carbonylation across all skin compartments, a hallmark of biological skin ageing. As carbonylated proteins cannot be repaired enzymatically, they accumulate, impairing collagen biosynthesis and barrier function. We evaluated in a validated model the protective efficacy of a day cream (P092) and reparative potential of a serum (P093) against UV-A- and PM10+UV-A-induced protein carbonylation and Collagen I depletion in human skin explants.
Skin explants (41-year-old female donor) were cultured in OxiProteomics® medium and exposed to UV-A (365 nm, 6 J/cm2) or PM10 (ERM-CZ100, 0.375 µg/cm2) + UV-A. P092 (antioxidant, osmoprotective, anti-pollution, barrier-reinforcing, and anti-inflammatory actives) was applied 2 days pre-stress, while P093 (proteasome-activating peptides, a wound-healing extract, and a hydrating agent) post-stress.
Carbonylation was quantified by epifluorescence (Oxi-View) in the stratum corneum, epidermis, dermis, and whole tissue, and Collagen I levels by immunofluorescence.
Stressors significantly increased protein carbonylation vs controls. P092 fully prevented carbonylation (100% inhibition; $p<0.001$) and preserved Collagen I levels (+34% vs. UV-A; +64% vs. PM10+UV-A). P093 reversed UV-A damage (74% to 95%; $p<0.001$), and PM10+UV-A damage (92-100%; $p<0.001$), restoring collagen I by 91% ($p<0.001$) and by 41%, respectively. These results support a combined protect-and-repair strategy to mitigate exposome-driven skin ageing.

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Hydrogen peroxide-induced senescence in dermal fibroblasts as a screening model for skin longevity actives

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Skin aging is closely linked to oxidative stress, which drives both intrinsic and extrinsic changes in dermal structure and function. Dermal fibroblasts play a central role in maintaining extracellular matrix homeostasis, yet reactive oxygen species (ROS) can disrupt redox balance, impair collagen production, increase matrix metalloproteinase activity, and promote the release of senescence-associated inflammatory mediators (SASP), ultimately leading to dermal senescence.
In this study, we aimed to establish a robust *in vitro* model of oxidative stress-induced premature senescence in normal human dermal fibroblasts (NHDF) that reproduces key features of dermal aging and can be used to screen compounds that support skin longevity. NHDF were exposed once to hydrogen peroxide (H_2O_2), a physiological ROS commonly used to induce oxidative stress-related premature senescence, at concentrations ranging from 250 to 800 µM depending on the endpoint. Intracellular ROS levels, β-galactosidase (β-gal) activity, DNA damage (COMET assay), the release of matrix remodeling and inflammatory mediators (MMP-1, MMP-3, procollagen I, IL-8), and collagen I expression were assessed.
A single H_2O_2 exposure increased intracellular ROS levels, β-gal activity, and DNA damage, and elevated the release of MMP-1, MMP-3, and IL-8, consistent with a senescence-associated secretory phenotype. In parallel, procollagen I release and collagen I expression (both extracellular matrix-deposited and intracellular) were reduced, indicating impaired extracellular matrix production. These senescence-associated changes were reversible with well-characterized cosmetic actives and reference compounds. Together, these data support the use of H_2O_2-challenged NHDF as a multi-endpoint platform to evaluate cosmetic or pharmacological compounds targeting dermal aging and fibroblast senescence.

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Target the osmolyte strategy to reinforce skin barrier and hydration

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Maintaining normal hydration level is essential for skin function. Normal human keratinocytes possess an osmolyte strategy to maintain cell homeostasis and skin hydration during osmotic stress. We have developed a cosmetic active plant extract from *Iresine weberbaueri*, rich in betaine, an organic osmolyte that protects cells from dehydration stress. We highlighted the beneficial effect of the extract to promote cellular osmoregulation and skin hydration by *in vitro* and *in vivo* clinical studies. Levels of osmolytes transporters (BGT-1, TAUT, SMIT) and skin barrier markers were analyzed in keratinocytes cultures and skin explant model by RT-qPCR and immunostaining. To evaluate protection effect against dehydration, an explant model stressed by infrared (IR) irradiation was used to evaluate claudin-1 and skin barrier function by fluorescent dye penetration (lucifer yellow). *In vivo*, hydration efficacy on forearm was assessed on 21 women with dry skin after D3 and D28 of application using corneometry and RAMAN spectroscopy for *stratum corneum* (SC) thickness. A second study on face was performed on 24 women with dry skin to assess skin barrier efficacy using TEWL evaluation. The extract increased the gene and protein expression of osmolytes transporters (TAUT, SMIT and BGT-1). It also increased the gene expression of barrier markers like tight junctions (claudin-1, occludin) and cornified envelope formation (transglutaminase-1, involucrin). The extract protected the explant from dehydration stress by increasing the claudin-1 staining decreased by IR and by limiting the penetration of lucifer yellow dye increased by IR. Clinical studies demonstrated improvement of skin hydration (+90% *vs* placebo at D28, $p<0.05$), increase of SC thickness (+1.8µm *vs* placebo, $p<0.05$) and improvement of skin barrier (-38% *vs* placebo at D28, $p<0.05$). Skin hydration is essential for healthy skin and its resilience. By increasing osmolytes transporters and skin barrier markers, the extract could promote skin hydration and resilience against stress.

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Epigenetic age reversal in fibroblasts using plant derived actives

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Cutaneous aging results from the progressive decline of cellular and tissue functions, largely driven by epigenetic mechanisms. Among these, DNA methylation is a key regulator of gene expression, particularly at CpG sites whose profiles change systematically with age. These epigenetic alterations affect essential biological pathways involved in keratinocyte proliferation, dermal homeostasis, extracellular matrix synthesis, and oxidative stress response. The accumulation of age associated methylation signatures contributes to reduced regenerative capacity, collagen disorganization, and increased inflammatory processes, which characterize both intrinsic and extrinsic skin aging.
The objective of this study was to investigate the capacity of a blend of plant derived bioactive compounds to modulate the epigenomic landscape of human dermal fibroblasts cultured *in vitro*. Genome wide DNA methylation profiles were quantified using the Illumina EPIC v2.0 array (>935,000 CpG sites), and epigenetic age was subsequently inferred using a skin specific epigenetic clock developed by the SkinOMIX platform. This clock, engineered specifically for human skin, provides a more accurate and sensitive estimation of cutaneous epigenetic age compared with existing methodologies.
Our results demonstrate a protective effect of the active combination on the fibroblast epigenome, reflected by a reduction of epigenetic age by 4.1 years. These preliminary results highlight the potential value of targeted cosmetic strategies capable of modulating the epigenetic mechanisms involved in skin aging. To further investigate these findings and confirm the *in vitro* data, a clinical study on volunteers using a finished product formulated with these actives will be conducted, with the objective of evaluating their impact on the skin's biological age.

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Artificial intelligence-driven protein clock to estimate epidermal biological age - results from the inspire-T cohort.

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Aging is characterized by the progressive accumulation of molecular and cellular damage, ultimately leading to functional decline and increased risk of chronic disease. Biological age, distinct from chronological age, reflects the dynamic interplay of cellular and biochemical processes and is strongly influenced by both intrinsic and environmental factors. In skin, most aging research has focused on the dermis, whereas the critical contribution of the epidermis to skin health and aging remains comparatively underexplored. Identifying molecular alterations that precede visible phenotypic changes is essential for the development of effective anti-aging interventions.
A skin "aging clock" aims to estimate biological age using molecular biomarkers. The recent integration of artificial intelligence (AI) with high-throughput proteomics has enabled the development of protein-based aging clocks, identifying specific biomarker signatures associated with skin aging.
The INSPIRE-T cohort (IHU HealthAge, Toulouse) includes 1,200 subjects aged 20–102 years and was designed to investigate trajectories of healthy aging using combined clinical and biological markers. In this study, we applied machine learning to define an epidermal proteomic signature of biological age within the INSPIRE-T cohort. Skin biopsies were obtained from 77 participants and subjected to proteomic profiling. An XGBoost model was trained on 62 subjects, optimized by cross-validation, and evaluated on an independent test set of 15 subjects. Using Recursive Feature Elimination, we reduced the model to a panel of 28 proteins, achieving a mean absolute error of 8 years for predicted biological age. Ingenuity Pathway Analysis revealed that these proteins are involved in five canonical pathways related to epidermal structure and function.
These findings support the feasibility of an AI-based epidermal protein clock and highlight its potential applications in the pharmaco-cosmetic field, particularly for the development of personalized anti-aging strategies and objective assessment of treatment efficacy.

329 [WITHDRAWN]

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Developing a 3D biologically aged epidermal model to address senescence

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Senescent cells are cells that have reached an irreversible cell cycle arrest due to internal or external stresses. These cells, often termed "zombie" cells can cause very detrimental effects to the surrounding tissue by the secretion of pro inflammatory cytokines, MMPs and growth factors.
Senescent cells have been detected in the basal layers of the epidermis and in the dermis of human skin in older people as well as actinic keratosis. Although we are still missing valuable information on the clinical consequences of senescent cells in skin, studies have described a decrease of wrinkles and an increase in epidermal thickness or decrease of TEWL (Transepidermal Water Loss) after senotherapeutic treatment, suggesting that elimination or silencing of senescent cells leads to an improvement of clinical signs linked to aging.
To best mimic chronic/biological senescence, we developed an *in vitro* 3D senescent RHE (Reconstructed Human Epidermis) model with cells from different aged donors showing different levels of senescent cells. We collected keratinocytes from donors of different ages that were seeded and left to grow and differentiate through air-liquid emersion.
We show a 3.2 and 3.75 fold increase of P21 and P16 respectively in the basal layer of the epidermis and 1.5 fold decrease of LaminB1 between donors aged 22 and 70 years confirming the prevalence of senescent cells in an older donor. Interestingly, the increase in senescent profile is matched with a decrease of Ki67 and Filaggrin expression.
Using combined innovative cell imaging methods to further characterize the 3D models, will allow us to identify novel cosmetic senotherapeutics.

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Topical RE.D Flavonoid, an extract of *Camellia japonica* seeds, increases procollagen 1 and reduces tyrosinase in photoaged skin.

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We previously showed that extract of *Camellia japonica* seeds [RE.D Flavonoid] can improve multiple clinical features of photoaging. It is unclear whether these changes correlate with biomarkers associated with photodamage. Facial skin biopsies were taken at baseline and after 24 weeks of treatment with RE.D. Flavonoid cream or placebo. Using immunohistochemical analysis, samples (10 treatment group and 5 placebo group) were evaluated for procollagen I, matrix metalloproteinase-2 (MMP2), tyrosinase, fibrillin-1, and Melan-A. Quantitative image analysis was performed in QuPath using compartment-specific regions of interest selected according to predefined criteria (intact dermis for dermal markers and interfollicular epidermis excluding adnexal structures for pigment-related markers). Expression was quantified as percent area positivity or percent positive cells within each defined region, as appropriate. Treatment resulted in a significant increase in procollagen I percent area positivity compared to placebo (mean change +4.1% vs +0.67%, $p<0.05$). Tyrosinase-positive cell percentage decreased in the treatment group but increased in the placebo arm (−1.62% vs +0.06%, $p<0.05$). There were no nonsignificant differences in the staining patterns of MMP2, fibrillin, and Melan-A between the two groups. Our data suggests that topical RE.D Flavonoid leads to meaningful changes in procollagen 1 and tyrosinase in photoaged skin.

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Regulation of oxidative dysfunction in senescent skin cells followed by ultra-weak photon emission

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The objective was to study how oxidative stress response is influenced by senescence in various skin cell types and its modulation by active ingredients, through the measure of ultra-weak photon emission.
Senescence was induced in NHKs and NHFs through replication, and in NHMs by UV exposure then followed by p16 or p21 expression. Oxidative stress response was evaluated by measuring ultra-weak photon emission (UPE) in non-senescent and senescent cells in the presence of exogenous chemical inducers. Non-senescent and senescent cells were then exposed to active ingredients, and the modulation of the oxidative response was again evaluated by UPE measurements.
In all cell types, the onset of senescence was confirmed by significant upregulation of p16 and p21. Upon oxidative stimulation, senescent cells also showed markedly increased UPE signal compared to normal cells, reflecting enhanced oxidative reactivity and diminished cellular regulation. This exacerbated oxidative reactivity to stress in senescent cells was successfully reduced in the presence of active ingredients.
In our study, UPE proved to be a relevant label-free marker of cellular dysfunction in the context of senescence and enabled the demonstration of active ingredient efficacy against senescence-associated oxidative stress dysregulation.

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Synergistic reduction of SASP markers in X-ray exposed fibroblasts by a combination of *Silybum marianum* extract, folic acid and sucralfat

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Senescence is a biological process that naturally occurs in cells but can also be induced by stress factors such as chronic inflammation or ionizing radiation. This study aimed to evaluate the effect of a combination of *Silybum marianum* fruit extract (A), folic acid (B), and sucralfate encapsulated in microspheres (S) on inhibition of senescence-associated secretory phenotype

(SASP) after irradiating cells with X rays. Additionally, the effects on cellular survival after exposure to particulate matter (PM4; particle size <4 µm) and UVB were evaluated.
Cells (primary dermal fibroblasts) were incubated with A, B, and S (50 µg/ml), as well as combination of all three substances (ABS) for 7 days before X ray irradiation (5 Gy). After 72 h, cells were lysed and lysates and media were collected and subsequently subjected to quantification of SASP (ELISA; IL-1β, IL-6, TNF-α, PAI1, IP10, CCL2, MMP3, CXCL10). For UVB and PM4 experiments, L929 murine dermal fibroblasts were used. In both cases, cells were preincubated with A, B, S, and ABS (at different concentration ratio) for 24 h and then subjected to UVB radiation (50 mJ/cm^2, single irradiation) or PM4 (100-300 µg/ml, 24 h incubation). After 24 hours, MTT cell viability assays were performed.
A reduction in SASP concentration in presence of ABS was observed in case of all evaluated proteins, with greatest effect observed for IL-6 (-76,9% vs. control). In PM4 exposure assay, synergistic effect of ABS was observed – cell survival in presence of PM4 and ABS was higher that PM4 alone (synergy index was 1,37 according to Kull's equation; values >1 indicate synergy). In case of UVB-irradiation assay, presence of ABS caused a 13,3% increase in cell viability. No increase in viability after UVB irradiation was observed in presence of A, B, and S separately.
The study showed that ABS reduces SASP secretion in X-ray irradiated cells and demonstrate cytoprotective effects against PM4 and UVB exposure, supporting their role in protecting cells from stress-induced damage and senescence-associated responses.

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In vivo and in vitro demonstration of the importance of an anti-dryness strategy for targeting pigmentation disorders

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Our study aimed to evaluate the correlation between environmental factors with dryness and pigmentation disorders, signs aging of high prevalence in Asian skin, both physiologically in vivo and mechanistically in vitro.
We first assessed the skin characteristics of 200 Chinese women aged 20-70 years, residing in rural and urban areas, across seasons, by rating hyperpigmentation and dryness. Environmental and lifestyle factors were collected for statistical analyses of associations between dyspigmentation and dryness, stratified by occupation, locality, and season. The crosstalk mechanism between skin dryness and pigmentation was then further explored by measuring melanogenesis in vitro in melanocytes exposed to osmotic stress, mimicking tissue dehydration.
In vivo results indicated significantly higher prevalence of dyspigmentation and skin dryness among rural participants in both seasons, correlating environmental exposure and lifestyle with signs of aging. This confirms the impact of exposome on accelerated skin aging as already described in the literature. Additionally, a significant correlation between skin dryness and dyspigmentation was also observed, which suggests interactions in underlying mechanisms. In vitro, melanocytes responded to osmotic stress, which was indeed associated with increased melanogenic activity.
This study demonstrates a significant correlation between skin dryness and dyspigmentation in vivo, which was further confirmed in vitro. This multi-level study gives a renewed overview of the crosstalk between pigmentation mechanisms and dehydration and emphasizes the importance of an anti-dryness approach for products targeting pigmentation disorders.

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Promoting skin longevity through humanin mitochondrial peptide with Iris pallida root extract

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The role of mitochondria goes beyond traditional powerhouses. Mitofusion & mitophagy are both implicated in mitochondrial restoration occurring preferentially during sleep. With aging, their decline led to increasing senescence. Interestingly, mitochondrial DNA encodes for multiple mitochondrial-derived peptides including humanin, a regulator of lifespan and healthspan, expressed at a higher level in Centenarians. However, the roles of humanin in the skin are still incompletely described, and particularly its role in nocturnal mitochondrial restoration. In line with these data, an innovative *Iris pallida* root extract (IPRE) has been developed using Ashland's PSR™ technology, with potential predictive activity against mitochondrial dysfunction.
In vitro studies showed a beneficial effect of IPRE on humanin expression in a model of senescent *ex vivo* skin biopsies. Additionally, IPRE application has been associated with an increase in mitophagy and mitofusion, studying respectively PINK1 and MFN-2, in a model of humanin-silencing reconstructed human epidermis. Finally, IPRE capacity to improve skin parameters and perceived age evaluated by IA-driven tool was assessed on 35 volunteers through a double-blind clinical study. To focus on nocturnal regulation, formulas placebo or containing 1% IPRE were applied following nighttime protocol, only in the evening. IPRE reversed perceived age by rejuvenating volunteer's appearance by one year *versus* placebo in only one month of application. Skin luminosity, firmness and wrinkles appearance were also significantly improved.
To conclude, this study emphasizes the role of humanin mitochondrial peptide as powerful longevity messenger involved in nocturnal mitochondrial restoration, through the testing of an innovative *Iris pallida* root extract that finally reverses perceived age clinically.

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Oval face restructuring in elderly panelists by a non-invasive cosmetic approach using a miniprotein

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One of the most easily recognized aspects of beauty is the shape of the face, which can convey age, gender, and attractiveness. In women, an oval facial shape is considered attractive; however, this structure is significantly altered with age due to skin laxity. One solution to preserve or restore an oval face shape is the use of dermal fillers as an invasive aesthetic approach. In our work, we propose a non-invasive cosmetic alternative based on the topical application of a miniprotein. The objective of this work was to demonstrate the efficacy of this miniprotein to preserve and/or restore the aesthetic aspects of the

faces of aged panelists. A double-blind, placebo-controlled, split-face clinical trial was conducted on 30 healthy Caucasian women before and after 14 days, 1 month, and 2 months of topical product use. The oval face improvement parameter was determined by volume reduction measurement determined by clinical grading assessment and by 3D image illustrations (Lifeviz Mini).The biomechanical properties of the face skin area were determined by skin stiffness parameter (EasyStiff® device). The study's results confirm the efficacy of the miniprotein in reversing visible signs of skin aging on the face. The clinical grading of oval shape showed that after two months of miniprotein application, the percentage of subjects without an increase in assigned score was higher than the placebo group, and the percentage of subjects that maintained the score was higher in the active ingredient group. These results confirm the ability of the miniprotein to combat the aging process. These outcomes were further substantiated by 3D images. Additionally, biomechanical assessments indicated that the miniprotein led to a consistent enhancement in skin stiffness across all measured time points. The results presented here demonstrates the effectiveness of the miniprotein in enhancing the aesthetic appearance of the facial skin in aged subjects.

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In vitro efficacy of Peauforia™ on hair follicles: implication for scalp health and hair well-being

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Maintaining scalp physiological balance is a key factor for optimal hair growth and quality, contributing to overall hair well-being. Scientific evidence indicates that alterations in scalp homeostasis—such as chronic inflammation, sebum excess and oxidative stress—are linked to hair disorders leading to an increasing need for products specifically formulated to preserve, strengthen, and restore scalp balance.
Peauforia™ is an innovative ingredient consisting of Fibroblast Growth Factor 2 fused to plant-derived oleosomes, which provide a biomimetic interface and support interaction with follicular environments while contributing to protein stability. It is involved in activation of the anagen phase of the hair cycle, promotion of skin barrier reinforcement and improvement of overall skin hydration.
In this in vitro study, the effect of Peauforia™ was evaluated on human scalp hair follicles, which were isolated from three donors and maintained ex vivo, preserving their three-dimensional architecture and follicular microenvironment. After 24 hours in culture medium, the follicles were treated with Peauforia™ for a total period of 36 hours. Subsequently, total RNA was extracted to evaluate the expression levels of key genes involved in scalp and hair health.
Results showed that Peauforia™ significantly increased aquaporin 3 and heme oxygenase 1 expression, supporting improved skin hydration as well as cytoprotective and anti-inflammatory effects, respectively. Similarly, a trend of decreased expression of peroxisome proliferator-activated receptor gamma was observed, suggesting a reduction in sebum production at the follicular level.
Furthermore, Peauforia™ positively influenced follicle growth, showing a tendency toward increased follicle elongation during the culture period.
Overall, the present findings support Peauforia™ as a promising bioactive ingredient for scalp care, acting on key pathways involved in hair follicle physiology and contributing to the maintenance of a healthy scalp environment.

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Ageing in South Asian Skin: Linking Biomechanical Decline to Histological Change

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South Asians (SA) are under-represented in skin ageing research despite comprising a large global population. This study aimed to characterise structural and functional ageing in SA skin using integrated biophysical and histological approaches.
A total of 109 healthy SA participants (18–90 years) were assessed for skin elasticity (cutometry, 4mm probe, F3 parameter) at photoprotected (buttock) and photoexposed (forearm) sites. Pigmentation was quantified using individual typology angle (ITA). Multiple linear regression evaluated associations with age, sex and pigmentation. A subset (n=10; 23–65 years) underwent paired biopsies (buttock and forearm) for histological analysis.
Elasticity declined significantly with age at both sites ($p<0.001$), with greater deterioration in photoexposed skin and in males. Lighter skin was associated with increased elasticity loss ($p=0.03$), while pigmentation declined with age ($p<0.001$). Histological analysis showed preservation of epidermal thickness with age, alongside progressive flattening of the dermal–epidermal junction, reduced rete ridge depth and disruption of elastin architecture, consistent with functional decline.
These findings demonstrate that SA skin undergoes significant age-related biomechanical and structural deterioration, despite higher constitutive pigmentation. Pigmentation appears to modulate, but not prevent, functional decline. Patterns broadly mirror those observed in Northern European skin, suggesting shared ageing mechanisms. This integrated approach highlights the importance of photoprotection and provides a framework for understanding skin healthspan across diverse populations.

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Analysis of risk factors and intervention effect of postherpetic neuralgia

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Our objective was to explore the independent risk factors of postherpetic neuralgia (PHN) and evaluate the efficacy of comprehensive intervention strategies based on these risk factors, so as to provide evidence-based basis for the early warning, prevention and treatment of PHN.
A retrospective cohort study was adopted, and patients diagnosed with herpes zoster were divided into two groups according to whether PHN occurred. Independent risk factors were screened by univariate and multivariate Logistic regression analysis. Then, a randomized controlled trial was conducted to divide PHN patients into an intervention group and a control group. The control group was given conventional analgesic and neurotrophic drug treatment, while the intervention group was additionally given nerve block, psychological intervention and rehabilitation training.
Seven factors including age ≥ 60 years old (OR=3.25, 95%CI: 1.52~5.31) and onset in the head, face and trunk (OR=2.47, 95%CI: 1.21~3.75) were independent risk factors for PHN (all $P<0.05$). After 4 weeks of treatment, the VAS score and course

of disease in the intervention group were significantly better than those in the control group (P<0.05), the scores of relevant dimensions of SF-36 were higher, and there was no significant difference in the incidence of adverse events between the two groups (P>0.05).
Advanced age, special onset site and other factors are key risk factors for PHN. Comprehensive intervention based on risk factors can effectively relieve pain, improve quality of life with controllable safety, and is worthy of clinical promotion.

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Targeting photoaging through unsaponifiable enriched grape seed oil: innovative insights into solar induced skin protection

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Solar radiation is a major contributor to skin damage, which can manifest in two distinct forms: (1) acute exposure, leading to immediate effects such as sunburn within minutes or hours of sun exposure; and (2) chronic exposure, resulting in long term consequences such as photoaging and skin cancers after years of repeated exposure. The primary mechanisms involved in solar induced skin damage, whether acute or chronic, include DNA lesions, oxidative stress, inflammation, increased production of matrix metalloproteinases (MMPs), and reduced collagen synthesis.
Different wavelengths of solar radiation (ultraviolet [UV], infrared [IR], and blue light) exert specific biological effects and contribute in various ways to cutaneous damage. Therefore, broad spectrum photoprotection is essential to ensure comprehensive skin defense.
In this study, we developed a grape seed oil specifically enriched in unsaponifiable compounds, a fraction less explored in previous studies, and evaluated its efficacy against solar radiation induced skin damage.
On human skin explants, the extract demonstrated protective effects against protein carbonylation induced by UVA, IR, and blue light stress.
On human skin explants, the extract protected against the reduction of type I collagen following IR stress.
On human skin explants, it also preserved barrier function by maintaining filaggrin levels after IR stress.
Finally, in vitro, the extract exhibited anti inflammatory activity, reducing IL6 levels in human keratinocytes subjected to thermal stress (45 °C, 30 min).
In summary, this enriched grape seed oil provides comprehensive protection against solar-induced skin aging by targeting oxidative stress, collagen loss, barrier integrity, and inflammation. These results position it as a promising and innovative ingredient for well aging skincare.

WOUND HEALING, REGENERATIVE DERMATOLOGY

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Radiotherapy-induced epigenetic memory impairs skin repair

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Radiotherapy is essential for cancer treatment, yet many survivors develop chronic skin fragility and poor wound repair months to years after exposure. Our study aimed to define how radiotherapy creates long-lasting regenerative failure in human skin and to identify targetable regulators of this process. We profiled paired irradiated and non-irradiated skin from breast cancer patients using *ex vivo* wound healing models, primary cell assays, DNA methylation profiling, and single-cell multi-omics integrating RNA-seq, ATAC-seq, and Nano-CUT&Tag. Key mechanisms were functionally validated in a murine radiation-wound healing model. Irradiated skin displayed an aging-like state with delayed wound closure, defective keratinocyte migration and proliferation, and impaired fibroblast function. In fibroblasts, chromatin accessibility profiling identified a primed thrombospondin 1 (THBS1) regulatory state, causing excessive THBS1 induction after injury; THBS1 inhibition improved *ex vivo* wound repair of previously irradiated human skin. In the epidermis, forkhead box M1 (FOXM1) was persistently reduced in proliferative basal keratinocytes. DNA methylation profiling revealed HCFC1 promoter hypermethylation, reduced HCFC1 expression, and diminished HCFC1 binding at the FOXM1 promoter, leading to sustained FOXM1 repression. Silencing HCFC1 or FOXM1 recapitulated irradiation-induced defects, whereas FOXM1 overexpression restored keratinocyte proliferation and accelerated wound closure. Single-cell multi-omics further revealed radiation-associated inflammation, cell-cycle suppression, and senescence-like states, with AP-1 linked to increased chromatin accessibility and KLF-family factors linked to reduced accessibility. Together, these findings reveal that radiotherapy imprints a durable epigenetic scar in human skin, locking regenerative cells into dysfunctional states and exposing new therapeutic opportunities to restore tissue repair.

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Targeting a polyamine metabolism-based molecular switch to restore re-epithelialization in chronic wounds

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A rapidly ageing global population faces an increasing burden of chronic, non-healing wounds, which contribute to substantial morbidity, mortality, and lower-extremity amputations. These wounds remain difficult to treat due to poorly understood age-related impairments in tissue repair. Wound healing is a tightly coordinated and self-limiting process governed by molecular switches that integrate proliferation, migration, and tissue remodeling; disruption of these programs leads to chronic wounds, fibrosis, or tumorigenesis. Emerging evidence identifies metabolic reprogramming as a central driver of repair, with metabolites functioning as active regulators rather than passive byproducts. Among these, polyamines have been implicated as critical upstream modulators of wound healing, yet their mechanistic roles remain unclear.
Here, we investigated the role of polyamine metabolism in epidermal repair using acute and perturbed rat wound models, human ex vivo wound systems, and clinical samples from chronic wound patients, combined with integrated transcriptomic, metabolomic, and proteomic analyses. We identify a polyamine metabolism-driven molecular switch that governs re-epithelialization. Polyamine-derived acrolein, traditionally considered cytotoxic, functions as a signaling mediator that transiently suppresses caspase-3 activity, thereby stabilizing the RNA-binding protein HuR to drive post-transcriptional repair programs

and reinforce polyamine metabolism. At later stages, elevated acrolein activates the KEAP1–NRF2 pathway to restore acrolein homeostasis and reactivate caspase-3, ensuring timely termination of repair. Disruption of this axis impairs keratinocyte migration and tissue regeneration in vivo, while pharmacological modulation restores healing. Notably, this pathway is profoundly dysregulated in chronic wound patient samples, highlighting its clinical relevance.
Collectively, our findings define polyamine metabolism as a master regulatory switch integrating the initiation, progression, and resolution of epidermal repair, and identify a targetable metabolic axis for chronic and age-associated non-healing wounds.

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The urokinase inhibitor BC-11 attenuates skin fibrosis via macrophage-fibroblast crosstalk modulation

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Fibrotic diseases are driven by persistent inflammatory activation and dysregulated crosstalk between macrophages and fibroblasts, leading to chronic tissue remodeling. Targeting this intercellular signaling represents a promising therapeutic strategy. We investigated the selective urokinase inhibitor BC-11 (Carbamimidothioic acid (4-Boronophenyl) methyl ester) for its effects on inflammation and fibrosis.
A murine full-thickness wound model was treated with BC-11 for eight weeks and analyzed using single-cell RNA sequencing to resolve cell type–specific responses. Complementary ex vivo human skin cultures were used to assess cytokine secretion, fibroblast activation and monocyte recruitment. Mechanistic readouts included Western blotting, ELISA and phosphoproteomics and proteomics.
BC-11 reduces inflammatory macrophage and activated fibroblast populations, as revealed by single-cell transcriptomics. Western blot analyses showed reduced levels of phosphorylated NF-κB, as well as decreased phosphorylated and total YAP/TAZ protein levels under BC-11 treatment. These changes were accompanied by reduced secretion of pro-inflammatory cytokines and chemokines and decreased levels of fibrotic markers including collagen and fibronectin. Conditioned supernatants from BC-11–treated fibroblasts reduced monocyte migration. Comparable effects were observed in ex vivo human skin cultures, including human hypertrophic scar tissue.
BC-11 attenuates inflammation-associated fibrotic responses and modulates macrophage–fibroblast interactions. These findings support further investigation of BC-11 as a potential therapeutic approach for fibrotic diseases.

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Modes of Programmed Macrophage Cell Death Govern Outcome of Cutaneous Wound Healing

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Timely clearance of macrophages from damaged tissues through regulated cell death (RCD) is essential for inflammatory resolution and repair; however, whether distinct modes of RCD in macrophages differentially determine healing quality remains undefined. Using inducible, macrophage-specific genetic models to trigger necroptosis ($FADD^{iMKO}$) or enhance apoptosis ($cIAP1^{iMKO}cIAP2^{-/-}$) during excisional skin wound healing, we investigated how each RCD mode shapes tissue architecture and cellular communication networks. Our findings demonstrated that macrophage necroptosis selectively reduced reparative macrophages, causing hemorrhagic granulation tissue, impaired myofibroblast differentiation, and sustained neutrophils in wound sites, thus delaying the resoution of inflammation. Single-cell transcriptomics and ligand-receptor interaction analysis revealed that necroptosis replaced a coordinated, growth factor-driven macrophage-fibroblast communication program with a damage-clearance state dominated by hemoglobin-scavenging, complement activation, and disorganized extracellular matrix remodeling. Interestingly, a Lyve1+ macrophage subpopulation expanded in necroptotic wounds, displaying a pro-regenerative interferon-stimulated transcriptional signature consistent with activation downstream of necroptotic danger signals. In contrast, enhanced macrophage apoptosis preserved fundamental tissue architecture and vascular integrity despite sustained macrophage reduction, and even reduced scar formation. These findings establish that the mode of macrophage death and the resulting paracrine signaling environment adds another critical component in how macrophages shape the tissue repair outcome.

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Extracellular Vesicles Expressing CD24 Enhance Skin Wound Healing

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Chronic non-healing wounds are driven by persistent inflammation, impaired cell migration, and defective tissue regeneration. CD24 is a highly glycosylated glycosylphosphatidylinositol-anchored protein with immunoregulatory functions and has been implicated in wound repair. This study aimed to evaluate glycosylated CD24-based topical therapeutics, including purified protein formulations and CD24-enriched extracellular vesicles (EXO-CD24), for accelerating acute and diabetic wound healing. Wound repair was assessed using in vitro scratch assays of an epithelial cell line, and in vivo full-thickness dorsal wound models in C57BL/6 mice, including a streptozotocin/nicotinamide-induced diabetes model. Animals received topical applications of either glycosylated murine CD24 (100–500µg or ointment 0.2–1mg/mL) or EXO-CD24 (10^9 particles/day). Wound area was quantified longitudinally using image analysis. Non-glycosylated CD24 derivatives were tested to assess structure–function requirements. Glycosylated CD24 enhanced cell migration in vitro, resulting in ~70% scratch closure at 48 h compared with ~35% in controls. In vivo, topical CD24 accelerated acute wound healing (~60% wound-area reduction by day 7 and ~90% by day 14, compared with ~35% and ~55% in placebo-treated animals, $p<0.05$). In diabetic mice, CD24 reduced wound area by ~50% at 72 h and ~85% by day 10, whereas placebo-treated wounds showed ~20% and ~45% reduction, respectively ($p<0.05$). Non-glycosylated CD24 derivatives showed no significant effect. EXO-CD24 further accelerated healing, achieving

~75% wound-area reduction by day 7 and 95% by day 9, compared with ~45% and ~55% in controls ($p<0.05$). Glycosylated CD24 accelerates wound healing in acute and diabetic models, and exosomal delivery further enhances efficacy through localized immunomodulation and regeneration. These findings support topical CD24 and EXO-CD24 as therapeutic candidates for the treatment of chronic wounds.

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Peripheral serotonin is a crucial regulator of skin homeostasis and repair

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Serotonin (5-HT) is best known as a central nervous system neurotransmitter, but it also plays an important role in peripheral tissue homeostasis, repair and regeneration. We thus sought to determine the role of 5-HT in skin homeostasis and healing. Tryptophan hydroxylase-1 knockout ($Tph1^{-/-}$) mice, which lack peripheral 5-HT, exhibited epidermal and dermal atrophy, reduced expression of genes involved in keratinocyte differentiation, barrier function, and extracellular matrix regulation, and increased transepidermal water loss. Upon skin wounding, $Tph1^{-/-}$ mice showed delayed closure, impaired angiogenesis and increased inflammation. After injury, *Htr1b* expression was increased in wounded skin. Single-cell data further revealed a significant upregulation in endothelial cells, fibroblasts, and basal keratinocytes. Topical applications of a 5-HTR agonist on wounded mice rescued healing, increased Ki67+ keratinocytes and CD31+ vessels, and reduced inflammatory infiltrates in $Tph1^{-/-}$mice, and in clobetasol-induced skin atrophy and sickle-cell mouse models. Spatial transcriptomics data from human wounds showed marked *HTR1B* upregulation, particularly in pericytes/vascular smooth muscle cells, fibroblasts, and migrating keratinocytes. We then analysed healing kinetics in a cohort of 484 patients with venous leg ulcers from our institution. SSRI exposure was associated with a 5-fold faster healing (adjusted HR 0.20, 95%CI 0.11−0.34), an effect absent with non-SSRI antidepressants. Overall, our results highlight a crucial role for peripheral 5-HT in maintaining skin homeostasis and promoting repair, and suggest that its therapeutic modulation may help counteract delayed wound healing, a major public health challenge.

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Adrenergic innervation fuels osteogenic matrix overproduction in keloids and presents a translatable target for topical therapy

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Keloids are fibroproliferative disorders driven by abnormal extracellular matrix deposition, yet the precise cellular mechanisms remain elusive. To elucidate the drivers of keloid pathogenesis, we comprehensively mapped the cellular landscape of these lesions. Through single-cell RNA sequencing of human keloid specimens, unbiased profiling revealed that neuronal-stromal interactions critically drive fibroblast dysregulation. We identified RUNX family transcription factor 2 $(RUNX2)^{+}$ osteogenic fibroblasts exhibiting ectopic integrin-binding sialoprotein (IBSP) deposition, a hallmark of pathological ossification. These fibroblasts displayed catecholamine response signatures correlating with excessive tyrosine hydroxylase-positive adrenergic innervation within the keloid stroma. Subsequent experiments showed that activation of the β1-adrenergic receptor (ADRB1) by epinephrine and norepinephrine induced IBSP production exclusively in keloid-derived fibroblasts via cAMP-PKA-CREB signaling. Mechanistically, CREB-activated translin (TSN) facilitated nuclear-to-cytoplasmic IBSP mRNA transport for efficient translation. *In vivo*, targeting catecholamine biosynthesis, adrenergic innervation, or ADRB1 via genetic or pharmacological inhibition prevented keloid-like pathology in rodent models. To translate these mechanistic insights, virtual screening of a natural compound library identified A7 as a topical ADRB1 antagonist. A7 bound ADRB1 directly with micromolar affinity and outperformed systemic β-blockers metoprolol and nebivolol in suppressing catecholamine-induced IBSP expression *in vitro*. Furthermore, topically applied A7 significantly mitigated dermal fibrosis and collagen over-deposition in bleomycin- and stretch-induced murine models. In conclusion, our results revealed that neuronal-adrenergic signaling drives fibrotic and osteogenic matrix overproduction via TSN-mediated RNA trafficking, establishing both a novel pathomechanism and A7 as a promising topical therapy for keloids.

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Recombinant Human Thrombomodulin Derivative Promotes Wound Healing in Diabetic Foot Ulcer by Modulating VEGF-dependent Angiogenesis and Extracellular Matrix Organization Pathway

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Diabetic foot ulcer (DFU) is a common and devastating complication of diabetes mellitus. Current treatment for DFU remains clinically challenged and requires specialized skills. Regranex, the only FDA-approved growth factor, has demonstrated efficacy in wound healing but is expensive and historically carries cancer risk, raising a need for more affordable, accessible while maintaining effective treatments. Thrombomodulin (TM) has gained attention for its role in tissue regeneration and wound healing. BB-101, a recombinant human thrombomodulin (rhTM) derivative, was developed and optimized for enhanced regenerative properties. This study aims to investigate the potentials of BB-101 in DFU.
Normal Human Dermal Fibroblasts (NHDFs) were used to assess the wound healing effect of BB-101 *in vitro* via migration assay. A diabetic mouse model was recruited to validate the results *in vivo*. Induced wound area was treated topically 24 hours after injury with vehicle, BB-101 or positive control once daily for 14 consecutive days. Subsequently, NHDFs were treated with vehicle or BB-101 for 4 hours and RNA-seq was employed to identify possible mechanism pathways of BB-101.
Results showed that BB-101 significantly improved cell migration in NHDFs. *In vivo*, BB-101-treated mice exhibited improvement in closure percentage of wound area and wound-half closure time compared to controls. RNA-seq revealed upregulated VEGF and other signaling pathways related to wound healing after BB-101 treatment. Additionally, gene network plot displays linkages between BB-101 induced genes and enriched biological functions, which include wound healing,

regulation of angiogenesis, extracellular matrix organization, and fibroblast migration. Consistently, these results were validated using single cell RNA sequencing.
Our findings suggested that rhTM derivative, BB-101, accelerated wound healing process and activated key regenerative pathways. This molecule represents a promising candidate for the development of new DFU therapies, offering an additional option with the potential to overcome the challenges of existing treatments.

349 [WITHDRAWN]

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Plasma activated media modulate key proteins involved in keratinocyte adhesion and migration: from *in vitro* proteomics to *ex vivo* burn models
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Cold atmospheric plasmas (ionised gases at low temperatures), particularly plasma activated media (PAM), have emerged as a promising therapeutic strategy for skin wound healing. However, the precise cellular and molecular mechanisms involved remain largely uncharacterized. This study aimed to identify proteins modulated by PAM that drive skin wound healing. Global proteomic analysis was performed on N/TERT-1 cells subjected to a scratch assay with or without PAM treatment. Some up-regulated proteins were functionally validated after siRNA-mediated knockdown using migration and adhesion assays. Their expression was assessed during wound healing using a 3D *ex vivo* model we developed: burned human skin explants were maintained in culture for 7 days; healing kinetics and epidermal differentiation were evaluated through immunohistochemical detection of proliferation (Ki67, KRT6) and differentiation (KRT10, FLG, CDSN, LOR) markers. Among the 76 proteins up-regulated during cell migration (6h versus 1h) after PAM treatment, and the 13 proteins up-regulated when comparing PAM to gas alone (control treatment), 6 were selected for further analysis in the migration and adhesion assays. Functional validation highlighted a critical role for KIF11 in cell adhesion, while the depletion of S100A6, NCAPH and SH3BGRL3 significantly enhanced keratinocyte migration. The developed *ex vivo* burn model, characterized as a second-degree superficial burn, exhibited complete re-epithelialization within 4 days of culture. Immunohistochemical analysis of the neo-epidermis confirmed progressive stratification and terminal differentiation within 7 days. In this model, KIF11 and SH3BGRL3 displayed robust expression in the neo-epidermis. Our findings demonstrate that PAM-modulated proteins, particularly KIF11, play pivotal roles in keratinocyte migration. These data provide mechanistic insights into PAM based therapies and highlight the translational potential of our *ex vivo* human skin model.

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Restoring Dysfunctional YAP Signalling in Junctional Epidermolysis Bullosa
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Junctional Epidermolysis Bullosa (JEB) is a devastating and often lethal inherited skin disorder characterized by severe blistering and chronic wounds. JEB is primarily caused by pathogenic variants in the *LAMA3*, *LAMB3*, and *LAMC2* genes encoding for the subunits that make up the basement membrane protein laminin-332. Despite the heavy clinical burden, effective regenerative therapies remain limited. The transcriptional co-regulator proteins YAP and TAZ are key drivers of epidermal stem cell renewal and tissue regeneration, and their activity was previously shown to be impaired in patients with JEB. Here, we have been investigating YAP/TAZ dysfunction in laminin-332-deficient JEB by using immortalized N/TERT-1 human keratinocytes with shRNA-mediated laminin-332 depletion. We show that *LAMA3* knock down is sufficient to recapitulate key features of the JEB phenotype observed in primary patient keratinocytes, including impaired clonal growth and increased terminal differentiation. N/TERT-1 parental keratinocytes displayed intact cell density-dependent activation of Hippo signalling, as evidenced by LATS1/2-mediated YAP S127 phosphorylation and decreased expression of downstream target genes at high cell densities. Of note, *LAMA3*-depleted N/TERT-1 keratinocytes phenocopied primary keratinocytes isolated from a *LAMA3*-mutant JEB patient by displaying a marked reduction in YAP expression at both mRNA and protein levels. Hence, qPCR analysis revealed downregulation of canonical YAP/TAZ target genes. Consequently, we also observed reduced YAP levels in 3D organotypic skin equivalents, as well as in skin wounds of an inducible JEB disease model ($Lama3^{flox/flox}$/K14CreERT). Intriguingly, we found that only depletion of the *LAMA3* chain, but neither of *LAMB3* or *LAMC2*, impacted on YAP expression, suggesting that other *LAMA3*-containing laminins may be involved in regulating YAP. Importantly, pharmacological modulation of the Hippo signalling pathway in shLAMA3 N/TERT-1 cells demonstrated that select compounds could partially restore YAP/TAZ activity and improve keratinocyte function, highlighting the therapeutic potential of targeting this signalling axis for improved wound healing in JEB.

352 [WITHDRAWN]

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miR-193b-3p Impairs Angiogenesis and Endothelial Metabolism in Diabetic Foot Ulcers
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Diabetic foot ulcers (DFUs) are chronic wounds characterized by impaired healing, increased risk of amputation, and substantial clinical and socioeconomic burden. Defective angiogenesis within the wound is a key driver of DFU persistence. MicroRNAs (miRs) are critical regulators of wound repair, and their dysregulation is a hallmark of chronic wounds. We previously identified miR-193b-3p as upregulated in DFUs and demonstrated its inhibitory effect on keratinocyte migration. Here, bulk RNA sequencing of DFU tissue and diabetic foot skin, integrated with predicted miR-193b-3p target analysis, identified angiogenesis as one of the most significantly downregulated biological processes associated with this miR. Among the predicted targets, CD34—mediator of endothelial cell migration and sprouting—was validated as a direct miR-193b-3p

target using 3′UTR luciferase assays in endothelial cells (HUVECs). miR-193b-3p overexpression suppressed CD34 expression, whereas its inhibition enhanced CD34 levels in HUVECs. We further identified a network of angiogenesis-regulating transcription factors as direct miR-193b-3p targets, suggesting an additional indirect mechanism by which this miR suppresses angiogenesis in DFUs. Single-cell RNA-Seq revealed endothelial metabolic dysfunction in DFUs, including reduced oxidative phosphorylation and ATP production, along with increased mitochondrial impairment. ATP5PB, an ATP synthase subunit and miR-193b-3p target, was downregulated in DFU endothelial cells, and modulation of miR-193b-3p correspondingly altered intracellular ATP levels in HUVECs. Functionally, miR-193b-3p overexpression reduced endothelial branching in vitro, while its inhibition enhanced angiogenic capacity. In diabetic murine wound models, miR-193b-3p overexpression impaired angiogenesis, whereas its inhibition promoted vascularization. Finally, Staphylococcus aureus, the most common DFU wound colonizer, induced miR-193b-3p expression in diabetic mouse wounds leading to supression of angiogenesis. Collectively, these findings establish miR-193b-3p as a central regulator of angiogenic impairment in DFUs and highlight its therapeutic potential to improve wound healing outcomes.

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Hyaluronic acid–driven immune-epithelial crosstalk: orchestrating macrophage immunomodulation and epithelial repair dynamics

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Wound healing is a highly coordinated process involving dynamic interactions between epithelial and immune cells. Impaired healing is often associated with macrophage-driven hyperinflammation, where persistent pro-inflammatory signaling disrupts keratinocyte function and tissue repair. Hyaluronic acid (HA), a key extracellular matrix component, has emerged as a promising biomaterial that modulates immune–epithelial interactions in a molecular weight–dependent manner.
This study investigates how different HA molecular weight fractions regulate cellular healing dynamics and the resolution of hyperinflammation. HA exerts size-dependent effects via receptor-mediated signaling, primarily through CD44 and Toll-like receptors (TLR2/TLR4), influencing macrophage activation and keratinocyte responses within the wound microenvironment. Low molecular weight HA (LMW-HA) modulates inflammatory activation via TLR4 signaling and interferes with lipopolysaccharide (LPS)-induced responses, attenuating NF-κB–dependent pro-inflammatory pathways. This regulates early inflammation while preserving controlled immune activation required for tissue repair. In contrast, high molecular weight HA (HMW-HA) exerts anti-inflammatory and tissue-protective effects. Through CD44-mediated signaling, it promotes macrophage polarization toward an M2-like pro-regenerative phenotype, associated with mediators such as TGF-β, supporting resolution of inflammation and preventing non-healing wounds. Furthermore, HMW-HA enhances epithelial repair via CD44-dependent pathways regulating cytoskeletal organization, thereby promoting keratinocyte migration.
In conclusion, HA is a dynamic biomaterial that actively orchestrates immune–epithelial crosstalk rather than serving as a passive structural component. By mitigating macrophage-mediated hyperinflammation in a molecular weight–dependent manner and promoting epithelial repair, HA fine-tunes the balance between inflammation and regeneration, highlighting its translational potential in regenerative dermatology.

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Biological effects of AKR101, a potential new drug for chronic wound therapy

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Exudates from chronic wounds (cWEs) transfer their clinical phenotype *ex vivo* onto primary human fibroblasts from healthy donors by impairing fibroblast proliferation. Drugs with potential for chronic wound therapy should be able to reverse this effect. Compound AKR101 rescued fibroblast proliferation in the presence of >200 cWEs. Gene expression studies were carried out to characterize the pathways affected by AKR101.
Human fibroblasts were cultured in 384- or 24-well plates for proliferation and gene expression studies, respectively. AKR101 was added to the cells in the absence or presence of cWEs. Incubation times were 72 hours for proliferation and 6 or 72 hours for gene expression. Cells were fixed and stained for cellular protein, and RNA was extracted for RT-PCR or sequencing on a HiSeq 2500 instrument (Illumina).
AKR101 rescued fibroblast proliferation with 80% of the 200 cWEs tested, indicating transition from a chronic/non-healing to a healing phenotype *ex vivo*. These rescue effects were independent of wound etiology and superior to the wound therapeutics, platelet-derived growth factor and silver sulfadiazine when tested with the same 101 cWEs samples (rescue effects 4% and 34%, respectively, compared to 90% for AKR101). Gene expression profiling revealed suppression of pro-inflammatory genes and genes associated with response to stress, such as the unfolded protein response, which were induced by cWEs. In addition, AKR101 enhanced expression of the extracellular matrix proteins collagen 1 and collagen 3.
AKR101 was identified as a promising candidate to reverse the inhibitory and damaging effect of exudates from a multitude of chronic wound patients. It acts *via* induction of tissue regeneration and suppression of inflammation and cellular stress. AKR101 is currently being developed for a Phase 2 clinical proof-of-concept study in chronic wound patients.

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Distinct Cellular and Matrix Dynamics Govern Palmoplantar Wound Repair

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Palmoplantar skin exhibits unique structural and functional characteristics that distinguish it from skin elsewhere on the body. Despite several cutaneous conditions affecting the palms and soles, the absence of a clinically relevant palmoplantar wound healing model often necessitates the use of back skin as a surrogate and currently the mechanisms of palmoplantar repair are largely unknown.
Here we developed a novel murine palmoplantar full-thickness excisional wound healing model on the ventral forepaw. Unwounded (control) and wounded skin samples collected at early and advances wound healing stages were analysed to assess the unique epidermal and dermal dynamics combining histology, immunofluorescence, second-harmonic generation

(SHG) imaging and extracellular matrix (ECM) fate mapping analysis.
Our results revealed that, in contrast to back skin, palmoplantar skin wounds show minimal contraction, quick reepithelization and partial regeneration of rete ridge like structures. Intriguingly, after the initial immune cell infiltration, papillary (CD39+) fibroblasts are predominantly activated, togeter with a moderate population of reticular (Sca1+) fibroblasts, both of which migrate and expand in the palmoplantar granulation tissue when blood vessels start reforming. During the early wound healing response, fibronectin and hyaluronan are the first ECM components to be deposited in the wound bed, and are progressively replaced by collagen fibres in the upper (papillary) dermis with tangential orientation to the epidermis. In contrast to back skin, ECM fate mapping experiments revealed that in palmoplantar skin there is minimal recruitment of existing ECM which may influence the scarring response.
By establishing a novel palmoplantar wound healing model in mice, we provided key insights into the distinct palmoplantar repair process and scarring behaviour supporting the development of effective therapeutic strategies for palmoplantar skin repair in health and disease.

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Association of statin use with reduced pressure injury incidence in adults: a population-based cohort study

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Pressure injuries (PIs) represent a major challenge in dermatology and geriatric medicine, arising from sustained mechanical loading, impaired tissue perfusion, and compromised skin integrity. In ageing populations, PIs are associated with pain, infection, delayed healing, and substantial healthcare burden. Using a large Japanese claims database, we previously identified a potential link between lipid-lowering therapy and PI incidence.
Our objective was to determine whether PI incidence differs according to lipid-lowering drug class and individual statins in a real-world population.
We conducted a retrospective, population-based new-user cohort study using the Shizuoka Kokuho Database, Japan, including adults aged ≥50 years between April 1, 2012 and September 30, 2022. Initiators of antihyperlipidaemic drugs were compared with noninitiators using propensity score-based inverse probability of treatment weighting. Incident PI was analysed using Fine–Gray competing-risk regression, with death treated as a competing event. A prespecified high-risk subgroup was additionally evaluated using a validated PI risk score.
In weighted analyses, statin initiation was associated with a significantly lower incidence of PI than noninitiation (subdistribution hazard ratio [sHR] 0.69, 95% confidence interval [CI] 0.67–0.72). In contrast, non-statin antihyperlipidaemic therapy was associated with a higher incidence of PI (sHR 1.28, 95% CI 1.13–1.46). Among the six statins available in Japan, pitavastatin was associated with the lowest PI incidence, although all statins were significantly associated with lower PI incidence.
Statin use was consistently associated with reduced PI incidence in this large real-world cohort, whereas non-statin lipid-lowering therapy showed an opposite association. These findings suggest that statins may exert skin- and soft tissue-protective effects beyond lipid lowering and highlight their translational relevance to PI prevention. Prospective and mechanistic studies are warranted.

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An IL-25-driven sensing-to-action pathway reprograms keratinocytes for wound repair

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Tissue repair is generally viewed as a systemic, immune-coordinated process. However, the molecular patterns that directly drives the epithelium to sense damage and initiate reepithelialization remains a central question in regenerative biology. IL-25 has so far primarily been regarded as a contributor to type 2 immune responses. Here, we report observations documenting its role as a major factor in wound healing. Through single-cell transcriptomics of human skin explants combined with functional assays in immune-cell-free epidermal models and in vivo wound models, we demonstrate that IL-25 directly reprograms basal keratinocytes into a migratory state, bypassing the requirement for systemic inflammatory stimuli. Mechanistically, IL-25 activates a rapid sensing-to-action pathway by triggering the nuclear translocation of the transcription factor GRHL3 and the induction of its downstream effector KRT17 which is linked to wound closure. Our findings define an epithelial-intrinsic rescue pathway whose impairment leads to the functional sensing silence and subsequent regenerative failure characteristic of chronic human wounds. This autonomous mechanism offers a clear translational pathway for restoring the paralyzed repair machinery in chronic wound complications.

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Reprogramming the follicular microenvironment to restore hair growth through multimodal pro-anagen mechanisms

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Hair loss arises from a progressive disruption of follicular homeostasis driven by inflammation, oxidative stress, impaired vascularization, and altered paracrine signaling from dermal papilla cells to follicular keratinocytes. This leads to premature catagen entry and follicular miniaturization. Restoring a supportive follicular microenvironment is therefore essential to sustain anagen and hair growth.
We investigated the biological activity of a dual botanical complex combining a proprietary sprouted lentil extract, enriched in peptides, amino acids, zinc, and B vitamins, and a yerba santa extract rich in bioflavonoids, on key pathways governing follicle physiology in human skin-derived models.

In PMA-challenged NHEKs, both extracts reduced IL-6, IL-8, and TNFα. Under oxidative stress, yerba santa induced a dose-dependent ROS reduction (up to −86%), supporting protection against stress-induced dysfunction.
Sprouted lentil stimulated angiogenesis in a NHDF/GFP-HUVEC model (+81% network connections, p<0.001), supporting vascular supply required for follicle metabolism.
At the signaling level, both extracts activated Wnt/β-catenin in HFDPCs (+71%, p<0.01), a key regulator of anagen initiation. In HFDPC–NHEK co-cultures, this translated into enhanced dermal papilla–keratinocyte paracrine signaling, as shown by increased FGF7 secretion (+111%, p<0.01), promoting matrix keratinocyte proliferation and hair shaft production, with effects comparable to 1 µM minoxidil.
qRT-PCR analyses showed upregulation of COL7A1, SDC1, and COL17A1 by yerba santa extract, indicative of reinforced follicular anchorage and stem cell niche maintenance.
Overall, protection of the follicular microenvironment enables reactivation of dermal papilla signaling, leading to Wnt/β-catenin-driven follicular regeneration and anagen initiation, amplified by FGF7 signaling, and stabilized by improved anchorage and niche integrity.
Consistent with the mechanistic effects observed in vitro, a formulation containing the complex increased the anagen/telogen ratio by +19% (p<0.05) after one month in an open-label exploratory study, supporting their biological relevance in vivo.

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A comparative split study on efficacy of Erbium glass non-ablative fractional laser (1565 nm) with Platelet rich plasma versus Erbium glass non-ablative fractional laser (1565nm) alone in the treatment of striae distensae

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Striae distensae (SD) are common dermal scars caused by mechanical stretching and extracellular matrix disruption, associated with puberty, pregnancy, and weight changes. Treatment outcomes remain inconsistent. The 1565 nm erbium glass non-ablative fractional laser induces dermal remodelling via controlled thermal injury. Platelet-rich plasma (PRP), containing growth factors such as PDGF, TGF-β, VEGF, and EGF, enhances tissue regeneration and may provide synergistic benefit when combined with laser therapy. Objective: To compare efficacy, safety, and patient satisfaction of 1565 nm erbium glass laser with PRP versus laser alone in SD. A prospective split-body study over 18 months included 30 patients (18–40 years) with bilateral SD. Each patient served as their own control: one side received laser alone, the other laser with PRP. Four sessions were performed at 4-week intervals. Outcomes included striae width reduction, quartile clinical grading (0–4), and patient satisfaction. Blinded and non-blinded assessments were done. Statistical analysis used paired t-tests and Wilcoxon signed-rank tests ($p < 0.05$). All patients completed the study. At 4 months, mean striae width reduction was greater in the combination group (75.34% ± 5.2%) than laser alone (53.34% ± 9.8%) ($p < 0.001$). Excellent improvement (>75%) occurred in 83.3% with combination therapy versus none with monotherapy. Blinded scores were significantly higher with combination therapy ($p < 0.001$). Patient satisfaction was greater (60% extremely satisfied vs 10%; $p < 0.01$). Adverse effects were mild, transient, and comparable, with no serious complications. PRP combined with 1565 nm erbium glass laser significantly improves outcomes and satisfaction in SD without increasing adverse effects. This synergistic approach is effective and safe, warranting validation in larger studies.

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FoxO3a prevents pathological scarring by coordinating neutrophil resolution and macrophage maturation

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Pathological scarring results from dysregulated inflammatory resolution and maladaptive immune–stromal remodeling during wound repair. However, the mechanisms that determine whether inflammation resolves productively or progresses toward fibrotic remodeling remain incompletely understood. Here, we identify FoxO3a as a critical regulator of neutrophil-driven inflammatory resolution during cutaneous wound healing.
Following full-thickness skin injury, FoxO3a-deficient mice developed excessive scar formation despite preserved intrinsic fibrogenic responsiveness of dermal fibroblasts. Instead, FoxO3a deficiency caused persistent accumulation of S100a8/a9-high neutrophils during the resolution phase, accompanied by impaired neutrophil apoptosis. Single-cell transcriptomic and trajectory analyses revealed defective macrophage maturation and altered immune–stromal remodeling programs, which were associated with emergence of profibrotic fibroblast programs in vivo.
Functionally, neutrophil depletion significantly reduced scar formation in FoxO3a-deficient mice. Pharmacological inhibition of S100A8/A9 with Paquinimod similarly attenuated pathological scarring. These findings demonstrate that FoxO3a prevents excessive scar formation by enforcing timely neutrophil resolution and limiting S100A8/A9-driven immune–stromal remodeling. Our study identifies inflammatory resolution as a key checkpoint controlling scar outcome during skin repair.

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Defining Wound Endotypes in RDEB Through Histology-Guided Transcriptomic Profiling

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Recessive dystrophic epidermolysis bullosa (RDEB) is a rare genetic disorder caused by type VII collagen mutations, leading to skin fragility, chronic wounds, and progressive tissue damage. Despite a shared genetic cause, RDEB wounds show marked heterogeneity, suggesting distinct biological states. We performed histology-guided transcriptomic profiling of 13 RDEB wound biopsies. Formalin-fixed paraffin-embedded samples were stratified into high vs low inflammation, epidermal hyperplasia, granulation tissue, and fibrosis. RNA sequencing was conducted using rRNA depletion (NovaSeq platform).
Inflammation emerged as the dominant transcriptional driver. Inflammation-high wounds showed 712 upregulated genes, including SELL, CXCL13, PAX5, and BANK1, with enrichment of leukocyte activation and B cell signalling (PPI p<0.0001), indicating coordinated adaptive immune responses. Epidermal hyperplasia-high wounds (518 genes) were enriched for keratinocyte differentiation, metabolism, and tissue remodelling, reflecting a hyperproliferative regenerative state. Fibrosis-high

wounds displayed a limited signature (24 genes), dominated by barrier-related genes (FLG, FLG2, KPRP, SPTLC3), suggesting differentiation-driven structural reinforcement. Granulation-high wounds (18 genes) showed a focused inflammatory profile (IL1B, CXCL8, CXCL1, CCL22, PTGS2, MMP1) with strong chemokine signaling (PPI p<0.0001), indicating active immune recruitment and matrix remodeling.
These data demonstrate that RDEB wounds segregate into distinct molecular programs aligned with histological features. We propose at least four wound endotypes: inflammatory, hyperproliferative/regenerative, barrier/fibrotic, and granulation/healing niche. This framework provides a novel basis for stratifying RDEB wounds beyond morphology and may inform targeted therapeutic strategies.

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Polyamine catabolism drives epithelial migration during tissue repair

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Efficient wound healing requires tightly regulated and self-limiting mechanisms to ensure precise tissue regeneration while preventing pathological outcomes. Here, we identify a metabolic regulatory pathway in which polyamine catabolism generates acrolein. Although traditionally viewed as a cytotoxic byproduct, acrolein functions instead as a transient and stage specific signaling molecule to promote healing.
Upon wounding, acrolein levels rise to suppress the activity of caspase 3. We demonstrate that the inhibition of caspase 3 is required for the promotion of healing. By preventing the premature cleavage of the RNA binding protein HuR, acrolein ensures stable HuR expression to post transcriptionally drive re epithelialization and cell migration. This metabolic flux creates a feed forward loop that reinforces polyamine metabolism during the initial stages of repair.
As healing reaches completion, accumulated acrolein activates the KEAP1 NRF2 detoxification pathway. This process restores metabolic homeostasis and removes caspase 3 inhibition which in turn drives HuR degradation and destabilisation of pro-migratory mRNAs. Disruption of this axis in a perturbed rat wound model results in impaired re epithelialization and delayed wound repair. Animal models of perturbed wounds show disrupted polyamine catabolism and elevated caspase 3 and caspase 3 inhibition significantly improves healing. We have further shown that this pathway is disrupted in human chronic wounds. Together, our findings identify acrolein as an essential signalling molecule that integrates metabolic state with post transcriptional regulation and identifies caspase 3 as therapeutic target for wound healing. This study provides a mechanistic framework for the initiation and termination of epithelial repair and identifies therapeutic targets for chronic wound management.

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Modelling cellular crosstalk driving extracellular matrix accumulation in fibrotic skin diseases

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Recent studies highlight Schwann cell–M2 macrophage interactions as key regulators of keloid pathogenesis, marked by abnormal wound repair and excessive extracellular matrix (ECM) deposition.
To further explore this process, we developed a novel 3D full-thickness in vitro model composed of self-secreted ECM from dermal cells of keloid and healthy skin, together with an epidermal layer. Peripheral blood–derived monocytes and polarized macrophages were introduced to evaluate their impact on fibroblasts and Schwann cells, alongside the reciprocal effects of Schwann cells on macrophage phenotype.
Keloid-derived models showed elevated extracellular matrix secretion and higher collagen fibre alignment than healthy skin equivalents, mirroring the in vivo keloid matrix architecture. Schwann cells had minimal influence on macrophage polarization; however, macrophages modulated Schwann cell transcriptional profiles in a polarization-specific manner. M1 macrophages triggered inflammatory gene expression in Schwann cells, whereas M2 macrophages promoted the expression of genes linked to TGF-β signalling and matrix synthesis. Exposure of Schwann cells to monocytes resulted in only minor transcriptional changes. However, both monocytes and M2 macrophages enhanced collagen deposition and fibre organization in ECM generated by healthy and keloid fibroblasts, while M1 macrophages suppressed matrix production in both conditions.
Overall, the model recapitulates key features of keloid pathology providing a platform for mechanistic studies and therapeutic testing. Our findings identify macrophages as central regulators of matrix remodelling, with M2 macrophages promoting ECM secretion, highlighting Schwann cell–M2 macrophage crosstalk as a potential therapeutic target.

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Matrix metalloproteinase profiles as indicators of microbial colonization, systemic inflammation, and healing trajectory in chronic wounds

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The transition from acute to chronic wound states is driven by a complex interplay of microbial colonization, local inflammatory signals, and systemic host factors.In this study, we characterized how specific pathogens and systemic inflammatory markers differentially modulate the host proteolytic pool to influence wound healing.To that end, we analyzed wound fluid concentrations of MMP-2, MMP-8, and MMP-9 in wound dressings of patients with chronic ulcers.These were stratified based on wound surface area, bacterial colonization with *Pseudomonas aeruginosa* and *Staphylococcus aureus*, and patient sera CRP levels.In addition, the wound healing trajectory was assessed as improved, stable, or worsened over a longitudinal follow-up period.Ulcers positive for *P. aeruginosa* exhibited a significant reduction in total concentrations of all measured MMPs ($p < 0.01$), suggesting an aggressive proteolytic short-circuit potentially driven by bacterial proteases. Conversely, *S. aureus* colonized ulcers showed no significant effect on extracellular MMP levels, consistent with an intracellular survival strategy.

While MMP-8 concentrations demonstrated a strong positive correlation with initial wound surface area ($p < 0.001$), it failed to predict healing outcomes. In contrast, MMP-2 emerged as a unique indicator of regenerative competency; significant depletion of MMP-2 was specifically associated with clinical deterioration ($p < 0.05$).Furthermore, an inverse relationship was observed between systemic C-reactive protein (CRP) and local gelatinase (MMP-2 and -9) levels, indicating that systemic inflammatory stress may blunt the local proteolytic response required for repair.Among the evaluated proteases, MMP-2 is the most consistent indicator of wound healing progression and systemic-local integration. While MMP-8 reflects the physical scale of the injury, MMP-2 serves as a critical biomarker for identifying stalled wounds.These findings suggest that monitoring specific MMP profiles, rather than total inflammatory burden, can guide targeted antimicrobial and systemic interventions to restore regenerative capacity in chronic cutaneous ulcerations.

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Prospective Cohort Study on Skin Microcirculation Monitoring and Preventive Nursing Interventions in High-Risk Patients with Pressure Injuries

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Our objective was to explore the predictive value of B-ultrasound-based skin microcirculation monitoring for pressure injury (PI) occurrence and to evaluate the effectiveness of targeted preventive nursing interventions in high-risk patients.
A prospective cohort study was conducted from January to December 2024. A total of 120 high-risk patients (Braden scale ≤18) were enrolled and divided into an intervention group (n=60) and a control group (n=60). Bedside B-ultrasound was used to assess subcutaneous tissue thickness and edema at pressure-prone sites (sacrum, heels, trochanters) upon admission and at 48 and 72 hours. The control group received routine nursing care, while the intervention group received individualized preventive protocols based on ultrasound findings, including adjusted repositioning schedules and pressure-redistribution measures. Primary outcomes were PI incidence and time-to-injury.
PI incidence was significantly lower in the intervention group than in the control group (9.5% vs. 23.8%, P=0.012). B-ultrasound-detected subcutaneous edema at 48 hours independently predicted PI development (OR=3.52, 95%CI: 1.58–7.84, P=0.002). The intervention group showed better subcutaneous tissue integrity and higher satisfaction scores (P<0.05).
B-ultrasound monitoring of skin microcirculation offers reliable early warning indicators for PI risk stratification. Ultrasound-guided preventive nursing interventions significantly reduce PI incidence, providing a practical approach for clinical nursing management of high-risk patients.

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Neutrophils in Wound healing: effect of calcium alginate-based dressings.

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During wound healing, inflammation is a crucial early phase that recruits innate immune cells, notably neutrophils, to remove pathogens and debris. Although a strong early neutrophil response helps prevent infection, excessive or prolonged activation can impair healing through the accumulation of reactive oxygen species (ROS) and neutrophil extracellular traps (NETs), as seen in chronic wounds.
Calcium alginate dressings are widely used to promote healing through exudate management and Ca2+ release. Ca2+ can activate several cell types in the wound bed. Depending on their composition, alginate dressings have been reported to stimulate innate immune cells (NK cells and macrophages).
This study examined the effects of three alginate dressings on neutrophil activation compared to a 3 mM Ca2+ solution: Algostéril® (ALG, 100% Ca2+ alginate, guluronic acid-rich), Biatain® Alginate (BIA, 80% Ca2+ alginate, 20% carboxymethylcellulose), and Algisite® M (SITE, 100% Ca2+ alginate, mannuronic acid-rich). Conditioned media (CM) from each dressing were tested on neutrophils isolated from healthy donors. The effect of neutrophil supernatants (NS) on fibroblast was also evaluated.
All dressings, but not Ca2+ alone, induced neutrophil activation, shown by reduced CD62L expression and increased degranulation markers CD63 and CD66b. Phagocytosis significantly decreased with BIA. ROS and NET production significantly increased with BIA and SITE, independently of neutrophil previous activation. In contrast, ALG and Ca2+ solution did not alter phagocytosis or NETosis. Fibroblast migration decreased in the presence of BIA NS, whereas ALG NS preserved migration. These findings suggest that Ca2+ is not responsible for the observed neutrophil effects; dressing composition is a key determinant as SITE followed by BIA strongly activate neutrophils compared to ALG. Therefore, selecting the appropriate dressing may be critical for hard-to-heal wounds where neutrophils are overactivated.

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Investigating the Intrinsic Regulation of the Hair Follicle Stem Cell Niche by MEF2C

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The mesenchyme-derived dermal papilla (DP) is a hair inductive niche that has the ability to instruct the ectodermal hair follicle stem cell compartment to drive hair follicle morphogenesis and regeneration, but the molecular mechanisms still remain largely unclear. While there is loss of DP inductivity when cultured in 2D, restoration is possible by culturing DP cells in 3D spheroids. To study chromatin arrangement differences between 2D vs 3D cultures of inductive DP cells and non-inductive, mesenchyme-derived papillary fibroblasts, Assay for Transposase-Accessible Chromatin (ATAC)-sequencing was conducted. This revealed an enrichment of Myocyte-Enhancer Factor 2C (MEF2C)-associated motifs in 3D DP cells as compared to papillary fibroblasts *in vitro*. Therefore, to investigate MEF2C function in the DP, a conditional knockout (cKO) of *Mef2c* in murine DP cells was generated using $Lepr^{Cre}$. Histological analysis revealed disrupted hair cycling during catagen, and also sebaceous gland size differences in the *Mef2c* cKO mice. Next, transcriptomic profiling of *Mef2c* cKO mice revealed transient upregulation of *Sox18* in telogen, causing dysregulation in the DP transcriptome during anagen. Additionally, hair inductive potential of *Mef2c* cKO DP was significantly compromised as shown by hair reconstitution assays. *Sox18* knockdown *in vitro* also suggested a Mef2c-Sox18 regulatory axis contributes to normal DP hair inductivity. Collectively, these findings highlight Mef2c as a key

transcriptional regulator of DP function and suggest that the *Mef2c-Sox18* axis may represent a potential therapeutic target in hair loss disorders such as androgenetic alopecia.

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Comprehensive evaluation of fractional CO_2 laser treatment's effects on ex vivo cultured human skin samples

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Fractional CO_2 laser resurfacing is a major dermatologic innovation in recent decades. The micro-injuries induced by the treatment usually heal without scarring, but the exact molecular mechanisms distinguishing this controlled repair from conventional wound healing remain not fully understood. Thus, we aimed to study the molecular effects of fractional CO2 laser treatment. *Ex vivo* laser-treated healthy skin samples, parallel-prepared cutting-type wound samples, and untreated controls were cultured on cell culture inserts at an air–liquid interface for 24 hours. Cytokine/chemokine expression was studied by protein array method from the culture media and analyzed using the String and Reactome databases. Compared to control samples, differentially expressed proteins (DEPs) with two-fold increase in both laser-treated and cutting-type wounds were the FGF2, IL-10 and PDGFA, which were mostly related to the wound healing response. DEPs that were exclusively upregulated in laser-treated samples, namely ANGPT2, TDGF1 and IL-31, were mainly implicated proliferation-related gene expression. Conversely, DEPs that were selectively upregulated in the cutting-type group, were primarily associated with IL-4 and IL-13 signaling and VEGF receptor activation. Consistent with these findings, hematoxylin-eosin staining indicated the initiation of re-epithelialization in both laser-treated and cutting-type wound samples. Using immunofluorescence staining, we observed at the micro-wound edges of laser-treated vs. cutting-type wounds, that keratin-17 exhibited a more extensive distribution, and actin expression was also more intense. Overall, our results suggest rapid activation of keratinocytes and wound healing process due to fractional CO_2 laser-treatment. Enhanced insight into the molecular effects of fractional CO2 laser treatment may guide not only its aesthetic but also potential broader clinical applications.

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An olfactory receptor–targeting DNA aptamer promotes wound repair in a skin-humanized model of epidermolysis bullosa

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Epidermolysis bullosa (EB) is a group of inherited blistering disorders characterized by chronic, difficult-to-heal wounds. Current management is largely palliative, underscoring the need for strategies that improve wound repair while definitive curative approaches are being developed. The purpose of this study is to evaluate a DNA aptamer agonist targeting olfactory receptor 2AT4 (OR2AT4), Ap.OR2AT4.17, as a modality to promote cutaneous wound repair in epidermolysis bullosa. OR2AT4 expression was examined by immunofluorescence in frozen skin sections from normal human skin and from patients with epidermolysis bullosa simplex, junctional epidermolysis bullosa, and recessive dystrophic epidermolysis bullosa, showing OR2AT4 immunoreactivity in epidermolysis bullosa samples comparable to normal human skin under identical staining conditions. Ap.OR2AT4.17 activity was assessed in human keratinocytes using HaCaT cells overexpressing OR2AT4, where the aptamer induced receptor internalization, increased cyclic adenosine monophosphate (cAMP) levels, and activated phosphoinositide 3-kinase/protein kinase B (PI3K/Akt) and mitogen-activated protein kinase/extracellular signal-regulated kinase (MAPK/Erk) signaling. Ap.OR2AT4.17 also promoted proliferative and migratory responses, including cytoskeletal remodeling and junctional reorganization, with increased scratch closure. Therapeutic relevance was evaluated in bioengineered three-dimensional organotypic cultures and in a skin-humanized mouse model generated from EB patient–derived cells, where Ap.OR2AT4.17 treatment showed higher re-epithelialization values compared to controls. These findings support aptamer-mediated activation of OR2AT4 as a promising pro-re-epithelialization approach for epidermolysis bullosa–associated wounds.

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3D Visualization of a Reconstructed Vascular Network in Skin Models Using Tissue Clearing Technology

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This study aims to develop a vascularized full-thickness skin model in a porous synthetic scaffold. Crucially, it introduces the first application of a modified iDisco+ tissue clearing technique to an *in vitro* skin model composed of a synthetic porous scaffold.
A comparative study of various synthetic porous scaffolds, involving different biomaterials and fabrication methods, was conducted using human fibroblasts and keratinocytes. Promising scaffolds were then evaluated for their capacity to host a vascular network by coseeding HUVEC cells with fibroblasts. For characterization, histological and immunostaining analyses were coupled with 3D visualization of cleared tissues.
Histological analysis confirmed excellent skin reconstruction across all tested scaffolds, showing homogeneously distributed fibroblasts synthesizing an accurate dermal extracellular matrix (ECM) and a uniformly stratified and differentiated epidermis. The vascular network proved to be highly scaffold-dependent, emphasizing the potential impact of scaffold material, porosity

and microarchitecture. A tailored bilayered electrospun/melt electrowritten scaffold successfully fostered a dense interconnected vascular network within an endogenous neosynthesized dermal ECM. Reconstructed capillaries displayed lumen and expressed correct differentiation markers. Tissue clearing appears to be a breakthrough technology to detect, visualize and characterize various spatial assets.
This work exemplifies tissue clearing as a powerful tool for unprecedented 3D visualization of the dermal vascular network, thus allowing a new comprehensive, tissue-scale exploration of dermal architecture in *in vitro* reconstructed skin models.

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Mechanism of action of triterpene extract in epidermolysis bullosa: Suppression followed by activation
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Birch bark-derived triterpene extract (TE) is a topical treatment approved in multiple jurisdictions for wounds in junctional and dystrophic epidermolysis bullosa (EB). While its clinical efficacy in promoting wound healing has been demonstrated, the molecular mechanism of action remains incompletely understood, with preclinical studies suggesting modulation of inflammatory mediators. This study aimed to further characterize cellular and signaling responses to TE in keratinocytes from healthy, dystrophic and laminin-332-deficient junctional EB donors. Primary keratinocytes were treated with TE, and signaling responses were analysed using receptor tyrosine kinase arrays, reactive oxygen species (ROS) measurements and pathway inhibition. TE induced an early reduction in activity of epidermal growth factor (EGFR) and insulin-like growth factor receptors (IGFR), followed by delayed EGFR activation selectively in dystrophic EB keratinocytes. This response was not associated with relocalisation of the candidate mediator of EGFR activation ADAM17. Next, we investigated ROS activity, as a common modulator of EGFR and IGFIR signaling. Keratinocytes from EB donors exhibited elevated baseline ROS levels, which were rapidly and transiently suppressed by TE across all genotypes. TE has previously been shown to also induce pro-inflammatory mediators which are linked to Janus kinase signaling. However, inhibition of JAK1 did not alter TE-associated wound healing activity. In conclusion, TE elicits both shared and genotype-specific responses in keratinocytes, including transient suppression of oxidative state and growth factor receptor signalling. Future studies will determine if the mediators and the ensuing initial suppression serve as a wound microenvironment resetting mechanism promoting wound healing.

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In vivo Efficacy of Sustained Release of Collagenase Clostridium Histolyticum for Dermal Fibrosis
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This study investigated the in vivo efficacy of a novel therapeutic to soften and remodel skin scars using sustained release of purified collagenase Clostridium histolyticum (CCH). Prior clinical studies have shown that injection of CCH in solution is ineffective in hypertrophic skin scars. We hypothesized that sustained local release would enhance efficacy by prolonging exposure and limiting enzyme washout. We have developed a sustained release CCH hydrogel using alginate and a biocompatible nanoclay additive to tune release kinetics. The effect on skin mechanics was first evaluated ex vivo in swine skin explants, where the CCH hydrogel significantly reduced tissue stiffness both via injection (38% reduction in modulus at day 5, $p<0.05$) and via topical application after CO2 fractional laser treatment (50% reduction in modulus at day 5, $p<0.05$). In vivo tolerance and efficacy were then evaluated in the mouse bleomycin model of dermal fibrosis. The CCH hydrogel caused significant collagen degradation at day 1 with mild inflammatory infiltrate. At day 7, dermal thickness was reduced by 42% (bleomycin – 425±35μm, bleomycin + CCH hydrogel – 247±10μm, $p<0.05$) with no apparent cytotoxicity. Injection of CCH in solution was ineffective in both the swine and mouse experiments, matching the previous clinical findings. These results validate the efficacy of sustained release of CCH as a prospective therapeutic for dermal fibrosis. Softening and reducing dermal thickness can provide symptom relief and improve the appearance of raised, contracted scars. There is also substantial evidence to suggest that reducing tissue stiffness can promote long-term regeneration of normal skin architecture. This treatment may have applications in the management of hypertrophic skin scars, keloids, scleroderma, and a wide variety of other fibrotic conditions occurring in the skin and throughout the body.

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Burn blister exudate as a dynamic pro-healing microenvironment in early wound repair
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The optimal clinical management of burn blisters remains unclear, with debate over whether blisters should be left intact, aspirated, or deroofed. The biological role of blister exudate in wound healing is poorly defined and lacks molecular evidence to guide clinical decisions. This study aimed to characterize the proteomic composition of blister exudate relative to plasma, investigate temporal changes during the first four days post-burn, and assess its role in wound repair.
Blister exudate and matched plasma samples were collected from 32 burn patients within 96 hours. Proteomic profiling was performed using liquid chromatography–tandem mass spectrometry (LC–MS/MS), followed by comparative and differential expression analyses.
Exudate showed a distinct proteomic profile compared with plasma. A total of 1,671 proteins were identified in exudate and 613 in plasma, including 509 shared proteins (129 upregulated, 135 downregulated). Notably, 1,162 proteins (~70%) were unique to exudate, indicating a predominantly local, wound-derived origin rather than a plasma filtrate. Temporal analysis showed a transition from early inflammation (day 1) to early proliferative repair (days 3–4). Early responses included activation of inflammatory, antimicrobial, and oxidative pathways alongside endogenous inhibitors, reflecting a balanced host response. From days 2–4, peak activity was observed in proteins involved in keratinocyte activation, extracellular matrix remodeling, angiogenesis, and immune defence.
Blister exudate is a biologically active microenvironment whose evolution supports antimicrobial defence, re-epithelialization,

and tissue remodeling. Early removal or deroofing may disrupt these processes, supporting preservation strategies. These findings provide a molecular basis for preserving blister integrity in early burn management.

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Laminin-332-derived motifs displayed on Caf1 promote organised re-epithelialisation in human *ex vivo* wounds

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Delayed re-epithelialisation remains a challenge in skin wound healing. Full-length basement membrane proteins provide important biological cues, but their structural complexity and limited stability restrict their therapeutic application. Here, we used Caf1, a stable and engineerable scaffold, to display short laminin-derived motifs in a defined and scalable format. These constructs were evaluated in a human *ex vivo* wound model for their ability to support re-epithelialisation.
Standardised wounds were generated in normal human skin explants from three donors and maintained at the air-liquid interface. Explants were treated with phosphate-buffered saline (PBS), recombinant human laminin-332, Caf1-WT, or Caf1 polymers displaying J3 and/or J4, two laminin-332-derived motifs from the α3 and γ2 chains, respectively. Repair was assessed at days 3, 5 and 7 by haematoxylin and eosin staining, with epithelial coverage and epidermal uniformity quantified. Keratin 16 and Ki67 immunostaining were used to assess epidermal activation and proliferation.
Caf1 polymers showed motif-dependent effects. At day 3, epithelial coverage was limited in PBS and Caf1-WT controls, but increased with Caf1-J3/WT, Caf1-J4/WT and Caf1-J3+J4/WT. By day 7, Caf1-J4/WT and Caf1-J3+J4/WT achieved near-complete epithelial coverage (99.4% and 99.6%, respectively), compared with 83.0% for PBS and 76.8% for Caf1-WT. Epithelial architecture differed at day 7, with the epithelial uniformity index lowest in PBS-treated wounds (0.32 ± 0.14) and increased in the laminin-332, Caf1-J4/WT and Caf1-J3+J4/WT groups. Caf1-J3+J4/WT produced the most uniform neo-epidermis, with the highest epithelial uniformity index (0.67 ± 0.12, $P < 0.0001$ vs. PBS). Keratin 16 staining at day 3 was increased in the J4/WT and J3+J4/WT groups, with levels approximately double those of PBS-treated wounds, whereas Ki67 remained modest, suggesting enhanced repair without excessive proliferation.
These findings indicate that engineered Caf1 scaffolds displaying short laminin-332-derived motifs can support organised human skin repair *ex vivo*, highlighting Caf1-based motif display as a promising approach for wound repair.

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HE1700 accelerates early wound closure and modulates ECM-remodeling transcripts in human full thickness 3D skin models

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Full thickness human 3D skin models closely resemble the native skin and allow reproducible investigations of wound healing at the molecular and histological level. We investigated whether HE1700, a multicomponent, multitarget medication made from natural ingredients, influences extracellular matrix (ECM) remodeling and wound closure after standardized laser injury. Differentiated full thickness 3D skin models generated from primary normal human keratinocytes and dermal fibroblasts were wounded using a fractional ablative Er:YAG laser (E10% mode, 60 J). Models were treated immediately after wounding and every second day with 20% HE1700 (verum) or 20% placebo in culture medium; untreated models served as controls. Histological analyses were performed at 16 h and day 5 post wounding, including quantification of lesion width and depth (≥20 lesions/group) at 16 h. Transcriptomic profiling by 3′mRNA Seq and gene ontology analysis was conducted at 16 h.
Untreated models exhibited lesions affecting the entire epidermis and upper dermis with a mean depth of 167 µm and width of 286 µm at 16 h. Placebo treatment moderately improved wound closure (51 µm/166 µm). HE1700 induced a significantly stronger effect with near complete dermal closure and further reductions in lesion depth (29 µm) and width (102 µm). At day 5 post wounding assessment, no residual lesions were detectable in any group. Transcriptomic analysis identified 88 differentially expressed genes ($p < 0.05$ and fold change ≥ 1.5) enriched in pathways related to collagen and elastin catabolic pathways, epithelial cell differentiation and regulation of cell population proliferation.
In conclusion, HE1700 significantly enhanced wound closure in standardized human 3D skin wounds and induced modest regulation of ECM remodeling and epidermal differentiation transcripts, partially explaining its wound healing activity.

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Bioactive inks as potential wound healing therapeutics: cellular responses in the presence of chronic wound exudates

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Exudates of chronic wounds (cWEs) contain pathophysiological factors which maintain the wound. In normal primary human cell cultures, cWEs have been found to inhibit fibroblast proliferation and induce pro-inflammatory cytokine secretion in macrophages. These *ex vivo* systems were used to investigate hyaluronic acid-based bioactive inks (BI) designed to i) combat inflammation and bacterial infections and ii) promote healing.
Exudates from chronic wound patients were harvested from polyurethane foam discs, used bandages or negative pressure wound therapy sponges. BIs or their individual components were added to primary human dermal fibroblasts or macrophages in the presence of cWEs. Cell proliferation was determined upon fixation and staining, and cytokines were quantified by ELISA or multiplex analysis.
BIs were more effective in rescuing fibroblasts and suppressing cytokine secretion in macrophages than their individual components. Pro-regenerative BI was superior to anti-bacterial and anti-inflammatory BIs. At least one of the BIs improved fibroblast proliferation in the presence of 12 of 15 cWEs, and at least one BI significantly suppressed the pro-inflammatory cytokines TNFα or IL-1ß with 6 of 15 cWEs. Results for 11 additional wound-related analytes (cytokines, chemokines, growth

factors) were inconclusive. With one cWE, all BIs significantly enhanced fibroblast proliferation and reduced macrophage TNFα and IL-1ß secretion.
In summary, BIs increased fibroblast proliferation and reduced macrophage TNFα and IL-1β secretion in ex *vivo* assays. Their effects showed marked variability across different cWEs. It is likely that the *in vivo* effect of the bioinks will depend upon the individual wound environment and ex-vivo pre-testing may be required. Future clinical studies will address the translatability of these results to wound therapy.

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Stem cell dynamics in human hair follicle development
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The hair follicle is a complex mini-organ crucial for skin regeneration and health. Understanding the molecular drivers of its development could inform future therapeutic strategies for follicular-related diseases such as alopecia and hidradenitis suppurativa. Human hair follicle morphogenesis occurs exclusively during prenatal development, and while many of its components are well described, little is known about the origins of several cell types relevant for skin regeneration, such as the stem cell populations in the dermal sheath, bulge, and sebaceous gland. Additionally, our understanding of these processes is mainly derived from rodents, where conservation across species remains unclear.
Here, we combine spatial transcriptomics, single-cell multiomics, and machine learning to map human hair follicle development from 8-19 PCW. We use this dataset, comprising 900k cells from 39 sections, to uncover an exciting dual fate of the dermal condensate, which gives rise not only to the dermal papilla but also the dermal sheath, a smooth muscle-like envelope surrounding the hair follicle with roles in wound healing and cell-cycle regulation. We further confirm the existence of vertical transcriptional segmentation in early shaft development that determines the positioning of the bulge and sebaceous gland. Finally, we describe the molecular drivers and phenotypes of the companion layer, sebaceous gland, and arrector pili muscle. Taken together, these findings show how species-specific differentiation processes in the epithelial component influence mesenchymal dynamics. We expect these changes to be crucial for inducing HFs in planar skin equivalents, thereby providing better pre-clinical models and moving towards hair-bearing skin grafts. Lastly, the dataset generated in this study will serve as a foundational resource for understanding developmental drivers in health and disease.

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Circulating Cytokine Profiling in Diabetic Foot Ulcer Patients Reveals Differential Angiogenic Signatures Associated with Cardiovascular Outcomes
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Diabetic foot ulcers (DFUs) are a major complication of diabetes, characterized by impaired wound healing and an increased risk of cardiovascular events. This study aimed to characterize circulating cytokine profiles in DFU patients and to investigate their association with cardiovascular outcomes, focusing on angiogenic mediators. Serum levels of 46 cytokines were measured using a multiplex Luminex assay in DFU patients (n=35) and healthy controls (n=7). Baseline cytokine levels were compared between groups and within the patient cohort, according to the presence or absence of major cardiovascular events, using non-parametric tests with false discovery rate correction. Compared to healthy controls, patients with DFUs exhibited significantly higher levels of vascular endothelial growth factor (VEGF) and interleukin (IL)-8, consistent with activation of hypoxia-driven angiogenic pathways. Within the patient cohort, both cytokines were significantly elevated in individuals without prior cardiovascular events compared to those with events, with moderate-to-large effect sizes, suggesting a more effective angiogenic response. These findings align with previous evidence linking angiogenic cytokines to improved tissue repair. Circulating angiogenic cytokines may serve as potential biomarkers for wound healing capacity and cardiovascular risk stratification in patients with DFUs, possibly reflecting compensatory mechanisms of collateral angiogenesis.

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Differential effects of dihydrotestosterone and minoxidil on angiogenic and profibrotic factors in dermal papilla cells
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Androgenetic alopecia involves changes in the signaling within the follicular microenvironment, however the mechanisms responsible for incomplete therapeutic responses remain poorly understood. We investigated how dihydrotestosterone (DHT) modulates the expression of angiogenic and profibrotic factors in human dermal papilla cells (DPC) and evaluated if these effects are counteracted by minoxidil.
DPC were exposed to DHT with or without minoxidil, and changes in VEGF and TGF β1 mRNA expression were quantified. Functional angiogenic activity was assessed through endothelial cell (HUVEC) migration assays using conditioned media from treated and control DPC. The expression of sulfotransferase 1A1 (SULT1A1), necessary for the bioactivation of minoxidil, was evaluated in DPC and HUVEC.
DHT significantly reduced VEGF expression while increasing TGF β1, indicating a shift towards reduced blood vessel formation and increased fibrous tissue production. Conditioned media from DHT-treated DPC impaired HUVEC migration, confirming a decrease in support for new blood vessel growth. Minoxidil partially restored HUVEC migration but failed to impact in VEGF downregulation and TGF β1 upregulation after DHT treatment. SULT1A1 was not found in DPC, but it was present in endothelial cells. There were no notable differences between treatments with minoxidil and minoxidil sulfate in DPC.
These results suggest that DHT creates a microenvironment in DPC that does not respond directly to minoxidil. The absence of

local bioactivation systems in DPC indicates that minoxidil's effectiveness may rely on neighboring cells, highlighting intercellular metabolic cooperation as a potential determinant of therapeutic response in androgenetic alopecia.

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Spatio-temporal dynamics of melanocytes following epidermal injury in neonatal mice

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During wound healing, the balance between stem cell differentiation and maintenance is tightly regulated to support tissue regeneration. We previously established a suction blister model that selectively injures the epidermis while preserving hair follicles, demonstrating that hair follicle-derived stem cells accumulate in the interfollicular epidermis(IFE) at wound sites. While studies in adult mice have shown that full-thickness wounding mobilizes melanocyte stem cells from hair follicles into the epidermis, it remains unclear how melanocytes respond to selective epidermal wounding, particularly in the early stages of development. We aimed to clarify melanocyte dynamics following epidermal wounding in neonatal mice using the suction blister model. Suction blisters were created on the dorsal skin of postnatal day 0 (P0) mice by applying negative pressure with a syringe, with unwounded skin serving as a control. Melanocyte behavior was assessed until P56 by immunostaining for premelanosome protein (Pmel) and tyrosinase-related protein 2 (TRP2) alongside Fontana-Masson staining to visualize melanin granules. Immunostaining revealed a transient increase in Pmel- and TRP2-positive cells in the IFE. The number of these marker-positive cells reached a peak and subsequently declined by P25, suggesting a potential shift in the cellular state or localization of the recruited melanocytes. Fontana-Masson staining demonstrated increased melanin granule-positive cells at P4 and P8 showing melanin presence in the wounded area. These findings demonstrate that epidermal wounding transiently mobilizes melanocytes from hair follicles into the IFE, providing a basis for further understanding the cellular dynamics involved in neonatal wound healing and pigmentary responses.

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A novel microbiota-inspired trace amine-derivative counteracts stress-impaired wound healing by targeting the β2-adrenergic receptor

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Psychological stress may alter skin homeostasis and delay tissue repair through cortisol or catecholamine release. Among catecholamines, epinephrine activates the β_2-adrenergic receptor (β2-AR), and excessive levels of this hormone are known to impair skin regeneration and to cause premature aging. While its effect on keratinocyte migration is well documented, its effects on dermal fibroblasts and extracellular matrix remodeling remain unclear.
This study investigates how epinephrine impairs dermal repair and whether β2-AR modulation can reverse these effects using timolol or a trace amine (TA)-mimic, a cosmetic-grade compound inspired by skin microbiota metabolites.
β2-AR involvement was explored by *in silico* docking and ERK phosphorylation analysis. Keratinocytes and dermal fibroblasts were exposed to epinephrine in the presence or in the absence of timolol (a β-AR antagonist) or the TA-mimic. Cell migration was assessed using a scratch assay and fibroblast function by measuring collagen I expression. The resulting tissue architecture was evaluated using human skin explants.
Epinephrine reduces keratinocyte and fibroblast migration (by 56% and 32% respectively) and decreases collagen I production, thus confirming a detrimental role in dermal repair. Timolol and the TA-mimic completely restored both skin cell migration capacities, and collagen synthesis and organization. The molecular analysis highlighted interactions between the TA-mimic and the β2-AR leading to a normalization of ERK signaling. Taken together, this suggests that the TA-mimic partially antagonizes β2-AR and thus opposes the effects of epinephrine on the skin regenerative capacities.
These results demonstrate that stress-related catecholamines impair epidermal and dermal repair. Functional modulation of β2-adrenergic signaling by TA-mimics appears as a relevant therapeutic strategy to preserve skin regeneration and improve repair under psychological stress.

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AI-assisted analysis enhances scratch assay quantification of ectoine-dependent migration in human skin cell lines

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Ectoine, a naturally occurring osmolyte, is widely used in dermatological applications due to its cytoprotective and stabilizing properties. While its effects on cell viability are well described, its influence on cell migration and wound healing dynamics remains poorly understood. Sratch assays represent a standard in vitro approach to assess these processes, but its evaluation is often limited by manual, low-resolution quantification. Two independent experiments were performed to investigate ectoine-dependent effects on cell migration in human skin cell lines and to directly compare manual versus AI-assisted analysis methods. Scratch closure of HaCaT keratinocytes was manually quantified at 0, 12, and 24 h after treatment with 100 and 200 mM ectoine. In parallel, HaCaT and A431 cells were analyzed under identical conditions including 10, 100, and 200 mM ectoine. For live-cell imaging an ImageXpress microscope was used with image acquisition controlled via MetaXpress software. The images were converted and analyzed using the AI-based software InCarta, enabling automated quantification every 30 min over 24h. Manual analysis revealed a concentration-dependent inhibition of cell migration in HaCaT cells, with stronger effects at 200 mM ectoine compared to 100 mM. AI-assisted analysis confirmed these findings and further identified subtle temporal differences in migration kinetics as well as minor effects at low ectoine concentrations (10 mM). A431 cells exhibited faster scratch closure compared to HaCaT but showed comparable responses to ectoine treatment. Importantly, the higher temporal resolution and increased data density of the AI-assisted approach resulted in reduced variability, enabling detection of smaller effect sizes and improving statistical sensitivity. Untreated controls displayed consistent scratch closure within 24 h, confirming assay robustness.
The combination of live imaging and AI-assisted quantification substantially enhances the analytical power of scratch assays,

allowing more precise characterization of ectoine-dependent effects on cell migration and providing a robust tool for future wound healing studies.

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Wound healing effects of birch bark and propolis extracts on epidermolysis bullosa keratinocytes

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Epidermolysis bullosa (EB) is a group of genetic skin fragility diseases and despite the current therapeutic options aim to achieve accelerated wound healing, there is still scope for improvement; therefore, birch bark and propolis were investigated due to their beneficial biological properties. A representative ethanolic extract was analyzed by RP-HPLC-DAD to characterize the chemical profile of the raw materials. A hydrophobic natural deep eutectic solvent (HNaDES) for birch bark extraction as well as a hydrogel and a bigel formulation, were prepared and characterized by FT-IR spectroscopy. Cytotoxicity and wound-healing potential were evaluated using MTT and scratch assays in six human keratinocyte cell lines: two from healthy individuals, two from recessive dystrophic EB patients, and two from laminin-332 deficient junctional EB patients. Chemical analysis revealed the presence of phenolic compounds and pentacyclic triterpenes in propolis and birch bark, respectively. FT-IR confirmed HNaDES formation and indicated physical interactions within the gels. All systems exhibited no cytotoxicity at 1 µg/mL and increased cell vitality. Moreover, in keratinocytes derived from JEB patients, hydrogel improved wound healing significantly at 24 h, whereas bigel showed significant improvement at 8 h. The developed systems could be promising topical treatments.

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Tobramycin promotes wound healing *via* antioxidant and anti-inflammatory effects

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Tobramycin (TOBRA) is an aminoglycoside antibiotic widely used to treat Gram-negative bacterial infections, including those caused by *Pseudomonas aeruginosa*. Notably, clinical use of TOBRA in infected chronic ulcers has been associated with improved wound healing outcomes.
Reactive oxygen species (ROS) are increased in diabetic conditions and contribute to diabetic foot ulcers (DFUs). We previously showed that ROS upregulate miR-200c, which promotes apoptosis, senescence, oxidative stress, nitric oxide reduction, and endothelial dysfunction. miR-200c impairs wound healing (WH), while its inhibition, especially combined with antioxidant catalase, improves diabetic skin repair.
Here we investigated whether TOBRA modulates ROS and miR-200c in cutaneous cells. We analyzed immortalized keratinocytes (HaCaT), primary fibroblasts (FBs) derived from diabetic patients undergoing amputations, as well as from healthy donors undergoing saphenectomy (controls), under basal conditions and H_2O_2-induced stress.
TOBRA significantly reduced ROS and miR-200c in all cell types both at basal and after oxidative stress. Analysis of pri-miR and pre-miR showed similar reductions, indicating no effect on miRNA processing, but an upstream action *via* ROS decrease. Consistently, miR-200c targets ZEB1 and SIRT1 were upregulated upon TOBRA treatment, while inflammatory genes (IL6, CCL2, PTGS2) were reduced under ROS and cytokine mix exposure in DFU FBs and healthy FBs.
Functionally, TOBRA strongly accelerated *in vitro* WH in FBs and HaCaT under basal and oxidative conditions.
In conclusion, beyond its antibiotic activity, TOBRA exerts antioxidant and anti-inflammatory effects in cutaneous cells by modulating the ROS/miR-200c axis strongly involved in the WH process, explaining at least in part the observed therapeutic role in chronic skin ulcers.

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Powerful study models to investigate the dynamic of skin healing

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Impaired wound healing remains a major public health concern. The proliferation phase, involving granulation tissue formation, angiogenesis, re-epithelialization, and inflammation resolution, is critical for tissue healing. This study aims to develop innovative multi-scale models mimicking the dynamic of this process and to characterize the efficacy of a natural active ingredient (NAI) on skin healing.
In this context, two advanced study models were developed:
1. A novel *in vitro* wounded, vascularized, and immunocompetent reconstructed skin (wounded VI SILABSKIN® FT) allowed the investigation of the healing markers.
2. An injured zebrafish larvae model was used to track the healing kinetics. Advanced 3D imaging and AI-based quantification were used to monitor biological events in real-time.
On these models, the NAI demonstrated a transversal action on the four pillars of the proliferation phase:
Granulation tissue: it induced TGF-β and dermal markers synthesis, as well as the principal fiber characteristics of this tissue, the collagen III.
Angiogenesis: it promoted VEGF secretion and significantly increased vascular network density.
Re-epithelialization: it stimulated growth factors secretion, enhancing basement membrane re-establishment and epidermal differentiation.
Inflammation**:** it limited pro-inflammatory markers while favoring the M2 pro-regenerative macrophage population.
Beyond the study of markers synthesis, these models enabled the analysis of skin healing dynamics. In wounded VI SILABSKIN® FT, the NAI promoted faster wound closure. A result confirmed in the zebrafish model, with a laser injury recovery rate significantly increased by 44%.

Hence, by combining 3D tissue engineering and latest generation imaging technologies, this study demonstrates that these models provide powerful tools for real-time visualization of healing dynamics and confirm the potential of this NAI to improve the rate and to visibly increase the quality of cutaneous repair.

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Pasteurella spp. lysate restores delayed wound healing in specific pathogen-free mice through activation of cathelicidin

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Chronic wounds represent a major global health burden and remain an importantunmet medical need. Studies investigating the mechanisms and treatment of impairedhealing are most often performed in mice. Over the past 50 years, housing conditionshave progressively shifted from conventional (Conv) environments to specificpathogen-free (SPF) facilities, which fail to mimic the natural colonization of the skinand mucosa by diverse microorganisms. However, recent studies have highlighted thecrucial role of microbiota in tissue repair. Therefore, we sought to compare cutaneouswound healing under Conv versus SPF housing conditions. We observed a significantdelay in wound closure in SPF mice compared with Conv mice, both in wild-type miceand in modelscharacterizedby impaired healing (K14-IGF1R knockout mice and sicklecell disease SAD mice). Impaired healing in SPF mice was associated with reducedcathelicidin production, decreased macrophage infiltration, diminishedneovascularisation, and lower cytokine expression. Interestingly, topical application ofaPasteurella spp. lysate,detected only in Conv facilities,accelerated wound closurein SPF mice, restoring activation of cathelicidin pathway. These findings suggest thatSPF housing may not adequately model wound healing and that conventionalconditions or microbiota supplementation may better support human-relevantconclusions.

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The Initation of Vesicant Induced Skin Injury

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Vesicants are blistering compounds developed for use in warfare, and many studies have examined their effects on various tissues, including the skin. Although there are many studies on these compounds, the initiation of vesicant-induced skin injury remains poorly studied. To better understand the mechanisms underlying vesicant-induced skin damage, we are studying the initiation of this injury at the single-cell level using various transcriptomic methods. We employ nitrogen mustard as an analog of sulfur mustard and phenylarsine oxide as an analog of lewisite. Our bulk and single-cell RNA-seq studies, as well as spatial transcriptomics studies, show that vesicant effects on the transcriptome are detected within the first hour, several hours before visible morphological changes appear. We observe an earlier loss of fibroblasts than epidermal keratinocytes, especially after nitrogen mustard application. Using spatial transcriptomics, we observe that neutrophils begin to appear in clusters in the dermis as early as three hours, then migrate throughout the skin and towards the epidermis over the next 21 hours. Single-cell RNA-seq and spatial transcriptomics data reveal two neutrophil populations that differ in their transcriptomes and localization. Additionally, we observe distinct neutrophil localizations in nitrogen mustard and phenylarsine oxide damaged skin, suggesting differences in innate immune cell dynamics between the two agents. The goal is to use these datasets to charcterize the effects of vesicants on skin and to idrentify pathways that can be targeted as means of countering vesicant-induced injury.

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A Type I Collagen Membrane as a Bioactive Scaffold for Skin Repair in Dystrophic Epidermolysis Bullosa

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Biomaterials capable of mimicking the skin extracellular microenvironment represent a promising strategy for Dystrophic Epidermolysis Bullosa (DEB), a rare disorder in which minimal trauma leads to skin lesions and tissue dysfunction. This study investigated whether a novel type I collagen membrane, derived from rat tail tendons, could support and stimulate reparative cellular responses in both healthy and DEB cells. Physical and morphological characterisation by scanning electron microscopy revealed the presence of microfibrils on both the external and internal surfaces of the membrane, observed following material fracture. Cell viability (MTT assay), adhesion, migration (scratch assay), and three-dimensional organotypic culture models were evaluated using human fibroblasts and keratinocytes from healthy and DEB donors, comparing cellular responses on intact and degraded membranes against an unseeded control. The membrane demonstrated biocompatibility, supporting adhesion, viability, and migration even in DEB-derived cells. Notably, degraded collagen fragments did not impair cellular performance, suggesting a favourable microenvironment for tissue repair. The organotypic model confirmed the substrate's capacity to reconstitute the dermal–epidermal interface. Taken together, these findings indicate that collagen degradation functions not merely as matrix breakdown, but as an active biological signal, highlighting this biomaterial's potential as a scaffold for novel therapeutic strategies in rare skin diseases.

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Randomized Phase 3 trial of spesolimab in patients with ulcerative pyoderma gangrenosum: A study protocol

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Pyoderma gangrenosum (PG) is a rare, inflammatory, neutrophilic dermatosis, usually manifesting as rapidly progressive and painful skin ulcers, although rarer, atypical presentations exist. Interleukin-36 (IL-36) is thought to play a key role in PG pathogenesis. This randomized, placebo-controlled, multicenter, Phase 3 study will assess spesolimab, a novel, humanized monoclonal antibody against the IL-36 receptor, in patients with ulcerative PG. The trial has been approved by Independent Ethics Committees of participating centers.

Approximately 90 participants aged ≥18 years with confirmed diagnosis of ulcerative PG, from 21 countries, will be included. In Part 1 of the trial (Weeks 0–27), participants will be randomized 2:1 to receive spesolimab + low-dose oral corticosteroid (n=60) or placebo + low-dose oral corticosteroid (n=30). In Part 2 of the trial (Weeks 28–52), participants with a non-complete response will receive spesolimab; those with a complete response will be re-randomized 1:1 to receive either spesolimab or placebo. The primary endpoint is complete closure and re-epithelization (PGAR-100) of the target ulcer up to Week 26, confirmed ≥2 weeks later. Secondary endpoints include PGAR-100 of the target ulcer at Week 26 and of any measurable ulcer at any time up to Week 26, confirmed ≥2 weeks later. Additional efficacy, safety and biomarker measures will also be assessed. The trial started in January 2025, with estimated trial completion in October 2026.

These results will provide safety and efficacy data on spesolimab as a potential treatment for ulcerative PG.

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Feasibility of indirect treatment comparisons for approved therapies in epidermolysis bullosa

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Newly approved therapies for epidermolysis bullosa (EB) lack head-to-head trials. We assessed feasibility of indirect treatment comparisons (ITCs) using available evidence.

A search identified studies in junctional/dystrophic EB treated with Oleogel-S10 (also known as birch bark extract/birch triterpenes), beremagene geperpavec-svdt (B-VEC) or prademagene zamikeracel (pz-cel), without limits on language, geography or design. We compared study design, eligibility criteria, patient/wound characteristics and outcomes definitions/timepoints. Prognostic factors and effect modifiers were EB subtype, wound size/age, infection and nutritional status. Eleven studies were eligible. The Oleogel-S10 study (EASE) was the only multi-country randomized controlled trial and only study including junctional EB (<12%). Others enrolled mostly recessive dystrophic EB (≥95%; EASE: 78%). Outcome definitions were not comparable: EASE reported incidence of first complete wound closure, B-VEC studies reported proportion of complete closure, and pz-cel studies reported wounds ≥50% healed. Few studies reported time to wound closure or percentage of wound surface area healed. Safety and tolerability reporting used inconsistent timepoints. Differences in baseline characteristics were substantial: median wound age was 5 years in pz-cel studies versus 39 days in Oleogel-S10 studies; median patient age for pz-cel was 21 years, for B-VEC was ~18 years and for Oleogel-S10 was ~13 years. The Oleogel-S10 control (sunflower oil and wax) was not comparable to placebo in other studies.

Heterogeneity in measured endpoints and baseline characteristics limits cross-study comparability. Any ITC would have major limitations. EB therapies are complementary and address distinct aspects of EB management.

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A new collagen biomimetic ingredient driven by AI technology to delay visible signs of aging

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The dermis, fundamental middle layer of skin, has important properties including mechanical strength, elasticity, and epidermal support due to its unique structure composed of collagen, elastin, extrafibrillar matrix, hyaluronic acid (HA), proteoglycans and glycoproteins. With aging, the dermal functions are reduced leading to appearance of wrinkles, loss of elasticity, dryness and sagging appearance.

Founded on the knowledge of the matrix components of dermis and of its architecture, a new innovative technology, named 2HP™ technology, was developed by artificial intelligence (AI) generating a new kind of ingredient. Composed of hydrolyzed HA and two collagen-peptides mimicking 20 skin-collagen chains, the interactions of these key elements were optimized and reinforced by a unique patented step of 2HP™ technology, requiring no chemical modification of these compounds and no additional chemical agents. This molecular complex, thus formed, had its own infrared spectrum determined by Fourier Transform Infrared Spectroscopy (FTIR) and provided a better stability compared to a simple blend containing the same quantity of HA and the two collagen-peptides. The efficiency of the molecular complex on *ex vivo* skin was also tested *versus* a simple blend on the expression of two major collagens, I and IV. These studies showed a beneficial effect of the molecular complex, significatively greater than the increase observed with the blend.

To bring a texturing action, the molecular complex was embedded in a cellulose gum matrix. The effectiveness of the whole ingredient has been evaluated on collagen fibers, among other things, by Picro-sirius red staining showing denser and elongated fibers compared to control.

In this study, we investigated the capabilities of this new technology to enhance the efficiency of a simple blend of different

components. The comparison between the molecular complex and the simple blend highlighted superior effects of the 2HP™ technology, bringing a new generation of dermal biomimetic ingredients.

396 [WITHDRAWN]

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Therapeutic Re-programming of p63 splicing: A novel splicing ASO-based strategy to rescue epidermal homeostasis in AEC Syndrome

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The *TP63* gene acts as a master regulator of epidermal commitment and homeostasis, but in Ankyloblepharon-Ectodermal defects-Cleft lip/palate (AEC) syndrome, heterozygous missense mutations in exons 13 and 14 of the ΔNp63α isoform trigger protein misfolding and toxic aggregation. These pathological aggregates exert a gain-of-function effect, sequestering p63 and disrupting the core transcriptional program essential for skin integrity. Since the p63β isoform naturally lacks exon 13 and is resistant to aggregation, we explored a splice-switching antisence oligonucleotide (ASOs) mediated strategy to bypass this molecular defect. Using a conditional mouse model, we first demonstrated that p63β is functionally sufficient to sustain epidermal stratification and adult skin homeostasis. Building on these findings, we developed ASOs designed to induce exon 13 skipping, effectively converting the pathogenic ΔNp63α into the functional ΔNp63β variant. Lead ASO candidates screened in human keratinocytes (NHEK N/TERT-2G) demonstrated high-efficiency isoform conversion without compromising cell viability. Crucially, in primary keratinocytes derived from AEC patients (carrying the I537T mutation), ASO treatment significantly reduced p63 aggregation and restored the expression of key p63-dependent transcriptional targets. Overall, these findings establish ASO-mediated p63 isoform switching as a potent and rational therapeutic approach for AEC syndrome, offering a promising strategy to restore epidermal function by neutralizing toxic protein aggregates.

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Lipid nanoparticles enable gene therapy in cellular and organotypic models of Hailey-Hailey disease

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Hailey-Hailey disease (HHD) is a rare skin blistering disorder lacking approved therapies despite discovery of its genetic etiology 25 years ago. Pathogenic *ATP2C1* variants cause haplo-insufficiency of Golgi calcium pump SPCA1, which disrupts epidermal cell-cell junctions, the hallmark of HHD. Because knockout (KO) mice failed to replicate HHD, we used CRISPR/Cas9 to ablate *ATP2C1* in human keratinocytes. SPCA1 deficiency compromised cell-cell junctions in *ATP2C1* heterozygous (HET) keratinocyte sheets and organotypic epidermis, producing HHD models amenable to therapeutic screening. Since lipid nanoparticles (LNPs) can deliver nucleic acids to intact tissues, we aimed to optimize LNP transfection of human keratinocytes for gene therapy. We first synthesized LNPs containing mRNA or DNA encoding GFP. Treatment of control or HET cells with 200 ng/ml mRNA LNPs produced visible GFP in 99% of cells by 24 hr with fluorescence peaking at 48 hr and decreasing over 10 days. Using DNA LNPs also at 200 ng/ml, GFP was highly expressed by 48 hr in only 30% of cells but remained stable beyond 11 days. Transfection efficiency was comparable in control and HET cells. To model subcutaneous delivery, we added 200 ng/ml LNPs to the medium underlying mature organotypic epidermis. After 48 hr, confocal microscopy revealed GFP in all layers of live control and HET tissues; mRNA LNPs again induced less GFP in a large majority of cells while DNA LNPs produced more GFP but in fewer cells. Based on these data, we synthesized LNPs containing FLAG-tagged *ATP2C1* mRNA to assess gene replacement in KO cells lacking SPCA1. Immunoblotting lysates from LNP-treated KO cells revealed a 100 kD band recognized by anti-SPCA1 that aligned with the endogenous protein in control lysates. FLAG immunostaining after 48 hr of LNP treatment showed peri-nuclear puncta consistent with Golgi localization. Importantly, treating KO cultures with *ATP2C1* mRNA LNPs rescued desmosomal cadherin localization and strengthened keratinocyte sheet integrity. Our results show that LNPs facilitate nucleic acid delivery to human keratinocytes and organotypic cultures, enabling epidermal gene therapy for orphan diseases like HHD.

399 [WITHDWRAWN]

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A targetable mitochondrial dependency in CYLD cutaneous syndrome

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The progressive growth of inherited CYLD cutaneous syndrome (CCS) hair follicle tumours, results in amongst the largest skin tumours in humans and is incompletely understood. Recently, we reported deregulated NF-kB signalling in CCS, a direct regulator of mitochondrial biogenesis, leading us to investigate mitochondrial dependency as a mechanism that may support progressive CCS tumour growth. Transcriptomic analysis of CCS and control RNAseq datasets (Bulk n=16 and single cell n=19, K=424,419) identified 115 overexpressed mitochondrial genes (FC>2, adj. $p<0.05$), that were upregulated in CCS keratinocytes at the single-cell level. Mitocarta 3.0 term mapping revealed these transcripts related to oxidative phosphorylation and ATP synthesis, mitochondrial ribosome assembly, mitochondrial dynamics, and pyruvate metabolism. CCS single cell proteomic (n=1, K=419 cells) analysis revealed metabolically heterogeneous cell states ranging from glycolysis predominant to oxidative phosphorylation predominant, detectable as a TFAM associated gradient through cell trajectory analysis.

Immunohistochemical TFAM protein expression (n=12) was highest at the edge of the tumour islands. Metabolomic profiling (n=14) further validated a metabolic shift in CCS tumours compared to healthy skin, where an increased NADH/NAD ratio supported aerobic glycolysis. Immunoblotting of CCS tumour lysates (n=7) revealed increased PGC1-alpha, TFAM, and complex I-III mitochondrial proteins in CCS tumour lysates compared to healthy skin. Quadruple staining (n=6) spatially located increased Complex I centrally in CCS tumour islands. Functional analysis in CCS primary keratinocytes utilising the Seahorse XF platform demonstrated increased respiratory capacity in CCS tumour cells. Dithranol, a clinical therapeutic known to target mitochondria in keratinocytes, killed CCS primary cells in culture, associated with an increase in cleaved caspase 3. Taken together, we report a mitochondrially driven tumour cell dependency in CCS that may be relevant for clinical targeting.

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Improving Skin Regeneration in Junctional Epidermolysis Bullosa by Targeting the Integrin αvβ6

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Epithelial integrin αvβ6 plays key roles in skin repair, particularly in activating latent TGFβ, and it is dysregulated in chronic wounds. αvβ6 is transiently upregulated in blistered skin, but becomes chronic in epidermolysis bullosa (EB), including junctional EB. Here, we explore how pathological αvβ6 upregulation in EB drives defective wound healing, inflammation, and fibrosis, and whether its targeted inhibition improves EB skin.
Using our JEB in vitro models, we investigated TGFβ/αvβ6 signalling upon *LAMA3* depletion by immunofluorescence, FACS, ELISA and western blot analysis. We utilized a tamoxifen-inducible JEB model with epidermis-specific LAMA3 depletion (eKO) to assess αvβ6 signalling by histology and immunofluorescence. Two novel αvβ6 blocking antibodies 264RAD and B686 with different affinities were tested in vitro and in vivo for their potential to improve JEB skin health and wound healing.
αvβ6 integrin expression is significantly upregulated in epidermis of JEB eKO mice and patients, especially in the blistering areas. Mechanistically we show that enhanced TGFβ secretion and an altered cell adhesion induces elevated αvβ6 integrin expression in JEB keratinocytes which was efficiently inhibited with both blocking antibodies. In vivo 264RAD treatment shows strong integrin αvβ6 inhibition and a modest increase in skin cell proliferation, angiogenesis and FoxP3+ Treg cells. Importantly, a reduction in skin fibrosis, reduced blistering and an overall improvement in wound healing is observed in JEB eKO mice treated with 264RAD.
In conclusion, elevated αvβ6 expression in JEB skin is associated with enhanced inflammatory and fibrosis markers in blistered and wounded skin, suggesting a key role in JEB pathogenesis. Our preclinical study indicates that αvβ6 inhibition is a promising novel therapeutic target to improve skin health and regeneration in JEB and possibly other EB subtypes.

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Keratin mutations associated with epidermolysis bullosa simplex induce nuclear fragility and inflammatory stress responses

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Epidermolysis bullosa simplex (EBS) is caused by mutations in cytokeratins 5 or 14 (K5, K14), which lead to keratin filament instability, protein aggregation, and epidermal blistering. In this study we aimed to understand how keratin mutations impact mechanotransduction, stress responses and contribute to pathogenesis in EBS.
We generated N/TERT keratinocyte cell lines stably expressing wild type (WT), R125H, or R416P mutant K14. WT cells retained normal keratin network organisation, whilst in both mutant K14 lines, keratin aggregates formed at the cell periphery. Mutant K14 keratinocytes displayed significant changes in nuclear architecture, including nuclear deformation (circularity<0.8), increased DNA damage, and increased expression of lamin B1. Mutant cells also displayed disorganised microtubule networks, and disruption of microtubules with nocodazole partially rescued nuclear deformity. By contrast, inhibition of acto-myosin tension with Y-27632 did not affect nuclear morphology, suggesting that interplay between keratins and microtubules promotes nuclear defects in EBS keratinocytes.
As nuclear fragility and rupture can activate the cGAS-STING innate immunity sensing pathway, we examined the effects of keratin mutations and mechanical stress on cGAS expression. Cells were subjected to equiaxial, cyclic stretch (0-10% strain, 01.Hz) for 1 hour using a Flexcell FX-5000 tension system. Stretching specifically increased recruitment of cGAS, to the nucleus in mutant K14 cells, and R125H mutants displayed higher baseline cGAS expression compared to WT. Similarly, transcriptional profiling revealed dysregulated inflammatory signalling networks in K14 mutant keratinocytes.
Together, these findings suggest that disruption of nuclear integrity due to keratin network failure activates the cGAS-STING pathway and downstream inflammatory signalling in EBS keratinocytes.

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Mitochondria-mediated COL7A1 delivery restores collagen VII expression in an epidermolysis bullosa patient cell xenograft model.

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Recessive Dystrophic Epidermolysis Bullosa (RDEB) is genetic disease resulting from mutations in the COL7A1 gene, which disrupts collagen VII expression, resulting in impaired dermal-epidermal adhesion, which presents as blistering and chronic impaired wound healing in patients.
Making use of the naturally occurring mitochondria uptake by cells through endocytosis, we developed a mitochondria-mediated delivery platform, using electrostatic interaction, capable of delivering multiple large payloads to cells, circumventing payload size limitations of AAV lentiviral vectors. Treatment with high doses of complex was well tolerated by cells, supporting expression of transgene *in vivo* up to 28 days.
Treatment of RDEB patient cells showed dose-dependent expression of collagen VII and significant thrombospondin-1 downregulation and lysyl-hydroxylase 3 upregulation. In wound healing assays *in vitro,* we demonstrated that the delivery of COL7A1 in RDEB patient fibroblasts and keratinocytes resulted in shortened wound closure time compared to vehicle. We then employed a xenograft model in which EB patient keratinocytes, fibroblasts, and HUVECs were grafted on the back of NSG

mice. Once the grafts were established, full thickness biopsy punch skin wounds were made and single doses of treatment or vehicle were administered. Staining for human CD44 confirmed human origin of the graft and human collagen VII deposition at the basement membrane zone was demonstrated by immunofluorescence after wound closure.
These findings demonstrate that mitochondria mediated-delivery of COL7A1 improves wound healing and restores collagen deposition in a clinically relevant model of RDEB, establishing a novel non-viral therapeutic modality for a severe unmet medical need.

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Inflammation–Pigmentation Crosstalk Drives Pigmentary Dysregulation in Xeroderma Pigmentosum-C Skin Organoids

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Xeroderma pigmentosum (XP) arises from a defective nucleotide excision repair (NER) manifesting an extremely sensitive skin to ultraviolet (UV) radiation, and eventually pigmentary abnormalities and elevated risk of developing skin malignancies at a young age. XP-C complementation group stems from mutations in the *XPC* gene encoding a key player in DNA damage recognition during the first steps of NER. The characteristic pigmentary dysregulation of XP-C remains poorly studied, primarily due to the lack of physiologically pertinent human-derived models. To address this gap, we integrated our pigmented 3D human skin organoids to investigate the mechanisms underlying the pathophysiology of the XP-C disease. Our model revealed that XPC-deficient epidermal microenvironment induces a hyperpigmented phenotype driven by darkly pigmented melanocytes. Such hyperpigmentation was validated molecularly by melanin solubilization and quantification, as well as the assessment of tyrosinase activity and the expression of main players within the pigmentary machinery. Analyzing the secretome of XPC-deficient keratinocytes revealed an augmentation in the secretion of proinflammatory cytokines, which is even more profound in response to UV irradiation. To identify whether the inflammatory status is linked to the visualized hyperpigmentation, we treated our organoids with FDA-approved anti-inflammatory drugs upon their reconstruction. The treatment remarkably counteracted the hyperpigmentation of the XPC-lacking organoids. Collectively, our organoids recapitulated the abnormal pigmentation of XP-C patients, serving as valid model to study the pathophysiology of XP-C, and revealed a strong correlation between the inflammatory XP-C microenvironment and its pigmentary abnormalities.

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Nirogacestat Ameliorates Fibrosis in Murine Recessive Dystrophic Epidermolysis Bullosa

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Recessive dystrophic epidermolysis bullosa (RDEB) is an inherited skin disorder hallmarked by mucocutaneous blistering and hard-to-heal wounds, with chronic inflammation and progressive fibrosis that cause severe complications and lack effective treatments. Our previous *in vitro* findings identified Notch as a key pathway in RDEB fibrosis, supporting its targeting as a novel therapeutic strategy. This study aims at confirming the anti-fibrotic activity of Notch inhibition by the γ-secretase inhibitor nirogacestat (NIR) in an *in vivo* model of RDEB, the type VII collagen hypomorphic mouse. After 13 weeks of treatment, NIR increased body weight, slowed the progression of mitten deformities and reduced TGF-β1 levels in forepaws. Notably, hair loss was markedly reduced, particularly at the abdomen, suggesting that NIR administration counteracts the development of alopecia associated with RDEB. Histological analyses of mouse forepaws showed reduced collagen deposition (Masson's trichrome) and improved bundle organization (Sirius red). Consistent with this NIR-driven fibrosis reduction, a targeted analysis of over 80 fibrosis-related genes by qRT-PCR panels showed a broad down-regulation of multiple transcripts involved in fibrosis and inflammation (*e.g. ACTA2, IL1B* and *TGFBR1*). The expression levels of selected targets are currently being validated at the protein level. RNA-seq analysis revealed that NIR treatment reversed the expression of a wide range of inflammation-associated genes, in particular TNF-α and interferon family members, compared with untreated mice. ELISA confirmed the reduction of TNF-α levels both in serum and forepaw lysates. In conclusion, these findings confirm the anti-fibrotic effects of NIR treatment *in vivo* and shed light on its anti-inflammatory activity, prompting further investigations on key aspects of the disease and supporting NIR repurposing as candidate therapeutics for RDEB.

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A Compensatory CASZ1–ZNF750 Axis Maintains Epidermal Differentiation and Barrier Function

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The epidermis is a self-renewing stratified epithelium whose barrier function depends on tightly coordinated transcriptional programs governing keratinocyte differentiation. Disruption of these programs underlies numerous skin pathologies, yet the regulatory circuitry that ensures their robustness remains incompletely understood. We identify Castor zinc finger 1 (CASZ1) as a differentiation-associated transcription factor required for keratinocyte maturation *in vitro*. Notably however, epidermal-specific deletion of murine *Casz1 in vivo* does not perturb differentiation, pointing to the existence of compensatory regulatory mechanisms.
Integrative ChIP-X analysis revealed that CASZ1 converges on a shared transcriptional program with ZNF750, a central driver of epidermal differentiation, co-occupying and co-regulating a common set of differentiation-associated target genes. To directly test functional redundancy between these two factors, we generated epidermal-specific double knockout mice lacking both *Casz1* and *Znf750*. Combined loss of these factors resulted in a dramatic exacerbation of the single-mutant phenotypes, leading to perinatal lethality. Functional assays revealed severe impairment of skin barrier function, affecting both inward and outward permeability. Histological analysis further demonstrated a markedly thickened epidermis with defective differentiation and persistent expression of progenitor markers.
Together, our findings define a compensatory transcriptional module in which CASZ1 and ZNF750 act in parallel on shared genomic targets to safeguard epidermal differentiation. This functional redundancy reinforces regulatory robustness, ensuring proper epidermal development and barrier integrity.

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Context-dependent iRhom2 signalling in skin and oesophagus

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Tylosis with Oesophageal Cancer (TOC), an inherited palmoplantar epidermal differentiation disorder (pEDD) associated with leukoplakia and oesophageal squamous cell carcinoma (OSCC), provides a powerful model to interrogate mechanisms governing squamous epithelium. TOC is caused by dominant, hyperactive mutations in *RHBDF2*, encoding iRhom2. iRhom2 regulates maturation and stability of ADAM17, a key sheddase responsible for releasing signalling molecules including EGF ligands and TNFα. The iRhom2-ADAM17-EGFR axis plays a central role in TOC and other pEDDs and our recent published data also support a nuclear role for iRhom2.
To determine whether iRhom2-mediated EGFR signalling similarly drives development of TOC oesophageal lesions, we performed RNA sequencing on human TOC oesophagus (bulk and spatial) and oesophagus from a TOC mouse model (bulk only) and compared to data from TOC mouse paw skin (bulk and spatial). Analysis revealed upregulation of keratinization and cell cycle pathways in both human and mouse TOC oesophagus and TOC mouse paw skin compared to control tissue. Inflammatory pathways, however, were differentially regulated: upregulated in TOC paw but downregulated in both human and mouse TOC oesophagus, including TNFα signalling. Metal ion response pathways were also selectively downregulated in TOC oesophagus.
Despite a more ‘skin-like’ transcriptional profile, human TOC oesophagus does not recapitulate the increased EGFR-mediated Transglutaminase 1 activity observed in TOC skin. We also found evidence of loss of the mutant *RHBDF2* allele in human TOC oesophagus, which may partly explain altered signalling between tissues. However, copy number variation (CNV) analysis of pre-dysplastic human TOC oesophagus did not identify significant alterations in *RHBDF2* copy number. Instead, recurrent CNVs were detected relative to control oesophagus, including amplification of *MYC*, *ERBB2*, and *KRT17*, suggesting early genomic events in TOC-OSCC development.
Together, these findings suggest that hyperactive iRhom2 functions in a context-dependent manner, promoting EGFR signalling and barrier-associated programs in skin while contributing to a distinct dysregulated epithelial state in the oesophagus.

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Exploring mevalonate pathway defects in porokeratosis and their role in systemic autoinflammation

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Porokeratosis is a heterogeneous spectrum of disorders defined by keratotic plaques histologically delimited by a “cornoid lamella”. Increasing evidence indicates that porokeratotic lesions arise from clonal defects in the mevalonate pathway, often through a somatic second hit in genetically predisposed heterozygous carriers.
We performed whole-exome sequencing of 46 porokeratosis patients, representing one of the largest cohorts reported to date, and the first European. We included 38 patients with disseminated superficial actinic porokeratosis, 4 with porokeratosis of Mibelli, 3 with linear porokeratosis and 1 with a drug-induced porokeratosis-like eruption. All patients underwent detailed clinical assessment, photographic documentation, review of family history and inheritance model, and line-field confocal optical coherence tomography (LC-OCT) evaluation. Furthermore, patients were treated with topical statins to achieve upstream inhibition of the mevalonate pathway, with response assessed clinically and through LC-OCT-based evaluation of histological changes.
Our cohort encompasses the clinical and genetic heterogeneity of porokeratosis, highlighting MVK, PMVK, MVD, and FDPS as key disease-associated genes. Genotype correlated with clinical morphology and variable response to topical statins. LC-OCT assessment of lesional in vivo architecture after statins supports a pathogenetic model in which accumulation of metabolic intermediates drives the observed keratinocyte abnormalities.
A major unexpected finding was the identification of biallelic pathogenic MVK variants in adults with disseminated superficial actinic porokeratosis and a history of childhood-onset autoinflammatory manifestations, leading to the recognition of previously undiagnosed hyper-IgD syndrome. These observations challenge the traditionally strict separation between porokeratosis and systemic inflammation due to mevalonate pathway alterations and suggest that porokeratosis may be a cutaneous marker of this metabolic abnormality. We propose that genetic testing should be considered in selected patients with porokeratosis and personal or family histories suggestive of autoinflammation.

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TRH receptor signaling drives intrafollicular TSH production and promotes hair growth in human hair follicles

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Human scalp hair follicles (HFs) engage in multiple neuroendocrine activities, whose physiological functions remain poorly understood. Here, we have asked if HFs operate a peripheral equivalent of the hypothalamic-pituitary-thyroid (HPT) axis under control of thyrotropin-releasing hormone (TRH) and thyrotropin (TSH). IF microscopy and in situ hybridization revealed mRNA and protein co-expression of TRH, TSH, and their receptors (TRHR, TSHR) in outer root sheath keratinocytes of anagen HFs. *Ex vivo* TRH treatment (3nM, 30nM) significantly upregulated TSH, TRHR, and TSHR, suggesting a self-amplifying positive feedback mechanism in HFs. T4 and T3 reduced intrafollicular TRH and TSH protein expression, indicating a local HPT-like negative feedback loop. TRHR knockdown showed that TRH-induced TSH production was mediated by TRHR activation.

However, given that TRH may also bind MC-1R, we checked its contribution using the MC-1R antagonist, agouti signaling protein (ASP), showing that TSH level in HF can be also MC-1R-dependent. Functionally, TRHR knockdown demonstrated that TRH-driven hair growth effects (prolonged anagen duration, enhanced hair matrix keratinocyte proliferation, reduced TGF-β2 expression) are specifically mediated *via* TRHR, as ASP failed to inhibit it, indicating that MC-1R activation is not involved. RNA sequencing of TRH-treated HFs also indicated downregulation of catagen-promoting factors (BMP4/6, BDNF, NGF). Thus, human HFs operate core elements of an autonomous, TRH-controlled, peripheral HPT-like axis with both negative and positive feedback regulation, which regulates HF growth. This system in human skin may represent an evolutionarily conserved ancestral neuroendocrine regulatory circuit that predates development of the central HPT axis.

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Knock-in of a patient-derived *ATP2A2* variant replicates Darier disease in organotypic epidermis to enable personalized medicine

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Darier disease (DD) is a rare skin blistering disorder caused by variants in *ATP2A2*, which encodes the endoplasmic reticulum calcium pump SERCA2. Though its genetic etiology was established 25 years ago, DD lacks any approved therapy. Ablating *Atp2a2* in mice did not replicate DD, thus we leveraged gene editing in TERT-immortalized human epidermal keratinocytes to build a pre-clinical DD model. After nucleofection of Cas9-gRNA complexes with a homology-directed repair template, we sequenced multiple knock-in (KI) clones to confirm an *ATP2A2* variant derived from a DD patient (c.115A>G; N39D). A key pathologic feature of DD is severing of adhesive cell-cell junctions, particularly desmosomes, which leads to epidermal blistering (acantholysis). We treated control and KI cells with 1.3 mM calcium upon reaching confluency to induce cell-cell junction formation. After 4 hr, KI cells exhibited impaired desmosome assembly shown by reduced immunostaining of desmosomal cadherins (DSG1, DSG3) and plaque proteins (plakoglobin, desmoplakin) at intercellular borders. While DSG1 localization improved by 24 hr, DSG3 was still not at cell-cell junctions in KI cells, which may explain the tendency of DD to exhibit pemphigus vulgaris-like epidermal splitting at the suprabasal level, where DSG3 function is most critical. Consistent with these desmosomal defects, a mechanical stress assay revealed that KI monolayers fragmented more easily than controls. To assess tissue-level pathology, we grew KI keratinocytes in an organotypic epidermal model, which exhibited acantholysis typical of DD. These cellular and tissue models confirm the pathogenicity of a patient-derived *ATP2A2* variant and offer pre-clinical platforms for therapeutic screening. Given emerging evidence for the role of MAP kinase overactivation in DD, we treated KI keratinocytes with a MEK inhibitor, cobimetinib, which bolstered their mechanical integrity, reducing KI monolayer fragmentation. In sum, we used gene editing in human keratinocytes to engineer a personalized model of DD, establishing a pipeline to enable drug repurposing studies for patients with rare genetic skin diseases.

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Myelin Protein Zero-like 3 (MPZL3) controls mitochondrial function in human skin *ex vivo* and murine skin *in vivo*

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Proper mitochondrial function is essential for skin physiology, while mitochondrial dysfunction is associated with skin aging and many skin diseases. Yet, the central controls of mitochondrial function in human skin remain incompletely understood. Here, we have examined one candidate regulator, the immunoglobulin v-type protein, Myelin Protein Zero-like 3 (MPZL3), which reportedly localizes to human keratinocyte mitochondria and regulates their ROS production in cell culture. We show that MPZL3 protein expression in human skin *in situ* is restricted to the epidermal granular layer, differentiated sebocytes, and the inner root sheath (IRS) of the hair follicle (HF). Here, MPZL3 signal showed partial co-localization with VDAC and MTCO1 (double–IF). In organ–cultured human scalp HFs, MPZL3 silencing (siMPZL3) did not alter the constitutive mitochondrial marker TOM20, while MTCO1 and VDAC were significantly reduced in siMPZL3 HF. Furthermore, increased TFAM expression suggests a compensatory response to mitochondrial dysfunction. These results suggest that MPZL3 controls both inner and outer mitochondrial membrane protein production in human HFs. In the skin of epithelia-specific *Mpzl3* knockout mice (via K14 promoter-driven Cre), bulk RNAseq analysis (FDR 0.05, 1.5x) showed that many of the top regulated pathways are involved in mitochondrial functions, e.g., mitochondrial respiratory chain complex assembly, oxidative phosphorylation, and fatty acid metabolic process, selected of which were validated by RT-qPCR. Skin extracts from global *Mpzl3* knockout mice also revealed altered mitochondrial enzymatic activities, suggestive of mitochondrial dysfunction. These results strongly suggest that MPZL3 is an essential regulator of mitochondrial function in human and murine skin, including in differentiated human keratinocytes and HF. Thus, MPZL3 deserves full exploration as a novel therapeutic intervention target in dermatoses and skin aging associated with mitochondrial dysfunction.

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Enhancing Repigmentation in Vitiligo Through Targeted Activation of Melanocyte Stem Cells

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Vitiligo is a chronic depigmenting disorder characterised by melanocyte loss and variable response to current therapies. Repigmentation depends on activation of melanocyte stem cells (MeSCs) residing in the hair follicle niche, yet the molecular mechanisms governing this process remain incompletely understood and limit therapeutic efficacy.
Here, we investigate the role of stem cell niche regulation in controlling melanocyte regeneration and repigmentation. Using in vitro systems and in vivo models, we identify bone morphogenetic protein (BMP) signaling as a key regulator of MeSC quiescence. Inhibition of this pathway promotes melanocyte activation, enhances melanin production, and drives increased cell migration.
Importantly, we demonstrate that small-molecule inhibitors of BMP signaling significantly enhance repigmentation outcomes.

Pharmacological inhibition of BMP activity increases melanocyte activation and pigment production in vitro, and robustly improves epidermal repigmentation in a UV-induced vitiligo mouse model, supporting the translational potential of this approach.
To further characterise the mechanisms underlying successful repigmentation, we apply transcriptomic and spatial analyses to resolve melanocyte subpopulations and their interactions within the niche. These data reveal dynamic changes in gene expression and cell–cell communication associated with melanocyte activation and migration, highlighting the critical role of niche-derived signals in coordinating regenerative responses .
Together, this work identifies BMP signaling as a central regulator of melanocyte stem cell quiescence and activation, and demonstrates that its pharmacological modulation can enhance repigmentation in vitiligo. These findings provide a strong foundation for the development of targeted, stem cell–based therapeutic strategies to improve clinical outcomes.

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Restoring the Epidermal Lipid Barrier in Junctional Epidermolysis Bullosa
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Junctional epidermolysis bullosa (JEB), caused by mutations in basement membrane genes such as Laminin332, is among the most severe form of EB. Affected individuals suffer from blistering from birth leading to scarring, granulation tissue and susceptibility to infection. We have identified a new pathway altered in JEB, the regulation of cholesterol biosynthesis. Loss of Laminin332 results in a reduced epidermal lipid barrier due to loss of cholesterol transport within keratinocytes.
We have developed a cholesterol transport screening assay to analyse the ability of targeted compounds to restore cholesterol transport in our *in vitro* model of JEB. Results of this small-scale screen identified 9 drugs that were able to restore cholesterol transport by up to 50% of control. Some of these drugs were further selected for screening in our 3D skin equivalent model of JEB. Skin equivalents of normal and JEB skin were treated for 7 days with either DMSO or 1µM of drug. In 3D, 4 drugs successfully increased stratum corneum lipids, shown by nile red staining, compared to the untreated JEB skin equivalents. Further analysis of skin differentiation and barrier were assessed to narrow drug selection for further in vivo testing.
Two of the drugs were further tested in the Laminin-α3 knockout mouse ($Lama3^{fl/fl}$/K14-Cre^{ERT}), where mice were treated with either drug (30mg/kg) or control, on alternate days for two weeks, before sacrificing. Changes to the epidermal skin barrier were analysed and showed a restoration in skin lipids compared to control treatment.
These data suggest that JEB is not just a disorder of epidermal-dermal adhesion, but a disorder of aberrant cholesterol trafficking causing a markedly impaired skin barrier. These findings suggests that drugs to target cholesterol transport could be a beneficial treatment for JEB, helping to improve the essential lipid barrier and prevent infection at the early stages of life.

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Dissecting pigmentary alterations in xeroderma pigmentosum using *Xenopus laevis* embryos
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Xeroderma pigmentosum (XP) illustrates the severe clinical impact of impaired nucleotide excision repair, resulting in extreme sensitivity of the skin to ultraviolet (UV) radiation and predisposing patients to pigmentary defects, premature photoaging, and a markedly elevated cancer risk. Mutations in *XPC* represent the most frequent genetic cause of xeroderma pigmentosum (XP). This genodermatosis, classified as xeroderma pigmentosum group C (XP-C; OMIM#278720). Despite decades of research, the molecular basis underlying XP-associated pigmentation abnormalities remains poorly understood, in part due to the lack of suitable *in vivo* models. Here, we take advantage of the distinctive properties of *Xenopus laevis*, including its optically transparent epidermis, accessible embryonic development, and conserved DNA repair mechanisms, to develop a novel model system for exploring the cutaneous manifestations of XP-C.
We demonstrate that UVB irradiation in Xenopus embryos induces a robust and conserved DNA damage response, characterized by activation of repair pathways, apoptotic clearance, epidermal hyperplasia, and enhanced melanocyte dendricity, closely paralleling responses in human skin. Targeted knockdown of xpc via antisense morpholinos recapitulated hallmark features of XP-C, including pigmentary and developmental abnormalities. This phenotype can be partially rescued by ectopic expression of human *XPC* mRNA *(hXPC)*, confirming a conserved and functional role for Xpc in pigmentation. Kinase activity profiling and global proteomic analysis of *xpc* morphants reveal widespread dysregulation of signaling pathways linked to cytoskeletal organization and cell migration. Together, these findings highlight a potential, previously unrecognized non-canonical role for Xpc in pigmentation and embryonic development making of *Xenopus laevis* a versatile model for studying pigmentary disorders and DNA repair-associated skin pathologies.

415 [WITHDRAWN]

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Development of immunecompetent full-thickness skin equivalents to study skin-T cell crosstalk
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Psoriasis ranks among the most prevalent human inflammatory skin disorders and is initiated and maintained through the interplay between T cells and epidermal keratinocytes. The strong association with the susceptibility allele HLA-C*06:02 (formerly HLA-Cw6), together with the abundance of CD8+ T cells in psoriatic lesions, highlights a central role for CD8+ T cells in disease pathogenesis. In contrast, murine models of psoriasis are predominantly driven by innate immune cells and γδ T cells, which limits their translational relevance and underscores the need for more suitable immunocompetent human experimental models. Here, we established a human experimental model incorporating different CD4+ and CD8+ T cell subsets into a human full-thickness skin equivalent (hFTSE) to investigate their individual and combined contributions to psoriasis pathogenesis. Across all conditions, inserted T cells migrated towards the epidermis and secreted psoriasis-associated cytokines. Immunohistological analysis revealed psoriasis-like alterations in epidermal differentiation as well as enhanced

expression of keratinocyte-derived antimicrobial peptides (AMPs) in T cell–incorporated hFTSEs. Notably, treatment with the psoriasis drug apremilast showed altered efficacy in the presence of CD8+ T cells. Overall, our findings contribute to the refinement of human skin models for studying disease pathophysiology and evaluating therapeutic responses, enabling more accurate predictions of drug efficacy and safety. Ongoing efforts to incorporate autologous skin and T cells will further increase the physiological relevance of this model and enable mechanistic studies of psoriasis-triggering events, including barrier disruption and infection.

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Melanin Transfer is Regulated by Fusogenic Proteins in Human Epidermis

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Transfer of melanin from melanocytes (MC) to keratinocytes (KC), whereby the MC-specific organelle - the melanosome - is donated to KCs, is crucial for the protection of our skin against harmful ultraviolet radiation (UVR). However, the regulation of this process remains enigmatic, despite several early hypotheses. For example, we have proposed a role for filopodia in melanin transfer, including under the control of the atypical motor protein Myosin-X. Here we investigate the role of membrane fusion in melanin transfer that involves MC filopodia and KC plasma membrane.
We investigated the expression of fusogenic proteins Syncytin-1; Myomaker; Myomerger; Alanine, serine, cysteine transporter-2 (ASCT-2); Phosphatidylserine receptor (PSR); and phospholipid scramblase 1 (PLSCR1) and the phosphatidylserine-interacting protein Annexin-A5, in human skin and in isolated primary human MC and KC by immunofluorescence (IF), western blot and RT-PCR. We also investigated whether knockdown of these membrane fusion-associated genes can affect melanin transfer in MC-KC co-culture.
We show for the first time that the fusogenic proteins Syncytin-1, Myomaker, Myomerger, ASCT-2, PSR, PLSCR1 are all expressed in human epidermis (MC and KC), as well as in primary human MC and KC *in vitro*. siRNA knockdown of *ASCT2, PSR, PLSCR1* and *Annexin A5* in MC-KC co-culture resulted in significant (40-60%) inhibition of melanin uptake by KC. This indicates a central role for membrane fusion between MC filopodia and KC in melanin transfer and supports our 'filopodia-cyto-phagocytosis' model of human skin pigmentation regulation. In summary, we propose an important role for fusogenic proteins in filopodia-mediated melanin transfer in human skin, which may be open to modulation in the context of hypo and hyperpigmentary conditions of the skin.

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Antisense RNA Strategy to Treat an Autosomal Dominant Form of Epidermal Differentiation Disorder Involving the *ELOVL1* Gene (*ELOVL1*-sEDD)

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Inherited ichthyoses belong to the Epidermal Differentiation Disorders (EDDs), a group of rare monogenic diseases affecting the epidermal barrier and characterized by scaling and/or hyperkeratosis, often accompanied by inflammation. Syndromic forms (sEDDs) may include extracutaneous manifestations, as observed in patients carrying *ELOVL1* variants, who develop moderate to severe ichthyosis associated with neurological and ophthalmological abnormalities. *ELOVL1* encodes a ubiquitously expressed enzyme that catalyzes the elongation of long-chain fatty acids, essential for the synthesis of ceramides playing a central role in both myelin sheath formation and epidermal barrier function.
We report a patient presenting dermatological and neurological features associated with a recurrent *de novo* heterozygous *ELOVL1* variant, c.494C>T (p.(Ser165Phe)). The clinical and biochemical phenotype is consistent with previously reported cases and resembles autosomal recessive forms, supporting a dominant-negative mechanism.
To specifically target the mutant transcript, we developed allele-specific antisense oligonucleotides (ASOs) designed to selectively degrade the mutated mRNA while preserving the wild-type allele. Sixteen ASOs were screened in patient-derived fibroblasts using droplet digital PCR, identifying two lead candidates that achieved efficient and preferential knockdown of the mutant transcript.
ELOVL1 deficiency is known to dysregulate other *ELOVL* genes, notably causing upregulation of *ELOVL2*. Using qRT-PCR, we showed that *ELOVL2* expression was partially normalized in patient fibroblasts treated with the most effective ASO. This phenotypic rescue is being further evaluated through analysis of fatty acid chain length in treated cells.
Further optimization of ASO design is ongoing to improve allele selectivity. The most promising candidates will be assessed in additional patient-derived cellular models, including induced pluripotent stem cell-derived neuronal progenitors and reconstructed epidermis generated from primary keratinocytes, to evaluate their therapeutic potential across relevant tissues.

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Clitocine is a Potent Readthrough Drug in Laminin-332 Deficient Junctional Epidermolysis Bullosa

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Junctional EB caused by laminin-332 deficiency (JEB-LAMB3) is a severe rare skin disorder without curative treatment. As nonsense mutations are common in this condition, translational readthrough has been proposed as a therapeutic way. Our study aimed to identify compounds capable of inducing readthrough of laminin β3 nonsense variants.
We used keratinocytes derived from JEB patients with the homozygous c.1903C>T (p.R635*) or compound heterozygous variants c.124C>T;1903C>T (p.R42*;R635*) or c.1903C>T;2500C>T (p.R635*;Q834*). The workflow optimizes the dosage and composition of translation readthrough inducing drugs to achieve a biologically relevant protein expression and validate its functional significance. The readthrough activity of artesunat, atazanavir, erythromycin, clitocine, gentamicin and TRID-C5 (gentamicin, paromomycin, CC-90009, apocynin and melatonin) was evaluated, and DMSO as a vehicle control.
Drug responses varied by mutation backgrounds, with certain drugs showing genotype-specific effects. Clitocine consistently restored the laminin β3 chain amount and was associated with improved keratinocyte adhesion across all genotypes examined. In JEB-LAMB3^{R635*} and JEB-LAMB3^{R42*R635*} keratinocytes, its effect was more pronounced than gentamicin and TRID-C5. When

compared to the NHK, the restored laminin β3 chain levels reached 10% of NHK levels in JEB-LAMB3^{R635*} and 29.2% in JEB-LAMB3$^{R42*/R635*}$ keratinocytes ($P < 0.01$). In JEB-LAMB3^{R635*Q834*} keratinocytes, which showed a residual laminin β3 chain amount, both clitocine and artesunate increased laminin β3 chain levels, suggesting a mutation-dependent response. Combination of clitocine with the enhancer CC90009 or with antioxidants did not increase readthrough. Gene expression studies showed clitocine restores the expression of a network of genes coding for extracellular matrix proteins associated with laminin-332 in JEB-LAM3 keratinocytes, but it has a broad off-target effect in particular in NHK.
Overall, clitocine showed the most consistent readthrough activity across different mutation *LAMB3* types, supporting its further evaluation as a candidate for topical translational readthrough therapy in JEB-LAMB3.

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Noncoding genetic risk from hidradenitis suppurativa GWAS converges on coherent regulatory programs in immune and epithelial cells

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Hidradenitis suppurativa (HS) is a debilitating inflammatory skin disease marked by recurrent lesions and limited therapeutic options. We and others have performed HS GWAS that cumulatively implicate 21 risk loci. Importantly, it remains uncertain how inherited genetic risk translates into dysregulated cellular programs in disease-relevant tissues. To address this challenge, we performed integrative epigenomic analyses to determine which HS risk variants are enriched within cell-type–specific cis-regulatory elements and how they converge on coordinated transcription factor (TF) networks across independent loci. We mapped risk-associated active regulatory elements to immune cells, epithelial cells, and cells derived to or from mesenchymal stem cells. We prioritized a set of transcription factors whose binding sites are disrupted by HS risk variants located across the genome, including STAT3, GATA3, TRIM28, JUND and FOS, among others. Using this framework, we revealed that imbalance at the MIF-CXCR4-CD74 signaling axis in HS keratinocytes drives aberrant epithelial signaling and inflammation, and identified the chemokine receptor CXCR4, which is an FDA-approved drug target, as a therapeutically actionable node in HS. More broadly, these results define coherent regulatory programs impacted by HS genetic risk and are guiding future multi-omic functional genomics studies integrating single-cell chromatin accessibility, transcriptional profiling, and transcription factor binding assays across genetically diverse HS patient cohorts to validate disease-relevant regulatory programs, genetic and molecular subtypes, and candidate drug targets.

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Spatial Transcriptomic Profiling of Recessive Dystrophic Epidermolysis Bullosa Skin

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Recessive dystrophic epidermolysis bullosa (RDEB), caused by pathogenic variants in basement membrane type VII collagen, is characterised by chronic wounding and high risk of aggressive cutaneous squamous cell carcinoma (SCC). We aimed to investigate spatially-resolved transcriptomic differences between RDEB and healthy skin. We applied high-resolution spatial transcriptomic analysis (10X Visium HD) to non-lesional RDEB skin (n=3), RDEB SCC (n=1), and healthy control skin (n=2). Spatial data were resolved to single-cell resolution for downstream analyses. Unsupervised clustering identified three shared epidermal keratinocyte populations (basal, spinous, granular/cornified) across all conditions. The RDEB epidermis showed a markedly stressed and inflammatory transcriptional profile, with pan-epidermal upregulation of stress-associated keratins, S100 alarmins, interferon-stimulated genes, and DNA damage/p53 response genes. Gene set enrichment analysis revealed coordinated activation of inflammatory signalling pathways (e.g. TNFα/NF-κB, IFN-γ, IL-6/STAT3, TGF-β, p53), alongside significant suppression of homologous recombination DNA repair pathways across RDEB basal and spinous keratinocyte clusters. Expression of damage-specific DNA binding protein 2 (DDB2), a regulator of homologous recombination via RAD51 stabilisation, was significantly reduced across basal and spinous RDEB epidermal layers. We present the first spatial transcriptomic comparison of RDEB and healthy skin. Our findings suggest that the RDEB epidermis is characterised by inflammatory activation and impaired DNA repair capacity, which may contribute to genomic instability and susceptibility to aggressive SCC.

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Reproducible and Efficient Generation of Complex, Hair-Bearing Skin Organoids from Human Pluripotent Stem Cells

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Physiologically relevant skin models are critical for studying human skin biology, disease, and drug screening. However, conventional 2D in vitro cultures, 3D organotypic cultures, and animal models do not adequately reproduce the structural complexity and appendage diversity of native skin. Additionally, reliance on primary human tissue is limited by restricted availability. To address these limitations, we developed serum-free STEMdiff™ Skin Organoid Kit to support the formation of structurally complex, hair-bearing skin organoids from human pluripotent stem cells (hPSCs). Our approach directs hPSCs sequentially through ectodermal and neural crest induction using temporally controlled signaling cues, before supporting maturation into the final organoids. Aggregates derived from 5 hPSC lines, maintained in mTeSR™ Plus on Corning® Matrigel®, were generated 2 days prior to induction. On day 0, aggregates > 300 µm in diameter were transferred into the induction medium for 12 days, followed by long-term culture in the maturation medium (up to day 150). By day 12, aggregates

(~4000, n = 20) formed cystic structures with a dense core. Hair placodes were observed on the surface of the cystic structure as early as day 50. Organoids harvested at ≥ 120 days (n = 24) were assessed by immunohistochemistry, demonstrating the presence of key skin cell populations, including dermal (PDGFRa+), epidermal (KRT17+, KRT5+), dermal papilla (SOX2+, PDGFRa+), neuronal (TUJ1+), Merkel (KRT20+, TUJ1+), and melanocyte (PMEL+) lineages. Complex appendages including hair follicles (PCAD+, LHX2+, KRT15+) and sebaceous glands (ECAD+, SCD1+) were observed. Overall, this platform enables consistent and scalable production of skin organoids from hPSCs, with efficiencies of > 80 ± 5% across lines, providing a physiologically relevant 3D model for research and testing applications.

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Visualizing type VII collagen mislocalization in dystrophic epidermolysis bullosa using structured illumination and expansion microscopy

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Type VII collagen (COL7) is essential for dermal-epidermal adhesion, and its deficiency or dysfunction leads to dystrophic epidermolysis bullosa (DEB). While previous immunoelectron microscopy (IEM) studies reported that residual COL7 in DEB skin is often mislocalized above the lamina densa, conventional fluorescence microscopy lacks the resolution to confirm these fine ultrastructural changes. In this study, we employed structured illumination microscopy (SIM) and expansion microscopy (ExM) to revisit COL7 localization in skin samples from patients with dominant DEB and intermediate recessive DEB. Using integrin α6 (ITGA6) and type IV collagen (COL4) as markers for hemidesmosomes and the lamina densa, we demonstrated that while COL7 is positioned beneath ITGA6 in normal skin, it is detected above ITGA6 within basal keratinocytes in DEB skin. Quantitative analysis by SIM revealed not only a significant upward shift of COL7 peaks but also increased variability in inter-protein distances, indicating a profound disorganization of the basement membrane zone architecture in DEB. Although ExM provided superior visual resolution, SIM proved more robust for quantitative assessment due to the technical variability in tissue expansion factors. These findings indicate that high-resolution light-based imaging can successfully reproduce classic IEM observations, offering a more accessible and quantitative complementary approach for the morphologic and diagnostic assessment of DEB.

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Identification of a deep intronic splice-site-creating variant in a patient with Hailey-Hailey disease using SpliceAI

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Hailey-Hailey disease is an autosomal dominant genodermatosis, characterized by pathogenic variants in *ATP2C1*. A number of variants in *ATP2C1* have been reported, most of which are located within exons or exon-intron boundaries. We report a patient with a deep intronic splice-site-creating variant in *ATP2C1*. Although the patient had clinical and histopathological findings of Hailey-Hailey disease, whole-exome sequencing from his peripheral blood DNA detected only a synonymous variant, c.2700G>A (p.Lys900=). Other detected variants were c.-108T>G, c.462+202dupA, c.858+98A>G, c.1126+67dupA, and c.2827-98dupT. Therefore, we considered the possibility of a deep intronic splice-site-creating variant. Using SpliceAI, a deep learning system that evaluates how likely variants are to affect splicing, we examined whether these five intronic variants have an effect on splicing. According to SpliceAI analysis, it was strongly suggested that the c.462+202dupA variant affects splicing and creates a novel exon between exons 5 and 6. We then performed RT-PCR with cDNA of the patient and wild-type control. Agarose gel electrophoresis showed two bands from the PCR sample of the patient, whereas there was a single band from the wild-type control, which suggested that the RT-PCR sample of the patient contained both mutant and wild-type cDNA. In order to verify this hypothesis, we performed TA cloning with the patient's RT-PCR products and separately sequenced the wild-type and mutant cDNA clones, identifying an aberrantly spliced transcript with a novel exon inclusion which was consistent with the SpliceAI prediction. The inserted exon between exons 5 and 6 is 68-bp long and causes a frameshift, leading to a premature termination codon in exon 6. From these findings, the patient was diagnosed with Hailey-Hailey disease with a deep intronic splice-site-creating variant.

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Identifying Signatures of Disease Heterogeneity in Epidermolysis Bullosa

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Epidermolysis Bullosa (EB) comprises rare inherited disorders marked by extreme skin and mucosal fragility. The most severe forms are dystrophic EB (DEB), caused by variants in type VII collagen, and junctional EB (JEB), caused by variants in laminin332 (α3, β3, γ2), type XVII collagen, or integrin α6β4. Despite clinical similarities, these subtypes differ greatly in life expectancy: individuals with DEB often survive into adulthood, whereas infants with severe JEB rarely survive beyond the first year. This study investigates molecular mechanisms underlying this disparity using transcriptomic and in vivo approaches.
Bulk RNA sequencing was performed on 22 DEB and 21 JEB infant skin samples (4 = LAMA3, 11 = LAMB3, 2 = LAMC2, 4 = COL17A1). Principal component, differential expression analyses and deconvolution analysis assessed relationships between subtypes, and immunohistochemistry validated findings in patient matched tissue and EB models.
Three transcriptional clusters emerged: one DEB and two JEB groups separated by LAMA3 and LAMC2 mutations. Pathway, Gene Ontology, and deconvolution analyses showed upregulation of cell death, pro inflammatory pathways, and interleukins (IL1, IL11, CXCL12, β defensins, IL31RA) in JEB (LAMA3) compared with DEB or JEB (LAMC2), indicating a heightened adaptive immune response. CellDIVE multiplex imaging (n=23) identified increased CD4+ T cell populations in LAMA3 patients, while the other JEB subtype showed elevated M1/M2 macrophages. Impaired epidermal differentiation DEGs were identified in LAMA3 patients, confirmed by reduced KRT14 staining. Transcriptional factors linked to wound healing (MMP2, COL1A1), collagen remodelling (LOX), and fibrosis (FN1, TGFβ) were also elevated in JEB, suggesting disrupted extracellular matrix synthesis, supported by aberrant fibronectin staining.
This work identifies early molecular differences between EB subtypes that may explain variation in severity and survival, highlighting pathways and cellular drivers that could inform future therapeutics.

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Defining the NFATC2-MITF axis in air pollution-associated skin pigmentation dysregulation

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The exposure of polycyclic aromatic hydrocarbons (PAHs), a key component associated with air pollution, has been shown to increase pigmentation. However, the molecular mechanisms underlying this response remains elusive. To investigate this, *in vitro* 2D and 3D melanocyte-keratinocyte co-culture models were exposed to PAHs and subjected to bulk RNA sequencing. In parallel, *in viv*o studies similarly demonstrated increased melanin content following PAH exposure, corroborating the pigmentation phenotype observed *in vitro*. Transcriptomic analysis of sorted *in vitro* melanocytes identified several differentially expressed genes, among which is NFATC2, which has not been previously associated with pigmentation regulation. NFATC2 is a calcium-responsive transcription factor involved in calcineurin-dependent signalling, and has been extensively studied in immune cells. To assess the functional role of NFATC2 in pigmentation dysregulation due to PAHs, loss- and gain-of-function approaches were performed in monocultured melanocytes in vitro. NFATC2 knockdown was associated with a reduction in melanin content, whereas overexpression resulted in an increase in melanin content. Next, given the central role of MITF as a master regulator of melanogenesis, subsequent work was focused on investigating a potential NFATC2-MITF regulatory axis. Preliminary *in silico* analyses suggest potential NFATC2 binding sites at the MITF promoter, providing a basis for mechanistic investigation. Current studies include chromatin profiling and protein-level investigations aim to confirm whether NFATC2 can directly regulate MITF. Also, ongoing work is done to extend these findings to *ex vivo* human skin models to assess physiological relevance. Overall, this study aims to better define the role of NFATC2 in air pollution-induced pigmentation.

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Selective HDAC inhibitors as anti-fibrotic agents in recessive dystrophic epidermolysis bullosa

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Recessive dystrophic epidermolysis bullosa (RDEB) is caused by biallelic variants in the *COL7A1* gene, which encodes type VII collagen (C7). C7 deficiency leads to skin blistering at the dermal–epidermal interface associated with heightened inflammation, scarring and progressive fibrosis, the latter being responsible for the development of severe disease manifestations. Increasing evidence indicates that epigenetic changes contribute to the RDEB disease activity. Specifically, histone acetylation is reduced in RDEB fibroblasts and skin, and treatment with a histone deacetylase (HDAC) inhibitor acting on several HDACs (pan-HDACi) ameliorates disease course in RDEB mice by counteracting progressive fibrosis. This study was aimed at identifying the role of individual HDACs in promoting the profibrotic phenotype of fibroblasts from RDEB patients. While individual HDACs were expressed at comparable levels in RDEB and non-RDEB fibroblasts, the activity of different HDAC classes was increased in nuclear extracts from RDEB fibroblasts. The HDAC isoforms most relevant to the profibrotic behavior of RDEB cells were identified by assessing changes in key profibrotic markers following HDAC isoform-specific RNA interference. Our results highlighted the role of HDAC1, 2 and 8 (Class I), HDAC5 and 7 (Class IIa) and HDAC6 (Class IIb) in mediating fibrotic features. Based on these findings, four selective HDAC inhibitors - CAY10683 (HDAC2), BRD6929 (HDAC1 and HDAC2), MS-275 (known as entinostat, HDAC1, HDAC2 and HDAC3), and CHDI00390576 (Class IIa) - were tested for their antifibrotic activity in RDEB fibroblasts. Each of these inhibitors effectively reduced and/or inhibited α-SMA expression, TGF-β release and cell contractility, with BRD6929 showing the strongest effect.
These results suggest that selective HDAC inhibition could minimize off-target effects and represents a promising strategy to treat fibrosis in RDEB, pending validation in a preclinical model.

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Targeting aberrant JAK/STAT signalling in junctional epidermolysis bullosa

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Junctional epidermolysis bullosa (JEB) is a severe, genetic disorder causing extreme skin fragility, blistering, and aberrant granulation tissue formation and inflammation due to defects in basement membrane proteins like laminin-332. JAK-STAT signalling plays a critical role for a wide range of cytokines and growth factors controlling skin homeostasis and inflammation. Dysregulation of the JAK-STAT pathway and its therapeutic potential have been investigated in other skin disease including Atopic dermatitis and Epidermolysis bullosa pruriginosa, but not in JEB.
Using our JEB in vitro disease models, we investigated JAK/STAT signalling activation upon LAMA3 depletion. We screened different classes of JAK/STAT inhibitors for their potential to suppress aberrant JAK/STAT signalling and improve the JEB phenotype by combining immunofluorescence, ELISA and western blot analysis. The most effective JAK/STAT inhibitor was selected for in vivo testing in our preclinical JEB disease model.
Depletion of *LAMA3* in keratinocytes leads to an increased JAK/STAT signalling activity in our in vitro and in vivo JEB disease models. Treatment of JEB cells with different JAK/STAT inhibitors demonstrated that selective JAK1 inhibition significantly decreased STAT3 phosphorylation in a concentration dependent manner. Our inducible JEB in vivo disease model shows prominent JAK/STAT signalling activation with increasing with disease severity, in blistered skin areas. Notably, treatment with selected JAK1 inhibitor not only decreased aberrant STAT3 phosphorylation, inflammation, cell proliferation and fibrotic granulation tissue formation, but also improved skin blistering, overall mouse health and skin regeneration.
Collectively, our study revealed that targeting JAK/STAT signalling pathways with selective JAK1 inhibitors show the potential to improve the skin health and regeneration in JEB and potentially other EB diseases.

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Hoxc13 regulates pigmentation of skin appendages in amphibians

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Human hair and nails depend on the transcription factor Hoxc13. In a recent study, we disrupted the *hoxc13* gene in clawed frogs to demonstrate that the role of Hoxc13 in the induction of hair keratin genes during the differentiation of cornified skin appendages is conserved in amphibians. Here, we investigated wildtype and hoxc13-deficient frogs to determine which other genes are regulated by Hoxc13. We identified a tyrosinase (TYR)-like gene which differs from the classical regulators of melanin synthesis, TYR, TYR-related protein 1 (TYRP1) and dopachrome tautomerase (DCT). However, the amino acid residues implicated in catalytic activity of tyrosinases are conserved in the TYR-like protein of the frog, and the recombinant TYR-like protein displayed tyrosinase activity. The *TYR-like* gene is specifically expressed in the pigmented claws of *Xenopus tropicalis*. The disruption of *hoxc13* suppressed the expression of the *TYR-like* gene and abolished the pigment formation on the toe tips. By contrast, mutations in canonical pigmentation genes, manifesting in an albino phenotype, do not abrogate the pigmentation of *Xenopus* claws. We conclude that a unique mechanism of pigmentation involving a non-conventional tyrosinase is controlled by Hoxc13 in amphibians.

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iSKID-TEN: A Next-Generation Immunocompetent Human Skin-in-a-Dish Platform to Model SJS/TEN Biology and Accelerate Therapeutic Discovery

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Current human skin models only partially capture the complexity of native tissue and remain limited for studying inflammatory skin disease. Many available 3D systems lack resident immune cells and fail to reproduce the dynamic crosstalk between epidermal, stromal, and immune compartments that governs tissue homeostasis and pathology. More physiologically relevant human models are therefore urgently needed for mechanistic studies and therapeutic development. To overcome these limitations, we developed iSKID, an immunocompetent 3D human skin equivalent incorporating induced pluripotent stem cell (iPSC)-derived macrophages (iMacs). These cells resemble primitive yolk sac-derived progenitors that colonize skin during development and give rise to dermal macrophages and Langerhans cells. Like during embryogenesis, integration of iMacs is essential to tissue formation. iMacs successfully engrafted within both dermal and epidermal layers, where the local niche instructed compartment-specific differentiation. Dermal iMacs retained a macrophage phenotype with proliferative capacity, whereas epidermal iMacs acquired Langerhans cell-associated markers. Single-cell transcriptomic analysis showed that the cellular composition of iSKID is similar to native human skin. Immune populations remained viable for more than two weeks and responded appropriately to external stimulation, consistent with in vivo function. To demonstrate disease-modelling potential, we generated a pathological iSKID version reproducing major hallmarks of Stevens Johnson Syndrome/Toxic Epidermal Necrolysis (SJS/TEN), including epithelial damage, immune activation, keratinocyte death, and epidermal barrier disruption. This system enables mechanistic interrogation of severe cutaneous adverse reactions and provides a human-relevant platform for preclinical therapeutic testing. Collectively, iSKID represents a next-generation skin-in-a-dish model combining structural fidelity, immune competence, and disease modelling capacity, offering a powerful tool for dermatology research, precision medicine, and drug discovery.

431 [WITHDRAWN]

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Hair-bearing skin organoids as a scalable source of functional 2D keratinocytes.

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A key advantage of induced pluripotent stem cells (iPSCs) is their ability to maintain a stable, patient-specific genetic background throughout the experimental workflow, providing a powerful platform for disease modelling. However, the generation of keratinocytes through directed differentiation remains challenging, often resulting in heterogeneous cell populations with variable maturation and limited proliferative capacity. To address these limitations, we established a method to isolate keratinocytes from hair-bearing skin organoids (SOs). We describe a reproducible workflow for keratinocyte isolation, including the experimental timeline, enzymatic dissociation strategy, and subsequent purification and characterization. The resulting cultures were benchmarked against primary basal keratinocytes. Immunofluorescence staining and FACS analysis of epithelial-specific markers, and single-cell RNA sequencing revealed that these basal keratinocytes display transcriptomic profiles closely matching those of primary human basal keratinocytes. These cells also exhibit strong proliferative potential and efficiently generated epidermal equivalents. The robustness of the method was confirmed across three independent hiPSC lines. By providing a scalable and patient-specific cell source, this approach supports the study of rare genetic skin disorders and enables therapeutic testing. As a proof of concept, we applied this platform to investigate the molecular consequences of a subtype of epidermolysis bullosa simplex, establishing a reliable system to study disease mechanisms.

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Identification of ERα-associated genes involved in sex-based differences in neuropsychiatric symptoms in a tuberous sclerosis model mouse

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Tuberous sclerosis complex (TSC) is a genetic disorder characterized by multisystem manifestations and is associated with neuropsychiatric symptoms, collectively referred to as TSC-associated neuropsychiatric disorders (TAND). It is known that the pathogenesis of TSC is caused by constitutive hyperactivation of the mechanistic target of rapamycin complex 1 (mTORC1). Although we have already reported sex-based differences in TAND in TSC model mice, the underlying mechanism has not yet been elucidated.
In previous study, we utilized TSC model mice that exhibit TAND-like phenotypes and found sex-based differences. Specifically, we observed that estrogen levels and ERα DNA-binding activity are higher in females with severe symptoms. We therefore hypothesized that ERα mediated transcriptional activation contributes to these sex-based differences in TAND. Focusing on this point, to further explore the molecular basis of these differences, we performed proteomic analysis and revealed distinct protein expression profiles between male and female TSC model mice. To identify specific genes linked to ERα among these proteins, we conducted chromatin immunoprecipitation (ChIP)-q-PCR.
We report on the roles of genes whose expression is upregulated in association with ERα in female TSC model mice, as well as the resulting the sex-specific signaling pathways involved.

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Sphingolipid metabolism orchestrates hair follicle stem cell compartment establishment and maintenance

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Sphingolipids constitute fundamental components of cellular membranes, ensuring subcellular compartmentalization and intercellular communication. However, the mechanisms by which cell type–specific lipid compositions are established, as well as their functional relevance for tissue morphogenesis and homeostasis, remain poorly understood. In this study, we identify a stem cell–specific function of ceramide synthase 4 (CerS4) in regulating cell fate decisions within the epidermis. Loss of CerS4 impairs the proper formation of the adult hair follicle bulge stem cell (HFSC) compartment, due to altered differentiation trajectories. Mechanistically, these HFSC differentiation defects result from an imbalance in key sphingolipids, leading to hyperactivation of the noncanonical Wnt/Ca2+ pathway, triggering stem cell differentiation through CaMKII. The defective establishment of the HFSC compartment disrupts hair follicle architecture and compromises skin barrier integrity, ultimately inducing a T helper 2 cell–dominated immune infiltration resembling human atopic dermatitis. Collectively, these findings reveal a fundamental role for cell state–specific sphingolipid profiles in maintaining stem cell homeostasis and preserving skin barrier function.

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Distinct molecular signatures define recessive dystrophic Epidermolysis Bullosa-associated and ultraviolet-induced cutaneous squamous cell carcinoma using AI-enhanced Raman spectroscopy

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Recessive dystrophic epidermolysis bullosa (RDEB) is a rare genodermatosis caused by mutations in COL7A1, characterised by mucocutaneous fragility, chronic inflammation and fibrosis, and a markedly increased risk of aggressive squamous cell carcinoma (SCC). RDEB-associated SCC arises earlier, progresses more rapidly and exhibits higher metastatic potential than conventional ultraviolet (UV)-induced SCC, suggesting fundamental biological differences beyond shared histological classification. However, the molecular basis of this divergence remains incompletely understood.
Raman spectra were acquired from formalin-free tissue biopsies (RDEB-associated SCC, n=4; non-EB SCC, n=3) and analysed using multivariate and machine learning approaches to interrogate tumour-associated biochemical signatures. SKiN-D analysis demonstrated robust separation between RDEB-associated and non-EB SCC, indicating distinct molecular phenotypes rather than a shared malignant signature. Spectral decomposition revealed differences in protein secondary structure, lipid composition and nucleic acid-associated features. RDEB-associated SCC showed enrichment of spectral bands consistent with altered protein conformations and extracellular matrix remodelling, whereas non-EB SCC exhibited stronger contributions from nucleic acid-associated and aromatic amino acid features, reflecting divergent mechanisms of genomic instability and tumour evolution.
These findings support the concept that SCC arising in RDEB represents a biologically distinct disease entity driven by a unique tumour microenvironment shaped by chronic inflammation and ECM dysfunction, in contrast to non-EB carcinogenesis. Raman spectroscopy, combined with machine learning-based spectral interpretation, provides a sensitive approach to resolving these disease-specific molecular differences and offers new insight into the divergent pathobiology of SCC subtypes.

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Pseudobulk Analysis Reveals Inflammatory Priming and Impaired Repair in Severe Epidermolysis Bullosa Simplex

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Severe epidermolysis bullosa simplex (EBS) is an inherited mechanobullous skin fragility disorder defined by basal keratinocyte cytolysis, arising from mutations in keratin 5 or 14. Building on our previously presented work demonstrating marked shifts in cellular composition between lesional and non-lesional severe epidermolysis bullosa simplex (EBS) biopsies and healthy controls using single-cell RNA sequencing, we performed pseudobulk differential expression analysis on the same dataset to further assess the contribution of inflammation to disease pathophysiology.
Within immune cell populations, two key patterns emerged. Firstly, non-lesional skin exhibited heightened inflammatory priming, characterised by upregulation of *CCL2*, *CALCA*, and *SPRED1*. In contrast, lesional immune cells showed enrichment of *SELL*, reflecting active leukocyte recruitment into sites of established tissue injury. Secondly, lesional tissue demonstrated loss of repair mechanisms, with reduced expression of genes involved in barrier maintenance and cellular resilience, including *RYR2*, *PPARG*, *KALRN*, and *CPED1*.
Two distinct patterns were also observed in non-immune compartments. Lesional samples showed extracellular matrix disruption, with downregulation of transcripts such as *WIF1*, *HAS2*, and *ABI3BP*. Non-lesional enrichment of *TNFSF14*, a cytokine known to impair epithelial differentiation and barrier integrity, further suggested that clinically unaffected tissue remains transcriptionally predisposed to barrier dysfunction. Secondly, lesional tissue exhibited reduced structural resilience, with downregulation of cytoskeletal and stress-response genes including *GSN*, *DCLK1*, and *CEBPD*.
Collectively, these findings support a model in which non-lesional severe EBS skin exists in a primed, inflammation-ready state, while lesional skin is characterised by immune recruitment alongside failure of barrier repair and structural resilience. Pseudobulk analysis therefore reinforces inflammation as a potential driver of severe EBS pathophysiology, rather than purely a secondary consequence of cytoskeletal dysfunction.

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Safflower Glucoside: A new approach based on Bound Ceramides biosynthesis for Enhanced Skin Barrier Repair

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Altered corneocytes-bound ceramides (bound-CER) profiles are linked to compromised barrier function in conditions such as atopic dermatitis, xerosis, UVB exposure and skin aging. The cornified lipid envelope (CLE) acts as a critical scaffolding for extracellular lamellar bilayers and *stratum corneum* (SC) cohesion. It functions also as a semi-permeable membrane restricting the loss of NMFs out of the corneocyte. We investigated Safflower glucoside (SG), an ester of glucose and linoleic acid, as a novel ingredient to enhance bound-CER synthesis.
In *ex vivo* delipidized SC, emulsified SG 0.5% significantly improved moisturization (+129% vs +74% vehicle) and barrier function (+25% TEWL improvement). SG upregulated Claudin-1 expression on monolayer cultures of normal human keratinocytes. In Reconstructed Human Epidermis (RHE), SG induced a dose-dependent increase in bound-CERs (+80%), which strongly correlated with reduced Bodipy penetration (-70%). SG blocked the response of the PAR-2 (Protease-Activated Receptor 2) receptor when challenged with reference agonists, in a dose dependent manner. In a Th2-induced inflammatory RHE model, SG reduced biomarkers TSLP (-50%), CCL2 (-40%), and IL1-α (-50%), while increasing loricrin. As a linoleoyl donor and activator of ALOXE3 synthesis, SG boosts the biosynthesis of bound-CERs. Results from a clinical study, using 0.5% SG on dry legs, confirmed a significant increase in total bound-CERs compared to baseline and an improved balance of saturated and unsaturated ceramide compared to vehicle.
These findings demonstrate that Safflower glucoside effectively boosts corneocyte-bound-ceramides synthesis and levels which strengthens the CLE. This mechanism offers a promising therapeutic approach for managing barrier-compromised skin through advanced repair formulations.

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Distinct Innate Immune Responses and skin Microbiota in the Facial Skin of Patients with Tuberous Sclerosis Complex

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Tuberous sclerosis complex (TSC) is an autosomal dominant genetic disorder characterized by multisystem hamartomas, hypomelanotic macules, epilepsy, and neuropsychiatric manifestations collectively referred to as TSC-associated neuropsychiatric disorders (TAND). Clinical severity varies widely, even among relatives carrying the same variant, but the mechanisms underlying this variability are not fully understood. To identify genetic modifiers, next-generation sequencing and single nucleotide polymorphism analyses were performed. However, no causative variants explaining phenotypic differences were identified.
We therefore hypothesized that acquired factors may contribute to the severity of neurological and cutaneous manifestations in TSC. To investigate this possibility, we analyzed skin and gut microbiota in patients with TSC and healthy controls using 16S metagenomic sequencing. Gut microbiota did not differ significantly between groups, whereas skin microbiota showed significant differences in both α- and β-diversity, indicating a TSC-specific skin microbiota. Skin microbial diversity was not associated with the severity of facial angiofibromas or epilepsy, but was correlated with hypomelanotic macules and developmental disorders. To assess the skin immune environment, we performed histological and immunohistochemical analyses of facial angiofibromas and measured serum cytokine levels. TSC skin exhibited innate immune responses distinct from those of control skin. These findings suggest that TSC-specific innate immune responses may alter the skin microbiota and may contribute to clinical variability in TSC.

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Intrafamilial phenotypic variability in endothelin receptor type B-related Waardenburg syndrome

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We report a 9-year-old boy with endothelin receptor type B-related Waardenburg syndrome showing marked intrafamilial phenotypic variability, underscoring the importance of careful family history assessment. He was first referred to our department at 2 months of age after bilateral retinal hypopigmentation was noted during follow-up for heterochromia iridis of the left eye, raising suspicion of oculocutaneous albinism. Although no apparent hearing loss was noted, physical examination revealed dystopia canthorum and patchy hypopigmentation of the hair, prompting clinical suspicion of Waardenburg syndrome. Whole-exome sequencing identified a premature termination variant in *EDNRB*, confirming the diagnosis. Further familial investigation revealed that his mother, who had initially been considered unaffected, also carried the same variant. Careful re-examination showed subtle hypopigmentation of her right iris. Detailed family history additionally disclosed that the two sons of the mother's sister had Hirschsprung disease. Genetic analysis demonstrated the same *EDNRB* variant in the mother's sister and in one of her two sons with Hirschsprung disease, whereas genetic testing was not performed for the other affected son. These findings demonstrate marked intrafamilial phenotypic variability associated with the same *EDNRB* variant, ranging from subtle pigmentary abnormalities to Hirschsprung disease. This case highlights that Waardenburg syndrome may be overlooked when pigmentary findings are mild and emphasizes the importance of thorough physical examination and detailed family history assessment for accurate diagnosis and appropriate genetic counseling.

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Newly identified p.E283X variant in *Keratin 9* causes stress-induced blistering

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KRT9-pEDD (OMIM 144200) is an autosomal dominant inherited disorder, characterized by massive thickening of the skin of palms and soles developing during the first weeks or months after birth. The majority of the underlying pathogenic variants are located in the 1A or 2B regions (hot spots), the remaining are synonymous mutations.
Here, we report a newly described heterozygous nonsense variant c.847G>T of *KRT9* that introduces the STOP codon p.E283X predicted to lead to *KRT9* loss of function (LoF). In contrast to previously reported missense variants in *KRT9*, individuals with c.847G>T exhibit only mild blistering. This variant results in less K9 IF instability when cultured keratinocytes derived from patient biopsies are exposed to heat. ~90% of K9-positive cells carrying a known missense mutation p.N161S in *KRT9,* known to cause severe palmoplantar thickening, show keratin aggregates throughout their cytoplasm due to a shortened sidechain of a serine residue as shown by 3-dimensional (3D) modelling. In contrast, cells containing the new *KRT9* p.E283X nonsense mutation show a significantly higher K9 IF stability after heat stress treatment. Thus, the p.E283X *KRT9* nonsense mutation shows a milder fragility phenotype as compared to the *KRT9*p.N161S mutation when exposed to heat stress. These findings suggest that a *KRT9* variant resulting in LoF causes a phenotype distinct from previously known *KRT9*-pEDD lacking a dominant-negative effect of abnormal K9, instead proposing haplosufficiency of wild-type K9. *KRT9* haplosufficiency stabilizes IF networks in a fashion that allows for normal daily activities, but is prone to mechanically-induced skin blistering. This haplosufficiency of *KRT9* informs the design of gene editing strategies for pEDD.

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Psoriasis flare-ups in non syndromic autosomal recessive epidermal differentiation disorders

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Epidermal differentiation disorders (EDDs) are a heterogeneous group of inherited keratinization disorders characterized by abnormal epidermal differentiation and impaired barrier function, including non-syndromic forms (nEDDs), previously termed autosomal recessive congenital ichthyosis.
Among 49 nEDD patients followed between 2011 and 2025, 13 (26.5%) experienced at least one psoriasis flare-up. Targeted next-generation sequencing of known EDD genes (*TGM1, ABCA12, ALOX12B, ALOXE3, CERS3, CYP4F22, NIPAL4, PNPLA1, SDR9C7, SULT2B1*) was performed, and 8 patients were additionally screened for monogenic psoriasis genes (*CARD14, AP1S3, IL36RN*).
The most common clinical presentation was ichthyosiform erythroderma (61.5%), while 38.5% had generalized thick scaling. *ALOX12B* was the most frequently mutated gene (30.7%). Median age at psoriasis onset was 28 years. Psoriasis phenotypes included plaque psoriasis (61.5%), generalized pustular psoriasis (46.2%), psoriasis inversa (38.5%), and localized pustular psoriasis (15.4%). Histopathology was consistent with psoriasis in all cases, with negative microbiological and PAS results. Erythrodermic flares occurred in 53.8% of patients. Among those genetically screened, one patient carried a likely benign *CARD14* variant, and two had non-coding AP1S3 variants; no IL36RN variants were identified in GPP cases.
Eleven patients required systemic therapy, including retinoids, methotrexate, cyclosporine, apremilast, and biologics (TNF, IL-12/23, IL-17, IL-23 inhibitors). All 7 patients treated with biologics achieved complete psoriasis control, although only 3 showed improvement of EDD.
Overall, psoriasis flare-ups occurred in 26.5% of nEDD patients, with an overrepresentation of pustular forms and *ALOX12B* mutations. These findings support a shared Th17/IL-23 inflammatory pathway between EDDs and psoriasis, highlighting the relevance of targeted therapies.

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Successful Treatment of Progressive Symmetric Erythrokeratoderma With Secukinumab

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Progressive symmetric erythrokeratoderma is a rare genodermatosis characterized by well-demarcated erythematous and scaly plaques with slow progression since childhood. Treatment is challenging and often limited to emollients, keratolytics and topical retinoids, while systemic retinoids may be effective but are restricted in women of childbearing potential because of teratogenicity. We report a 28-year-old woman with diffuse scaling lesions since birth and a history of collodion baby. Previous biopsy showed lamellar hyperkeratosis, acanthosis and papillomatosis, consistent with progressive symmetric erythrokeratoderma. She had used acitretin 60 mg/day with initial improvement, but relapsed after dose reduction due to reproductive planning. Cyclosporine 200 mg/day was then introduced, with marked worsening of scaling and pruritus. Examination revealed diffuse xerosis and multiple well-demarcated, mildly keratotic, scaly plaques over erythematous-brown macules, symmetrically affecting the abdomen, inguinal region, cubital and popliteal fossae and elbows. Excoriations were present on the upper back, where Koebner phenomenon was observed. Given refractoriness and desire for future pregnancy, secukinumab, a selective interleukin-17A inhibitor, was started at 300 mg weekly at weeks 0, 1, 2, 3 and 4, followed by 300 mg monthly. The patient was advised to wait three years after acitretin discontinuation before attempting pregnancy. From the fourth application, significant clinical improvement was observed, with reduction of scaling and pruritus. After four months, she achieved near-complete remission, with no adverse events. This response supports the hypothesis that inflammatory pathways may coexist with primary keratinization defects in severe progressive symmetric erythrokeratoderma. Barrier dysfunction may promote interleukin-17 activation, contributing to persistent erythema and scaling. This case suggests that interleukin-17A blockade may be a promising off-label option for refractory progressive symmetric erythrokeratoderma, particularly when systemic retinoids are contraindicated or undesirable.

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Expression of skin integrity-related proteins in a Recessive Dystrophic Epidermolysis Bullosa *in vitro* CRISPR/Cas9 model is enhanced by TGFβ2

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Recessive Dystrophic Epidermolysis Bullosa (RDEB) causes defective production and function of collagen VIIA1 protein leading to severe skin blistering, abnormal wound healing and a heightened risk of aggressive squamous cell carcinoma. Here, n-TERT-1 keratinocytes were CRISPR/Cas9-edited to knockout (KO) *COL7A1,* using an Origene-delivered construct, to mimic severe RDEB disease conditions. We stimulated these edited keratinocytes with TGFβ2 [5 ng/ml for 24h & 10 ng/ml for 30min] to check how this wound-healing factor may affect production of collagens, integrins, laminins and keratins, all of which are important for optimal skin integrity. Differential protein expression analysis (fold-change) was performed using LC/MS proteomics.
We detected statistically-significant changes in expression of skin integrity/wound-healing proteins after stimulating the edited keratinocytes with TGFβ2 [5 ng/ml for 24h] vs non-stimulated control keratinocytes. These included: COL4A2 (3.47-fold), ITGB1 (4.13-fold), LAMB2 (2.46-fold), MXRA7 (2.63-fold); KRT76 (5.53-fold). Moreover, IPA canonical pathway analysis showed increased/upregulated signaling for the *Wound healing signaling pathway* (Z-score=1, p-value=2.34E-02) and the *Epithelial membrane protein signaling pathway* (Z-score=2, p-value=6.77E-03).
Separately, stimulation of edited keratinocytes with TGFβ2 at 10ng/ml for 30 min resulted in statistically-significant changes in expression of several proteins related to skin barrier formation including: ITGB1 (2.81-fold), IVL (3.19-fold), KRT1 (2.39-fold), KRT10 (7.20-fold), KRT13 (10.05-fold), KRT4 (4.57-fold). Here, the IPA canonical pathway *Keratinization pathway* showed significantly increased signaling (Z-score= 1.633, p-value= 5.24E-04).
In summary, our results suggest that skin integrity properties in *COL7A1-KO* human keratinocytes may be improved after stimulation with TGFβ2.

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Generation of Functional Reconstructed Human Epidermis for In Vitro Applications

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The epidermis forms the body's outermost barrier, protecting against environmental insults, such as pathogens and chemical exposure. Increasing demand for predictive and physiologically relevant in vitro skin models in dermatological research has driven the transition toward defined culture systems that minimize animal-derived components. Here, we introduce DermaCult™ Keratinocyte Differentiation Medium (KDM), a novel component of a two-stage, serum- and bovine pituitary extract-free workflow for generating reconstructed human epidermis (RHE) at the air-liquid interface (ALI). Primary keratinocytes isolated from neonatal (n = 2) and adult (n = 2) donors were expanded for up to five passages in DermaCult™ Keratinocyte Expansion Medium (KEM), then seeded onto collagen I-coated polycarbonate inserts. After two days in submerged conditions using KEM, cultures were transitioned to KDM; ALI was established by removing apical medium after two more days. Following 10 days at ALI, all donor- and passage-derived cultures formed consistent RHE tissues, exhibiting well-organized, multi-layered epidermal architecture that include basal, spinous, granular, and cornified layers. Immunostaining confirmed correct localization of basal markers (KRT5, KRT14, p63) and suprabasal differentiation markers (KRT10, involucrin, loricrin). Functional barrier integrity was supported by high transepithelial electrical resistance (TEER) values (> 1500 Ωxcm2) and limited caffeine permeability (< 1.5% after 30 minutes at 10 mg/mL). Exposure to 1% Triton X-100 resulted in time-dependent reductions in tissue viability (ET50: 2-10 hours), consistent with established irritancy benchmarks. Together, these

findings demonstrate that the DermaCult™ KDM system reproducibly generates barrier-forming RHE suitable for in vitro skin toxicity and irritation assessment.

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In vitro application of the Warthin-Starry Stain for high-contrast melanin visualisation

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Melanin quantification in cultured cells is most performed using chemical extraction followed by spectrophotometric analysis or liquid chromatography-mass spectrometry. Although widely used, these approaches require specialised instrumentation, technical expertise and involve cell lysis, thereby eliminating spatial information and cellular morphology. Alternative methods that preserve cellular structure may provide additional insight into melanin distribution and transfer *in vitro*. The Warthin-Starry silver impregnation method has historically been used for microorganism detection and has also been applied to visualise melanin in *ex vivo* skin tissue. Here, we describe an adapted Warthin-Starry staining protocol optimised for melanocyte monocultures and melanocyte-keratinocyte co-cultures. This method enables high-contrast visualisation of intracellular melanin while preserving cellular morphology and allowing assessment at a single-cell level. This morphology-preserving approach enables imaging-based assessment of melanin content and distribution in cultured cells. In addition to providing a complementary alternative to extraction-based assays, this method may support quantitative image analysis and could serve as a useful tool for studying melanin production, melanin transfer and screening of pigmentation-modulating compounds *in vitro*.

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Biological responses of human skin to 5G electromagnetic waves

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The increasing deployment of 5G electromagnetic waves has raised concerns about their potential health effects. The skin is directly exposed to 5G frequency range 2 (FR2) waves and thus represents the first biological target for this wavelength range. This study evaluates the biological effects of 5G FR2 exposure on human skin at the cellular and molecular levels using a discovery oriented single cell strategy that does not rely on predefined gene or pathway targets.

We recruited healthy volunteers and individuals with genetic predisposition to skin cancer, photoaged skin, or inflammatory skin disorders. Male and female participants received a single 27.5 GHz exposure for 20 minutes at 20 W/m2, the maximum dose currently allowed in Switzerland. Skin biopsies were collected after 1h and 24h from both exposed and sham-exposed sites and analyzed using customized single-cell RNA sequencing (scRNA-seq).

Our protocol allows us to record transcriptomic changes related to wound regeneration and gender-specific differences, independently of 5G irradiation, confirming the technique sensitivity. While overall cohorts' cell proportions remain stable, subtle effects of 5G waves can be detected in specific skin cell types. These findings align with parallel cell and animal studies within our research consortium (Horizon-SEAWave).

Using well-defined exposure parameters and state-of-the-art techniques, our study provides a comprehensive cellular and molecular landscape of human skin under different conditions, including varied time points, ages, genders, and disease states. By integrating our findings with ongoing research, we contribute scientific evidence to the safety assessment of 5G FR2 electromagnetic waves.

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Drawing the Skin: A Visual Arts-Based Educational Supplement for Researchers and Dermatologists

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Drawing is not a widely developed activity in the curricula of biology researchers or dermatologists, despite its potential to enhance observation and understanding of skin structures. At Harvard Medical School, drawing techniques are used in *visual literacy* courses to strengthen attention, observation, and critical reflection. Various drawing modalities can be explored in scientific education: drawing from existing references, simple contour drawing, and blind contour drawing, which involves reproducing a model without looking at the paper. These exercises develop concentration, attention to detail, and fine perception of biological structures.

We selected skin diagrams from reference books and online sources, which participants reproduced using a fine-tipped black pen, at both tissue and cellular level. The exercise was conducted in a calm and focused setting, following the instructions for contour and blind contour drawing.

An exhibition of 20 drawings will be organized at ESDR 2026, including tissue- and cell-level reproductions. The goal is to raise awareness among participants of the benefits of drawing for learning and research, highlighting that the time spent drawing promotes concentration and personal reflection, creating a space conducive to scientific analysis.

A collective drawing of a giant epidermis will be offered to participants, accompanied by a questionnaire to gather impressions and assess the educational impact, particularly participants' ability to adopt a new perspective on drawing and their willingness to represent biological structures studied in their own research.

448 [WITHDRAWN]

INFECTIOUS SKIN DISEASES, DYSBIOSIS, THE SKIN MICROBIOME

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Cx26 mutation skews candidalysin-induced keratinocyte death toward corneoptosis, driving chronic cutaneous candidiasis

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Human genetic disorders revealing the role of non-hematopoietic cells in host defense are rare. Keratitis–Ichthyosis–Deafness (KID) syndrome is caused by *GJB2* mutations encoding the epithelial protein connexin 26, yet how KID patients develop chronic cutaneous candidiasis (CCC) is unknown.
Skin samples from six KID patients with CCC showed significant hyperkeratosis with abundant hyphae in the stratum corneum. Compared to acute cutaneous candidiasis and CCC in patients with STAT3 mutations, KID-CCC showed significant infiltration of $CD4^+$, $CD8^+$, and IL-$17A^+$ cells, indicating that immune cell responses are likely maintained.
To determine if an epithelial-intrinsic defect alone drives KID-CCC, we generated keratinocyte-specific Cx26S17F mice (epiKID). Following *C. albicans* application, control mice rapidly accumulated neutrophils in the stratum corneum and cleared *Candida*, whereas epiKID mice developed progressive hyperkeratosis with invading hyphae, and reduced epidermal neutrophil recruitment, recapitulating KID-CCC. Bulk RNA sequencing showed attenuated epithelial responses to *Candida* in epiKID mice, including reduced gene expression of pattern recognition receptors, chemokines, and antimicrobial peptides (AMP). Notably, candidalysin-deficient *C. albicans* (ece1Δ) failed to induce CCC-like response in epiKID mice. Consistently, epiKID 3D epidermal cultures showed blunted chemokine and AMP induction after candidalysin stimulation, accompanied by increased keratinocyte death. While candidalysin is known to induce inflammatory cell death in epithelial cells, pharmacologic screening identified corneoptosis inhibition as sufficient to restore epiKID 3D epidermis transcriptional responses to candidalysin.
In conclusion, we identify keratinocyte-intrinsic dysfunction as a central driver of CCC in KID syndrome and demonstrate an essential role for epithelial innate immunity in skin antifungal defense.

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Spatial organization of skin immunity drives antiviral responses during human MPOX infection

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MPOX is an epidermatotropic orthopoxvirus that induces a strong and long-lasting protective immune response in humans and serves as a valuable model to study antiviral immunity in the skin. However, the contribution of skin immunity in coordinating durable systemic protection against skin-tropic viruses remains poorly understood.
Here, we define the immune architecture of human MPOX infection by integrating single-cell transcriptomics, T cell receptor sequencing, and spatial analyses of matched lesional skin and blood during the exanthematic phase.
MPOX lesions exhibited a structurally segregated immune infiltration characterized by enrichment of plasmacytoid dendritic cells and B cells in the epidermis and inflammatory macrophages and newly recruited circulating T cells in the dermis. Clonal tracking revealed extensive coupling between blood and skin T cell compartments, with influx of polyclonal circulating central memory T cells into lesions. Concomitantly, immune and stromal cells reorganized into lymphoid niches supporting chemokine-guided positioning, antigen presentation, and costimulatory signaling, with features of tertiary lymphoid structures and local immune activation.
Together, MPOX lesions are spatially organized immune microenvironments that integrate systemic and local antiviral responses. A deeper understanding of these tissue-specific immune programs may inform strategies to elicit robust and long-lasting protective immunity not only against MPOX but also across a broader spectrum of skin-tropic viral infections, with potential implications for vaccine design and immunotherapeutic approaches targeting barrier tissues.

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STAT3 regulates IFNγ-induced necroptosis in human keratinocytes

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Keratinocytes play a central role in epidermal antiviral defense by integrating cytokine signaling with regulated cell death pathways such as necroptosis, which limits viral propagation but requires tight control to prevent tissue damage. Interferon gamma (IFNγ) induces RIPK3-dependent necroptosis in keratinocytes; however, the upstream regulatory mechanisms remain incompletely defined. Here, we identify signal transducer and activator of transcription 3 (STAT3) as a key regulator of IFNγ-mediated necroptosis in human keratinocytes.
Expression analysis of IFNγ-stimulated primary keratinocytes demonstrated upregulation of STAT3 and necroptosis-associated mediators, including Z-DNA binding protein 1 (ZBP1) and receptor interacting protein kinase 3 (RIPK3). To assess the functional relevance of STAT3, keratinocytes were stimulated with IFNγ in the presence or absence of the selective STAT3 inhibitor Stattic. STAT3 blockade prevented Tyr705 phosphorylation, suppressed ZBP1 induction, and inhibited RIPK3 activation. While RIPK3 inhibition (GSK'872) blocked downstream execution of necroptosis, STAT3 inhibition prevented upstream induction of necroptotic signaling. Flow cytometry confirmed a dose-dependent reduction in IFNγ-induced necroptotic cell death upon STAT3 inhibition.
Immunohistochemical analysis of varicella-zoster virus (VZV)-infected human skin revealed STAT3 phosphorylation predominantly in uninfected keratinocytes adjacent to infected epidermal regions, spatially associated with areas exhibiting necroptotic signaling. In contrast, infected keratinocytes and healthy control skin showed no detectable STAT3 activation. These findings suggest that STAT3 contributes to priming necroptotic signaling in neighboring uninfected keratinocytes, supporting coordinated antiviral tissue responses.

Taken together, STAT3 signaling contributes to the regulation of IFNγ-induced necroptotic priming in keratinocytes. Pharmacological inhibition of STAT3 suppresses ZBP1 expression, RIPK3 activation, and necroptotic cell death. Notably, therapeutic JAK inhibitors may impair STAT3-dependent antiviral responses and thereby increase susceptibility to herpes zoster.

452 [WITHDRAWN]

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Candida albicans-driven itch is associated with mast cell expansion and type 2 neuro-immune signaling in skin

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Candida albicans asymptomatically inhabits skin as a commensal fungus that provokes inflammation via Th17-mediated response upon epithelial barrier disruption. Itch is a burdensome symptom of various inflammatory skin disorders that severely impacts patients' quality of life. However, the neuro-immune mechanisms underlying itch sensation in *Candida albicans* skin infection are yet to be discovered. To address this question, we established a *Candida albicans* skin-infected murine model for evaluation of itch behavioral phenotype and analysis of interactions between neuronal and immune cells. In the established model, itch-related behavior was increased on day 10 ($p<0.0001$) under daily epicutaneous application of *Candida albicans* (10^5 CFU/mL). scRNA-sequence analysis revealed upregulation of type 2 immune receptors' markers in itch-specific sensory neurons (NP1, NP2, NP3) ($p<0.001$) and mast cell expansion in skin in the experimental group, which was further confirmed by flow cytometry and immunofluorescence staining. Moreover, whole-mount imaging showed close anatomical association between nerve fibers and mast cells in skin. Also, increased thickness and density of nerve fibers ($p<0.01$) together with enhanced excitability of DRG cells were observed in *Candida albicans*-treated group. Furthermore, serum *Candida albicans*-specific IgEs were elevated on day 10 ($p<0.001$) in *Candida albicans*-treated mice, while increased expression of FcεRI in mast cells and heavy chain of IgE and IL-4 in lymph nodes was observed in the transcriptomic data. Taken together, this research shows the upregulation of type 2 immune response in *Candida albicans*-induced itch murine model.

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UV radiation–induced changes in the skin microbial metabolome enhance AhR signalling and barrier marker expression

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Ultraviolet radiation (UVR) impairs the skin barrier. Microbiome-derived tryptophan metabolites regulate barrier function via the aryl hydrocarbon receptor (AhR). We recently showed that UVR alters metabolite production in skin bacteria, enhancing AhR activation and barrier function, suggesting a role for the microbiome in barrier recovery following UVR exposure. Here, we extend this work using an *ex vivo* human skin microbiome model to assess the impact of UVR on microbial metabolite production and downstream responses.
Human skin explants were sterilised with 70% ethanol and microbiomes collected from the forearms of healthy donors (*n=5*) were applied for 24h. Biopsies were exposed to 37.5mJ/cm^2 of solar simulated radiation (SSR) and incubated for 24 or 120h alongside microbiome (MB) and SSR negative controls. MB composition and tryptophan metabolome profiles were analysed by shotgun sequencing and LC-MS, respectively. Barrier function was assessed by TEWL and immunofluorescence, while AhR activation was determined by qPCR of *CYP1A1*.
SSR exposure led to observed increases in the abundance of *Brevibacterium* (120h) and decreases in *Kocuria* (24 and 120h) and *Staphylococci* (120h). MB-positive biopsies had significantly enhanced metabolite abundance compared with MB-negative biopsies. The composition of the metabolome was significantly altered by SSR. At 24h, SSR-irradiation increased tryptamine and reduced indole-3-acetamide (IAM), while at 120h, IAM was increased and 5-hydroxyindoleacetic acid was reduced. The MB increased AhR activation in unirradiated samples at both timepoints. Irradiation also activated the AhR and this was significantly enhanced by the presence of MB at 24h, but not 120h post SSR exposure. TEWL was unchanged in all conditions. However, irradiation increased involucrin expression at 24h which was further enhanced by the presence of MB.
Exposure to UVR reshapes the microbiome and its metabolome with associated changes in AhR signalling and expression of involucrin. These data highlight microbial metabolites as potential regulators of the epidermal response to UVR.

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Role of Malassezia secreted aspartic proteases in scalp barrier intergrity

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Dandruff is a prevalent scalp disorder characterized by epidermal barrier dysfunction and microbial dysbiosis, where the abundance of Malassezia, particularly *M. restricta*, correlates significantly with disease severity. While the role of fungal lipases is well documented, the contribution of fungal proteases to barrier impairment remains less understood. This study aimed to characterize the expression and secretion of Malassezia Secreted Aspartic Proteases (SAPs) and evaluate their molecular impact on the stratum corneum. We analyzed five Malassezia species (*M. furfur*, *M. restricta*, *M. globosa*, *M. sympodialis*, and *M. pachydermatis*) cultured in an optimized modified Leeming and Notman medium. Standardized transcriptomic (qPCR) and proteomic analyses were performed to quantify SAP1-5 gene expression and secreted protein levels during the exponential growth phase. Functional proteolytic activity was measured in conditioned media across varying pH levels, with SAP specificity validated using the aspartic protease inhibitor pepstatin. Furthermore, LC-MS/MS proteomics was employed on ex-vivo human corneocytes treated with Malassezia supernatants to identify specific structural proteins targeted for degradation. We observed that *M. restricta* expressed and secreted the highest levels of SAPs, followed by *M. furfur* and *M. globosa*, whereas *M. sympodialis* and *M. pachydermatis* showed no detectable SAP activity. The functional proteolytic capacity was strain-, time-, and pH-dependent, showing potent activity against stratum corneum proteins. Proteomic profiling of treated corneocytes identified the degradation of several key structural proteins essential for epidermal integrity and cohesion. These results

demonstrate that Malassezia SAPs are significant contributors to barrier damage through the targeted proteolysis of essential structural proteins. This biological pathway provides new insights into the mechanisms of scalp dysbiosis and highlights these enzymes as important microbiological factors for maintaining scalp barrier integrity.

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Keratinocyte response to dermatophytosis: the joint role of TLR2 and Dectin-1

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Skin infections caused by various dermatophyte species are characterized by the development of an inflammatory response of varying intensity. Keratinocytes, the main cell type in the epidermis, are the first cells to detect dermatophyte invasion and release pro-inflammatory cytokines and antimicrobial peptides that initiate the immune response. On the surface of keratinocytes, Toll-like receptors (TLRs) and C-type lectins (Dectin receptors) are involved in the recognition of these fungi. However, the significance of their role in cytokine release by keratinocytes and in initiating this response remains to be fully elucidated. To this end, reconstructed human epidermis (RHEs) produced with immortalized human keratinocytes (N/TERTs) were infected with two dermatophyte species, *Trichophyton benhamiae* and *Trichophyton rubrum*. Before and after infection, the expression levels of various target cells were analyzed. Infection of wild-type N/TERTs revealed that TLR2 and Dectin-1 expression increases after infection, suggesting that these two receptors play a predominant role in the response to dermatophyte infection. Following infection, the expression of various pro-inflammatory cytokines is also increased. Preliminary results indicate that the absence of TLR2 or Dectin-1 does not impact the immune response, as infection with dermatophytes of RHE produced with TLR2$^{-/-}$ N/TERT and Dectin-1$^{-/-}$ N/TERT obtained using CRISPR/Cas9 exhibits a similar induction of gene expression encoding different pro-inflammatory cytokines. Interestingly, in these TLR2$^{-/-}$N/TERT, Dectin-1 expression is increased, and the opposite is also observed in the absence of Dectin-1. These observations suggest potential compensatory mechanism that could explain why the induction of pro-inflammatory cytokines is unaffected by the absence of TLR2 or Dectin-1. The relationship between both receptors is being analyzed after infection of N/TERTs simultaneously deleted for Dectin-1 and TLR2, hoping to improve our current understanding of dual involvement of TLR2 and Dectin-1 receptors in keratinocyte release of pro-inflammatory cytokines during dermatophytosis.

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Tracking herpes simplex virus 1 infection during tissue regeneration using three-dimensional human skin equivalents

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Viral infections are a main contributor to dermatological pathologies, which can affect the integrity of the physical, chemical, microbial, and immunological barrier components of the skin. Herpes simplex virus 1 (HSV1) can infect the skin leading to herpes outburst on the site of exposure. Although symptoms are temporary, the presence of HSV1 is long-lasting, and flare treatment strategies can become ineffective upon resistance development. The aim of this study is to investigate how formation of the skin barrier affects HSV1 infection and to identify mediators of HSV1 infection that serve as target for novel host-directed antivirals. We developed a medium throughput host-virome interaction platform using Leiden Epidermal Models (LEMs) infected with HSV1 at multiple time-points during skin regeneration to uncover keratinocyte-specific pathways mediating viral infection. Multimodal read-outs included assessments of HSV1 replication, cytologic evaluation of epidermal morphogenesis, and gene expression profiling using RNAseq. In LEMs, HSV1 infection and replication reached highest levels prior to stratum corneum development. RNAseq revealed separate clustering of HSV1 infected versus non-infected control LEMs at early phases of epidermal regenerations. Main host transcriptomic mediators of HSV1 viral infection in LEMs were mitotic nuclear division and RNA splicing on biological process, cadherin binding and structural constituent of chromatin on molecular function, and chromosomal region and nuclear speck on cellular component. In conclusion, the regenerating epidermis exhibits heightened susceptibility to HSV1 infection at early stages, mediated by keratinocyte-specific host factors that represent potential targets for pharmacological intervention.

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Nutrient-dependent public goods-cheater dynamics shapes *Staphylococcus aureus* evolution in healthy and inflamed skin

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Staphylococcus aureus (SA) resides on 30-50% of the world population, but its high abundance is associated with the onset and severity of chronic inflammatory skin diseases, such as atopic dermatitis (AD). SA secretes numerous virulence factors that contribute to exacerbating AD symptoms and their expression is regulated by the accessory gene regulator quorum-sensing (Agr-QS) system, comprising *agrA, agrB, agrC,* and *agrD*. SA's evolution on healthy infant skin shows emergence of *agrC/A* mutant (agr-) non-virulent strains while *agr+* strains are conserved on atopic dermatitis skin, but the mechanisms underlying their emergence and selection remain unclear. In our study, we took *in vitro*, *in silico*, and *in vivo* approaches to address this question. *In vitro* serial passaging in TSB medium revealed frequent emergence of *agrC/A* mutations, suggesting their competitive advantage in the media. *In silico* model fitting to growth curves of *agr+* and the *agr-* strains predicted a nutrient-dependent strain fitness advantage, and subsequent *in vitro agr+* vs *agr-* strain competitions, inoculated at 1:1 ratio, in nutrient rich (TSB) and nutrient poor conditions (RPMI + Triolein), validated the *in silico* predictions – *agr-* mutants become dominant in nutrient poor conditions but depleted in nutrient rich conditions. This suggests *agr-* strains act as a public goods-cheater. Co-infection of *agr-* and *agr+* strains *in vivo* on mice skin also confirmed *in vivo* and *in silico* predictions. In nutrient poor conditions (bacteria + PBS), *agr-* mutants significantly reduced bacterial burden and skin inflammation compared to *agr+* infections alone, but in nutrient rich conditions (bacteria + 1xTSB), *agr-* mutants failed to prevent skin damage and inflammation by *agr+* strains. Altogether, SA *agr* evolution on the skin is shaped by a nutrient-dependent public goods-cheating.

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Similarities and differences in herpesviruses' interaction with the epidermal terminal differentiation programme

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Varicella-zoster virus (VZV) and Herpes Simplex Virus (HSV)-1 are skin-tropic human alphaherpesviruses, which have in the skin epidermis their main location of replication. Both primary infection by VZV, which causes chickenpox, and VZV reactivation, which causes herpes zoster, manifest with a skin blistering rash. Infection by HSV-1 causes orofacial or genital skin blistering lesions.
We have previously demonstrated that VZV profoundly alters the expression of epidermal differentiation markers for replication, namely VZV downregulates the expression of keratin 10 and desmosome proteins such as desmocollin-1 and desmoglein-1. Here we show that HSV-1 too disrupts keratin 10, desmocollin-1 and desmoglein-1 expression, although likely through different mechanisms.
Additionally, we investigated the other side of the virus-host interaction, namely how epidermal differentiation affects both VZV and HSV-1 replication and life cycle. For this, we developed models of epidermal differentiation based on primary human keratinocytes that mimic three distinct stages of differentiation; undifferentiated, early differentiated and late differentiated. We found that both VZV and HSV-1 were able to infect keratinocytes at all the differentiation levels, but they optimally replicated when infecting undifferentiated keratinocytes. Interestingly, differently from VZV, HSV-1 still had some level of replication when infecting already differentiated keratinocytes. Moreover, HSV-1 did not require the infected undifferentiated keratinocytes to undergo differentiation after infection to fully replicate, indicating that overall HSV-1 is less stringent than VZV in its interaction with epidermal differentiation.
Taken together these data identify similarities and differences in how alphaherpesviruses interconnect with the host skin, which provide the basis for the identification of novel antivirals targeting skin pathways.

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Neonatal UVB exposure remodels early life cutaneous immunity and atopic risk

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Children with reduced ultraviolet (UV) exposure during early infancy, particularly those born in autumn or winter, are at higher risk of developing atopic diseases, including atopic dermatitis (AD) and food allergy. Prior studies link low serum vitamin D (VitD) levels to pediatric AD, yet how early-life UV exposure shapes cutaneous immunity remains unclear. Furthermore, while neonatal skin dysbiosis is associated with AD development, UV's role in shaping the microbiome remains unexplored. Using a human infant cohort and mouse models, we investigated how the early life skin environment is shaped and how it affects the risk of AD in later life. In our human cohort, serum VitD levels at 6 months negatively correlated with total IgE at 6 months and with egg white- and ovomucoid-specific IgE at 12 months. Additionally, 6-month serum VitD positively correlated with skin microbial a diversity, suggesting a role in healthy microbiome development. In mice, neonatal UVB exposure suppressed cutaneous expression of Th2-associated chemokines *Ccl17* and *Ccl22* at postnatal day 21. It also increased skin microbial a diversity at postnatal day 28 and reduced the relative abundance of *Staphylococcus sciuri*. Single-cell RNA sequencing at postnatal day 28 revealed reduced abundance and transcriptional reprogramming of dermal fibroblast subsets following neonatal UVB, indicating persistent stromal remodeling. Notably, these changes occurred under VitD-sufficient diets where serum VitD concentrations were unaltered by UVB. suggesting neonatal UVB exerts local cutaneous effects extending beyond systemic VitD modulation. This indicates that immunological and microbiome changes are not mediated by circulating VitD but reflect direct local effects of UVB on the cutaneous microenvironment. Together, neonatal UVB remodels the skin microbiome and cutaneous signaling networks, establishing an early life skin environment that shapes long-term immune homeostasis.

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Impact of HIF-1α expression on human keratinocyte infection by Usutu virus

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Usutu virus (USUV) is an emerging arbovirus in Europe, infection with which may cause severe neurological complications. Mainly inoculated within the extravascular compartment of the dermis and the epidermis by an infected mosquito, USUV replicates in skin cells. Recent data suggest that hypoxia and activation of the Hypoxia-Inducible Factors (HIF)-1α, transcription factor whose expression is regulated by oxygenation levels can modulate the immune response and cellular metabolism, and could therefore influence viral replication.
This study aims to evaluate the impact of hypoxia and HIF-1α expression on human keratinocyte infection by USUV.
Control (Ctrl) and HIF-1α knockdown (HIF-1KD) keratinocytes were cultured under normoxia (20% of partial pressure of O_2 (ppO_2)) or hypoxia (1% ppO_2) for 16h, then infected with USUV or stimulated with poly(I:C), a synthetic double-stranded RNA mimicking viral infection for 48 h.
In both oxygenation conditions, a significant increase in basal expression of antiviral gene mRNA was observed in HIF-1KD compared to Ctrl cells. A significant decrease in viral RNA and viral particle production as well as in the induced antiviral response was noticed in HIF-1KD compared to Ctrl cells. In addition, the antiviral gene mRNA expression induced by poly(I:C) was potentiated in HIF-1KD compared to Ctrl cells suggesting a key regulatory role of HIF-1α in keratinocyte antiviral defences. WB analysis revealed that HIF-1α down expression modulated the expression of innate immune receptors as well as IRF3 and IRF7 pathways.
These results suggest that HIF-1α expression levels impact both viral replication and the innate immune response induced during keratinocyte infection. Given the ability of HIF-1α to mediate cellular metabolism reprogramming, studies are underway to determine the metabolic modulations induced outside and during viral infection.

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Leveraging machine learning to determine mechanisms of mTOR inhibition and barrier repair by *Lacticaseibacillus rhamnosus GG* in eczema models

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Probiotics are attractive candidates for eczema therapy, but molecular mechanisms remain poorly defined. Our aim was to understand how an established probiotic, *Lacticaseibacillus rhamnosus GG* (LGG) restores skin barrier function in two well-defined eczema models, shRNA knockdown of Filaggrin (shFLG) and Interleukin 3/14 treated skin explants.
shFLG keratinocytes showed increased mTORC1 signalling, coupled with disruption in both tight junctions and desmosomes, also observed non-lesional eczema epidermis. Conversely, keratinocytes treated with an LGG-derived lysate showed reduced mTORC1signalling and restoration of tight junction and desmosomal protein expression. In shFLG organotypic cultures and IL3/14 treated explants, lysate treatment reduced hyperactive mTORC1 signalling. As mTORC1 inhibition was not prevented by heat treatment or trypsin digestion treatment of the lysate, we hypothesised that a bacterial metabolite was responsible for mTORC1 inhibition.
We used machine learning to compare the genomes of 56 bacteria with reported beneficial effects on eczema skin to 56 of non-beneficial bacteria. We identified ~100 core beneficial clusters including carbohydrate metabolism enzymes functioning through the aryl hydrocarbon receptor (AhR), and a hypothetical peptidoglycan hydrolase functioning via muramyl dipeptide and increased NOD2 signalling. Consistent with these predictions, LGG lysate treatment led to a modest increase in the AhR target CYP1A1, and increased NOD2 signalling. By machine learning, we can rapidly identify potential beneficial metabolic pathways and metabolites that inhibit key signalling pathways upregulated in eczema, suggesting an alternative therapeutic approach whereby skin commensal bacteria produce key actives to restore skin barrier before disease flare.

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Staphylococcus aureus lipoproteins drive TLR2-mediated skin inflammation.

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Staphylococcus aureus (SA) commonly colonizes atopic dermatitis skin lesions and contributes to disease exacerbation. SA expresses multiple pathogen-associated molecular patterns, among which cell wall lipoproteins are prominent and are recognized by Toll-like receptor 2 (TLR2) in host cells. We aimed to investigate how innate immune sensing of SA lipoproteins drives skin inflammation. To specifically define the role of lipoproteins, we used an SA mutant strain (SA-Δlgt) lacking mature lipoproteins. In vitro stimulation of murine bone marrow–derived macrophages and dendritic cells with SA-Δlgt failed to induce pro-inflammatory cytokine/chemokine production, including IL-6, IL-18, and CXCL1, compared to wild-type SA. Similar results were observed in the human keratinocyte cell line HaCaT stimulated in vitro with either live or heat-killed SA. In vivo, application of SA-WT or SA-Δlgt to tape-stripped skin of wild-type mice revealed markedly reduced skin inflammation and immune cell infiltration in SA-Δlgt–treated mice compared to SA-WT. Moreover, TLR2 expression in $CD45^+$ skin immune cells was increased in SA-WT–treated mice but remained unchanged in SA-Δlgt–treated mice.
Together, these findings identify SA-derived lipoproteins as key drivers of TLR2-dependent skin inflammation during SA colonization.

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Higher frequency of natural killer NK cells in atopic skin is due to the colonization by *Staphylococcus aureus*

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Having identified a reduction in the number of NK cells in the circulation of atopic dermatitis (AD) patients, we were curious whether a similar alteration can be observed in atopic skin. Cells were isolated from atopic (lesional and non-lesional) or healthy control (HC) skin and subjected to FACS analysis. The frequency of NK cells ($CD45^+CD3^-7AAD^-CD56^+$) in atopic skin was not reduced but even higher than in HC skin. NK cells obtained from atopic lesional skin expressed higher levels of the homing receptor cutaneous lymphocyte antigen (CLA) in comparison to cells from non-lesional or HC skin. To address whether *Staph. aureus*, a major driver of AD, is responsible for the attraction of NK cells into atopic skin, chemoattraction analysis was performed using Boyden chambers. This revealed that NK cells were highly attracted when placing either *Staph. aureus* supernatant or a *Staph. aureus* strain into the lower chamber. One of the most important activating receptors on NK cells NKG2D (natural killer group2, member D) appeared to be involved in the migration since the majority of NK cells attracted by *Staph. aureus* expressed high levels of NKG2D and the migration could be abrogated by addition of an antibody blocking NKG2D. On the other hand, expression of the inhibitory receptor ILT2 on NK cells isolated from the lesional atopic skin was significantly upregulated compared to HC and non-lesional skin. The enhanced expression of the negative regulatory ILT2 receptor may give rise to the speculation of an impaired activity of NK cells which remains to be proven by functional assays. Together, the data indicate that *Staph. aureus* may attract NK cells into the skin from the periphery which, however, could be impaired in their function due to enhanced expression of negative regulatory receptors.

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Skin bile acids – Investigation of body site distribution and metabolism by skin microbes

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Beyond their role in digestion, bile acids act as signalling molecules that regulate metabolism and immunity. Primary bile acids are produced in the liver and biotransformed by the gut microbiome into secondary bile acids. We posit that bile acids can distribute to the skin and be further metabolized by the skin microbiome to yield skin health effects. In this study, we aim to

characterize skin bile acid distribution and perform detailed investigations of skin microbial metabolism on bile acids. Via untargeted liquid chromatography-mass spectrometry, we showed the presence of more than 30 bile acid classes distributed across genders and four skin sampling sites (scalp, cheek, axilla, antecubital fossa) in healthy Southeast Asian subjects. Glycodeoxycholic acid (GDCA) and taurodeoxycholic acid, are amongst the most abundant bile acids measured. In further *in vitro* experiments using defined media spiked individually with bile acids, we demonstrated that two skin commensals, *Micrococcus luteus* and *Staphylococcus epidermidis*, were distinct and selective in their capacity to biotransform bile acids. *M. luteus* was only able to oxidise deoxycholic acid (DCA) putatively into 7-ketolithocholic acid or 12-ketolithocholic acid, while *S. epidermidis* could perform glycine conjugation on DCA and chenodeoxycholic acid into GDCA and glycochenodeoxycholic acid (GCDCA) respectively. To our knowledge, this is the first reported evidence of skin microbial metabolism of bile acids. DCA was differentially metabolized by two microbes, oxidation by *M. luteus* and conjugation by *S. epidermidis*. Studying their roles on the skin will illuminate the diverse landscape of bile acids and their metabolizing microbes. Altogether, this study opens new pathways to utilise skin microbiome-derived bile acids to promote skin health or treat diseases.

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Investigating the Role of Bacterial Metabolites as Regulators of the Epidermal Barrier in 3D models

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The skin microbiome is a complex community of microorganisms that plays a critical role in maintaining barrier integrity. *Cutibacterium acnes*, the most prevalent member of the skin microbiome contributes to barrier function by metabolizing sebum into free fatty acids as well as interacting with keratinocytes and immune cells to modulate antimicrobial peptide production and inflammatory responses.
The role of individual microbial metabolites in supporting skin barrier function has not been fully elucidated. It is also not yet fully understood how different bacterial species and strains interact with the skin barrier. A greater understanding of the skin microbiome and its metabolites is essential to identify bacterial species and strains that can act as regulators of the skin barrier. This project aimed to generate a model for evaluating the effect of relevant bacterial metabolites on epidermal differentiation and the skin barrier. Two *C. acnes* strains, ATCC11828 (considered a commensal strain) and ATCC6919 (an acne-associated strain), were grown in artificial sebum to collect physiologically relevant metabolites. Metabolites, alongside metabolites studied in previous literature, were screened using 2D differentiation assays with nTERT cell-lines. Then further investigated in a fully immortalized 3D skin organotypic model using nTERTs and HCA2s. Tryptophan metabolites were found to alter keratinocyte differentiation patterns in 3D models. Metabolites triggered early differentiation with altered patterns of ki67, Involucrin, K10, and TGM1 expression, suggesting a direct role in shaping skin barrier architecture. Metabolites also promoted more consistent tight junction expression in 3D skin models. These findings highlight the importance of microbial metabolites as active regulators of skin homeostasis and provide a foundation for future research into microbiome-based strategies for enhancing barrier function and preventing skin disorders.

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Natural sulfated polysaccharides and glycolipids inhibit the adhesion of an atopic dermatitis *Staphylococcus epidermidis* strain to the stratum corneum

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In atopic dermatitis (AD) the abundance of *S. aureus* and *S. epidermidis* on lesions can exacerbate underlying inflammatory condition and itch. Thus, it is desirable to inhibit the adhesion of *S. aureus and S. epidermidis* to the stratum corneum (SC) to provide a therapeutic improvement of AD.The objective of this study was to obtain a better understanding of *S. epidermidis* adhesion to SC and to discover potential natural inhibitors of this interaction. We isolated several strains of *S. epidermidis* from both lesional and non-lesional sites in a clinical study of mild to moderate AD.Using mutagenesis strategies, we demonstrated that *in vitro* adhesion assays *S. epidermidis* AD strains bound to the periphery of corneocytes via its adhesin SdrF and across their surface via the Aap protein. Furthermore, while it is known that the Aap protein binds to glycans on the corneocyte surface, we showed that in bacterial adhesion assays SdrF interacted specifically with Desmoglein 1 (DSG1). However, few of the isolated *S. epidermidis* AD strains bound to DSG1 suggesting that corneocyte glycans are preferred binding sites for their adhesion. Thus, we evaluated the inhibitory potential of a natural sulfated polysaccharide, carrageenan, and a glycolipid, rhamnolipid, in an *in vitro* corneocyte adhesion assay.They significantly inhibited (> 80 % $p<0.001$) the adhesion of a *S. epidermidis* AD strain to the surface of corneocytes. Thus, natural sulfated polysaccharides and glycolipids could be used in cosmetic formula in *vivo* to modulate the adhesion of staphylococcus epidermidis to the SC in moderate AD and consequently treat the symptoms of inflammation and itch associated with this condition.

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Promising synergistic effects of skin commensals on keratinocytes

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Maintenance of epidermal homeostasis depends on a balanced skin microbiome, where commensal bacteria modulate antimicrobial peptides (AMP), barrier proteins, and immunoregulatory factors. While individual strain effects are well-described, it remains unclear whether bacterial combinations act additively or synergistically; a key question in atopic dermatitis (AD), where dysbiosis, barrier dysfunction, and immune dysregulation are interconnected. This study aimed to identify synergistic bacterial strains enhancing epidermal defense (e.g., via AMP induction, barrier improvement, or anti-inflammatory effects) and determine if benefits persist under AD-like conditions.
Primary human keratinocytes were stimulated with supernatants of selected commensals, singly or in combination, with or without IL-4/IL-13 pretreatment to simulate an AD milieu. Gene expression of the AMP human beta defensin (hBD)-2 and -3 and RNase 7, barrier proteins, and inflammatory markers was analyzed via qPCR.
Applied individually, supernatants from selected strains showed differential AMP induction; in some cases, this was accompanied by inhibition of the eosinophil-recruiting chemokine CCL26, which plays an important role in inflammatory

processes. Notably, specific combinations produced markedly stronger AMP induction than single supernatants, suggesting additive or synergistic interactions. This gain occurred under basal conditions and, partially, in the AD model. Certain combinations also elicited higher filaggrin (FLG) expression than individual strains. Beyond supra-additive effects on individual targets, we also assembled combinations with complementary functional profiles; for example, pairing a strong AMP-inducing yet FLG-suppressive supernatant with an FLG-inducing counterpart mitigated this trade-off and yielded a more balanced response.
These results suggest a potential approach for microbiome-based combination strategies to support immune homeostasis on atopic skin, though validation in differentiated skin models is required.

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Human-relevant skin-on-chip platform integrating microbiome: from validated applications to mechanistic investigation of ageing and barrier dysfunction

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Skin ageing and microbiome dysbiosis are tightly interconnected processes contributing to barrier impairment, chronic low-grade inflammation, and altered response to topical interventions. Conventional in vitro models fail to reproduce dynamic exposure conditions and host–microbiome interactions, limiting mechanistic understanding and translational predictivity. Millifluidic organ-on-chip platforms enable physiologically relevant dynamic culture, improving human response prediction. This work presents a human-relevant skin-on-chip platform with validated applications in permeation and irritation testing, and outlines its capability to support microbiome integration and mechanistic studies of ageing-associated barrier dysfunction, leveraging approaches established in other epithelial organ-on-chip models.
A patented millifluidic system was used to culture reconstructed human skin under perfusion, ensuring physiological nutrient exchange and long-term viability. The platform was validated for barrier function assessment through permeation studies and for irritation testing under dynamic exposure. Additional capabilities are supported by cross-model validation, including: (i) microbiome co-culture and probiotic interventions demonstrated in epithelial organ-on-chip systems (e.g., vaginal models); (ii) inflammatory and barrier dysfunction readouts (TEER, permeability, IL-6 and IL-8) established in gut-on-chip models. Dynamic culture supported robust tissue viability and reproducible barrier function, enabling reliable permeation and irritation assessment versus static systems. Complementary studies in other models demonstrated controlled microbiome integration, modulation of barrier integrity, and context-dependent effects of probiotic interventions, supporting transferability to skin applications.
In conclusion, the skin-on-chip platform provides a robust system for dynamic permeation and irritation testing. Building on validated approaches in other models, it enables future mechanistic investigation of skin ageing and microbiome-driven barrier dysfunction, supporting predictive, animal-free dermatological research.

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Altered immunophenotype may explain the persistence of psoriasis on the lower legs after highly effective biological therapy

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Psoriasis is a chronic, immune-mediated inflammatory skin disease with significant variability in treatment response across different anatomical sites. While biologics have revolutionised psoriasis management, a strong resistance to this therapy in lower-leg (LL) versus non-lower-leg (NLL) lesions suggests underlying molecular differences driving recalcitrance.
This study compares the proteome of LL and NLL psoriasis to identify molecular mechanisms that may explain this difference. A total of 75 non-invasive biopsies were collected from 25 patients at the Department of Dermatology, Westmead Hospital, Australia. Tape stripping was used to collect samples from LL and NLL psoriatic lesions, as well as normal skin (NS). Proteins were extracted and prepared before their identification and quantification using a data-independent acquisition mass spectrometry-based proteomics. Statistical and bioinformatic analysis were performed to identify differentially abundant proteins and pathways altered between the lesion sites.
A total of 4,587 unique protein groups were identified across all samples. Differential abundance analysis revealed a trend of downregulated proteins related to innate immune defence in LL lesions. Pathway analysis predicted a strong link to a weakened antimicrobial barrier through downregulation of antimicrobial peptides, and revealed potential additional mechanisms behind psoriatic inflammation, distinct from the traditional IL-23/Th17 pathway in the LL.
Overall, this study proposed novel mechanisms contributing to the recalcitrant nature of LL psoriasis, revealing proteomic signatures that drive persistent inflammation and keratinocyte differentiation. By identifying key deficiencies in innate immune defence and alternative inflammatory pathways, this research may inform the development of site-specific therapeutic strategies to improve outcomes for patients with challenging LL psoriatic lesions.

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The Cottontail Rabbit Papillomavirus Model Recapitulates Key Features of Human Cutaneous Warts

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Cutaneous warts are common contagious skin lesions caused by human papillomaviruses (HPV), with high prevalence across children, adolescents and adults. Current therapeutic options show limited efficacy, require prolonged treatment periods, present tolerability issues, and recurrences are common, highlighting a clear unmet medical need for persistent and recalcitrant warts. Because papillomaviruses are species specific, HPV does not infect standard laboratory animals, and mouse papillomavirus models are constrained by spontaneous wart regression. In contrast, rabbits infected with Cottontail Rabbit Papillomavirus (CRPV) develop persistent and sizeable cutaneous lesions resembling HPV induced warts. In this study, we established a CRPV infection model using a DNA plasmid encoding the full CRPV genome, instead of infectious virus, to reduce biosafety risks while maintaining robust lesion formation. Resulting warts were characterized by using proteomic and transcriptomic analysis methods and align with human HPV infection. CRPV induced lesions showed features typical of HPV

induced warts, including low immune infiltration and limited antigen presentation. However, they exhibited a highly proliferative and aggressive phenotype that aligns more closely with HPV driven malignancies than with benign human warts. Despite this limitation, the plasmid based CRPV model constitutes a valuable in vivo platform to investigate the biology of persistent papillomavirus lesions and to evaluate novel therapeutic strategies aimed at achieving clearance and reducing recurrence.

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Staphylococcus aureus colonization of the nasal cavity is frequently observed in patients with atopic dermatitis despite dupilumab therapy

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The skin microbiome of patients with atopic dermatitis (AD) is often characterized by an excessive overgrowth of the pathogen *Staphylococcus aureus*. Numerous studies have meanwhile shown its importance as a driver of AD symptoms and skin colonization is associated with more severe clinical symptoms. Treatment of AD with the IL-4 receptor alpha chain blocking antibody dupilumab has been shown to reduce *S. aureus* on the skin and to improve the disease symptoms. Interestingly, approximately 30% of the general population harbour *S. aureus* in their anterior nares, suggesting that the nose is a reservoir niche for this bacterium.
This study aimed to investigate the presence of *S. aureus* in the nose and on the skin in a cross-sectional clinical cohort of 91 adult AD patients with regard to therapy. Clinical severity of the disease was assessed by SCOring Atopic Dermatitis (SCORAD), as well as the local SCORAD of the lesion which was tested.
Overall, our study is consistent with previous research indicating an association between *S. aureus* skin colonisation and higher SCORAD. This was also evident in the local SCORAD of the target lesion. However, nasal colonisation was not associated with severity. In our cohort, the most common systemic therapy was dupilumab, and it was associated with lower *S. aureus* burden in general and specifically the lowest number of *S. aureus* skin positive patients. Nasal carriage under dupilumab was however unexpectedly frequent, and detectable in 53% of patients. Noteworthy, the duration of dupilumab treatment had no effect on the nasal carriage of *S. aureus*.
In conclusion, our dataset confirms that dupilumab reduces the *S. aureus* burden on the skin of patients with atopic dermatitis, but suggests that its effect on *S. aureus* in the nasal cavity is weaker or absent. Although nasal carriage does not appear to be associated with AD severity, it still merits attention, as it presumably acts as a reservoir for the pathogen.

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Novel colorimetric method evaluating sweat composition and its association with skin microbiome in patients with atopic dermatitis

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Atopic dermatitis (AD) involves altered sweating function and skin dysbiosis, but in vivo links between sweat composition and the skin microbiota remain poorly understood. Using a novel colorimetric method for evaluating sweat, we phenotyped sweat in AD patients and explored associations with bacterial and fungal skin communities.
Twenty moderate-to-severe AD adults and 20 healthy controls (HC) were enrolled. Five-analyte strips (Glucose, Na, K, Cl, pH) were worn at the antecubital fossa and abdomen for 24–36 h; after removal, strips were imaged by stereomicroscopy with an in-frame color reference chart. Bacterial 16S rRNA and fungal ITS1 amplicon sequencing for microbiome analyses were performed by swabbing on contralateral antecubital and abdominal skin.
Sweat glucose levels at both the antecubital fossa and abdomen did not differ between AD and HC. Sweat sodium also showed no significant between-group difference (antecubital AD/HC median 32.5/22.8, p=0.344; abdomen 6.7/20.0, p=0.365); potassium, chloride, and pH are currently under analysis. In skin microbiome analyses, S. aureus was selectively detected in 8/19 (42%) at the antecubital fossa and 6/20 (30%) at the abdomen in AD patients, whereas in 1/19 (5%) and 0/20 (0%) of HC, respectively (p=0.019 and p=0.020). An α-diversity index, Pielou evenness, for bacterial microbiome was significantly reduced at the AD abdomen (p=0.019). At the antecubital fossa, genus Staphylococcus and genus Malassezia abundances inversely correlated with sweat glucose (ρ=-0.35, p=0.033 and ρ=-0.42, p=0.019, respectively; within-AD Malassezia subgroup ρ=-0.55, n=15, p=0.034).
Thus, sweat glucose concentration in sweat in patients with atopic dermatitis (AD) may be associated with skin bacterial and fungal microbiota. Sweat glucose may have a negative correlation with the genus Staphylococcus, which provides a new perspective on AD management by focusing on the properties of sweat.

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The differential effect of dupilumab and JAK inhibitors on the skin microbiome in patients with moderate-to-severe atopic dermatitis in daily practice: data from the BioDay Registry

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The skin microbiome of atopic dermatitis (AD) patients is characterized by decreased bacterial diversity and increased colonization with *Staphylococcus aureus*. This study evaluates the differential effect of dupilumab and JAK1-selective inhibitors (JAK1-i) on the skin microbiome of adult AD patients.
Skin swabs were collected from adult AD patients: for dupilumab (n=10) lesional/non-lesional at baseline (T0) and week 16 (T16); for JAK1-i (n=15) lesional/non-lesional/nasal at T0, week 4 (T4) and week 28 (T28); and for healthy controls (HCs) (n=17) at one single time point. Relative abundance and microbial diversity were analyzed using shotgun sequencing.
In both groups, Eczema Area Severity Index (EASI) scores significantly decreased over time, reflecting clinical response. For lesional skin, a significant decrease of *S. aureus* abundance was observed at T16 compared to T0 for dupilumab-treated patients; however, in JAK1-i treated patients, a less apparent, non-significant decrease of *S. aureus* abundance was observed at T28 compared to T0. Additionally, during dupilumab treatment, a shift in microbial profiles and increase in diversity was

observed, which revealed a shift towards that of HCs. This was less evident in JAK1-i treated patients. For non-lesional skin, the relative abundance of the most abundant species shifted towards HCs over time in the dupilumab group, while the relative abundance remained stable and significantly different from HCs in the JAK1-i group.
Although both dupilumab and JAK1-i treatment resulted in a comparable clinical effect, both the lesional and non-lesional skin microbiome of AD patients treated with dupilumab shifted towards that of healthy skin, while this effect was less apparent during JAK1-i treatment.

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Construction of a minimal skin microbiome as a tool to study microbe-microbe interactions
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The skin microbiome plays a key role in maintaining skin health, and its imbalance contributes to inflammatory skin diseases such as atopic dermatitis, often marked by *Staphylococcus aureus* dominance. To investigate microbe-microbe interactions within a minimal skin microbiome, our aim is to develop selective culture methods that allow quantification of individual bacterial strains from mixed communities after co-culture with skin models.
Four skin-associated bacteria were selected, three commensal species (*Cutibacterium acnes, Staphylococcus epidermidis, Corynebacterium striatum*), and one pathogenic strain (*Staphylococcus aureus*). Mixed samples were plated on selective agar plates under varying conditions to enable differentiation of colony-forming units per species. Tested parameters included pH modification, temperature, culture duration, antibiotics, and colorimetric selection.
Acidification of brain heart infusion (BHI) agar to pH 4.6 selectively inhibited *C. striatum* and slowed growth of *S. epidermidis,* thereby allowing early scoring of *S. aureus* colonies. Supplementation of BHI agar with lysostaphin reduces *S. aureus* growth, while incubation at 45 °C suppresses *C. striatum*, thereby enabling selective counting of *S. epidermidis* colonies. The antibiotic afabicin inhibits staphylococcal species, thereby allowing quantification of *C. acnes* and *C. striatum*. Because *C. acnes* forms colonies only under anaerobic conditions, *C. striatum* was quantified aerobically, while *C. acnes* colonies were visually enhanced and identified using X-gal overlays, which stain them bright blue.
By combining abiotic, antibiotic, and colorimetric selection strategies, we established a reproducible method to quantify individual alive bacterial populations within a defined minimal skin microbiome. These optimized separation techniques can support future studies on microbe-microbe interactions and the growth dynamics of microbes on various *in vitro* models such as the human epidermal equivalents.

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Skin Microbiome Manipulation as a Probiotic Strategy to Resolve Underarm Body Odor
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Body odor is primarily driven by microbial dysbiosis in the underarm, where odor-causing bacteria such as *Corynebacterium* species metabolize sweat into malodorous volatile compounds. This study investigated whether targeted skin microbiome editing, both on skin and on textiles, can provide a lasting reduction in body odor by restoring a health-associated microbial community.
Participants with body odor received a probiotic spray containing live *Staphylococcus* strains, administered over multiple weeks in a placebo-controlled design. Microbiome composition was tracked longitudinally via DNA sequencing. Volatile organic compounds were identified by GC/MS to objectively assess odor reduction. Probiotic-infused textiles were additionally evaluated through wear trials using both panel and self-assessed odor scoring.
The interventions successfully shifted the underarm microbiome toward *Staphylococcus*-dominance, displacing odor-associated *Corynebacterium* species. Microbiome changes persisted throughout the applications, and persistent when the placebo treatment was applied, thus leading to a durable shift in microbiome. Odor assessments marked significant improvements in hedonic value and odor intensity. GC/MS confirmed a significantly altered volatile profile, with marked reduction in malodorous molecules in treated versus placebo subjects. Probiotic textile trials demonstrated significant odor reduction ($p<0.05$) by both panel and self-assessment after one day of wear.
These results demonstrate that skin microbiome editing is an effective, natural, and durable strategy for managing chronic body odor, with translational potential across topical probiotics and functional textiles as alternatives to conventional antiperspirants.

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Ultraviolet Fluorescence Dermoscopy (UVFD) at 365 nm as a Novel Adjunctive Tool for the Bedside Diagnosis of Scabies
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Ultraviolet fluorescence dermoscopy (UVFD) at 365 nm is an emerging non-invasive tool that enhances bedside diagnosis of scabies by improving visualization of the mite-gallery unit (MGU).
A 24-year-old male presented with a 1-week history of intensely pruritic papular lesions over the wrist with nocturnal exacerbation. Examination revealed multiple erythematous papules coalescing into linear burrow-like lesions over the flexor aspect of the left wrist, with no other sites involved. Polarised dermoscopy (10×, contact mode) demonstrated a curvilinear burrow with a dark triangular structure at the leading edge—the classic “delta wing” sign—corresponding to the mite head, along with subtle scaling.
Switching to 365 nm UV mode revealed a continuous bluish-white to yellow-green fluorescent tract (“white wave”)

corresponding to the burrow, with a focal bright terminal “green dot” indicating the mite. This markedly enhanced lesion contrast and enabled rapid, non-invasive diagnosis without skin scraping.
The patient was treated with topical permethrin 5% cream (two applications, one week apart) and oral ivermectin 12 mg, with simultaneous treatment of household contacts. Complete resolution was observed at 4-week follow-up.
Scabies, caused by the mite *Sarcoptes scabiei*, affects millions worldwide. UVFD allows visualization of both components of the MGU—the fluorescent burrow (body) and mite (head)—and may be especially useful in early, excoriated, genital, or darker skin lesions where conventional dermoscopy is limited.
This case highlights UVFD as a rapid, contact-free, and diagnostically superior adjunct to conventional dermoscopy, facilitating prompt diagnosis and treatment of scabies in routine clinical practice.

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Zinc for Molluscum Contagiosum: An Integrative Review of Efficacy and Safety
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Molluscum contagiosum is a self-limited cutaneous poxvirus infection, predominantly affecting children. Although spontaneous resolution is frequent, treatment may reduce autoinoculation, transmission, inflammation, secondary infection, scarring, pigmentary changes, psychosocial burden and repeated medical visits. Zinc is an essential micronutrient involved in epithelial integrity and innate and adaptive immunity, and has been investigated for viral skin infections. This integrative review evaluated the efficacy and safety of zinc in molluscum contagiosum. A search was conducted in PubMed, Embase and Scopus from inception to April 2025 using “zinc” and “molluscum contagiosum”. Clinical trials, observational studies and case reports assessing zinc were included, and outcomes were analyzed descriptively. Three studies comprising 85 children aged 3 to 11 years were included. Zinc was administered orally as zinc sulfate in two studies and topically as zinc oxide in one. All studies reported lesion resolution. Complete clearance was observed in 66%, 43% and 47.5% of patients, while partial response ranged from 25.4% to 33%. Treatment failure occurred in two studies, affecting 13% and 28% of participants. Among studies evaluating serum zinc levels, oral zinc sulfate significantly increased zinc concentrations. Recurrence was described in one patient among six followed in one study. Adverse events were mild and limited to gastrointestinal symptoms, including nausea, abdominal pain and constipation, in one oral zinc study. Although current evidence suggests that oral and topical zinc may be safe and potentially effective for molluscum contagiosum in children, interpretation is limited by few studies, heterogeneous formulations, variable doses, different durations and lack of robust controlled trials. Zinc may be particularly relevant in patients with atopic dermatitis, in whom molluscum contagiosum may be more extensive and zinc deficiency has been described. Randomized controlled trials are needed to define optimal formulation, dose, duration, durability of response and long-term safety.

TRANSLATIONAL SKIN RESEARCH, AI

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Single cell multi-omic analysis of IL-4 receptor-alpha blockade demonstrates reduction in ex-tissue-resident memory T-cells precedes the resolution of atopic dermatitis
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Atopic dermatitis (AD) is the most common inflammatory skin disease. While IL-4 receptor (IL4Rα) inhibitor biologic drugs are highly effective at inducing AD remission, patients commonly experience disease recurrence upon treatment withdrawal. Evidence suggests that pathogenic memory T lymphocytes can persist in the skin and circulation even after clinical AD resolution and retain a proinflammatory phenotype. However, the sequence of immune events across the skin and blood that underpins AD remission and recurrence remains poorly defined. To address this, we serially sampled 35 patients with AD (20 males; mean age 39.1) receiving the IL4Rα inhibitor dupilumab at baseline, day 3, day 14, week 12 and week 24 of treatment. We profiled circulating immune populations using single cell transcriptome, surface proteome and TCR repertoire analysis of PBMCs. In parallel, we analysed matched skin samples from a subset of individuals using 10x Xenium to spatially map these immune populations in eczema skin at single cell resolution. We defined 18 broad immune populations in >1.2 million PBMCs and further resolved 13 CD4+ T cell populations. We observed an early expansion of memory regulatory T cells during IL4Rα inhibitor-induced AD resolution that was transient in blood but sustained in skin. Conversely, we observed a significant decrease in the abundance of circulating CD4+ ex-tissue resident memory (Trm) cells after just three days of treatment (p<0.05), but no detectable change was observed in skin Trm cells during AD resolution. Analysis of a separate AD cohort of 8 individuals undergoing treatment discontinuation (3 males; mean age 44) showed that the frequency of circulating CD4+ ex-Trm cells rebounds upon AD recurrence. Our study provides an integrated view of blood-skin immune dynamics and suggests that changes in circulating ex-Trm cells precede clinical remission and accompany recurrence, highlighting their potential as therapeutic targets for immune monitoring and long-term disease modification in AD.

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Recombinant Protein Therapy for the Treatment of Junctional Epidermolysis Bullosa
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Junctional epidermolysis bullosa (JEB) is a rare genetic skin disorder leading to severe skin fragility from birth and widespread blistering. It is caused by mutations in genes encoding the skin basement membrane proteins, Laminin 332, type XVII collagen or the basement membrane binding integrin α6β4, which anchor the epidermis to the dermis. The most severe form, JEB

generalised severe, is caused by loss of function mutations in the basement membrane protein Laminin 332. Patients suffer from failure to thrive, poor wound healing, anaemia, respiratory complications, and infections, with babies diagnosed with this form generally not surviving past their first birthday. The aim of this study was to explore the efficacy of delivering recombinant laminin 332 (rLM332) to skin for the treatment of JEB.
In JEB eKO mice, a $K14^+$ cell specific, tamoxifen inducible knockout of the laminin α3 gene ($Lama3^{flox/flox}K14^{CreERT}$) results in progressive blistering of the skin and oral mucosa, mirroring the human disease. Using subcutaneous injection and topical application, we examined the impact of rLM332 on JEB eKO mouse skin health and wound healing.
Both topical application of rLM332 and subcutaneous injection results in localisation of the protein to the skin basement membrane. rLM332 treatment reduced skin blistering, increased wound healing rate and improved the organisation of the basement membrane, including type VII collagen deposition. These improvements were durable, persisting at least 10 days after protein application.
These results demonstrate for the first time that recombinant protein therapy presents a promising therapy for the treatment of JEB in patients. In mice, we observed durable improvements in skin health and accelerated wound healing. Topical application of recombinant protein is a non-invasive, off-the-shelf approach to JEB therapy.

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Single-cell profiling reveals melanocyte maturation arrest and sustained immune activation in treatment-resistant vitiligo skin

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Therapeutic repigmentation of vitiligo lesions is frequently incomplete, yet the biological features distinguishing responsive from refractory lesions remain unclear. We sought to define the cellular programs that favour or impede pigment restoration in vitiligo.
We conducted a clinical trial enrolling 10 patients with active vitiligo who received oral mini-pulse corticosteroids and phototherapy for 6 months. Skin biopsies were collected from the perilesional area at baseline, and subsequently from repigmented and non-repigmented areas of the same lesion at month 3 and at the end of treatment. 5′ single-cell RNA sequencing with paired T-cell receptor sequencing was performed on 50 skin samples.
Repigmented skin displayed restoration of melanocyte maturation within progenitor-like and immature melanocyte populations. In contrast, non-responsive lesional skin retained melanocyte-lineage cells in progenitor-like and migratory immature states, indicating a block in terminal differentiation. Treatment-resistant areas also preserved activated immune niches, characterized by activated Langerhans cells and dendritic cells expressing *IL1B*, *IL23A*, and *CXCL8*, alongside persistent activated T-cell compartments supported by clonal continuity. Conversely, repigmented skin was enriched for regulatory and reparative myeloid states expressing *CCL22, IL1RN*, and *TNFAIP3*. Cell–cell interaction analysis further showed enrichment of immune-regulatory signaling in repigmented skin, involving regulatory T cells, dendritic cells, and the IL-10 and TGF-β pathways, whereas activation-associated interactions predominated in non-repigmented skin.
These data identify two converging drivers of failed repigmentation: sustained immune activation and melanocyte differentiation dysfunction. In contrast, successful repigmentation is associated with immune regulation that enables progenitor-like melanocytes to mature into functional pigment-producing cells.

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The Skin Genetics Consortium: a large-scale meta-analysis of genome-wide association studies across skin diseases and traits

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The Skin Genetics Consortium (SGC) is an international collaboration leveraging population-scale biobanks to systematically resolve the genetic architecture of skin disease. Digital biobanks combining genetic data with electronic health records (EHR) remain underutilised in dermatology, despite collectively including millions of ancestrally diverse participants.
The SGC has engaged over 20 biobanks worldwide, encompassing approximately 4.8 million research participants, to conduct genome-wide association study (GWAS) meta-analyses for 106 skin conditions captured in structured EHR data. Standardised workflows have been developed and distributed to contributing sites for phenotype harmonisation, quality control and association analysis.
Here we present an update on the progress of the first phase of the SGC meta-analysis programme, including preliminary findings from the initial rounds of data collection and harmonisation across contributing biobanks. We describe the scope of the phenotypes under investigation, which span inflammatory, autoimmune, infectious and neoplastic skin diseases, and report on the range of sample sizes expected and the ancestral diversity of the combined dataset.
Results from interim GWAS meta-analyses demonstrate the potential of this approach to substantially increase the number of identified genetic susceptibility loci for many skin conditions, including in previously understudied ancestry groups. Summary statistics from the SGC will be made publicly available through the Skin Knowledge Portal, an open-access platform for dissemination and visualisation.
This initiative represents an unprecedented effort to generate a comprehensive genetic resource for skin disease, with the aim of galvanising translational research and informing therapeutic strategies across dermatology.

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Artificial intelligence (AI)-based whole-slide image analysis combined with clinical parameters improves the prediction of metastatic risk and outcomes in Cutaneous Squamous Cell Carcinoma

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Cutaneous squamous cell carcinoma (cSCC) is the most common metastatic skin cancer. However, identifying aggressive primary tumors with metastasizing potential remains difficult to predict at the time of diagnosis. Metastatic disease is associated with increased morbidity and mortality, underscoring the need for improved risk stratification. In this study, we present a proof-of-concept approach utilizing artifical intelligence (AI)-based analysis of histopathological whole-slide images to distinguish non-metastatic cSCC (non-mcSCC, n=59) tumors from metastatic cSCC (mcSCC, n=54) tumors. The AI-based framework employed in this study consisted of two primary components: feature extraction was done with UNI2 model, a histopathology foundation model, and for classification attention-based multiple-instance learning was adopted as the aggregation and prediction head. We show a novel approach to AI-based image analysis by providing detailed information about regions of interest identified by the AI as prognostically significant within cSCC tumors and tumor microenvironment. The AI model used in this study was able to separate mcSCCs of non-mcSCCs with high accuracy (AUC 0.858 ± 0.102). By combining AI-prediction of metastatic risk with AJCC (8^{th}) T3-T4 stages, the model predicted metastatic risk more accurately compared to conventional risk stratification methods. In addition, survival analysis of patients with AI-prediction of metastasis combined with Clark 5 class and AJCC (8^{th}) T3-T4 stages were linked to poor outcome. Our findings highlight the potential of AI-assisted image analysis as a complementary tool for improved risk stratification of metastatic risk and prognosis of cSCC patients at the time of primary diagnosis.

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LCK and HOMER1 gene expression-based classifier distinguishes early mycosis fungoides from eczema and psoriasis

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Mycosis fungoides (MF) is a rare form of cutaneous T cell lymphoma that often mimics eczema and psoriasis in its early stages. This frequently leads to misdiagnosis and delayed treatment, highlighting the need for improved diagnostic strategies. Therefore, we aim to establish and validate a gene expression-based classifier with high accuracy already at the early disease stages. We developed a biomarker discovery framework based on bulk RNA sequencing of skin biopsies (19 MF, 105 eczema, 112 psoriasis), which identified seven candidate diagnostic genes. RT-PCR analysis of formalin-fixed and paraffin-embedded (FFPE) tissue specimens (65 MF, 101 eczema/psoriasis) verified *LCK* and *HOMER1* as robust discriminator genes. A logistic regression model based on *LCK* and *HOMER1* gene expression was able to differentiate between MF and eczema or psoriasis with 91% sensitivity and 94% specificity. The robustness of the classifier was confirmed in seven independent international cohorts (58 MF, 55 eczema/psoriasis). Additionally, case studies demonstrated earlier detection of MF with the classifier compared to conventional histopathology. Spatial and single-cell transcriptomic analyses were conducted to reveal the biological underpinnings of classifier accuracy. *LCK* was found to be enriched in malignant and specific T cell subsets in MF, whereas *HOMER1* was confined to keratinocytes in eczema and psoriasis but was nearly absent in MF.
This molecular diagnostic approach enables earlier and more reliable distinction of MF from common inflammatory dermatoses, offering a clinically applicable tool to reduce diagnostic uncertainty, accelerate appropriate treatment, and improve patient outcomes.

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Proteomic Profiling of Benign and Dysplastic Nevi Using a Non-Invasive Scarless Biopsy Approach

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Melanoma may arise from pre-malignant lesions, including benign and dysplastic nevi, and distinguishing these lesions is critical for improved patient outcomes. Histopathology remains the gold standard but is invasive and may not always reliably distinguish them. This study aimed to apply a non-invasive scarless biopsy technique (tape stripping) combined with proteomics to identify protein biomarkers distinguishing benign and dysplastic nevi. Scarless biopsy using tape stripping was performed to collect samples from patients with benign (n=46) and dysplastic nevi (n=49) attending dermatology clinics at Westmead, Alfred, and Princess Alexandra Hospitals. Proteins were analyzed using data-independent acquisition liquid chromatography–mass spectrometry (DIA LC–MS), followed by data processing and molecular pathway analysis. Participant characteristics were similar: age (benign: 60.2 ± 12.8 vs dysplastic: 55.9 ± 15.4 years; p = 0.14) and sex distribution (female: 56.5% vs 53.1%, male:

43.5% vs 46.9%; p = 0.89). A total of 4,065 proteins were identified. Principal component analysis showed limited separation (PC1: 15.5%, PC2: 9.1%). Differential abundance analysis identified 13 significantly altered proteins (adj. P value < 0.05, |logFC| ≥ 0.5), including C16orf89 and RBBP6. Biological function analysis indicated enrichment of cancer-related processes and protein metabolism in dysplastic nevi, with inhibition of RNA processing, cell viability, and migration, and activation of extracellular matrix organization and tissue remodeling. Molecular pathway analysis showed strong inhibition of EIF2AK4 (GCN2)-mediated amino acid response and translation-related pathways. Upstream regulator analysis showed inhibition of MYC family members and MLXIPL, with activation of LARP1 and FMR1. This study highlights the potential of non-invasive scarless biopsy combined with proteomics to identify biomarkers such as C16orf89 and RBBP6 for distinguishing benign and dysplastic nevi, with dysplastic transformation involving targeted protein synthesis and metabolic alterations.

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Community-level microbial balance captures treatment-relevant skin microbiome structure beyond individual taxa in atopic dermatitis

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Skin microbiome studies in atopic dermatitis (AD) have largely focused on individual taxa such as *Staphylococcus aureus*, yet taxon-centric analyses may miss clinically meaningful variation at the community level.
We profiled skin microbiomes from four body sites in 215 systemic therapy-naive patients with AD and 98 healthy controls by 16S rRNA sequencing. After amplicon sequence variant (ASV) inference by DADA2, multisite samples were integrated at the patient level using compositional tensor factorization (CTF) followed by unsupervised clustering. This analysis identified two clusters: a lower-severity group enriched in healthy controls and a predominantly AD group with higher objective SCORAD, serum IgE, and TARC. To capture community-level ASV patterns underlying this separation, we computed a microbial balance score as a log-ratio derived from 20 ASVs that best distinguished the clusters. This microbial balance—reflecting *Cutibacterium/Corynebacterium*-associated versus *Staphylococcus*-associated ASVs—significantly distinguished the groups, whereas individual taxa, including *S. aureus*, did not. This indicates that clinically relevant microbiome variation was better captured by coordinated community structure than by individual taxa alone. Consistent with this, higher baseline microbial balance was associated with lower post-treatment objective SCORAD at 2–3 months among dupilumab-treated patients (n = 14), after adjustment for baseline severity.
These findings suggest that AD-associated skin microbiome variation is hierarchically organized: CTF-derived patient-level structure reflects disease burden, whereas microbial balance captures additional treatment-relevant variation beyond individual taxa. Microbial balance may represent an interpretable biomarker for treatment-response stratification in AD, as exemplified by dupilumab.

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Photoacoustic Tomography as a Next Generation Tool for Topical Bioequivalence Assessment

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Topical bioequivalence (BE) assessment is essential for ensuring both pharmaceutic and therapeutic equivalence of topical products, whose complex microstructure, especially in semisolid dosage forms, highly influences drug release, skin permeation and bioavailability. Clinical endpoint studies are the gold standard, however due to scientific and economic constraints, alternative mechanistic and performance-based approaches, such as *in vitro* permeation testing, are now accepted. Yet, this approach requires human skin, thereby introducing ethical, logistical, and variability constraints. Reconstructed and synthetic membranes, though more reproducible, fail to mimic physiological settings, motivating interest in innovative methodologies that capture drug behaviour within the skin under controlled, clinically relevant conditions.
The purpose of this study was to evaluate photoacoustic tomography (PAT) as a non-invasive method for topical BE by visualising drug permeation and distribution across skin layers. A controlled experimental design was implemented using heat-separated human epidermis and four formulations: a reference corticosteroid cream, a test generic cream, a placebo base, and the isolated active drug clobetasol propionate, within a solvent formulation. Photoacoustic signals were acquired under wavelength-dependent laser excitation to capture the drug optical absorption within the tissue and reconstructed into depth-resolved images for quantification. The isolated active drug served as calibration reference for identifying characteristic absorption peaks and validating the analytical strategy. Reference and generic formulations produced comparable spectral patterns, with amplitude differences suggesting permeation kinetics variations, while placebo exhibited only baseline tissue absorption, confirming method specificity.
These findings demonstrate that PAT may be used to map drug distribution in skin, with sufficient sensitivity to differentiate formulations, supporting its potential as a complementary, real-time, non-destructive imaging method for topical BE assessment capable of capturing mechanistic drug permeation differences beyond conventional *in vitro* methods.

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Data-driven biomarkers for metastatic cutaneous squamous cell carcinoma using artificial intelligence

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Cutaneous squamous cell carcinoma (CSCC) patients at high risk of metastasis are insufficiently identified by current staging systems. Advances in artificial intelligence (AI) offers opportunities to improve risk stratification by extracting prognostic histomorphological features from hematoxylin and eosin (H&E) slides. We evaluated whether an attention-based multiple instance learning approach (AddMIL) can distinguish primary CSCC that metastasized from those that did not, and generate slide-level metastatic risk scores. The AddMIL model development used a large pretrained CSCC model (7743 H&E samples) and was further trained and evaluated on 838 H&E whole slide images from a nationwide Dutch nested case-control study (D-SQUAME), with cases and controls matched for metastatic risk using the Erasmus MC model. For interpretability, image patches were morphologically clustered and ranked based on slide-level predictions. The risk-matched design enables direct interrogation of prognostic morphology while minimizing clinic-pathological confounding, and is complemented by parallel data-driven workflows – slide-level prediction and independent morphological clustering using a frozen foundation model (UNI) – to reduce model-specific bias. Ranked clusters are being reviewed by pathologists to align with established and candidate metastasis-associated features. Preliminary results showed a highest AUC of 0.74 (median 0.694; IQR 0.05). Ongoing work focuses on the generation and validation of morphological concepts. This interpretable, data-driven approach enables identification of metastasis-associated regions and supports the discovery of novel histopathological biomarkers in metastatic CSCC.

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Leveraging the UK Biobank to derive proteomic signatures representative of disease biology of dermatological indications

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The identification of robust, disease-specific biomarkers remains challenging in dermatological diseases such as vitiligo and lichen planus (LP). This project investigates whether real-world proteomic data from the UK Biobank can be used to derive plasma-based disease signatures that reflect underlying disease biology and support scalable biomarker discovery that can potentially be applied to any indication.
Patients with at least one ICD10 diagnosis of vitiligo (L80) or LP (L43), a diagnosis preceding baseline, and available OLINK proteomic data were included. For each case, approximately five controls were matched by age, sex, year of birth and ethnicity. Differential protein abundance was assessed using linear modeling in 105 vitiligo patients (490 controls) and 138 LP patients (662 controls). Plasma signatures were compared with published transcriptomic data from lesional and nonlesional skin, and longitudinal patterns were explored relative to sampling time and disease diagnosis.
We identified 232 differentially abundant plasma proteins in vitiligo and 112 in LP compared with controls. Type I immune–associated cytokines and chemokines, including IL12, CXCL9, and CXCL10, were consistently upregulated in both diseases. The LP plasma signature showed concordance with lesional skin transcriptomic signatures. In vitiligo, some proteins (e.g., GZMB) were selectively altered within one year of diagnosis, whereas others (e.g., CXCL9/10) remained elevated at later time points.
These findings demonstrate the feasibility of using real-world proteomic data to derive disease-relevant plasma signatures in dermatology. Temporal differences in protein regulation suggest dynamic disease biology and potential treatment effects, supporting the use of minimally-invasive proteomic biomarkers for diagnosis and disease monitoring.

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Olfactory Receptor Signaling as a Potential Therapeutic Target in Acquired Idiopathic Generalized Anhidrosis

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Acquired idiopathic generalized anhidrosis is a rare sweating disorder characterized by impaired thermoregulatory sweating and limited therapeutic options. Because the molecular mechanisms underlying sweat gland dysfunction remain poorly defined, the development of mechanism-based treatments has been challenging. To identify novel therapeutic targets, we performed RNA sequencing–based transcriptomic analysis of eccrine sweat glands isolated from paired hidrotic and anhidrotic skin areas of patients with acquired idiopathic generalized anhidrosis.
Differentially expressed genes were validated by in situ hybridization and immunohistochemistry. Among the molecules downregulated in anhidrotic sweat glands, specific olfactory receptors were selected as potential ligand-responsive and druggable targets. Functional receptor activity was assessed using a G-protein–dependent reporter assay, and the physiological relevance of receptor activation was examined by quantitative sudomotor axon reflex testing after topical application of an odorant ligand.
Transcriptomic analysis revealed reduced expression of selected olfactory receptors in anhidrotic sweat glands. OR51A7 and OR51E2 were consistently detected in human eccrine sweat glands. Functional assays confirmed that these receptors responded to an odorant ligand via G-protein–dependent signaling. Furthermore, topical ligand application significantly modulated acetylcholine-induced sweating in healthy subjects, with sex-dependent effects: sweating was attenuated in females and augmented in males.
These results identify olfactory receptors as functional regulators of human eccrine sweat glands and suggest that altered olfactory receptor signaling may contribute to sweat gland dysfunction in acquired idiopathic generalized anhidrosis. Given their ligand responsiveness and pharmacological accessibility, olfactory receptors may serve as novel therapeutic targets for mechanism-based drug development in anhidrosis and related sweating disorders.

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A Global Atlas of Digital Dermatology to Map Innovation and Disparities

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The adoption of artificial intelligence in dermatology promises democratized access to healthcare, but model reliability depends on the quality and comprehensiveness of the data fueling these models. Despite rapid growth in publicly available dermatology images, the field lacks quantitative key performance indicators to measure whether new datasets expand clinical coverage or merely replicate what is already known. Here we present SkinMap, a multi-modal framework for the first comprehensive audit of the field's entire data basis. We unify the publicly available dermatology datasets into a single, queryable semantic atlas comprising more than 1.1 million images of skin conditions and quantify (i) informational novelty over time, (ii) dataset redundancy, and (iii) representation gaps across demographics and diagnoses. Despite exponential growth in dataset sizes, informational novelty across time has somewhat plateaued: Some clusters, such as common neoplasms on fair skin, are densely populated, while underrepresented skin types and many rare diseases remain unaddressed. We further identify structural gaps in coverage: Darker skin tones (Fitzpatrick V-VI) constitute only 5.8% of images and pediatric patients only 3.0%, while many rare diseases and phenotype combinations remain sparsely represented. SkinMap provides infrastructure to measure blind spots and steer strategic data acquisition toward undercovered regions of clinical space.

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Personalized readthrough therapies for junctional epidermolysis bullosa and a new enhancer

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This study aimed to develop a personalized readthrough-based therapeutic strategy for junctional epidermolysis bullosa (JEB) with collagen XVII (C17) deficiency (JEB-C17) caused by *COL17A1* nonsense mutations, by combining translation readthrough–inducing drugs (TRIDs) with nonsense-mediated decay (NMD) inhibitors.
Patient-derived keratinocytes with the following nonsense mutations were tested: p.R145*, p.R169*, p.W464*, p.R584*, p.R688*, p.G803*, and p.R1226*. Amlexanox, artesunate, ataluren (PTC124), atazanavir, clitocine, 2,6-diaminopurine, ELX-02, erythromycin, gentamicin (G), and paromomycin (P), which have been shown to promote readthrough activity in other diseases, were evaluated. The eRF1 inhibitor N-Oxalylglycine (NOG) and the eRF3 inhibitor CC90009 were combined with TRIDs. Readthrough efficacy was analyzed by C17 immunoblotting. MTT assay and *in vitro* wound closure assay were conducted to reveal the effects of the drugs on cell viability, cell proliferation, and cell migration.
We found that each mutation demonstrated an individual response pattern to TRIDs in spite of sharing the same stop codon, UGA. JEB-W464* responded to G and P, JEB-G803* responded to G, and JEB-R145* responded to G and PTC124. The other mutations did not respond with readthrough to any of the tested drugs. Notably, NOG combined with CC90009, G, and P significantly enhanced the C17 mRNA and protein level in the responsive mutations without any negative impacts on cell functions.
Our findings reveal mutation-specific readthrough patterns in JEB-C17, suggesting that this approach represents a form of individualized therapy. NOG emerged as a novel enhancer of readthrough in combination with aminoglycosides and CC90009, without significant cell toxicity. Based on the current data, the stop codon and its surrounding sequence cannot predict the readthrough response to TRIDs in JEB-C17. The observed variability in responses to TRIDs and enhancers among nonsense mutations highlights the importance of patient-derived cellular systems for evaluating readthrough efficacy for personalized therapy.

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Genetic fine-mapping of frontal fibrosing alopecia enables accurate prediction and early intervention

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Frontal fibrosing alopecia (FFA) is an increasingly common cicatricial alopecia predominantly affecting postmenopausal women. Early intervention can curtail disease progression and substantially limit its high burden of psychosocial morbidity and autoimmune comorbidities.
FFA risk is associated with four class I major histocompatibility complex (MHC) alleles and epistasis with *ERAP1* locus risk alleles. However, the non-additive contribution of individual MHC alleles, and their utility for clinical decision making, remain unclear.
We characterise the joint distribution of FFA-associated MHC alleles in an unselected UK population and estimate FFA risk attributable to common MHC profiles in four female FFA studies (UK and Spain; 1,585 cases, 5,083 controls). Using a leave-one-study-out prediction modelling framework, we test various models for their ability to discriminate FFA cases from controls. Finally, we validate the optimal model in a newly genotyped dataset of 115 Italian FFA cases and 493 controls.
We find that 42.5% of the unselected population carry at least one MHC risk allele, most commonly one copy of HLA-B*07:02 (21.6%). The FFA risk attributable to this MHC profile relative to participants with no risk alleles is much higher than previously assumed (odds ratio [OR] 14.2; 95% CI: 11.5-17.4). However, participants carrying at least one copy of HLA-A*33:01 are at substantially higher risk (OR 53.0; 95% CI 37.6-74.8) and a higher proportion of Spanish FFA cases (37.1%) than UK cases (8.4%) are HLA-A*33:01 driven.
The optimal penalised regression model includes additive terms per MHC risk allele, non-MHC loci lead SNPs, and an MHC × *ERAP1* interaction term (average area under the ROC curve [AUC] = 0.847). It is independently validated in the Italian dataset (AUC = 0.857).
We conclude that FFA risk varies based on the profile of MHC risk alleles carried, with HLA-A*33:01 carriers – more numerous in Southern European countries – at exceptionally high risk. We demonstrate a highly accurate genetic prediction model facilitating risk profiling and prompting early diagnosis when presenting clinical features are subtle.

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Skin bile acids as anti-inflammatory bioactives

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The skin hosts a dynamic metabolite landscape shaped by intrinsic biochemical activity, systemic exchange, and metabolism by resident microbiota. We performed untargeted metabolomics analysis using liquid chromatography-mass spectrometry on skin tapes and swabs from four regions of skin (antecubital fossa, scalp, cheek and axilla) and unexpectedly detected the presence of numerous primary and secondary bile acids on skin, including glycocholic acid, taurocholic acid, glycodeoxycholic

acid, taurodeoxycholic acid and lithocholic acid (LCA). Beyond digestive functions, bile acids are pharmacologically active in the gut, liver, brain, muscle and adipose tissue; but their presence and activity in skin have been neglected. Using an N/TERT keratinocyte cell line modified to express IL-1β carrying a luminescent HiBiT tag, we assessed the impact of specific bile acids on the NLRP3 inflammasome. N/TERT cells cultured in monolayers were first primed using TNFα and IFNγ, then activated with supernatant derived from a pathogenic *S. aureus* strain containing the α-hemolysin toxin. Using this assay design, we screened a panel of skin bile acids and identified LCA as strong suppressor of IL-1β secretion. Mechanistic studies revealed that 10 µM LCA was able to completely abolish the transcription of *NLRP3*. LCA is a dual agonist of both the Takeda G protein-coupled receptor 5 (TGR5) and vitamin D receptor (VDR), which are expressed in keratinocytes. Unlike LCA, single agonists of TGR5 (10 µM INT-777) and VDR (10 nM MC903) could only partially suppress *NLRP3* expression. Additionally, 1 µM LCA maintained a stronger suppression of IL-1β secretion as compared to 1 µM INT-777 and 1 nM MC903 without inducing cytotoxicity, suggesting dual agonism of TGR5 and VDR results in greater potency. In a 3D full-thickness skin equivalent model in which *NLRP3* expression was induced, LCA reduced spongiosis supressed the expression of *GATA3, STAT3* and *STAT6*, which mediate the response to Th2 cytokines. Our data therefore indicates bile acids are active in skin as potent anti-inflammatory agents.

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Pre-clinical evaluation of a pan-KRAS inhibitor on cutaneous neurofibromas in neurofibromatosis type 1 mouse model

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Cutaneous neurofibromas (cNFs) are a hallmark of Neurofibromatosis type 1 and impair quality of life. No medical therapies are approved. We evaluated a systemically administered pan-KRAS inhibitor (pan-KRASi) in cNFs using the *Prss56-Nf1* knockout (KO) mice.
Prss56-Nf1-KO mice with growing (Phase 1; n=15/group; mean age 5 months) or mature cNFs (Phase 2; n=4/group; mean age 7 months) received pan-KRASi (30 mg/kg, BID) or vehicle for 1 month. Body weight and behavior were monitored. Whole-back epifluorescence imaging (tdTomato-labeled tumor Schwann cells [SCs]) was performed at baseline and endpoint. Tumors and healthy-looking skin (HLS) were collected, weighed, and analyzed by immunofluorescence (IF), Western blot, and single-cell RNA- sequencing (scRNA-seq).
Pan-KRASi was well tolerated and reduced P-ERK. In Phase 1, fluorescence intensity and area increased in controls but remained stable with treatment. Excised treated-cNFs weighed ~20% less than controls, while adjacent HLS was unaffected. IF confirmed reduced tumor SC area (p=0.010) and proportion (p=0.012), with more bipolar/tripolar morphologies in treated tumors compared with the typical multipolar one in controls. Fibroblast (Fb) density (p=0.024) and fibrosis (collagen I, p=0.035), were decreased in treated mice. No significant differences in senescence-associated β-galactosidase, apoptosis (cleaved caspase-3, p=0.051) or proliferation (EdU incorporation, p=0.200) were observed, suggesting reduced tumor cell size underlies lower fluorescence. In Phase 2, pan-KRASi did not induce SC regression: fluorescence intensity, total area, and SC density remained unchanged. However, Fb density remained reduced (p=0.021). ScRNA-seq confirmed fewer pro-fibrotic Uni-Fb (Ly-6C+) Fb in treated tumors, with no significant immune cell changes.
This first preclinical evaluation of pan-KRAS inhibition in cNFs shows good tolerability and tumor-selective activity. Pan-KRASi suppresses early tumor growth, altering SC morphology, and reducing fibrosis through effects on pro-fibrotic Fb. These findings support further development of pan-KRASi for early intervention, potentially via systemic or topical delivery.

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Predicting infection following biologic therapy for psoriasis using an uncertainty-aware machine learning approach on real-world national registry data

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Infection risk following biologic therapy in psoriasis varies by biologic class and patient factors. Infections may trigger disease flares and lead to treatment interruption or discontinuation. Predictive tools to estimate infection risk and subsequently facilitate a more personalised approach to treatment selection are lacking. We applied machine learning (ML) and conformal prediction (uncertainty quantification) to predict infection risk following biologic initiation in psoriasis.
Adult psoriasis patients in the British Association of Dermatologist's Biologics and Immunomodulators Register initiating biologic therapy (2007–2024) were included. The outcome was first infection (MedDRA coded), excluding the common cold. Data were split 80/10/10 for training/calibration/testing and a series of ML models were evaluated. Conformal prediction, a method that quantifies prediction uncertainty, generated prediction sets with guaranteed coverage (evaluated at 85-95% certainty). Shapley values explained model predictions.
Overall, 12,889 patients were included and 55% experienced ≥1 infection. A light gradient boosting machine outperformed other ML models (F1 0.69; AUPRC 0.72 [95% CI 0.69-0.75]; AUROC 0.70 [95% CI 0.68-0.72]). Features contributing most to predicting infection included current and prior biologic therapy, sex, body mass index and an asthma diagnosis. The conformal prediction coverage guarantee held on the test set, where restricting predictions to 85% certainty reduced the model error rate from 31% to 14%. Our findings support that this explainable, uncertainty-aware ML framework enables robust infection prediction. By presenting predictions only when the model is certain, and supported by clinical justification, our model supports clinical translation aligned with regulatory requirements for interpretability and uncertainty quantification.

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Pre-clinical development of a CD200R agonist antibody for type 2 immune diseases

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CD200R (*CD200R1*) is an inhibitory receptor expressed at high levels by type 2 immune cells. Engagement of CD200R by its ligand CD200 or by agonistic antibodies suppresses immune cell activation, and published *in vivo* studies support a role for CD200R signalling in limiting inflammation. CD200R is under investigation as a therapeutic target for type 2 immune diseases, including atopic dermatitis (AD), where mutations in *CD200R1* are associated with disease risk and elevated expression is observed in affected skin.
Here, we describe the preclinical evaluation of a non ligand blocking CD200R agonist antibody, which binds to human and cynomolgus CD200R with high affinity and induces CD200R phosphorylation and DOK2 recruitment in reporter cell line assays. The structure of the antibody in complex with the extracellular domain of CD200R was resolved to 2.7 Å by cryo electron microscopy, revealing a membrane proximal epitope.
Functionally, the CD200R agonist antibody potently inhibited the activation of primary human T cells from both healthy donors and patients with AD, reducing the release of multiple Th cytokines, and also suppressed activation of *in vitro* differentiated mast cells. In a murine oxazolone induced dermatitis model, treatment with the CD200R agonist antibody significantly reduced ear thickening.
Together, these data highlight the therapeutic potential for CD200R agonist antibodies in type 2 immune disease, in particular AD.

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Artificial intelligence-mediated determination of human skin tone demonstrates strong agreement with in vivo spectrophotometry individual typology angle results.

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Assessment of skin pigmentation is important in dermatology and in clinical medicine, for example in relation to use of pulse oximetry. Accurate and reproducible quantification of skin tone in clinical and research settings remains challenging. Spectrophotometry has been shown to measure skin tone more reliably than Fitzpatrick skin typing or skin colour charts therefore this study aimed to train a neural network to classify skin tone using spectrophotometric data.
Spectrophotometric measurements of skin tone, recorded in the CIELab* colour space for calculation of individual typology angle (ITA), were obtained from four anatomic regions, with corresponding photographs taken at each site. Participants were recruited from each of the six skin tone groups and a convolutional neural network (CNN) was trained to determine ITA directly from photographs using paired spectrophotometric measurements as the ground truth. Agreement between CNN-predicted and spectrophotometry-derived ITA was assessed using regression-based metrics and correlation across six skin tone categories. A total of 3,621 paired spectrophotometric measurements and photographs were obtained from 106 individuals. CNN-derived ITA estimates showed strong agreement with in vivo measurements, achieving a root mean square error of 13.87±0.56 ITA units and coefficient of determination of 0.81±0.02. Categorical agreement between CNN-predicted and spectrophotometry-derived skin tone group was excellent, with a quadratic-weighted Cohen's kappa of 0.84±0.02. In addition, the CNN was able to assign 95.9% of cases to within the correct or immediately adjacent skin tone category.
A CNN can estimate skin tone from photographs, with category-level predictions demonstrating close agreement with spectrophotometric measurements. This artificial intelligence based approach has the potential to provide an objective skin tone quantification in clinical practice in dermatology and general medicine when accurate assessment of the patient's skin tone is required.

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Artificial intelligence for detection and longitudinal monitoring of actinic keratosis on clinical photographs

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The appearance of actinic keratosis lesions heralds the emergence of cutaneous field cancerization in chronically ultraviolet-exposed skin areas. The available clinical scoring systems to date provide an adequate global estimate of facial actinic keratosis severity, yet do not capture the dynamics of the temporospatial evolution of the highly unstable, morphologically discrete disease lesions.
To address this challenge, we implemented a transformer-based deep network that integrates fine-grained features with contextual cues from the surrounding photodamaged skin. A proprietary annotated dataset of 569 clinical face photographs from 115 patients was used to train and evaluate the model, achieving a median Dice score of 65% in whole-image assessment, comparable to human-level performance.
For global facial assessment, we applied a multi-view framework based on MediaPipe Face Mesh to establish dense anatomical correspondence across facial views. Facial anatomical landmarks and mesh triangulation were used to map lesions into a common canonical space via barycentric coordinates, enabling confident cross-view registration and avoiding duplicate lesion counts. Using frontal, 45° right, and 45° left facial views, a unified lesion count was obtained in a cohort of 25 patients with follow-up at 3, 6, and 12 months. For consistent lesion tracking across sequential visits, local surface orientation derived from mesh normals identifies the most informative views for longitudinal alignment.
Our approach demonstrated excellent agreement with expert-derived global lesion counts (Intraclass Correlation Coefficient >0.90). Beyond enabling reliable global scoring, the proposed framework provides objective, spatially explicit quantification of

actinic keratosis kinetics, with direct implications for clinical decision-making and for elaboration of standardized therapeutic endpoints in translational skin research.

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Beyond the Lesion: Circulating Proteomic Signatures for Skin Cancer Risk Prediction in UK Biobank

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Skin cancer prevention must balance ultraviolet carcinogenesis with the physiological benefits of sunlight. Yet population-level risk prediction remains limited, and skin cancers are often treated as lesion-based diseases, while circulating signals of tumour–host interaction, immune surveillance and inflammation remain underexplored.Dermoscopy-based AI models are usually trained on clinically selected lesions, limiting their relevance for pre-clinical risk stratification. Plasma proteomics may provide a complementary approach.
our objective was to develop and interpret plasma proteomic prediction models for incident basal cell carcinoma, squamous cell carcinoma and melanoma in UK Biobank.
We analysed approximately 50,000 UK Biobank participants with baseline plasma proteomics. Training and test sets were strictly separated. Feature selection was performed within the training set using Elastic-Net Cox regression, bootstrap stability ranking and 5-fold cross-validation of top-ranked protein panels, capped at 30 proteins. Logistic regression, Random Forest, Extra Trees, Gradient Boosting, HistGradientBoosting and XGBoost were evaluated. SHAP was used for interpretation.
Final panels included 30 proteins for basal cell carcinoma, 30 for squamous cell carcinoma and 25 for melanoma, satisfying events-per-variable requirements. Melanoma and squamous cell carcinoma shared MCTS1, while squamous cell carcinoma and basal cell carcinoma shared epidermal proteins including KRT5. In test-set evaluation, squamous cell carcinoma performed best, with an AUC of approximately 0.75; basal cell carcinoma and melanoma showed AUCs around 0.61. Performance was broadly similar across classifiers. SHAP identified EDA2R as the leading squamous cell carcinoma predictor.
Plasma proteomics provides an interpretable approach for skin cancer risk prediction beyond lesion-centred imaging. Squamous cell carcinoma showed the strongest performance, potentially reflecting stronger systemic signal than basal cell carcinoma and greater diagnostic stability than melanoma. EDA2R warrants further investigation as an inflammatory and ageing-related biomarker.

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Scarless Biopsy to Discriminate between Atopic Dermatitis, Psoriasis, and Actinic Keratosis using Proteomic Analysis

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Atopic dermatitis (AD), psoriasis and actinic keratosis (AK) are common skin diseases that present as inflamed, red scaly patches. They often have overlapping clinical features, making their diagnosis and classification difficult. The objective of the current study is to establish proteomic signatures for reliable, non-invasive diagnosis and classification of AD, psoriasis, and AK while providing insights into the underlying molecular pathways and biological functions of these conditions. A total of 67 scarless skin samples were collected from patients with AD (n=20), psoriasis (n=10), AK (n=20), and normal skin (n=17) using adhesive discs. These samples were analysed using data-independent acquisition mass spectrometry-based proteomics (DIA-MS) to identify and quantify proteins. Differential abundance analysis and bioinformatic analysis were then performed on the proteomic data. A total of 2202 protein groups were identified from the samples studied, pinpointing three protein specific signatures, such as protein 1, protein 2, and protein 3 that could differentiate between AD, psoriasis, or AK compared to other lesion groups, respectively. Principal component analysis correctly classified a significant proportion of the samples based on their clinical diagnosis, while support vector machine analysis correctly classified 76.5% of AD, 77.8% of psoriasis and 78.9% of AK samples according to their clinical diagnosis. Pathway Ingenuity Pathway Analysis (IPA) of the data revealed significant dysregulation of key pathways related to inflammation, immune responses, and endocytosis in different lesions. This study successfully identified lesion-specific biomarkers and biological functions through proteomic analysis of scarless skin samples from AD, psoriasis, and AK patients. These findings offer promise for the development of a non-invasive diagnostic and classification method for these skin conditions.

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Raising the standard for skin tone annotations: a comparison of the Fitzpatrick and Monk scales in a diverse multiethnic cohort

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Image-based AI models in dermatology often underperform on darker skin tones, partly due to the lack of reliable skin tone labels in training and benchmarking datasets. The Fitzpatrick skin type (FST) scale is the current labeling standard, despite having been developed for photosensitivity rather than skin tone. The Monk skin tone (MST) scale is a promising alternative, but lacks validation against objective pigmentation measures. We validated the MST against spectrophotometry and compared it to the FST in a diverse, multiethnic cohort. We conducted an observational study in two dermatology outpatient clinics. FST and MST were rated by one of three trained researchers. Participant-reported FST and MST were also recorded. Spectrophotometry on the dorsal forearm yielded the individual typology angle (ITA), an objective measure of pigmentation. The Spearman correlation (ρ) between each scale and the ITA was determined; participant-investigator agreement was evaluated using the quadratic weighted kappa. In total, 518 participants were analyzed, born in 44 countries, with 28.2% being born abroad. FST distribution was relatively balanced (I–II: 29.5%, III–IV: 42.9%, V–VI: 27.6%). Investigator-rated MST correlated more strongly with ITA than FST (ρ = −0.87, 95% CI [−0.89, −0.84] vs. ρ = −0.79, 95% CI [−0.82, −0.75]). For participant-reported measures, MST again outperformed FST (ρ = −0.85, 95% CI [−0.87, −0.81] vs. ρ = −0.67, 95% CI [−0.72, −0.61]). Participant-investigator agreement was substantial for FST (κ = 0.76) and excellent for MST (κ = 0.91). Combined with

the FST's known limitations, our findings support the MST as a more reliable scale for capturing pigmentation in dermatologic datasets. Whether this translates into improved model fairness remains to be demonstrated.

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Scalp morphology alterations under menopause and Female Pattern Hair Loss using line-field confocal optical coherence tomography

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Menopause is well-known to impact hair quality and hair loss, yet the structural evolution of the scalp tissue itself remains poorly characterized. Historically, imaging has focused on facial skin, leaving the scalp's histological environment underexplored. This study uses line-field confocal optical coherence tomography (LC-OCT) to assess the impact of menopause as well as Female Pattern Hair Loss (FPHL) on scalp internal structure.
A cohort of 77 women was categorized into four groups: pre-menopausal (mean age 46.8; N=20 no FPHL, N=18 with FPHL) and post-menopausal (mean age 55.2; N=20 no FPHL, N=19 with FPHL). Vertex measurements were compared against a facial skin reference database (temple, N=100).
Results reveal that the scalp possesses a distinct histological signature: the stratum corneum (SC) (17±4µm *vs* 13±1µm) and epidermis (87±18µm *vs* 51±4µm) were 31% and 71% thicker respectively than facial skin in age-matched groups. In menopausal women *vs* non-menopausal, a decrease in SC (15±2µm *vs* 17±4µm, p≤0.005) and epidermal thickness (76±17µm *vs* 87±18µm, p≤0.005) was observed. Interestingly, we show that menopause predominated over chronological age in driving structural change. FPHL also induced a significant decrease in SC and epidermal thickness (p≤0.05).
Dermal fiber reorganization was analyzed and showed FPHL and menopause deteriorated network integrity, with decreased bundle length and higher fragmentation in papillary dermis. This suggests that dermal fibers remodeling occurs under FPHL before any menopause characteristics are present yet.
By using LC-OCT, this study highlights the impact of menopause modulations on scalp integrity. FPHL might induce similar morphological alterations, even before menopause, suggesting that premature hair loss acts as a driver of anticipated scalp aging.

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3D human keratinocyte sheet culture on an oxygen-permeable membrane for customizable skin generation

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Oxygen availability is a critical determinant of cellular physiology; however, its role in standard cell culture systems has been underappreciated compared to temperature and CO_2 concentration. While oxygen levels in most tissues are substantially lower than atmospheric conditions, epidermal keratinocytes are uniquely exposed to ambient oxygen, suggesting a distinct sensitivity to oxygen availability.
In this study, we investigated the culture of human keratinocytes on an oxygen-permeable polyolefin membrane that provides enhanced oxygen diffusion compared to conventional plastic culture plates. We found that keratinocyte proliferation was significantly enhanced on the oxygen-permeable membrane. Moreover, long-term culture led to the formation of a stratified structure consisting of a basal-like layer and a differentiated cell layer, representing a simplified three-dimensional epidermal architecture.
Notably, this approach does not require feeder cells or air–liquid interface culture, indicating a simple and scalable method for generating three-dimensional keratinocyte sheets. The membrane substrate allows for the fabrication of customizable skin sheets with various shapes that can conform to wrinkles and joints of the human body. Transcriptomic analysis further demonstrated that the gene expression profiles of the membrane-cultured sheets were comparable to those obtained using conventional MilliCell insert-based systems.
These findings suggest that oxygen-permeable membranes provide a promising platform for the efficient generation of customizable skin constructs and may offer advantages for both basic research and translational applications.

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A model-informed precision dosing Bayesian dashboard for biologic therapies in psoriasis: Development, validation and clinical translation

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Fixed-dose biologic regimens for psoriasis overlook individual variability in drug exposure and response. Conventional therapeutic drug monitoring (TDM) adjusts dosing using serum drug levels and population thresholds, ignoring prior patient data. Model-informed precision dosing (MIPD) dashboards integrate TDM with pharmacokinetic/pharmacodynamic (PK/PD) models, patient-specific characteristics and Bayesian forecasting to personalise therapy.
Published population PK/PD models for risankizumab, adalimumab, and ustekinumab were externally validated using UK real-world data and integrated into an R-Shiny dashboard, which used Bayesian forecasting, serum drug levels, and patient covariates to recommend doses. Clinicians user-tested the dashboard, and we evaluated clinical impact i) in a feasibility study of risankizumab dose reduction and ii) in silico against routine TDM for adalimumab in a national psoriasis service.
The dashboard comprised a 2-compartment PK (risankizumab), 1-compartment PK with turnover PD (adalimumab) and 1-compartment PK with mixture turnover PD model (ustekinumab). Bayesian prediction showed good performance (mean prediction error 15% for risankizumab). Clinicians rated dashboard usability highly (n=29, System-Usability-Scale 74/100), averaging 2 mins/calculation. In the feasibility study (n=94; 14 UK centres; remission over 1 year on risankizumab), according to dashboard-guided dosing, 49% received longer intervals than standard (mean 16 weeks; range 12–23), saving £3,326/patient annually. *In silico*, the dashboard recommended longer adalimumab intervals for 96% patients (n=73, 66% male;

median weight 84kg; median PASI 0.8) than routine-care TDM (mean 5 vs 2 weeks; range 1-10 vs 1-4).
Our end-to-end MIPD translational framework is scalable across drugs and dermatology, paving the way for precision dosing in routine care.

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Real-Time Automated Diagnosis of Skin Tumors: An AI Approach Using Dynamic Video Imaging
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Artificial intelligence (AI) using static clinical images has demonstrated high performance in diagnosing skin tumors. However, in real-world clinical settings, AI predictions can fluctuate based on camera angle or lighting, leading to diagnostic inconsistency. To address this, we developed an AI system utilizing dynamic video imaging, which captures continuous morphological features to provide more robust diagnostic support.
To evaluate the stability and consistency of a video-based AI diagnostic system for skin tumors and to compare its performance with conventional static image analysis through a multi-center clinical study.
A multi-center study is being conducted across six institutions in Japan. We are collecting high-definition video sequences of various skin tumors, including malignant melanoma and basal cell carcinoma. The AI model processes these video streams, aggregating frame-by-frame predictions to generate a stabilized output. The study focuses on evaluating how temporal data integration reduces "prediction flickering" compared to single-frame analysis.
We will present the results of our ongoing multi-center study, specifically focusing on the comparative analysis of diagnostic variance between video-based and static image-based models. Preliminary data suggests that video sequences may offer higher consistency by accounting for varying imaging conditions. Detailed metrics, including concordance rates among the six participating institutions, will be provided.
This study explores the potential of dynamic video imaging to enhance the reliability of AI in dermatology. By utilizing multi-center clinical data, we aim to demonstrate that a video-based approach can bridge the gap between experimental AI performance and practical clinical utility, providing a more "clinician-like" assessment.

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Machine learning-guided computational immunophenotyping identifies malignant circulating T-cell states in cutaneous T-cell lymphoma
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Flow cytometry of circulating T cells in cutaneous T-cell lymphoma (CTCL) is conventionally interpreted by manual, sequential gating, which is operator-dependent and does not exploit the full multiparametric structure of the data. We developed a reproducible, open-source pipeline based on self-organising maps (FlowSOM) to characterise circulating T cells without prior gating and benchmark it against expert analysis. Sixty-eight peripheral blood samples from 37 subjects (14 controls, 19 mycosis fungoides, 4 Sézary syndrome) were acquired on a EuroFlow-compatible 8-colour panel (CD2, CD3, CD4, CD7, CD8, CD26, CD45, TCR-Cβ1). After quality control (FlowJo, Infinicyt), 4.24×10^6 events were analysed in R with FlowSOM: a self-organising map (10×10 grid, 100 clusters) trained with a fixed random seed, consensus-metaclustered into eight metaclusters, and embedded by t-SNE and UMAP. TCR-Cβ1 clonality was assigned per cluster; cluster-derived features were compared across groups (Kruskal-Wallis, Benjamini-Hochberg), ranked by ROC, and benchmarked against expert gating. Eight metaclusters matched recognisable immune compartments, including a dominant CD3-low compartment (MC1, 77% of events) and a $CD4^+$ T-cell compartment (MC7, 13% of events). Of 216 cluster-derived features, 92 differed between groups after correction. The malignant CD3-dim, $CD4^+CD7^-CD26^-$ TCR-Cβ1-restricted cluster was assigned to MC1 rather than the $CD4^+$ T-cell metacluster, so the clonal signal emerged at fine-cluster rather than metacluster level. C14 was the strongest de novo discriminator of clonal cells (AUC 0.89), ahead of the aberrant $CD4^+CD7^-CD26^-$ cluster (AUC 0.87); a combined two-cluster model reached AUC 0.91. Cluster-derived classification agreed with expert gating in 95.6% of samples (Cohen's κ=0.85; McNemar p=1.000; cluster-manual correlation r=0.843). Beyond the malignant clone, multiple immune states shifted with disease burden, with expansion of the CD3-low compartment and progressive contraction of $CD4^+$ T-cell and NK-associated compartments. This reproducible, open-source FlowSOM pipeline recovers the clonal Sézary-type signature de novo with high concordance to expert analysis, providing an objective analytical framework and a data-driven candidate marker for further study.

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Gene expression profiling for prognostication in cutaneous melanoma: evaluation of current molecular models

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Current staging systems for cutaneous melanoma rely on clinicopathological factors such as Breslow thickness, ulceration and sentinel lymph node status. However, a substantial proportion of patients with early-stage disease still experience recurrence or death, highlighting the need for more accurate prognostic tools. Gene expression profiling (GEP) has emerged as a potential molecular method for improving risk stratification in melanoma.
A narrative review was conducted evaluating the current role of GEP models in melanoma prognostication. Published literature examining the two most widely studied models, the 31-gene expression profile (31-GEP) and clinicopathologic-gene expression profile (CP-GEP), was analysed. Studies were reviewed for their ability to predict recurrence risk, sentinel lymph node metastasis and clinical utility compared with conventional staging methods.
Both GEP models demonstrated high negative predictive value when identifying patients at low risk of nodal metastasis or recurrence. The 31-GEP showed predictive value in identifying high-risk stage II melanoma patients, while CP-GEP integrates clinicopathologic variables with gene expression to predict sentinel lymph node positivity. These models may reduce unnecessary sentinel lymph node biopsies and associated morbidity. However, limitations remain including false-positive results, variability between validation studies and limited prospective evidence supporting routine clinical implementation.
Gene expression profiling represents a promising adjunct to traditional melanoma staging systems. While current models demonstrate potential for improving risk stratification and guiding surveillance strategies, further prospective validation is required before widespread incorporation into clinical guidelines.

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Androgen receptor antagonism by topical clascoterone modulates androgen-driven inflammatory and fibrotic pathways in a human male pattern hair loss scalp skin organ culture model

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Male pattern hair loss (MPHL) is a progressive androgenetic condition in which androgen receptor (AR) activation, inhibits hair growth, triggers perifollicular microinflammation and fibrotic remodelling, forming a self-reinforcing loop that promotes follicular miniaturization. Clascoterone, a topical AR antagonist currently in Phase 3 development for the treatment of MPHL, was applied to affected scalp skin *ex vivo* to determine the mechanistic impact of AR inhibition on the MPHL microenvironment by analysing the secreted proteome. Scalp biopsies from five MPHL patients (Hamilton-Norwood stages III-VI) were cultured with 30 nM testosterone to reproduce androgen exposure, then treated topically with clascoterone 5% solution for 4 hours every other day over 5 days; conditioned media collected on days 1, 3, and 5 analysed using the Omni1000 nELISA platform (998 analytes). Of 550 consistently detected proteins, clascoterone reduced the androgen-regulated proteins thrombospondin-1 (TSP1) and matrix metalloproteinase-9 (MMP9), and concurrently their downstream target, the TGF-β precursor, TGF-β LAP domain. Modulation of IGF-binding proteins further supports preservation of DP inductivity and anagen maintenance, consistent with prior tissue-level findings. AR blockade also broadly suppressed the pro-inflammatory secretome, reducing IL-6, IL-1β, LIF1, CXCL9, CCL5, and CCL18, downregulating immune-activating receptors (CLEC5A) and macrophage inflammatory regulators (TREM1, Periostin), as well as inhibited multiple other MMPs (MMP 2, 3, 10, 12, 13), collectively indicating stabilization of the perifollicular extracellular matrix and reduced perifollicular inflammation. These data demonstrate that AR blockade by clascoterone disrupts the AR-driven inflammatory-fibrotic axis providing mechanistic support for its therapeutic potential in MPHL.

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Multimodal Deep Learning for Melanoma Staging: Added Value of Clinical Metadata to Image-Based Prediction

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Accurate staging of melanoma is essential for treatment decision-making; however, automated staging solely based on clinical images remains challenging, as prognosis often depends on factors beyond visible morphology. We investigated whether integrating clinical metadata with clinical images improves staging performance within a deep learning framework. A vision–language model based on LLaVA (Large Language-and Vision Assistant) was developed to integrate heterogeneous clinical and imaging information, incorporating patient age, sex, and lesion site. A total of 187 images from 125 patients with melanoma treated at our institution were retrospectively analyzed. Model performance was evaluated using five-fold cross-validation (train 70%, validation 10%, test 20%), and accuracy was compared across three settings: image-only, metadata-only, and multimodal models for four-stage classification (stages 0–III). Whereas the image-only model achieved an accuracy of approximately 49%, the multimodal model achieved higher accuracy of approximately 58%. These findings suggest the additive value of clinical metadata, highlighting its substantial contribution to melanoma staging and indicating that a multimodal approach outperforms image-only models. Further validation with larger and more diverse datasets is warranted to improve generalizability.

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Triglyceride-glucose Index as a novel insulin resistance biomarker and high-risk phenotype identification in Adult Male Acne

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Adult-male-acne (AMA) represents an underexplored dermatologic-metabolic condition, increasingly recognized for its systemic implications. Insulin resistance (IR) has a central pathophysiological mechanism, yet gold-standard HOMA-IR assessment remains inaccessible in resource-limited healthcare systems. This study validates TyG index diagnostic performance against HOMA-IR, identifies significant IR clinical predictors in AMA, and develops machine-learning (ML) models for non-invasive IR pre-identification.
This analytical comparative study enrolled 100 AMA patients (≥25 years) and 100 age-BMI matched controls. Assessment included Global-Acne-Grading-System (GAGS) scoring, anthropometric profiling, and dual IR evaluation using TyG & HOMA-IR

index. Statistical analysis employed chi-square testing, correlation coefficients and multivariate risk stratification. Finally, ML algorithms (DecisionTree, Support-Vector-Regression, Logistic-Regression) were trained on high-risk phenotypic features to predict IR status prior to biochemical confirmation.
TyG-index demonstrated high IR (58% AMA versus 31% controls,1.87-fold risk, $X^2 = 8.84$,p = 0.003) with significant HOMA-IR correlation (r= 0.72, ρ=0.76, p <0.0001) and 81.25% cost-reduction. High-risk predictors were: GAGS ≥19 (77.4% IR, $X^2 = 5.85$,p = 0.016, RR=1.57, r= 0.4502,p < 0.001) and grades 3-4 acne (67.3% IR, RR=1.44, r= 0.4391,p < 0.001); facial-arc site (71.4% IR, RR= 2.86, $X^2 = 9.4$,p < 0.05) versus axis (25.0%). Anthropometric parameters: WHR >0.90 (r= 0.491, p <0.001; <0.90: 42.9% IR; 0.90-0.95: 68.8% IR; >0.95: 82.1% IR), waist-circumference ≥90cm (r= 0.409, <0.001, 64.6% IR), and BMI ≥25 (r=0.4201, p <0.001, 77.3% IR, 3.45-fold risk). Also, 50% IR-prevalence in normal BMI necessitates central adiposity assessment. ML-model achieved 85% accuracy for IR prediction using these clinical phenotypes only, enabling non-invasive pre-screening.
This study highlights AMA as a cutaneous marker of metabolic-syndrome and validates TyG-index as a cost-effective, novel IR marker, with pioneering work on ML-model to pre-screen using high-risk predictors.

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Skin Barrier Changes in Atopic Dermatitis Treated with Biologics and JAK Inhibitors
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Atopic dermatitis (AD) is a chronic immune-mediated skin condition, marked by recurrent eczema and impaired skin barrier function. Disruption of the skin barrier, particularly due to an altered stratum corneum (SC) ceramide profile, plays a central role in AD disease pathophysiology. Treatment with Janus kinase inhibitors (JAKi) and biologics, targeting type 2 immune responses can reduce aberrant immune responses and improve barrier function. This study characterizes skin barrier changes during these targeted therapies using electrical impedance spectroscopy (EIS) and ceramide profiling.
Stratum corneum impedance and deeper layer impedance were measured using EIS on lesional and non-lesional volar forearm skin of adult AD patients starting biologics (n=31) or JAKi (n=10) at baseline, week 4, and week 16. SC ceramides were collected using tape strips followed by liquid chromatography-mass spectrometry.
At baseline, lesional skin showed a distinctly altered ceramide profile compared with non-lesional skin. Treatment with both JAKi and biologics restored ceramide subclass distribution, including normalization of the average chain length toward non-lesional levels, and improved barrier function. By week 16, both SC impedance and deeper layer impedance improved significantly. Deeper layer impedance showed stronger associations with ceramide composition than SC impedance.
In conclusion, this study is the first to demonstrate an association between EIS-measured skin barrier function and ceramide profiles in AD. Combining non-invasive EIS with lipidomic analysis provides complementary insight into barrier restoration and supports the potential of ceramide profiling as a biomarker for treatment monitoring in AD.

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Dynamically monitor human skin barrier integrity by engineering in-vitro epidermal models on the organic electrochemical transistors
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In this study, we aimed to develop a method for dynamically monitoring the skin barrier properties, with particular emphasis on the barrier integrity. To achieve this, we first fabricated *in-vitro* three-dimensional (3D) epidermal models and characterized their structural and functional integrity using hematoxylin and eosin staining, immunofluorescence staining, and scanning electron microscopy. The *in-vitro* 3D epidermal models were then integrated with organic electrochemical transistors (OECTs) to enable real-time, non-invasive assessment of barrier integrity. Electrochemical measurement results obtained from the OECT device were further compared with transepidermal electrical resistance (TEER) assays to evaluate performance and reliability. The results demonstrated that the *in-vitro* 3D epidermal models successfully recapitulated key structural and functional characteristics of *in-vivo* human epidermis. Moreover, the OECT measurements revealed a progressive decrease in ion permeability as epidermal differentiation advanced. Upon chemical treatment with sodium dodecyl sulfate (SDS), the *in-vitro* 3D epidermal models exhibited significantly enhanced ion transport, reflecting the disruption of skin barrier integrity. The findings confirm that the OECT measurement can sensitively monitor both formation and disruption of skin barrier function, with superior time resolution compared to traditional methods. In conclusion, the integration of *in-vitro* 3D epidermal models with OECT device provides a platform for dynamically evaluating skin barrier integrity. This approach offers significant advantages over conventional techniques, enabling continuous, high-resolution monitoring while reducing reliance on animal models. The platform holds considerable potential for applications in dermatological research, drug testing, and the development of point-of-care diagnostic technologies.

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AI-assisted integrative transcriptomic analysis supports mechanistic delineation of nodular and extensive keloid subtypes with therapeutic implications
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Keloids are heterogeneous fibrotic skin disorders in which nodular and extensive forms differ in growth behavior, recurrence, and treatment response. While transcriptomic studies have identified subtype-specific signatures, the mechanistic architectures underlying these divergent phenotypes remain unresolved.
To achieve a system-level dissection of nodular and extensive keloids to support subtype-adapted treatment using AI-assisted transcriptomic analysis.

We reanalyzed published transcriptomic data from primary dermal fibroblasts of nodular, extensive keloids and healthy skin using OCEAN (Omics-to-Clinical Evidence & Action Navigator), an AI-assisted framework integrating differential expression, pathway enrichment, and protein–protein interaction networks into coherent mechanistic architectures with traceable evidence. Cultured fibroblasts from normal skin biopsies or extensive/nodular keloids were used for biological experiments aimed to validate IA underlined pathways and new pharmacological target molecules.
AI-assisted analysis confirmed known subtype differences, including a dominant extracellular matrix program in nodular keloids and a more inflammatory profile in extensive keloids. System-level integration reconstructed distinct mechanistic architectures: nodular keloids displayed a compact, self-reinforcing fibrotic network integrating TGF-β signaling, hypoxia adaptation, transcriptional stabilization, and autocrine inflammation, consistent with reduced therapeutic responsiveness. In contrast, extensive keloids showed an adaptive, expansion-oriented program marked by immune responsiveness, stromal plasticity, angiogenic signaling, and metabolic stress adaptation, revealing subtype-specific therapeutic vulnerabilities.
AI-assisted integrative transcriptomics transforms descriptive omics profiles into clinically actionable mechanistic insight, enabling precision stratification and mechanism-informed therapeutic targeting in keloid disease.

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Artificial Intelligence in Mohs Micrographic Surgery for Basal Cell Carcinoma in an Asian Cohort
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Mohs micrographic surgery is the gold standard for treating basal cell carcinoma (BCC) due to precise intraoperative margin assessment, but interpretation of multiple frozen sections is labour-intensive and subject to variability. Artificial intelligence has shown promise in histological BCC detection yet data in Asian populations remain limited. Tissue section completeness, a prerequisite for accurate interpretation, is also underexplored.
This study aimed to develop and validate an artificial intelligence model for BCC detection and tissue section completeness assessment in frozen sections from an Asian cohort.
A retrospective single-centre study was conducted in a tertiary centre using archived Mohs frozen sections from 130 patients treated between 2017 and 2025. Tissue sections were classified as complete or incomplete based on predefined histologic criteria. Convolutional neural network models were trained for patch-level tumour detection, section-level region-of-interest detection and section completeness assessment. Model performance was evaluated by accuracy, sensitivity, precision, F1-score, Area Under Curve (AUC) for the Receiver Operating Characteristic (ROC) and Precision Recall Curve (PRC). A total of 734 whole slide images were analysed. The BCC detection model performed comparably to existing models, achieving accuracy 0.993, sensitivity 0.91, precision 0.89, F1-score 0.90, AUC-ROC 0.992, and AUC-PRC 0.957, with comparable region-of-interest sensitivity (0.91). The section completeness model achieved accuracy 0.890, sensitivity 0.524, precision 0.611, F1-score 0.564, AUC-ROC 0.923 and AUC-PRC 0.674.
These findings demonstrate the feasibility of artificial intelligence for simultaneous basal cell carcinoma detection and section completeness assessment in Mohs surgery within an Asian population. Such models may support intraoperative decision-making and improve workflow efficiency, warranting further prospective validation.

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Multimodal large language models in dermatology education: divergence between knowledge retrieval and image-integrated clinical reasoning
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Large language models (LLMs) are increasingly used in dermatology training, yet their capacity for multimodal clinical reasoning remains unclear. We evaluated three state-of-the-art LLMs – ChatGPT, Gemini, and Claude – across 506 dermatology assessment items, comprising 150 multiple-choice questions (MCQs) and 61 image-based case studies scored against predefined mark schemes. Performance was analysed using mixed-effects logistic and linear mixed-effects models accounting for repeated measures. Overall performance differed significantly between models ($p<0.001$), with ChatGPT (79.3%) and Gemini (78.5%) performing comparably, and Claude performing lower (61.0%). Accuracy was high across models for MCQs (92.7%, 94.7%, and 80.0%, respectively) but declined substantially in image-based case studies (75.3%, 73.7%, and 55.3%; all $p<0.001$). A significant Model x Format interaction ($p=0.002$) demonstrated that Claude's performance deficit was specifically amplified in case-based tasks, suggesting a model-specific vulnerability to multi-step clinical reasoning. Within case studies, between-model differences were restricted to higher-order tasks (diagnosis, management), while knowledge-retrieval domains (investigations, pathophysiology) showed no significant model effect. Across all models, performance was consistently lower for image-containing MCQs compared with text-only MCQs ($p<0.001$), with no model-modality interaction, suggesting that image-associated difficulty represents a class-wide limitation. These findings identify a reproducible dissociation between factual knowledge retrieval and image-integrated clinical reasoning in contemporary LLMs. In dermatology – where diagnosis and management are inherently multimodal – this gap limits current LLMs as clinical reasoning tools. Their performance profile supports use as knowledge-retrieval aids rather than decision-support systems, with important implications for their integration into dermatology education and assessment.

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Transcriptome-guided identification of candidate antifibrotic compounds in localized scleroderma
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Effective therapies for localized scleroderma are limited, and novel approaches are needed. Differential expression analysis results derived from plaque morphea, generalized morphea and eosinophilic fasciitis were used to identify disease-associated gene expression signatures. By comparing the diseases' pathway-level profiles with KEGG enrichment profiles derived from L1000 perturbational signatures from the Broad Institute's Connectivity Map L1000 resource, we aimed to identify drugs that

have the potential to interfere with morphea-associated transcriptional programs.
This approach predicted in silico the anti-fibrotic effect of drugs such as calcitriol and estrogen signaling blockers and also identified novel compounds with anti-fibrotic and anti-inflammatory activity. Histone deacetylase inhibitors like PCI-34051, tacedinaline and panobinostat were found to be promising due to their role in chromatin-regulatory mechanisms involving protein homeostasis and fibroblast activation. Most candidate compounds were shared across disease subsets, reflecting similar underlying transcriptional programs rather than subtype specific targets. Data obtained from spatial transcriptomic analysis also allowed to identify candidate compounds inhibiting pathways specifically found in the inflammatory, fibrotic, and also in unaffected areas in the skin biopsies. This spatial specificity could suggest that effective intervention may require combination therapy.
These results provide a first transcriptome-guided framework for identifying potential therapeutic candidates in patients with localized scleroderma and highlights how spatial transcriptomics combined with in silico drug screening can support the translation of molecular insights into therapeutic strategies for fibrotic skin disease.

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Transcriptomic profiling of inflammatory skin challenge models and inflammatory skin diseases

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Early-phase clinical studies that include human inflammatory challenge models are used to show proof-of-mechanism and support the selection of target patient population(s). To improve the translatability from model to disease, we aimed to compare specific transcriptomic features between two skin challenge models, the intradermal lipopolysaccharide (ID LPS) and topical imiquimod (IMQ) models, and three inflammatory skin diseases: cutaneous lupus erythematosus (CLE), atopic dermatitis (AD), and psoriasis (PsO).
Bulk RNA sequencing data from skin biopsies was available for IMQ, LPS, AD, and PsO from clinical studies, and a publicly available cohort was used for CLE. Modules reported by Seremet et al. (2024) were applied to characterize the main drivers of inflammation across sample groups. To further characterize these groups, we used a subset of signatures collated by Martinez et al. (2022). Signature enrichment scores were obtained using ssGSEA and GSVA. We subsequently performed differential gene expression analysis followed by pathway analysis on MSigDB sets (GSEA), and CIBERSORTx was used to estimate the abundance scores of 22 immune cell types.
The dominant immune modules were Th17 for PsO and Th2 for AD, consistent with the literature. The dominant immune modules were IFN, neutrophilic, and macrophagic in the ID LPS model and mainly IFN in the IMQ model. Th2 and Th17 were also positively enriched in both models, more pronounced in ID LPS. Since the IFN pathway is a key driver of CLE pathophysiology, we have included a cohort of CLE patients into our analyses, which are currently in progress. Moreover, we identified significantly enriched features across the 4 datasets including MSigDB pathways, gene signatures, and cell types based on CIBERSORTx.
By describing features per model and disease within a single framework, we revealed shared characteristics that could enhance the translatability between these challenge models and inflammatory skin diseases.

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A Human Hair Follicle–Preserving *Ex Vivo* Model to Study Scalp Inflammation for Immunotoxicology and Therapeutic Testing

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Scalp inflammation contributes to disorders such as seborrheic dermatitis, psoriasis, and alopecia, yet physiologically relevant models remain limited. We present a bio-stabilized *ex vivo* human scalp model designed to mimic bacterial-induced inflammation and assess effects on hair follicle integrity and immune activation.
Ex vivo models consisted of 6 mm human scalp biopsies from five female donors (51–72 years) with comparable hair density. Biopsies were embedded in a proprietary matrix and cultured at an air–liquid interface in serum-free medium. Inflammation was induced by topical lipopolysaccharide (LPS) application for 24 h; PBS served as control. Tissue integrity was evaluated by H&E staining on days 1, 2, and 5. Inflammatory mediators were quantified using a 36-plex cytokine panel. Hair follicle responses were assessed by co-immunostaining of Ki67 and active Caspase-3 to quantify proliferation and apoptosis.
Baseline samples showed preserved epidermal, dermal, and follicular architecture. LPS induced mild superficial epidermal pyknosis in one donor. Cytokine profiling revealed increased GM-CSF, IL-8, and MIP-1β (p=0.062). LPS reduced proliferation (decreased Ki67;) and increased apoptosis by day 5 , while overall tissue and follicle morphology remained preserved.
This *ex vivo* scalp platform models LPS-induced inflammation, triggering localized immune responses and modulating hair follicle cell turnover while maintaining tissue integrity. It provides a relevant tool to study scalp inflammation and evaluate anti-inflammatory therapies in human tissue.

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A Core Human Skin Cell Atlas

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Understanding skin disease requires a precise, high-resolution reference map of healthy human skin. We generated a core Human Skin Cell Atlas by uniformly reprocessing raw FASTQ files from publicly available single-cell RNA-sequencing datasets representing healthy skin from 71 individuals across diverse ages, ethnic backgrounds, and anatomical sites. This integration produced an atlas of more than 120,000 cells spanning all major skin lineages, harmonised across three hierarchical annotation levels. The atlas also resolved rare cell populations not typically reported in healthy skin, including B cells and plasmacytoid dendritic cells. Projection of disease datasets onto this reference achieved high annotation accuracy, particularly at the first two

hierarchical levels. To maximise accessibility, we will develop a user-friendly online resource for researchers and clinicians to explore the data and generate hypotheses.

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Proteogenomic Detection of Melanoma-Associated Mutations by Mass Spectrometry-Based Proteomics

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Detection of genetic mutations is widely used for tumour diagnosis and classification. However, genomic testing identifies DNA-level alterations irrespective of their translational status, is relatively costly, and may require prolonged turnaround times. On the other hand, proteomics offers a functional alternative by detecting protein products of mutated genes, thereby confirming the translation of genomic alterations.

In this study, we investigate the feasibility of proteomic-based mutation detection using four human melanoma cell lines: PT101, PT111, PT129, and SK-MEL-28. Gene mutation profiles for the cell lines were used to computationally generate customised mutation-augmented protein reference libraries. These libraries incorporated missense mutation–derived protein sequences alongside orthogonal positive and negative control peptides. Matched high-resolution liquid chromatography-tandem mass spectrometry (LC–MS/MS) proteomic datasets for the cell lines were searched against the customised reference library to identify mutation-specific peptides.

Using a stringent threshold of ≥3 peptide-spectrum matches (PSMs), we identified 83, 55, 168, and 48 unique mutation-derived peptides in PT101, PT111, PT129, and SK-MEL-28, respectively. Positive control peptides were consistently detected across all samples (>55% detection), whereas no negative control peptides were identified, supporting analytical sensitivity and specificity.

This study demonstrates proof of concept that genetic mutations can be detected directly at the protein level using mutation-informed proteogenomic libraries. This approach confirms translated mutations and represents a scalable and potentially cost-effective complementary strategy to genomic testing in melanoma and other malignancies.

528 [WITHDRAWN]

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Linking Sleep Disturbance to Skin Inflammation in Atopic Dermatitis

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Sleep disturbances are a highly prevalent comorbidity in atopic dermatitis (AD), affecting up to 80% of children and 90% of adults, and significantly impairing quality of life as well as neurocognitive function. Despite their clinical relevance, objective data remain scarce, and the impact of poor sleep on skin pathology remains poorly understood. In previous work, we used polysomnography to demonstrate altered sleep architecture and reduced sleep efficiency in patients with AD.

To further characterize sleep disturbances and their effects on AD pathology, we classified a cohort of 20 AD patients into groups with relatively good or poor sleep based on polysomnography and compared them to 6 healthy controls. Transcriptomic and PCR analyses of lesional and non-lesional skin biopsies were performed in parallel to identify molecular patterns associated with poor sleep in AD.

This approach revealed differential expression of genes involved in circadian regulation (so-called “clock genes,” e.g., NR1D1), inflammation and tissue damage (upregulation of HIF1α, TIMP1, IL6), as well as a gene linked to obesity and fatigue (downregulation of NHLH2), which were particularly dysregulated in the skin of AD patients with poor sleep. Notably, these molecular signatures distinguishing good and poor sleepers were independent of disease severity.

In summary, sleep disturbance is common in AD, yet its objective assessment and molecular characterization remain under development. There remains a need for standardized and validated clinical outcome measures, as well as further research to identify actionable biomarkers of poor sleep in AD. Our findings suggest that impaired sleep directly influences the characteristics and severity of AD lesions. Future therapeutic strategies should therefore include assessing and improving sleep quality to positively impact disease progression.

530 [WITHDRAWN]

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Untangling the web: A systems-based approach at single-cell resolution reveals druggable targets in atopic dermatitis

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Treating and preventing atopic dermatitis (AD) flare-ups appropriately requires extensive knowledge of molecular drivers. Investigating deregulated genes using bulk sequencing misses tightly regulated interactions from particular cell types and forfeits overlapping knowledge between diseases. The aim of this study is to integrate single-cell RNA sequencing data of inflammatory skin diseases to identify disease regulators through gene correlation networks to select potential drug candidates for localized treatment. Data of over 300.000 cells from AD patients were integrated with psoriasis and hidradenitis suppurativa data through the Harmony algorithm. The gene networks were created using hdWGCNA on a publicly available single-cell RNA sequencing data set. In keratinocytes we identified 7 network modules in healthy control samples representing different epidermal skin layers. In AD lesional keratinocytes there was one additional network module representing inflammation together with mitochondrial and metabolic deregulation, which is associated with a chronic stress adapted cell state. A central hub gene within this disease module is PDZK1IP1, correlating with multiple well-known AD hallmark genes, such as several S100 and SERPIN genes. The PDZK1IP1 protein is located in epithelial cells where it acts as a carrier of PDZ-domain proteins, which are primarily receptor proteins. Additionally, we found that stimulating a 3D in vitro epidermal model with AD cytokines significantly upregulates PDZK1IP1. Literature notes a decrease in proliferation in cancer model after silencing of PDZK1IP1, and a link to psoriasis for which we find similar results to AD. Disease hallmarks could be induced by modulating the amount of receptor availability by upregulation of PDZK1IP1 which may allow for increased signaling. As this module is keratinocyte

specific, a topical drug can be considered, reducing toxicity caused by systemic option. Future research will focus on finding the right drug treatment options suitable for this network, which will require integration with curated drug libraries.

CLINICAL DERMATOLOGY, CASE REPORTS, EPIDEMIOLOGY

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Prognostic determinants of early and late relapse in cutaneous squamous cell carcinoma

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Cutaneous squamous cell carcinoma (cSCC) carries a significant risk of recurrence, yet factors influencing the timing of relapse remain poorly defined. We aimed to identify clinicopathological determinants associated with early and late recurrence patterns. We conducted a multicenter retrospective cohort study including 23,166 cases of cSCC. Recurrences were classified as early (≤4 months), intermediate (4–24 months), or late (>24 months). Windowed competing-risk models were applied to evaluate associations between tumor features and recurrence timing, with death as a competing event. Multinomial logistic regression was used to assess factors associated with recurrence timing among patients with recurrence.
Among 669 tumors with recurrence, 189 were early, 374 intermediate, and 106 late. Several established high-risk features were associated with recurrence overall, including tumor size, depth, poor differentiation, lymphovascular invasion (LVI), and large-caliber/deep perineural invasion (LCDPNI). However, their impact varied over time.
LVI (SHR 3.01) and LCDPNI (SHR 2.55) were independently associated with early locoregional metastasis, with diminishing influence in later periods. Poor differentiation showed a sustained prognostic impact, being associated with both early (SHR 3.26) and late (SHR 4.63) metastasis. Tumor diameter and head and neck location were consistently associated with recurrence across time windows.
Multinomial analysis supported these findings, identifying LVI and LCDPNI as predictors of early metastasis, while tumor diameter and immunosuppression were associated with earlier local recurrence. Some variables demonstrated stable effects over time, whereas others exhibited clear temporal patterns.
These findings suggest that determinants of recurrence risk and recurrence timing are not equivalent. LVI and LCDPNI identify tumors prone to early dissemination and may support baseline nodal staging, whereas poor differentiation remains relevant for late relapse and may justify prolonged follow-up. Incorporating temporal dynamics into risk stratification could improve personalized management strategies in cSCC.

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Early phase reduction of CCR6+CCR5+ CD8 effector memory T cells implies better response to baricitinib in severe alopecia areata

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Clinical responses to Janus kinase inhibitors (JAKi), such as baricitinib, in severe alopecia areata (AA) are heterogeneous and usually evaluated after prolonged treatment. To identify early immunological features associated with therapeutic response, we investigated longitudinal changes in chemokine receptor expression patterns on CD8 T cell subsets during baricitinib treatment. Peripheral blood mononuclear cells were retrospectively analyzed from 10 patients with severe AA treated with baricitinib. Samples were sequentially collected at baseline, 3 weeks, 3 months, and 1 year and analyzed by flow cytometry. Patients achieving a ≥50% reduction in the Severity of Alopecia Tool (SALT) score at 1 year were defined as SALT50 responders (n=7), while the remaining patients were classified as non-responders (n=3).
CCR6 expression was preferentially observed on CD8 effector memory T cells (TEM). At 3 months, the percentage change from baseline (%Δ) in the frequency of CCR6+ TEM and their mean fluorescence intensity (MFI) showed a tendency toward reduction in responders compared with non-responders (frequency: −20.1 ± 7.1 vs. 0.8 ± 16.7 %Δ; MFI: −26.9 ± 10.2 vs. 12.0 ± 20.1 %Δ). In contrast, CCR5 expression relatively remained unchanged (frequency: −4.1 ± 1.8 vs. −2.5 ± 1.5 %Δ; MFI: −14.0 ± 5.2 vs. −1.9 ± 5.1 %Δ). This tendency was more pronounced in the CCR6+CCR5+ TEM population, which showed a greater reduction in the frequency of positive cells (−17.2 ± 7.4 vs. −0.8 ± 15.6 %Δ).
Functional analysis demonstrated that granzyme B and perforin were predominantly expressed in CCR6− TEM, whereas CCR6+ TEM were enriched for granzyme A expression, suggesting their distinct effector characteristics. In a preliminary stimulation assay using the AA-associated autoantigen trichohyalin, antigen-reactive cells persisted within the CCR6+ TEM fraction in non-responders but not in responders.
Although limited by sample size, these findings suggest that early changes in peripheral blood CCR6+CCR5+ CD8 TEM are associated with distinct immunological states and may serve as an early biomarker reflecting response to JAKi treatment in severe AA.

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Association of Urinary Arsenic Metabolites with Melanoma and Nonmelanoma Skin Cancer: National Health and Nutrition Examination Survey 2003–2020

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Arsenic is a recognized skin carcinogen, yet evidence at U.S. population exposure-levels remains limited, and whether specific metabolites such as dimethylarsinic acid (DMA) confer differential risk remains unclear. We aimed to evaluate associations between urinary arsenic metabolites and the risk of melanoma and nonmelanoma skin cancer (NMSC), with a focus on identifying metabolite-specific DMA cutoffs not previously examined in U.S. populations. We conducted a cross-sectional analysis of 14,802 adults aged ≥20 years in the National Health and Nutrition Examination Survey (NHANES) 2003–2020 with urinary arsenic measurements. Skin cancer outcomes were self-reported from the medical conditions questionnaire. Total urinary arsenic (TUA) was dichotomized at >50 µg/L; among speciated metabolites, only DMA had adequate detectability and was dichotomized at >3 µg/L based on the median. Survey-weighted logistic regression adjusted for age, sex, race, education, income, body mass index, smoking status, sunburn history, urinary creatinine, and sun exposure. Among participants, 99 reported melanoma and 230 reported NMSC. TUA >50 µg/L was associated with twice the odds of NMSC (OR = 2.02; 95% CI: 1.11–3.66), with comparable estimates in males and females. DMA >3 µg/L was associated with 76% higher odds of NMSC (OR = 1.76; 95% CI: 1.24–2.51). Associations with melanoma were positive but non-significant for both measures. Our findings are consistent with prior studies reporting associations between arsenic and keratinocytic carcinomas, and extend prior NHANES analyses by incorporating additional survey cycles, evaluating a novel DMA cutoff, and conducting sex-stratified analyses. To our knowledge, this is the first U.S. study to identify a potential metabolite-specific DMA threshold associated with NMSC risk, positioning DMA as a potential biomarker for arsenic-related skin cancer risk even at low-to-moderate concentrations.

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Reduced Cord Blood Regulatory B10 Cells in Newborns Later Developing Atopic Dermatitis: Results from The Munich Atopy Prediction Study

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A complex interaction between environmental and lifestyle factors, immune dysregulation, and skin barrier integrity is believed to contribute to the development of atopic dermatitis (AD). However, the precise mechanisms underlying disease onset in infants remain largely unclear.
The Munich Atopic Prediction Study is a comprehensive clinical and biological investigation of a prospective birth cohort from Munich, Germany. Information on pregnancy, child development, environmental influences, parental exposure to potential allergens, as well as illnesses affecting both children and parents is gathered through questionnaires. This is complemented by thorough clinical examinations conducted by trained dermatologists, with a particular focus on allergies and skin health. Biomarker analyses were performed, for example, on cord blood immune cells using flow cytometry (FACS analysis).
Maternal AD (aOR = 3.06, p = 0.020) and affected siblings (aOR = 4.80, p = 0.039) are associated with an increased risk of AD, whereas cold-remedy intake showed a protective association (aOR = 0.11, p = 0.047). Infants later diagnosed with AD (total 74 infants, AD n = 27, healthy n = 47) are characterized by reduced frequencies of $CD4^+$ T cells (p = 0.0247) and increased B-cell counts (p = 0.0067). Moreover, for the first time, we could identify a significant reduction in regulatory B (Breg)-cell frequencies in these infants (p = 0.0015). Furthermore, our findings suggest that maternal allergen-specific immunotherapy may have a beneficial effect on the development and frequency of Breg cells (p = 0.0497).
Our study identifies early immune alterations, particularly a reduction in cord blood Breg cells, as potential contributors to AD pathogenesis. Incorporating Breg-cell measurements into neonatal immune panels, along with key perinatal and familial risk factors, may enhance early risk stratification and enable more personalized prevention of atopic diseases.

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JAK inhibitors as a novel treatment option in palmoplantar pustulosis: results from a single-centre feasibility trial (JAKPPPOT)

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Palmoplantar pustulosis (PPP) is a rare, inflammatory dermatosis with substantial unmet clinical need. Janus kinase inhibitors (JAKi) are an attractive option in PPP; however, trial data are lacking and safety concerns (malignancy, cardiovascular risk) may affect acceptability in a population with high smoking prevalence. We evaluated the feasibility of JAKi use in PPP.
This single-centre, open-label feasibility trial evaluated upadacitinib in adults (≥18 years) with moderate-to-severe PPP. Participants received upadacitinib 30 mg once daily for 8 weeks; historic placebo data from a closely matched randomised controlled trial (RCT) in PPP were used as a comparator. The primary endpoint was a composite feasibility outcome of recruitment (proportion consenting), adherence (≥80% proportion of days covered), and acceptability (5-point Likert scale). Secondary outcomes included an indication of efficacy (Palmoplantar Pustulosis Area and Severity Index [PP-PASI]).
Of 67 individuals approached, 28 (41.8%) consented, of whom 20/28 (71.4%; average age 46.0 years, 85% female) initiated treatment. Adherence was high, with 19/20 (95%) achieving ≥80% adherence (mean 95.7%, SD 9.7), and acceptability was favourable (18/19, 94.7%). All feasibility criteria (recruitment ≥40%; adherence ≥80%; acceptability ≥80%) were met.
Mean PP-PASI decreased from 20.3 (SD 10.6) at baseline to 4.6 (SD 4.5) at week 8 with upadacitinib, vs 18.0 (10.4) to 15.4 (10.1) with placebo. The baseline-adjusted between-group difference in PP-PASI change over 8 weeks was −12.3 (95% CI −15.8 to −8.9). At week 8, response rates were higher with upadacitinib than placebo (PP-PASI50: 100% vs 16.1%; PP-PASI75: 65.0% vs 3.2%). Upadacitinib was well tolerated; one unrelated serious adverse event (retinal detachment) occurred, and one participant discontinued treatment (weight gain).
These findings demonstrate that JAKi treatment is feasible in patients with PPP, with a large treatment effect relative to placebo. These data support progression to a multicentre RCT and position JAK inhibition as a promising strategy to address the substantial unmet therapeutic need in PPP.

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Associations between circulating metabolites and psoriasis risk in the UK Biobank and HUNT Study

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Psoriasis management is increasingly shifting toward preventative approaches. Given the link between psoriasis and systemic metabolic dysregulation, circulating metabolites may serve as susceptibility markers, supporting earlier prevention. Here, we aimed to identify circulating metabolites associated with psoriasis risk, using two large population-based cohorts: UK Biobank and HUNT. These cohorts comprise 5,343 incident psoriasis cases among 458,940 participants of White ethnicity/European ancestry (n UK Biobank = 4,558/443,041; n HUNT = 785/15,899), with mean follow-up times of 16.8 and 15.8 years in the UK Biobank and HUNT, respectively. We identified psoriasis cases using a combination of self-reports and medical records. In both cohorts, metabolite levels were quantified using Nightingale Health Nuclear Magnetic Resonance spectroscopy, enabling comparability. We performed Cox proportional hazards regression and meta-analyzed results across cohorts. The strongest associations were observed for albumin in the UK Biobank (HR [95% CI]: 0.91 [0.89 - 0.94]) and Glycoprotein acetyls in HUNT (HR [95% CI]: 1.15 [1.06 - 1.23]). The meta-analysis identified 28 associations, including metabolites related to inflammation, fluid balance, and glycolysis, as well as amino acids, ketone bodies, and lipid compositions in lipoprotein fractions and subfractions. Glycoprotein acetyls (HR [95% CI]: 1.10 [1.07 - 1.14]) and albumin (HR [95% CI]: 0.91 [0.89 - 0.94]) showed the strongest association, followed by triglyceride measures across multiple lipoprotein subfractions, including XS-VLDL-TG, IDL-TG, L-LDL-TG, and LDL-TG. In conclusion, we identified circulating metabolites that may be useful for identifying individuals at higher risk of developing psoriasis. Further studies are needed to assess causality and evaluate their potential for disease prediction.

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Genetic evidence that smoking initiation and intensity causally increase hidradenitis suppurativa risk

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Hidradenitis suppurativa (HS) is a chronic inflammatory skin disease with a complex and incompletely understood aetiology. Observational studies demonstrate a strong association between smoking and both the development and severity of HS, and smoking cessation is widely recommended in clinical practice. However, it remains unclear whether smoking is a causal driver of HS or a correlated behavioural marker, and the biological mechanisms underlying this relationship are poorly defined.
We used two-sample Mendelian randomisation (MR) to test the hypothesis that smoking behaviours causally increase HS risk and to explore potential mechanistic pathways. Genome-wide significant variants ($p < 5\times10^{-8}$) from large GWAS were used as instruments for smoking initiation (248 variants), lifetime smoking (126 variants), smoking heaviness (59 variants) and smoking cessation (22 variants). To account for collider bias, instruments for smoking heaviness and smoking cessation were adjusted for smoking initiation. HS outcome data were obtained from the largest available multi-ancestry GWAS (6361 cases). Sensitivity analyses were undertaken to evaluate directionality, heterogeneity and pleiotropy.
Genetically predicted smoking initiation showed strong evidence of a causal effect on HS susceptibility (OR 1.69 [95%-CI:1.28–2.22]). Comparable or larger effect estimates were observed for lifetime smoking exposure (OR 2.25 [1.50–3.36]) and smoking heaviness (OR 1.81 [1.35–2.41]). Findings were consistent across sensitivity analyses. In contrast, evidence for smoking cessation was inconclusive, likely reflecting limited instrument strength.
These findings provide genetic evidence supporting smoking as a causal driver of HS. To build on these results, we are undertaking ancestry-stratified analyses and two-step MR mediation analyses integrating proteomic and metabolomic data to identify biological pathways linking smoking to HS risk. This study strengthens the evidence base for smoking as a modifiable risk factor in HS and establishes a framework for mechanistic interrogation of downstream pathways relevant to prevention.

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Do Biologics and Janus Kinase Inhibitors Perform Consistently Across Patients with Atopic Dermatitis with Diverse Skin Tones? Real-World Data from a Tetriary Centre

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Non-White patients with atopic dermatitis (AD) remain underrepresented in clinical trials of advanced therapies. Although response patterns to biologics and JAK inhibitors appear similar across skin tones, further real-world data are needed. We aimed to compare real-world efficacy, safety, and treatment patterns of all EMA-approved advanced therapies in White vs. non-White patients with moderate-to-severe AD, and to explore whether therapy class influenced outcomes differently across groups.
We conducted a retrospective single-centre study at a UK tertiary dermatology service (Feb-Dec 2025). One hundred patients were stratified by Fitzpatrick phototype into White (I-II, n=50) and non-White (IV-VI, n=50) cohorts. All received advanced systemic therapy (80% biologics, 20% JAK inhibitors). Outcomes included EASI improvement, DLQI changes, EASI≤1, DLQI≤1, adverse events, and treatment switching.

Both cohorts demonstrated substantial clinical improvement. Mean EASI improvement was 89.0±18.7% in White and 70.5±72.6% in non-White patients (p=0.040), with greater variability in the latter reflecting true outliers and occasional negative responses. Mean DLQI improvement was 16.3±7.6 vs. 12.6±7.3 (p=0.025), EASI≤1 was achieved in 62% vs. 56%, and DLQI≤1 in 46% vs. 36% in the White vs. non-White group, respectively. Non-White patients were more frequently initiated on biologics as first-line advanced therapy, yet JAK inhibitors demonstrated higher EASI improvement in this group (95.6% vs. 64.2%, p=0.004), whereas response was more uniform across therapy classes in White patients (85.7% vs. 89.9%, p=0.594). Adverse events were comparable between groups, with eczema herpeticum more frequent in non-White patients (12% vs. 6%). Treatment switching occurred more often in non-White patients (24% vs. 14%).
Advanced therapies were effective across both groups, though real-world patterns diverged in terms of efficacy, safety, and treatment behaviour. Immunological endotype variation across phototypes may influence therapeutic response and support the urgent need for inclusive large-scale prospective studies in AD.

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Polygenic risk selectively predicts isotretinoin escalation but not antibiotic exposure in acne: a multi-ancestry real-world study

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A multi-ancestry genome-wide association meta-regression for acne recently delivered a polygenic risk score (PRS) validated against self- and clinician-assessed acne severity in research cohorts. Acne treatment typically escalates from topical therapy to systemic antibiotics and ultimately to oral isotretinoin, yet objective predictors of escalation are lacking. We conducted an exploratory pharmacoepidemiological analysis using prescribing trajectories as a proxy for disease severity, to test whether the validated PRS predicts real-world treatment intensity and to provide initial evidence for prospective implementation.
SBayesR-derived PRS weights were applied to whole-genome sequencing data from 16,928 participants with acne diagnoses in the All of Us Research Program (CDRv8 Controlled Tier). Four outcomes were defined from prescription records: any tetracycline exposure; chronic tetracycline use (dispensing span >=180 days); oral isotretinoin exposure; and combined tetracycline-plus-isotretinoin escalation. Logistic regression was adjusted for age, gender, and ten ancestry principal components, with ancestry-stratified sensitivity analyses.
Per-SD PRS was strongly associated with oral isotretinoin (OR 1.44, 95% CI 1.32 to 1.57, p=2.4x10-16) and escalation (OR 1.41, 1.27 to 1.57, p=4.4x10-11), but showed no association with chronic tetracycline use (OR 0.99, p=0.65). The isotretinoin association transported across European (OR 1.46), African (OR 1.53), and Admixed American (OR 1.40) ancestries (all p<0.01), with non-significant heterogeneity. Highest-quintile participants were 2.6-fold more likely to receive isotretinoin than lowest-quintile (3.9% vs 1.5%). Among participants receiving both treatments, 86% had a tetracycline-then-isotretinoin sequence (median 517 days).
The acne PRS selectively predicts real-world escalation to retinoid therapy across diverse ancestries, while remaining null for antibiotic exposure. These findings provide initial retrospective evidence to justify prospective evaluation of genetic risk stratification for earlier, more equitable access to isotretinoin.

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Genetic evidence implicates metabolic dysfunction as a driver of hidradenitis suppurativa: a bidirectional Mendelian randomization study

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Hidradenitis suppurativa (HS) is associated with a high burden of cardiometabolic comorbidity, including metabolic syndrome (MetS) and cardiovascular (CV) risk factors. While epidemiological studies demonstrate strong associations, the directionality of these relationships and the relative contributions of individual metabolic components remain unclear.
We conducted a two-sample bidirectional Mendelian randomization (MR) study using summary statistics from the largest available (cases=6300) HS genome-wide association study (GWAS) and large GWAS meta-analyses of MetS, its components, and CV risk factors.
Genetically predicted MetS was associated with increased HS risk (odds ratio [OR] 1.55, 95% CI 1.22-1.96, $p=3.52\times10^{-4}$), with no evidence supporting an association in the reverse direction for MetS or any of its components. Among MetS components, genetic predictors of body mass index-adjusted waist-hip ratio showed a strong association with HS risk (OR 2.04, 95% CI 1.83-2.27, $p=6.40\times10^{-35}$). Genetic predictors of type 2 diabetes were also associated with increased HS risk (OR 1.11, 95% CI 1.06-1.17, $p=1.56\times10^{-5}$), as was genetically predicted LDL cholesterol (OR 1.13, 95% CI 1.04-1.24, p=0.004). In addition, genetic liability to smoking, a key CV risk factor, was associated with increased HS risk (OR 1.44, 95% CI 1.26-1.64, $p=6.03\times10^{-8}$). No MR associations were observed between genetic liability to coronary artery disease, alcohol use, or other components of MetS and HS risk.
These findings suggest that the observed epidemiological associations between HS and cardiometabolic disease are driven predominantly by metabolic dysfunction, particularly central adiposity, dysglycemia, dyslipidemia, and smoking. This genetic evidence provides a mechanistic framework for HS comorbidity patterns and highlights cardiometabolic risk modification as a potential strategy to reduce HS risk and disease burden.

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Effect of microRNA milk-derived exosomes on objective markers of hair growth in androgenetic alopecia and/or telogen effluvium: a randomised placebo-controlled study

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In vitro, bovine milk-derived exosomes enriched in specific microRNAs (MEV-miRNAs) increased dermal papilla cell viability and modulated WNT-related gene expression in human hair follicles, supporting a potential role in follicular growth dynamics. Our objective was to investigate whether this MEV-miRNAs-based topical approach translates into measurable changes in

objective markers of follicular growth dynamics and hair shaft quality in adults with androgenetic alopecia and/or telogen effluvium.
In this multicentre, randomised, double-blind, placebo-controlled study, 40 adult men and women were enrolled, of whom 39 completed the study (MEV-miRNAs gel n=19; placebo n=20). The product was applied once weekly for 12 weeks. Efficacy was assessed at baseline, Week 8, and Week 12 by digital phototrichogram and epiluminescence microscopy. Key endpoints included the percentage of anagen hair follicles, the anagen/telogen ratio, and hair shaft diameter. Safety was evaluated through investigator examination and subject-reported tolerability.
MEV-miRNAs gel significantly ($p<0.001$) increased anagen hair follicles from 58.15±1.67 at baseline to 70.37±1.05 at Week 8 and 77.73±0.86 at Week 12, with significant ($p<0.001$) between-group differences versus placebo in change from baseline at Week 8 and Week 12. The anagen/telogen ratio increased from 1.45±0.09 to 2.46±0.13 and 3.63±0.20, respectively, versus minimal change with placebo (between-group $p<0.001$). Hair shaft diameter increased from 47.54±2.64 µm to 65.26±3.10 µm and 70.63±2.27 µm, with significant differences vs placebo at both time points. Subject-reported outcomes at Week 12 favoured MEV-miRNAs/iSFRP1 gel, with 89.47% reporting stronger hair, 89.47% higher hair density, 94.74% improved hair quality, and 89.47% satisfaction with the product. No product-related clinical signs or discomfort were reported.
The MEV-miRNAs topical strategy, applied once weekly, improved objective indicators of follicular growth dynamics and shaft quality over 12 weeks, supporting its relevance as a translational approach for hair loss disorders.

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Reduced sugar exposure in the first 1000 days of life and risk of psoriatic disease

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Psoriatic disease (PsD) is a chronic immune-mediated inflammatory condition characterised by skin inflammation and joint pain. High sugar intake has been linked to systemic inflammation. We examined whether sugar rationing during the first 1000 days of life was protective against developing PsD. We used a quasi-experimental design based on the natural experiment created by sugar rationing in the United Kingdom (UK), which began in January 1940 during World War II and ended in September 1953. The study included 29,075 UK Biobank participants with linked primary care data who were born between October 1951 and March 1956. Individuals born outside the UK, those who were adopted, and multiple births were excluded. Follow-up was defined using age, starting at birth and ending at the earliest of first outcome or age 66 years (censoring). The outcome was PsD, including cutaneous psoriasis (PsO) and psoriatic arthritis (PsA). Negative control outcomes (NCOs) included rheumatoid arthritis, fracture, trauma and appendicitis. Exposure was defined as sugar rationing during early-life, from in-utero through 24 months postnatally. Exposure to sugar rationing from in-utero through 24 months postnatally was associated with a lower risk of PsD (adjusted hazard ratio [aHR], 0.74; 95%CI, 0.65-0.85). Sensitivity analyses showed similar associations for PsO (0.88; 0.76-1.04) and PsA (0.60; 0.37-0.98). Absolute risk differences at the end of follow up demonstrated 18 fewer cases of PsD per 1000 individuals exposed postnatally. Early-life exposure to sugar until 24 months postnatally delay PsD for up 8 years (4.90-10.39). No associations were observed for NCOs. Reduced sugar exposure during critical developmental periods may contribute to a lower risk of psoriatic diseases, supporting early-life sugar reduction as a potential population-level prevention strategy.

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Perioperative imaging in high-risk cutaneous squamous cell carcinoma: impact on survival outcomes and clinical decision-making

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The role of perioperative imaging in high-risk cutaneous squamous cell carcinoma (cSCC) remains uncertain. We aimed to evaluate its association with survival outcomes and clinical management.
We conducted a retrospective cohort study including 762 patients (851 tumors). After exclusions and a 60-day landmark analysis, 725 patients (808 tumors) were analyzed. Perioperative imaging (CT, MRI and/or ultrasound) was compared with clinical observation. Baseline characteristics were assessed using unweighted and inverse probability of treatment weighting (IPTW-ATT) analyses. Survival outcomes (progression-free survival [PFS] and overall survival [OS]) were evaluated using weighted Kaplan–Meier curves and Cox models with time-varying effects.
Imaging was performed in 88 tumors (10.9%) and was associated with more adverse clinicopathological features, including larger size, deeper invasion, perineural invasion and higher BWH stage. After ATT weighting, covariate balance improved substantially.
In weighted analyses, imaging was not associated with improved OS at baseline (HR 1.52, 95% CI 0.32–2.50; p=0.10), but showed a significant time-dependent effect (interaction HR 0.79, 95% CI 0.65–0.96). For PFS, imaging was associated with worse early outcomes (HR 2.01, 95% CI 1.32–3.06; p=0.001), with attenuation over time (interaction HR 0.72, 95% CI 0.56–0.93).
Among imaged tumors, abnormal findings were frequent, identifying deep invasion (>25%) and occult metastases (~10%), often impacting clinical management. In a subcohort with imaging dates, no significant association was observed between time to imaging and survival outcomes, although a trend toward worse outcomes with delay was noted.
Perioperative imaging is preferentially used in higher-risk tumors. Although not associated with improved survival at baseline, its time-dependent effect and diagnostic yield suggest a potential role in selected patients. Further prospective studies are warranted.

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Baseline serum thymus and activation-regulated chemokine and one-year relapse in bullous pemphigoid: a retrospective cohort study

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Bullous pemphigoid is an autoimmune blistering disease with a high one-year relapse rate. Although serum thymus and activation-regulated chemokine (TARC/CCL17) reflects disease activity, its usefulness as a baseline prognostic marker for subsequent relapse remains unclear. This study aimed to determine whether baseline serum TARC is associated with relapse within 1 year after treatment initiation in bullous pemphigoid. We conducted a retrospective cohort study of 109 Japanese patients with bullous pemphigoid treated at a tertiary referral center between 2006 and 2025. Relapse after disease control was assessed over 365 days. Time to relapse was analyzed using Cox proportional hazards models adjusted for dementia, anti-BP180 NC16A antibody titer, and Bullous Pemphigoid Disease Area Index severity. Competing-risk and additional sensitivity analyses were also performed. During follow-up, 31 patients relapsed and 9 died. Higher baseline TARC was associated with relapse in univariable analysis (hazard ratio 1.06 per 1000 pg/mL, 95% confidence interval 1.01–1.12; P = 0.023) and remained associated after multivariable adjustment (hazard ratio 1.06, 95% confidence interval 1.00–1.11; P = 0.047). The direction of association was generally consistent across the competing-risk and sensitivity analyses. Baseline serum TARC levels did not differ significantly by exposure to DPP-4 inhibitors or by inflammatory phenotype. These findings suggest that baseline serum TARC levels may offer modest additional prognostic information at treatment initiation in bullous pemphigoid and may reflect an underlying type 2 inflammatory milieu that predisposes patients to relapse.

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Persistent diagnostic delay in patients with mycosis fungoides but no associations with overall survival: findings from a multicenter cohort study

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The diagnosing of mycosis fungoides (MF) is often challenging, both clinically and histologically, with reported delays of several years. In this multicenter retrospective cohort study, we describe diagnostic and investigational delay in MF, investigate risk factors for longer delays, and the association between diagnostic delay and survival. Adult patients diagnosed with histologically verified MF at two academic referral centers in Sweden between year 2000 and 2024 were included in the study. Clinical, histopathological, and demographic data were extracted from medical records. Group differences were analyzed using nonparametric tests, Spearman correlations, and Cox regression. Among 132 MF patients, median diagnostic delay was 58 months, investigational delay was 0.91 months, and 53.8% required multiple biopsy samplings. Longer diagnostic delay was associated with earlier clinical stage at diagnosis (stage IA – IIA: median 63.1 months [IQR 101.6] vs stage IIB – IVB: 20.8 months [IQR 45.9], p=0.006). Patients with dermatological comorbidities had longer investigational delay compared to patients with no dermatological comorbidities (5.23 [IQR 25.0] vs 0.0 months [IQR 3.13], p=0.004). There were no associations with sex, age, or calendar year. Neither diagnostic delay (p=0.17) nor investigational delay (0.80) predicted overall survival. In conclusion, diagnostic delay in MF remains substantial, particularly in early-stage disease, but was not associated with overall survival.

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Longitudinal Trends in Management and Outcomes of Stevens–Johnson Syndrome and Toxic Epidermal Necrolysis: A Decade-Long Population-Level Cohort Study using Epic Cosmos

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Stevens–Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN) are rare, life-threatening mucocutaneous reactions most commonly triggered by medications. Despite their severity, no standardized evidence-based management protocol exists. Using Epic Cosmos, a multi-institutional, de-duplicated electronic health records database that includes 1,762 hospitals and 300 million patients, we conducted a population-based cohort study of 15,943 patients diagnosed with SJS/TEN between January 1, 2015, and December 31, 2025. We examined the use of systemic corticosteroids, cyclosporine, etanercept, infliximab, and intravenous immunoglobulin (IVIG) within 10 days of diagnosis, and evaluated skin biopsy rate, hospital length of stay, disposition, and mortality.
Systemic corticosteroid use doubled from 18.1% in 2015 to 38.9% in 2025, while cyclosporine use rose modestly from 3.1% to 11.1% during the study period. Etanercept use remained limited, reaching 5.1% in 2025, while both IVIG and infliximab utilization persisted below 2% across all years. The most frequent combinations for treatment of SJS/TEN were corticosteroids and cyclosporine (7.2%), followed by corticosteroids and etanercept (2.1%). Skin biopsy rates increased from 1.5% in 2015 to 15.8% in 2025. Mean hospital length of stay remained stable during the study period, with SJS patients hospitalized for 7–8 days and TEN patients for 11–13 days. In-hospital mortality consistently ranged from 7-11%, which a slight increase to 13% during the COVID-19 pandemic. Hospital disposition to inpatient or hospice facilities rates remained stable at 10-13%.
Despite increased use of systemic corticosteroids, adoption of non-steroidal immunomodulating therapies remains limited. The slow adoption of immunomodulators and lack of improvement in mortality and hospitalization outcomes underscore uncertainty regarding efficacy and the absence of standardized management protocol. Future efforts should focus on developing targeted therapies and prospective multicenter trials to define the role of immunomodulators in improving clinical outcomes.

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Once-daily Oral Zasocitinib in Moderate-to-Severe Plaque Psoriasis: Results from Two Randomized Phase 3 Trials

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Zasocitinib efficacy and safety in adults with moderate-to-severe plaque psoriasis (PASI≥12, sPGA≥3, BSA≥10%) was evaluated in two phase 3 randomized, double-blind, multicentre trials: LATITUDE-PsO-3001 (3001, NCT06088043, n=693) and LATITUDE-PsO-3002 (3002, NCT06108544, n=1108). Zasocitinib 30mg once daily (QD) was compared with placebo (PBO) or apremilast (APR) 30mg twice daily over 52 (3001) or 60 (3002) weeks (W). Coprimary endpoints were sPGA 0/1 (≥2-point improvement) and PASI75 vs PBO (W16). At W16, in 3001/3002, zasocitinib met co-primary endpoints (sPGA 0/1: zasocitinib 71.4%/69.2%, PBO 10.7%/12.6% [p<0.001]; PASI75: zasocitinib 75.7%/71.4%, PBO 12.1%/12.3% [p<0.001], superiority seen at W4 [3002]), and showed superiority over APR (sPGA 0/1: APR 32.1%/29.7% [p<0.001]; PASI75: APR 37.2%/33.0% [p<0.001]). Progressive improvement was observed through W24. Zasocitinib safety was consistent with prior studies. TEAEs to W16: zasocitinib 66.1%/59.6%, PBO 45.0%/48.0%, APR 54.0%/48.4%. The most common (≥5%) TEAEs observed with zasocitinib were upper respiratory tract infection, nasopharyngitis and acne. Zasocitinib QD showed rapid and reproducible skin clearance with a consistent safety profile.

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Prurigo Nodularis in Children in Finland from 1995 to 2024: A Nationwide Registry Study

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The incidence of prurigo nodularis (PN) increasing. While PN is most common in elderly people, it also occurs in children. As the body of published data on PN in children is sparse we designed the present study to elucidate the incidence of childhood PN, its preceding dermatological conditions and the use of systemic treatments.
Data from the database of the Finnish Care Register for Health Care were retrieved for all individuals aged <15 years whose record contained at least two entries of the International Classification of Diseases version 10 (ICD-10) code L28.1 for a diagnosis of PN during the period of 1995-2024. To analyse preceding conditions, we then searched each of the retrieved records for entries of ICD-10 codes with the prefix 'L', which denotes dermatological diseases. Data on the frequency of use of methotrexate, cyclosporine and dupilumab, medications most commonly used to treat PN, were gathered from the Drug Reimbursement Registry of Finland.
Crude and age-standardized incidence rates of PN were calculated using annual population-at-risk data from Statistics Finland and the 2013 European Standard Population. Categorical variables were presented as proportions and compared using the Pearson Chi-square test. P-values <0.05 were considered statistically significant.
In total 48 individuals fulfilled the inclusion criteria. The total crude incidence of PN was 1.99/1,000,000 (95% confidence interval [CI] 1.46–2.63), and it remained similar (2.06/1,000,000, 95% CI 0–2.87) when the European standard population was used as a reference. Over three-quarters of individuals had at least one dermatological diagnosis that preceded that of PN, with AD being the most common (52.1%). There were no statistically significant differences between sexes in occurrences of any of the other conditions. A quarter of individuals had received systemic medication for PN, and dupilumab (n=7, 14.6%) was the most frequent.
Based on our findings, PN is uncommon in children in Finland, and, while AD was the most common dermatological comorbidity, not all our population had any preceding dermatological condition.

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Psychiatric comorbidities in patients with atopic dermatitis: the importance of the multidimensional impact of the disease

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Atopic Dermatitis affects approximately 9 -10% of the world's population. The prevalence of psychiatric comorbidity is high among patients with skin disease, including those with atopic dermatitis (AD). Clinical variables play a proven, yet still minor, role in explaining patients' mental health, and the role of patient-reported disease burden needs to be further explored.
Using the new Patient-Reported Impact of Dermatological Diseases (PRIDD), this study examined the associations between sociodemographics, clinical variables, disease impact, and anxiety and depression scores.
A global online survey was conducted between 06.2023 and 01.2024. Adults with a self-reported skin condition were recruited through patient organizations and social media. Participants completed the PRIDD questionnaire (16 items assessing physical, life responsibilities, psychological and social impact domains), the Patient Health Questionnaire (PHQ-9), the General Anxiety Disorder Screener (GAD-7), and provided sociodemographic and clinical information.
From the 275 participants with AD (72.7% female, 39.4±13.4 years, from 32 different countries), 99 (36.0%) scored above the threshold for clinical depression (PHQ-9≥10) and 73 (26.5%) for clinical anxiety (GAD-7≥10). In hierarchical regression analyses, sociodemograhics (younger age, darker skin type) explained 10.2% and 10.4% of the variance in depression and anxiety scores. Clinical variables explained 14.6% of depression and 14.7% of anxiety scores, with higher disease severity and presence of comorbidities associated with worse mental health. PRIDD physical and social dimensions explained an additional variance of 20% for depression, and psychological and social dimensions explained 15.8% for anxiety.

These results emphasize the importance of capturing the multidimensional burden of AD, as an important risk factor for psychiatric comorbidity. This helps inform the need for integrated psychological supports into overall treatment plans.

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Zumilokibart (APG777), a half-life extended monoclonal antibody, provides rapid and sustained inhibition of type 2 biomarkers in moderate-to-severe atopic dermatitis

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Type 2 inflammation is a hallmark of atopic dermatitis (AD) pathogenesis, characterized by elevated serum and tissue levels of multiple cytokines. Quantifying pathway-level changes is critical for understanding therapeutic impact; however, many Type 2 mediators circulate at concentrations below the detection limits of conventional assays.
To address this limitation, Part A of the APEX Phase 2 trial (NCT06395948)—a randomized, double-blinded, placebo-controlled trial of zumilokibart (APG777) in moderate-to-severe AD—used an ultrasensitive proteomics platform. The platform evaluated pharmacodynamic effects of zumilokibart, a half-life–extended, anti-IL-13 antibody, on Type 2 inflammatory biomarkers. Serum samples were collected from patients (zumilokibart n=72; placebo n=39) at Baseline and Weeks 2 and 16.
Patients on zumilokibart showed early and sustained reduction in an inflammation score composed of 18 Type 2-related biomarkers. Reductions were observed as early as Week 2 and sustained through Week 16 (mean z-score: 0.354 baseline; -0.117 week 2; -0.241 week 16; $p<0.0001$) in the zumilokibart arm vs. no significant changes in the placebo arm (0.042 baseline; 0.009 week 2; -0.054 week 16; $p>0.05$). The most notable changes from baseline at Week 16 were observed in IL-13 (Log2FC -7.23), TARC (Log2 FC -2.00), CCL26 (Log2 FC -1.72), and MCP-4 (Log2 FC -0.840).
Zumilokibart's rapid and sustained effects on Type 2 biomarkers are consistent with the clinical benefits observed in patients with AD (reported earlier), with only 4 dosing days during induction.

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Responders to Zumilokibart (APG777) Show Durable Maintenance and Deepening of Response Beyond Week 16: Results from the Phase 2 APEX Part A Study in Atopic Dermatitis

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Zumilokibart (APG777) is the first half-life-extended anti-IL-13 antibody to be evaluated in atopic dermatitis (AD), testing 4 induction dosing days and 2 or 4 maintenance dosing days per year. The phase 2 APEX AD Part A study (NCT06395948) met the primary endpoint at Week 16 (W16). Here, maintenance and deepening of responses achieved at W16 with zumilokibart are presented at completion of the Part A maintenance period at W52.
Adults (N=123) with moderate-to-severe AD were randomized 2:1 to zumilokibart or placebo. Upon completing W16, participants initially randomized to zumilokibart (N=72) were re-randomized 1:1 to zumilokibart Q12W or Q24W. Maintenance of response analyses were conducted with multiple imputation combined with non-responder imputation. Deepening of response analyses were as-observed.
At W16, EASI-75 was achieved by 66.9% of zumilokibart participants (vs. 24.6% placebo; $p<0.001$). Maintenance of EASI-75 at W52 was achieved by 75.2% (Q12W) and 85.3% (Q24W) of W16 responders. Deepening of response was observed among those achieving EASI-75 at W16: EASI-90 response rates increased from W16 to W52 (Q12W: 61.5% to 84.0%; Q24W: 41.7% to 54.5%). Treatment was well-tolerated over 52 weeks; AEs in ≥5% zumilokibart-treated participants included noninfective conjunctivitis, upper respiratory tract infection, nasopharyngitis, and atopic dermatitis.
Both Q12W and Q24W dosing with zumilokibart led to durable maintenance and deepening of response through W52, supporting further clinical investigation.

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Zumilokibart (APG777) Provides Robust, Progressive Improvement in the Signs and Symptoms of Atopic Dermatitis: 52-Week Results from the Phase 2 APEX Part A Study

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Zumilokibart (APG777) is a half-life-extended anti-IL-13 antibody with 4 induction dosing days and 2 or 4 maintenance dosing days per year. The phase 2 APEX Part A study (NCT06395948) met its primary endpoint at Week 16 (W16). Here, we report clinician- and patient-reported outcomes with zumilokibart over the full 52-weeks of APEX Part A.
Adults (N=123) with moderate-to-severe AD were randomized 2:1 to an induction regimen of zumilokibart or placebo. W16 zumilokibart completers (N=72) were re-randomized 1:1 to zumilokibart Q12W or Q24W in maintenance. W16 placebo completers (N=37) crossed over to receive a modified zumilokibart maintenance regimen. Clinician assessments included change from Baseline in EASI and proportions achieving EASI-75 and vIGA-AD 0/1. Patient-reported outcomes included Itch (I)-NRS. All analyses were as-observed.

Continued zumilokibart treatment led to progressive reduction in EASI; the LS mean (LSM) change from Baseline in EASI was -90.8% (Q12W) and -86.0% (Q24W) at W52. Placebo-zumilokibart crossover participants achieved a -85.2% LSM change from Baseline in EASI at Week 52. Among participants continuing zumilokibart in maintenance, 87.5% (Q12W) and 80.6% (Q24W) achieved EASI-75 at W52; 71.9% (W12) and 51.6% (W24) achieved clear/almost clear skin (vIGA-AD 0/1). Zumilokibart treatment led to progressive improvement in itch (LSM change from Baseline in I-NRS -72.5% [Q12W] and -64.1% [Q24W]) at W48. Treatment was well-tolerated over 52 weeks.
These results support further clinical investigation with zumilokibart in APEX Part B.

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Low-dose oral minoxidil for persistent alopecia after hematopoietic stem cell transplantation in children with hematological malignancies

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Persistent chemotherapy-induced alopecia (pCIA) - non-scarring alopecia persisting more than 6 months after chemotherapy - is a recognised but understudied late effect of haematopoietic stem cell transplantation (HSCT) in pediatric oncology. Myeloablative conditioning regimens destroy hair follicle stem cells, leading to permanent/prolonged alopecia. While low-dose oral minoxidil (LDOM) treats pCIA in adults, pediatric data remain scarce. This study evaluated the efficacy and safety of LDOM in children with persistent post-transplant alopecia.
This retrospective study included 8 pediatric patients (5 females, 3 males; median age 7 years, range 5-17) with persistent non-scarring alopecia after HSCT for hematologic malignancies. Patients received LDOM at 0.5-1.25 mg/day. Outcomes were assessed after 12 months via clinical evaluation, trichoscopy and physician/patient/parent global improvement.
At baseline, trichoscopy showed markedly reduced follicular unit pattern, dominated by single-hair follicular units, reduced density and yellow dots, without clinical features of scarring alopecia. Oral minoxidil was well tolerated in all cases. No patient discontinued therapy due to adverse events. No systemic side effects, including cardiovascular adverse events, were documented. After 12 months, regrowth was partial rather than complete, but all assessments indicated clinically relevant benefit. The physician-rated response was mild, whereas patients and parents reported moderate improvement. The most consistent changes were increased hair shaft thickness and a modest increase in hair density, documented on paired photographs and trichoscopy.
LDOM may represent a feasible and well-tolerated therapeutic option for persistent post-HSCT alopecia in pediatric cancer survivors. Although regrowth was partial, increased hair thickness and visible density were meaningful to patients and families. These real-world data support systematic dermatologic follow-up of pediatric HSCT survivors and prospective studies for new therapeutic options.

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Evaluating the durability of psoriasis control on systemic immunomodulatory therapy: A Systematic Review and Meta-analysis

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As targeted therapies become more widely used in psoriasis, more patients are achieving disease control. Understanding the durability of control and its determinants is needed to personalise long-term care.
We conducted a systematic review and meta-analysis to estimate, among individuals achieving well-controlled psoriasis, the proportion maintaining control (defined as mild psoriasis or better) on systemic immunomodulatory therapy and to identify factors associated with sustained control (PROSPERO CRD420251089461). MEDLINE, Embase, CENTRAL and Web of Science were searched to 01/04/25. Data were extracted in duplicate and study quality assessed using a modified Newcastle-Ottawa Scale. Thresholds across disease activity measures were mapped to clear, nearly clear or mild psoriasis categories. Studies defining control using a disease activity measure at one timepoint and non-responder imputation for missing data were pooled in a random-effects meta-analysis to estimate the proportion maintaining the same level of control over time.
Of 12,487 studies identified, 65 were eligible. Across overlapping groups, 2,524 patients achieved clear, 9,642 achieved nearly clear and 7,771 achieved mild psoriasis. Evidence for small molecule and conventional systemic therapies was insufficient for pooling. Of 57 biologic studies, 41 met meta-analysis criteria. At >=24 months, pooled estimates showed that 73% (95% CI 2–100; 2 studies), 68% (95% CI 47–84; 5 studies), and 70% (95% CI 56–81; 4 studies) of patients achieving clear, nearly clear and mild psoriasis, respectively, maintained the same level of control. IL-17 and IL-23 inhibitors were associated with better maintenance of nearly clear and mild psoriasis than TNF inhibitors. No TNF inhibitor studies defined control as clear, precluding comparison for this outcome. Factors associated with sustained control included lower BMI, shorter disease duration, and biologic-naïve status.
Over two-thirds of patients achieving psoriasis control on biologic therapy maintained control at >=24 months, providing a benchmark for counselling and a foundation for developing prognostic tools to support risk-stratified care.

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Upadacitinib in Real-World Practice: A Three-Month Analysis of Clinical and Patient-Reported Outcomes focusing on body regions in the Large German Atopic Dermatitis Registry TREATgermany

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Atopic dermatitis (AD) is a chronic inflammatory skin disease that manifests with regional variation, impacting the head/neck, trunk, arms, and legs with differing degrees of severity. This analysis aimed to evaluate the effectiveness of upadacitinib on the signs of AD across the four key body regions in the large TREATgermany registry cohort.
This retrospective, multicenter analysis included data from 61 therapy episodes (63.9% of the patients were male, mean age 39.1 years) with moderate-to-severe AD who were initiated on upadacitinib therapy. Clinical effectiveness was assessed using IGA (0–4) and EASI (0–72), with regionally weighted subscores. Patient-reported outcomes at therapy start visit and after 3 months of upadacitinib included BSA (%) and 0–10 NRS for pain, itch (average/peak), and sleep impairment (0-10 NRS).
Initially, patients demonstrated high disease activity and a severe symptom burden. A vast majority (95.1%) presented with an IGA of 3 (moderate, 60.7%) or 4 (severe, 34.4%), or 5 (very severe, 3.3%). The mean EASI was 19.0, and the mean patient-reported BSA was 23.5%. Regionally, the arms were most severely affected relative to their maximum score (mean EASI 4.7, 32.6% of max), followed by the head/neck (2.0, 27.8%), trunk (5.7, 26.3%), and legs (6.6, 22.9%). The subjective burden was substantial, with high mean NRS scores of 6.4 for average itch, 6.1 for peak itch, 4.9 for sleep impairment, and 4.3 for pain.
After three months of treatment, the following improvements were observed: The proportion of patients achieving an IGA of 0 (clear) or 1 (almost clear) was 49.2%. The mean total EASI decreased by 75.8% to 4.6, and the mean patient-reported BSA decreased by 77.2% to 5.4%. This clinical improvement was consistent across all body regions, with mean EASI subscore reductions of 77.2% for the trunk (to 1.3), 72.7% for the legs (to 1.8), 78.2% for the arms (to 1.0), and 70.0% for the head/neck area (to 0.6). This real-world analysis provides evidence that upadacitinib delivers rapid improvement across all body regions for patients with moderate-to-severe AD.

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Improvement of Pruritus and Pain in Chronic Hand Eczema Treated with Delgocitinib: Correlation with HECSI in a Real-World Cohort

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Chronic hand eczema (CHE) is a frequent and debilitating inflammatory skin disease characterized by persistent lesions, pruritus, and pain, significantly impairing quality of life and daily functioning. The Hand Eczema Severity Index (HECSI) is widely used to assess clinical severity; however, symptom burden, particularly pruritus and pain, is not always fully captured by objective scores. Delgocitinib, a topical pan-Janus kinase (JAK) inhibitor, has emerged as a promising therapeutic option for CHE due to its broad anti-inflammatory effects.
We conducted a real-world observational study in a specialized eczema unit at a tertiary care hospital in Barcelona (Hospital de la Santa Creu i Sant Pau), including 25 patients with severe CHE treated with topical delgocitinib. Clinical severity was assessed using HECSI, while pruritus and pain were evaluated using numerical rating scales (NRS). Assessments were performed at baseline and during follow-up.
Treatment with delgocitinib was associated with a significant reduction in HECSI scores, reflecting improvement in clinical signs. Importantly, patients also reported a marked decrease in pruritus and pain intensity. Symptom improvement paralleled the reduction in HECSI, although in some cases, relief of pruritus and pain was observed earlier than visible clinical improvement.
These findings highlight the effectiveness of delgocitinib in improving both objective and subjective dimensions of CHE. The correlation between HECSI reduction and symptom relief supports its utility in clinical practice, while also underscoring the importance of incorporating patient-reported outcomes in disease assessment. Delgocitinib represents a valuable therapeutic option for addressing the multifaceted burden of CHE.

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Pedi AD in the ED: Real-world Management Practices in Atopic Dermatitis in Pediatric Emergency Room Visits: A Population-level Cohort Study Using Epic Cosmos

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Atopic dermatitis (AD) affects ~15-30% of children, and its chronic nature is marked by recurrent flares. Limited access to specialty care and absence of guidelines for managing flare in the emergency department (ED) contribute to substantial variability across practice settings. This study aims to evaluate management patterns for AD in pediatric ED encounters, including medications and bacterial culture.
We conducted a retrospective cross-sectional study using Epic Cosmos, a population-level electronic health record database encompassing 300 million patients. We analyzed data from January 1, 2021 to December 31, 2025. ED encounters for AD with patients <18 of age were included. Medications based on Anatomical Therapeutic Chemical (ATC) codes were queried.
Based on 41,853 ED encounters, topical corticosteroids were most frequently prescribed, with weak (26.5%) and moderately potent (31.6%) agents comprising nearly 60% of encounters. High-potency corticosteroids were infrequently used (Group 3: 4.9%; Group 4: 1.7%). Antibiotics were prescribed in 8.5% (topical) and 12.4% (systemic) of encounters. Bacterial cultures were obtained in only 15.9% and 25.8% of these cases, respectively. Non-steroidal topical therapies (tacrolimus, roflumilast, ruxolitinib, tapinarof) and other immunomodulators (methotrexate, cyclosporine, dupilumab) were rarely prescribed (<1% each).
In this population-level cohort, patterns demonstrate a predominance of weaker topical corticosteroids, suggesting undertreatment of more severe presentations. Antibiotic use frequently occurs without microbiologic confirmation, underscoring

opportunities for improved antimicrobial stewardship. The minimal use of non-steroidal topicals, methotrexate, cyclosporine, and dupilumab is unsurprising given these medications often require the expertise of dermatologists, which are limited in the ED settings. Aligning ED prescribing practices with disease severity and evidence-based guidelines is crucial for improving outcomes. Timely outpatient follow-up and referral to dermatology remain paramount for achieving sustained disease control in pediatric AD.

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Beyond conventional histology: Hybrid open-top light-sheet microscopy for 3D dermatopathology of inflammatory and neoplastic skin diseases

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This pilot study evaluates hybrid open-top light-sheet microscopy for three-dimensional characterization of inflammatory and malignant skin diseases. Conventional dermatohistopathology relies on two-dimensional sections that disrupt spatial relationships and limit assessment of complex tissue architecture.
Formalin-fixed, paraffin-embedded skin samples from psoriasis, atopic dermatitis, basal cell carcinoma and malignant melanoma were analyzed using a hybrid open-top light-sheet microscopy platform integrating orthogonal and non-orthogonal illumination geometries. Immunofluorescent labeling with disease-specific markers was performed to visualize immune cells, tumor cells and vascular structures. Volumetric datasets were reconstructed to assess spatial cellular distribution and microvascular organization within intact tissue blocks.
Three-dimensional imaging enabled preservation of native tissue architecture and revealed distinct spatial patterns across disease entities. In inflammatory dermatoses, immune cell populations showed characteristic distribution patterns, including perineural immune cell aggregation in atopic dermatitis. In malignant tumors, volumetric reconstruction demonstrated complex blood and lymphatic vessel networks and their spatial relationship to tumor cell clusters and immune infiltrates. In melanoma samples, vascular structures were screened for intravascular tumor cells.
Hybrid open-top light-sheet microscopy allows volumetric assessment of intact skin specimens and provides insights into inflammatory microenvironments and tumor–vascular interactions beyond conventional histology. This approach may support mechanistic research and future biomarker development in dermatopathology.

563 [WITHDRAWN]

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Eczema subtypes in a multiracial Asian population

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Eczema encompasses heterogeneous inflammatory skin conditions with distinct aetiologies and risk factors, yet is often analysed as a single entity. Examining eczema by subtype is crucial for elucidating pathophysiology and informing basic research and public health strategies. This study characterised epidemiology, temporal trends, and predictors of eczema subtypes in a multiracial Asian population. A retrospective cohort study was conducted using anonymised outpatient data from the National Skin Centre from 2012-2023. Patients were identified using diagnostic codes and grouped into 13 eczema diagnoses. Multivariate logistic regression was performed for each subtype, adjusting for age, sex, race and year of attendance. A total of 167396 patients were included; 20.6% aged 0-20 years, 62.6% 21-64 years, and 16.8% ≥65 years. Males comprised 51.2% and females 48.8%. The cohort comprised Chinese (76.7%), Malays (6.2%), Indians (8.7%), and others (8.4%). A significant annual increase in the odds of diagnosis was observed for atopic dermatitis, discoid eczema, and unspecified eczema, while a decrease was seen for contact dermatitis and hand-feet eczema. Increasing age was associated with asteatotic eczema (OR 1.09, 95% CI 1.087-1.091) and stasis dermatitis (OR 1.07, 95% CI 1.065-1.071), while younger age was associated with atopic dermatitis (OR 0.916, 95% CI 0.915-0.917). Female sex was associated with facial eczema (OR 3.73, 95% CI 3.46-4.06) and contact dermatitis (OR 1.84, 95% CI 1.79-1.94), while male sex was associated with exfoliative dermatitis (OR 3.19, 95% CI 2.75-3.69) and asteatotic eczema (OR 1.55, 95% CI 1.50-1.59). Malays had higher odds of discoid eczema than both Chinese (OR 1.48, 95% CI 1.35-1.62) and Indians (OR 2.01, 95% CI 1.86-2.17). Chinese had higher odds of atopic dermatitis than both Malays (OR 1.10, 95% CI 1.04-1.16) and Indians (OR 1.62, 95% CI 1.53-1.72), and higher odds of facial eczema than Malays (OR 1.64, 95% CI 1.41-1.90) and Indians (OR 1.29, 95% CI 1.14-1.46). Indians had higher odds of seborrhoeic dermatitis than both Chinese (OR 1.64, 95% CI 1.56-1.73) and Malays (OR 2.64, 95% CI 2.39-2.91). Eczema subtypes vary in epidemiology, with implications for clinical practice and research.

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Risk Factors for Paronychia in Anti-epidermal Growth Factor Receptor Therapy: A Retrospective Cohort Analysis from a Tertiary Referral Medical Center in Taiwan

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Paronychia is a common dermatologic toxicity of anti–epidermal growth factor receptor (EGFR) therapy significantly affecting quality of life and treatment adherence, so the purpose of this study is to identify clinical predictors of EGFR inhibitor-induced paronychia in patients with non–small–cell lung cancer or colorectal cancer.
This retrospective cohort study reviewed electronic medical records from a Taiwanese tertiary center (from January 2014 to June 2023). Eligible treatment episodes were analyzed with multivariable logistic regression; discrimination was assessed by the area under the receiver–operating–characteristic curve (AUROC).
Among 3,898 episodes, paronychia occurred in 704 (18.1%), highest in second–generation tyrosine kinase inhibitors (TKIs) (35.5%) and lowest in third–generation TKIs (6.8%). Onset was significantly later with third–generation TKIs (median 147 vs. 73–75 days; P ≤ 0.002). Independent risk factors were younger age (adjusted odds ratio [aOR] 1.013/year decrement; 95% CI, 1.005–1.020), female sex (aOR 1.35; 95% CI, 1.12–1.59), and second–generation TKIs (aOR 3.26; 95% CI, 2.69–3.97). Third–generation TKIs were protective (aOR 0.42; 95% CI, 0.28–0.62). The model demonstrated acceptable discrimination (AUROC

0.71).
We found female sex, younger age, and second-generation EGFR–TKIs significantly increase paronychia risk, aiding clinicians in patient risk stratification and preventive care.

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The German S3 guideline 'Mental health for skin patients' is launching modern, integrative and evidence-based care for patients with psychodermatological conditions

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In patients with skin disorders, psychosomatic conditions are frequent but underrecognized in routine care. International expert groups call for specialised, multidisciplinary care structures. To improve psychodermatological care, 39 experts from 29 professional and patient organisations developed an *S3* guideline of the Association of the Scientific Medical Societies in Germany (AWMF). *S3* guidelines represent the highest standard of guideline development in Germany. 'Mental health for dermatologic (skin) patients' (MenDaSkin) highlights psychodermatology as an evidence-based interdisciplinary field at the intersection of mental health care and somatic medicine. First, a systematic literature search shows prevalences between 18% (melanoma) and 37% (alopecia areata) for depression and 25% (hidradenitis suppurativa) and 40% (lupus erythematodes) for anxiety among patients with skin conditions. This leads to clear recommendations for routine screening for psychosocial distress as a central component of integrated dermatological care. Second, when symptoms are identified, patients should receive information, appropriate diagnosis and treatment recommendations for mental health improvement. Evidence levels for psychosocial interventions vary. The guideline also identifies research gaps, including the lack of cost-effectiveness studies on integrated care models. In summary, guideline development in interdisciplinary areas can yield both, practical recommendations for healthcare and guide future research funding.

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Teledermatology in Ethiopia: providers' perspectives, readiness, and barriers to implementation in a resource-limited setting

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The disparity in access to specialist dermatological care is a pressing public health issue in resource-limited settings like Ethiopia, where the dermatologist-to-population ratio is approximately 1:570,000. Teledermatology offers a viable solution, yet its success depends heavily on the readiness of frontline healthcare professionals. This study analyzed healthcare professionals' perspectives in Hawassa, Ethiopia, to evaluate the need for teledermatology, technological readiness, and barriers to implementation. A mixed-method approach was employed, combining a quantitative cross-sectional survey of 144 healthcare professionals from ten public health centers with qualitative in-depth interviews of 30 health professionals, IT specialists, and administrators. Quantitative data were analyzed using descriptive and inferential statistics, while qualitative data underwent thematic analysis. The study revealed an overwhelming need for teledermatology, with 93% of respondents requiring specialist dermatology consultations at least weekly. While 81.3% of respondents possessed internet-enabled smartphones, only 37.9% had prior telemedicine experience. Despite this, 93.1% held positive attitudes toward teledermatology, primarily driven by the potential to improve patient access and enhance professional knowledge. However, significant structural barriers were identified. The top three impediments were insufficient management support for funding and policy, unreliable internet connectivity, and limited access to dedicated devices. Qualitative findings reinforced these hurdles, highlighting frustration with unpredictable connectivity. Sustainability was significantly linked to continued training and reliable technology. In conclusion, healthcare professionals in Hawassa demonstrate high readiness and positive attitudes toward teledermatology as a critical tool for health equity. However, successful implementation is contingent upon a strategic plan that prioritizes investment in reliable internet infrastructure and comprehensive, continuous training programs to surmount systemic barriers.

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Epidemiology of autoimmune bullous diseases in Finland 2000-2024

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Autoimmune bullous dermatoses (AIBD) include pemphigus group, pemphigoid group and dermatitis herpetiformis (DH). The aim of this national retrospective registry study was to investigate the epidemiology of all AIBD in Finland and analyze the trend of incidence. We included all patients over 16 years of age who were diagnosed with an AIBD in Finland between 2000-2024. Data was collected from the Finnish Care Register for Health Care using specific ICD-10 codes.
A total of 7940 patients met our inclusion criteria. More than half of the cases (73.6 %) were diagnosed with pemphigoid diseases, 20.9 % with DH and only about 5.5% had pemphigus diseases. There was a female predominance in all other AIBD except in DH and pemphigus foliaceus (PF), the female to male ratio varying from 1.04 to 1.65. The largest patient groups were bullous pemphigoid (BP) with 66.9% (n=5315), DH with 20.9% (n=1662), mucous membrane pemphigoid with 3.8% (n=302), pemphigus vulgaris with 2.1% (n=168) and PF with 2.5% (n=196) of all included patients. We calculated crude incidence of each disease in 5-year periods. The incidences of PV, PF and BP continued to rise until 2020, after which their incidence began to decrease. The incidence of DH was steadily declining during the study period. MMP and pemphigus erythematosus also had a slightly decreasing incidence. For the other AIBD the incidence remained constant. BP had the highest incidence being 58 cases/million/year during 2020-2024.
In conclusion, all AIBD are rare or extremely rare in Finland. BP was by far the most common AIBD. Interestingly, we found a recent decline in the incidence of BP; further research is needed to understand the reasons behind this observation. Pemphigus diseases and other pemphigoid diseases excluding BP are very rare, and there were no changes in their incidence during the study period, although the small number of cases limits the interpretation of the results.

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Beyond the Surface: High Awareness but Low Implementation of Psychosocial Care in Dermatology

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To assess dermatologists' awareness, attitudes, and practices regarding psychological and psychosexual comorbidities in patients with chronic skin diseases and to identify barriers to holistic care.
Methods: An anonymous 25-item online survey was distributed to members of the Swiss Society of Dermatology and Venereology (October 2024–February 2025). The questionnaire evaluated perspectives on psychological well-being and sexual health, as well as perceived barriers.
Sixty-two dermatologists participated (59.7% women; median age 39 years; median experience 11.5 years). Most respondents acknowledged the negative impact of skin disease on psychological well-being (98.4%) and sexual life (96.7%). However, only 69.4% routinely assessed psychological burden and 16.2% addressed sexual health. Main barriers included lack of time (68.8% vs 71.7%), insufficient training (43.5% vs 50.9%), and lack of standardized tools (41.0% vs 57.4%).
Despite high awareness, psychosocial aspects of skin disease—particularly sexual health—remain underassessed in dermatological practice. Systematic use of brief screening tools and targeted psychodermatology training is needed to improve holistic care.

570 [WITHDRAWN]

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Divergent trends in dermatology hospital admissions in England over 25 years: the impact of biologics and evolving care pathways

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This study describes temporal trends in hospital admissions for inflammatory skin diseases in England over 25 years, examining how advanced systemic therapies and evolving care pathways have reshaped hospital activity.
We conducted a retrospective analysis of NHS Digital Hospital Episode Statistics (April 1998–March 2023), identifying admissions by primary ICD-10 diagnosis for psoriasis, urticaria, atopic dermatitis, hidradenitis suppurativa, and immunobullous diseases. Crude admission rates per 100,000 were calculated using ONS mid-year population estimates. Overnight admissions were defined as total admissions minus day cases.
Overall admission rates were stable (41.2 to 41.9 per 100,000), masking a fundamental transformation in case-mix. As advanced therapy administration is increasingly captured as planned day cases in administrative datasets, headline rates are a poor proxy for disease burden. Psoriasis admissions fell 38% (18.7 to 11.5 per 100,000) as biologics shifted management to outpatient settings: day cases rose from 42% to 70%, overnight admissions fell correspondingly, and mean length of stay halved. Urticaria admissions rose 183% (4.2 to 11.8 per 100,000), accelerating sharply from 2015 following UK approval of omalizumab for chronic spontaneous urticaria, with day cases rising from 5% to 63% and overnight admissions declining as a proportion. Where established infusion therapies are absent, trends more directly reflect clinical need: immunobullous admissions rose 62% (1.9 to 3.2 per 100,000), likely driven by population ageing, while emergency hidradenitis suppurativa admissions rose 250% in absolute terms (229 to 802 per year), with the emergency proportion more than doubling (12.7% to 28.7%), suggesting growing unmet need and delayed specialist access.
Despite stable overall dermatology admission rates, the underlying case-mix has been profoundly transformed by therapeutic advances and shifting care pathways, with important implications for interpreting administrative hospital data.

572 [WITHDRAWN]

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Agent-based modelling of the two major patterns of human congenital melanocytic diseases supports the existence novel melanocyte precursor population

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Two recurrent patterns of human congenital melanocytic disease have been described. The first, the "segmental" pattern, is compatible with animal data on neural crest melanocytic development and migration. The second, "non-segmental" pattern, is not compatible with what is known of neural crest melanocytes and was hypothesised to be of mesodermal origin. Whether both contribute to human pigmentation in healthy conditions is not yet known, however evidence of the patterns of pigment loss in segmental and non-segmental vitiligo, which closely mimic the patterns of the two populations, would suggest that they do. This study used a form of computational modelling, known as agent-based modelling, to understand how cell migration and proliferation result in these two recurrent patterns seen in humans. Cells are represented as autonomous individuals on a grid, following simple, yet accurate rules of local interaction with each other and nearby surroundings, such that experiments on the scale of tens of thousands of cells are possible. By varying model parameters: precursor cell number and positioning, cell cycle length, migratory cell speed, the number of migratory cells, and chemoattractant strength, we can recreate various real-world pigmentation patterns, different in shape, size, perceived density, and number of foci. Thus, the models provide a way to associate cell-level behaviours with human congenital macroscopic patterns, suggesting underlying causes of real-world pattern variation. Furthermore, the model supports the different origin hypothesis, as the two categories of patterns cannot be recreated under similar conditions, requiring the restriction of parameter values and differing initial conditions.

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Targeting *Staphylococcus aureus* in Atopic Dermatitis – Evaluating Safety and Efficacy of Emollients Containing Acetyl Heptapeptide-4 and Oligofructans from *Ophiopogon japonicus*

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The severeness od AD is associated with excessive skin colonization by *St. aureus*. To date, the basis of treatment for this disease is emollient therapy. A novel generation of emollients contain non-pharmacological ingredients targeting mechanisms in AD pathophysiology. The aim of this study was to evaluate the safety and effectiveness of three emollients containing different concentration complex of oligofructans from *Ophiopogon japonicus* and acetyl heptapeptide-4 targeting *St. aureus* by inhibiting its skin surface adhesion and biofilm formation. The safety of tested products was evaluated by agarose overlay MTT test *in vitro*. The irritation potential was assessed using a human *ex vivo* skin model. In order to investigate the sensitization potential, culture media was collected and the IL-18 level was evaluated using ELISA test. *In vivo* study was conducted on a group of patients with symptomatic AD lesions recruited from Polish Association for Atopic Diseases. Photos were taken before and after using the product (8-28 days). All three emollients presented good safety profiles. No irritation or sensitization responses were detected in *ex vivo* model: tissue viability was higher than the negative control: 102,9% in 16924, 113,5% in 16925 and 103,5% in 16926 and the average amount of IL-18 level was below the negative control (16924, 16925) or on the similar level (difference statistically insignificant). Products safety was confirmed also in agarose overlay test. The effectiveness of the products was confirmed in an *in vivo* study. Photographic documentation of skin lesions with features of atopic dermatitis proved that the skin condition had improved after using the tested emollients (visual reduction of erythema and inflammatory lesions). In conclusion, regardless of the concentration of the two-ingredient complex (oligofructans from *Ophiopogon japonicus* and acetyl heptapeptide-4) contained in the products, all the tested emollients were found to be safe and efficient in alleviating the symptoms of atopic dermatitis. Targeting *St. aureus* appears to be effective in the event of AD flare-ups

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The role of supportive conversation for patients undergoing cancer diagnostics

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Diagnostic process of cancer disease is very challenging for patients, who experience uncertainty about possible diagnosis. The aim of our study was to investigate the effect of supportive conversation in this group of patients. The study included 30 patients undergoing diagnostics of lung cancer, admitted to the ward for bronchoscopy and other procedures. Almost all the patients experienced xerosis and required skin moisturization also due to augmented stress.
Supportive conversation comprising active listening, open questions, psychoeducation and stress management was carried out with every patient. DASS-21 questionnaire (stress, anxiety and depression) and VAS (1-10) for stress and tension levels were collected. Additionally, in half of the patients cortisol and DHEA levels in saliva were measured before and after intervention. DASS-21 showed that high numbers of patients suffer from increased stress, anxiety and depression (76.7%, 53.3% and 30% respectively). Stress reduction after our intervention was observed both in VAS ($p<0.001$) and cortisol levels ($p=0.028$). We also demonstrated that cortisol/DHEA ratio decreased after intervention ($p=0.003$), indicating an improvement in neuroendocrine balance in stress response.
Additionally, patients described their subjective feelings about intervention. Of interest, patients who initially did not express the need for emotional support also benefited from our intervention.
The study shows that our supportive intervention may be effective in reducing stress and improving overall wellbeing, leading to better psychological functioning and reduction of skin-related issues.

576 [WITHDRAWN]

577 [WITHDRAWN]

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Reconciliation of Topical Treatment Prescriptions Upon Hospital Admission

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Accurate medicines reconciliation on admission is essential for patient safety, however, topical therapies are frequently overlooked despite their importance in managing chronic conditions. This audit aimed to evaluate and improve the reconciliation of such when admitted to King's Mill Hospital, ensuring that chronic conditions managed this way remain free of flare ups
A prospective audit reviewed patients admitted to three wards between 12/01/26 and 14/02/26 (to minimize speciality bias). SystemOne was used to identify regular topical medications, which were cross-checked against admission documentation and inpatient drug charts. For reconciled medications, prescribing details and administration were assessed. For omissions, documentation of reasons was assessed.
Fifty patients (aged 37–94) were included, all using at least one topical medication at home. A total of 68 medications were identified, most commonly emollients, ibuprofen gel, barrier creams, and topical corticosteroids. Indications included eczema, osteoarthritis, and pressure sores. On admission, 78% had medications documented, but only 54% were formally reconciled. Among these, 93% had complete details and 81% were administered. Of those not reconciled, 35% had a documented reason. Additionally, 22% had no topical medications recorded and received no treatment.
Reconciliation was accurate when performed, but overall rates were suboptimal, indicating a gap in medicines optimization and

potential patient safety risk. Interventions including educational posters and clerking sticker prompts have been introduced to improve recognition and prescribing of topical therapies on admission.
Please note this audit is currently awaiting a second cycle to see the impact of these interventions

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Molecular profiling-guided baricitinib treatment in refractory generalized granuloma annulare: A case report
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Generalized granuloma annulare is often persistent and poorly responsive to conventional systemic therapy. We report a refractory case in which molecular profiling helped guide targeted treatment. A 70-year-old man with type 2 diabetes presented with a two-year history of intensely pruritic, erythematous, non-scaly papules and plaques on the lower abdomen, buttocks, and thighs. Punch biopsy showed interstitial and perivascular dermatitis with dense lymphohistiocytic infiltrates between degenerated collagen fibers. Colloidal iron staining was positive for mucin, consistent with granuloma annulare. Additional work-up for infectious, inflammatory, and malignant causes was unremarkable. The patient was initially treated with potent topical corticosteroids, followed sequentially by isotretinoin, hydroxychloroquine, and dapsone, without adequate disease control. Because prior systemic therapies failed, molecular profiling was performed to further characterize the inflammatory milieu. This demonstrated a mixed inflammatory signature with co-dominant myeloid and Th2 pathways and a subdominant Th1 component. Given emerging evidence linking granuloma annulare to JAK-STAT pathway activation, baricitinib 4 mg/day was initiated. After 3 months, the patient achieved complete clinical resolution with relief of pruritus, and repeat biopsy confirmed histologic clearance. Baricitinib was well tolerated, and the patient remained in drug-free remission for 2 months at the time of writing. These findings support molecular profiling as a useful tool for identifying actionable inflammatory pathways and guiding targeted therapy in refractory generalized granuloma annulare.

580 [WITHDRAWN]

581 [WITHDRAWN]

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Management Challenges of Cutaneous T-Cell Lymphoma in a Resource-Limited Setting: A Case Report from Tanzania, Sub-Saharan Africa
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Cutaneous T-cell lymphoma (CTCL) requires specialised diagnostics and multidisciplinary care, often unavailable in sub-Saharan Africa.
We present a case of a 34-year-old male with a 3-year history of progressive pruritic skin lesions evolving from patches to plaques, nodules, and tumours, with recent scalp masses and alopecia. Prior corticosteroid therapy was ineffective.
Examination showed generalised dermatosis with papules, plaques, and nodules sparing the face and firm, non-tender scalp/neck tumours (up to 3×2 cm).
Laboratory findings: Hb 9.4 g/dL, WBC (lymphocyte predominant), ESR 30 mm/hr; no Sézary cells. LDH unavailable. Skin biopsy showed epidermotropism, atypical lymphocytes, Pautrier microabscesses, and increased mitoses.
Immunohistochemistry and molecular studies were not performed due to lack of reagents.
He was staged clinically as advanced CTCL (Stage III, nodular type) and treated with six cycles of CHOEP, achieving partial clinical response. Second-line therapy options remain limited.
This case reflects key challenges in Tanzania: delayed presentation; limited dermatologist (48 specialists for more than 68 million); and restricted access to immunophenotyping and molecular diagnostics, leading to diagnostic uncertainty and suboptimal staging. Essential tests such as LDH and imaging are inconsistently available. Treatment is largely confined to conventional chemotherapy, with minimal access to targeted agents or advanced modalities.
In contrast, European settings enable early diagnosis through specialist access, routine immunohistochemistry, flow cytometry, and molecular assays, allowing precise classification and risk-adapted therapy, including biologics and stem cell transplantation.
From a physician's perspective, care in this context relies heavily on clinical judgement under constrained resources, often managing advanced disease with limited therapeutic options.
CTCL outcomes in resource-limited settings are compromised by late presentation, diagnostic gaps, and restricted treatment access, underscoring the need for improved infrastructure, specialist capacity and partnership in expert collaboration.

583 [WITHDRAWN]

584 [WITHDRAWN]

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Beyond Lipids: Topical Simvastatin Modulates Psoriatic Inflammation in an Immunometabolic Context
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Psoriasis is a systemic inflammatory disease characterized by immunometabolic heterogeneity, which may influence therapeutic response. Statins exert pleiotropic anti-inflammatory effects, but the impact of topical formulations on local inflammatory and microvascular changes remains insufficiently explored.
Our objective was evaluate the clinical and microstructural effects of topical simvastatin in psoriasis and to explore differences in response according to metabolic status.
Two patients with plaque psoriasis were included: one with cardiometabolic comorbidities (obesity, hypertension, dyslipidemia,

impaired glucose metabolism) and one metabolically healthy young adult. A single psoriatic plaque per patient was treated with topical simvastatin 2% in a lipophilic cholesterol-enriched base for 12 weeks. Clinical, dermatoscopic, optical coherence tomography (OCT), and vascular mapping assessments were performed.
Both patients improved. In the patient with cardiometabolic comorbidities, OCT showed marked baseline epidermal thickening, severe hyperkeratosis, and architectural disorganization, followed by significant improvement, including reduced hyperkeratosis, normalization of epidermal contour, and partial restoration of the dermo-epidermal junction. Dermal vessels appeared more visible at follow-up, likely due to improved optical penetration rather than increased inflammation; vascular mapping showed an apparent increase in vessel density (0.3% to 8.9%). Clinical improvement was evident. In the metabolically healthy patient, OCT showed partial normalization of epidermal architecture and reduced surface undulation. Vascular mapping indicated decreased vessel density (5.5% to 0.2%). Clinical improvement was less pronounced.
Topical simvastatin may improve psoriasis, with response potentially influenced by metabolic status. OCT and vascular mapping provide complementary insights, although vascular findings should be interpreted cautiously due to possible imaging artifacts. Further studies are needed

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Integrated Therapeutic Strategy Using Brodalumab and Semaglutide for Multi-Morbidity: Psoriasis, Obesity, and Type 2 Diabetes Mellitus

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Psoriasis is a chronic, relapsing inflammatory skin disease frequently associated with metabolic comorbidities, including type 2 diabetes mellitus and obesity. Emerging evidence indicates that long-acting glucagon-like peptide-1 (GLP-1) receptor agonists, such as semaglutide, may confer therapeutic benefits not only for metabolic dysregulation but also for psoriatic pathology. Herein, we present the case of a 37-year-old obese Caucasian male with severe plaque psoriasis and type 2 diabetes mellitus who demonstrated amelioration of all three disease states following combination therapy with semaglutide and brodalumab—a fully human monoclonal antibody directed against the interleukin-17 (IL-17) receptor, approved for moderate to severe plaque psoriasis. Adjunctive initiation of semaglutide to ongoing brodalumab therapy resulted in a substantial reduction in glycosylated hemoglobin (HbA1c) from 8.3% to 5.7% over 12 months, fasting plasma glucose from 184 mg/dL to 99 mg/dL, and body mass index (BMI) from 50.9 kg/m^2 to 40.7 kg/m^2. The Psoriasis Area and Severity Index (PASI) concomitantly declined from 15.4 to 1.2 within the same interval. In summary, the addition of semaglutide to established biologic therapy with brodalumab in a patient with concomitant psoriasis, obesity, and type 2 diabetes mellitus achieved marked, sustained improvements in all clinical domains without adverse events. Larger-scale studies are warranted to corroborate these findings and inform future clinical practice.

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Audit on Deucravacitinib for treating moderate to severe plaque psoriasis against NICE Guidelines

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This audit aimed to evaluate the use of deucravacitinib at Lincoln County Hospital between December 2024 to December 2025 for patients with moderate to severe plaque psoriasis, assessing compliance with NICE guidance.
Specifically, we assessed adherence to documentation of baseline Psoriasis Area and Severity Index (PASI) and Dermatology Life Quality Index (DLQI) prior to treatment initiation, baseline eligibility criteria (PASI and DLQI ≥10), completion of 16-week PASI and DLQI assessments, appropriate treatment response at 16 weeks, with discontinuation if <75% reduction in PASI or <50% reduction in PASI with <5-point reduction in DLQI.
A retrospective audit cycle was conducted from December 2024–December 2025 (n=21).
Data included prior systemic treatments, treatment initiation and discontinuation dates and clinical outcomes.
Outcomes were assessed using PASI and DLQI scores at baseline and at 16 weeks post-treatment initiation.
Baseline assessment (n = 21) showed 91% had both PASI and DLQI recorded at baseline with PASI and DLQI ≥10. Hence, 91% of patients fulfilled NICE criteria for initiation of deucravacitinib.
At 16-week follow-up, only 48% had documented PASI and DLQI scores at 16 weeks.
Treatment response (n = 10 with follow-up data) showed 90% patients achieved ≥75% reduction in PASI and 10% patients did not meet response criteria.
5/21patients discontinued treatment due to adverse effects and lack of efficacy.
In conclusion, 48% of patients had complete 16-week PASI and DLQI documentation while 52% were non-compliant due to missing follow-up documentation. In patients with available follow-up data, deucravacitinib demonstrated high effectiveness, with 90% achieving PASI75 at 16 weeks.
This audit demonstrates that initiation of deucravacitinib at Lincoln County Hospital is largely compliant with NICE guidance, with appropriate patient selection in 91% of cases.
However, a significant gap was identified in 16-week outcome monitoring, with only 48% of patients having documented PASI and DLQI scores.
The main limitation of this audit is incomplete documentation, which may underestimate true clinical response thus limiting full evaluation of treatment effectiveness

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Significance of Sectioning (Breadloafing) the Entire Skin Tissue When Pathology Does Not Match Clinical Appearance

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Melanocytic nevi, or moles, are benign melanocyte proliferations, often presenting diagnostic challenges when clinical and histopathologic findings are inconsistent. Melanoma in situ (MIS), the earliest melanoma stage, remains confined to the epidermis, making early diagnosis critical for optimal outcomes. This case report highlights the diagnostic utility of the breadloafing, a serial sectioning technique, in cases where conventional histopathology might miss malignancy. This case presents a 35-year-old woman with a recurrent pigmented lesion initially diagnosed as a compound melanocytic nevus. Ten months postbiopsy, the lesion recurred, and subsequent histopathology revealed MIS. The lesion's complete excision showed an intradermal nevus without atypia, raising concerns of misdiagnosis. However, breadloafing confirmed MIS adjacent to the intradermal nevus. This case highlights the importance of integrating serial sectioning techniques, such as breadloafing, to reconcile clinicopathologic discrepancies and enhance diagnostic accuracy for atypical pigmented lesions.

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Efficacy of a Novel Bacteriostatic Cleansing Solution in the Management of Post-Acne Scarring: An 8-Week Controlled Study

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Acne vulgaris is a chronic inflammatory skin condition that may lead to permanent scarring. Chemical peeling supports treatment by controlled exfoliation and skin regeneration. The aim of the study was to determine the extent to which the novel bacteriostatic cleansing solution 14006D (containing copper gluconate, salicylic acid (0,5%), lactobionic acid (0,5%), glycolic acid (4%) and sodium hyaluronate) improves the appearance of existing post-acne scars. Bacteriostatic properties were confirmed against *C. acnes* and *S. aureus* after 1, 3, and 5 minutes of contact between the bacterial inoculum and the tested product. Sixty volunteers (18–70 years) were divided into two groups using the product twice daily for 4 or 8 weeks. Scars, hyperpigmentation, inflammation, and pores were assessed using instrumental methods (Aram Huvis ASW 300F light microscopy, SkinColorCatch Delfin Tech., VisioFace) and subjective scales. A self-assessment questionnaire and a single-application test (exfoliation index, sebum content, smothness and pH) in 5 subjects were also conducted. After 8 weeks, improvements in post-acne scars were observed in some participants. In 6/10 subjects, scars became smoother, softer, and less visible due to improved pigmentation. Instrumental measurements showed discoloration reduction in 9/10 subjects (up to 12%). Image analysis revealed reduced inflammation in 8/10 participants, with visible soothing effects. The same group showed improved skin tone uniformity and overall skin condition. After a single application, sebum content and exfoliation levels were reduced by 7% and 11%, respectively. Skin pH was restored, and skin smoothness improved. These findings indicate that the tested solution demonstrates multidirectional activity, combining exfoliating, sebostatic, and anti-inflammatory effects. Regular use may not only support the reduction of post-acne scars and discoloration but also contribute to improved skin barrier balance, normalization of sebum production, and enhanced overall skin texture.

590 [WITHDRAWN]

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Biocompatibility of leave-on emollients in relations to intended use area – does formulation impact skin tolerance?

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Emollient formulations differ in composition and texture, which may influence their interaction with the skin, especially in sensitive populations. This study aimed to compare the biocompatibility of three leave-on emollient medical devices with different intended use area.
Three products were evaluated: a face and eyelid cream (16912B), a light body balm without paraffin (16914A), and a richer body cream containing paraffin (16915A). All tested formulations including hemp seeds and canola oils. Biocompatibility assessment was performed according to ISO 10993, including cytotoxicity, skin irritation, and sensitization potential. *In vivo* application studies were caried out in subjects with sensitive and atopic dermatitis skin.
All tested products demonstrated a good safety profile *in vitro*. The body products (16914A and 16915A) showed no cytotoxicity, while the face cream (16912B) exhibited slight cytotoxicity (grade 1). None of the products induced irritation in reconstructed human epidermis models, with high tissue viability observed across formulations (108.5% for 16912B, 105.6% for 16914A, 95.5% for 16915A). Neither sensitization potential *in vitro*, nor irritation in patch tests were reported.
In vivo, all products were well tolerated, with no significant adverse reactions reported. A single transient reaction was observed for 16914A. Application studies confirmed improvements in skin hydration, smoothness, and reduction of dryness and pruritus. The paraffin-containing creams showed pronounced effects on skin barrier support and reduction of transepidermal water loss, while the lighter balm was associated with high user acceptance and good tolerability. The face cream showed suitability for sensitive areas, including eyelids.
Despite differences in formulation, all emollients exhibited high biocompatibility and good skin tolerance. Minor differences observed *in vitro* did not translate into clinically relevant effects. The results suggest that well-designed emollient formulations can ensure consistent safety and efficacy profiles, regardless of their richness, supporting their use in sensitive and atopic dermatitis skin.

592 [WITHDRAWN]

593 [WITHDRAWN]

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MELTUMP: A Diagnostic Trap with Major Clinical Impact

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Borderline melanocytic lesions, particularly MELTUMP (melanocytic tumor of uncertain malignant potential), remain a major diagnostic challenge due to their uncertain biological behavior and lack of standardized management. These difficulties are amplified in patients with complex oncologic history, where accurate diagnosis directly impacts staging and therapeutic decisions.

We report a 56-year-old male with a complex oncologic background, including dermatofibrosarcoma protuberans, currently under targeted therapy. During follow-up, the patient developed multiple melanocytic neoplasms: a melanoma in situ, an invasive melanoma (stage IIB), and a subsequent lesion initially diagnosed as MELTUMP. Given the clinical context, histopathological slides were reevaluated, leading to reclassification as a primary cutaneous melanoma (pT2a). This prompted appropriate reexcision and sentinel lymph node biopsy (SLNB). Although SLNB initially suggested subcapsular melanocytic proliferation suspicious for metastasis, immunohistochemistry confirmed a benign capsular nevus, establishing a final stage IB. This case highlights the diagnostic uncertainty surrounding MELTUMP and the importance of expert histopathological reassessment, particularly in patients with prior malignancies. Reclassification to melanoma significantly influenced surgical management and staging. In addition, distinguishing nodal nevi from melanoma metastases represents a critical diagnostic pitfall, with major implications for staging and the avoidance of unnecessary systemic therapy.

MELTUMP remains a challenging entity requiring careful clinicopathological correlation and, when necessary, expert second opinion. In patients with complex oncologic histories, including DFSP, accurate diagnosis and staging are essential to guide management and to prevent both under- and overtreatment.

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Evaluation of an Atopic Dermatitis emollient by high phototype subjects

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Atopic dermatitis (AD) in dark skin presents specific clinical characteristics and lacks tailored management recommendations. The objective of this study was to evaluate the efficacy and the user perception of emollient cream designed for AD care after 28 days of use in children with AD and adults with dry skin, all with phototypes IV to VI.

An open-label, monocentric clinical study was conducted in Brazil among 34 children (1–7 years old) with AD and 34 adults (19–64 years old) with dry skin. The emollient cream was applied twice daily for 28 days. Evaluation criteria included: SCORAD, POEM, clinical dryness scoring, a quality-of-life questionnaire (QoL), and a subjective perception questionnaire. After 28 days, a significant improvement of 50.7% in SCORAD was observed, as well as a 76.7% improvement in POEM recorded in 100% of children. A significant 43.6% reduction in skin dryness was measured in 98.5% of subjects (children and adults, $p<0.05$). A significant borderline ($p<0.1$) 11.9% improvement in QoL was also noted. Overall perception of the cream was very positive. However, discreet fragrance and slightly sticky texture were rated lower than in previous studies.

The tested emollient cream demonstrates significant clinical efficacy on atopic dermatitis and skin dryness in high-phototype subjects living in a tropical climate. Despite some sensory reservations that may influence reapplication, overall satisfaction and clinical performance confirm that this emollient is adapted to the specific needs of this population.

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Recessive dystrophic epidermolysis bullosa complicated by massive ascites, profound malnutrition, and growth failure

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Severe recessive dystrophic epidermolysis bullosa is a rare autosomal recessive genodermatosis caused by biallelic COL7A1 variants, leading to type VII collagen deficiency and defective anchoring fibrils. This causes generalized skin fragility, blistering, scarring, nail loss, pseudosyndactyly, mucosal involvement, growth failure, chronic anemia, nutritional compromise, and high lifetime risk of squamous cell carcinoma.

Case Presentation: A 7-year-old boy, fourth child of a consanguineous marriage, presented with generalized recurrent blistering and erosions since birth, triggered by minimal trauma, healing with scarring. He had painful oral ulcerations causing feeding difficulties and poor nutrition. Esophageal assessment was not done due to financial limits and pain, but upper GI involvement was suspected. An older brother died from similar disease. Examination showed severe wasting, growth failure, marasmus, widespread erosions, ulcerations, atrophic scarring, complete nail loss, pseudosyndactyly with mitten deformities, and ascites. Diagnosis was clinical with skin biopsy/immunofluorescence confirming dystrophic EB. Labs: severe anemia, hypoalbuminemia. Ultrasound confirmed ascites. Liver enzymes were twice normal (possibly due to herbal medications); renal function normal. Supportive care included wound cleansing, petrolatum gauze, analgesia, antibiotics, nutritional rehabilitation, and nasogastric feeding. Despite these measures, the child developed sepsis and died.

This case illustrates the mutilating phenotype of severe RDEB with severe extracutaneous involvement and fatal outcome. It highlights profound malnutrition, hypoalbuminemia, anemia, growth failure, and massive ascites as markers of advanced systemic disease—likely due to protein depletion, chronic inflammation, and cutaneous/GI protein loss. Early diagnosis, meticulous wound care, aggressive nutrition, infection prevention, and multidisciplinary care are critical; genetic counseling is essential for consanguineous families.

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Acute ichthyosiform eruption revealing HIV infection

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Human Immunodeficiency Virus (HIV) is associated with a broad spectrum of inflammatory and infectious dermatoses. Over 90% of individuals affected by HIV will experience a dermatologic condition.These conditions frequently exhibit exaggerated phenotypes resulting from systemic immune dysregulation. Consequently, clinicians must include HIV in the differential diagnosis for any rash displaying an atypical or recalcitrant clinical course.This report presents the case of a 64-year-old man who presented to the emergency department with a 6-day history of a generalized rash, following two weeks of fever and asthenia. His recent medications included intranasal corticosteroids for nasal congestion, which he had gradually discontinued over the preceding week.Physical examination revealed an ichthyosiform eruption characterized by lamellar plaques on the face and trunk, along with fine, erythematosquamous, polygonal papules on the limbs. The patient was initially discharged from the emergency department with topical corticosteroids and a referral to the dermatology clinic.Four days later, he was evaluated in our clinic, where partial improvement of the facial lesions was noted. Given the atypical nature of the eruption and the systemic prodrome, a detailed history was obtained. The patient's sexual history was significant for unprotected intercourse with male partners over the previous three years. Immediate comprehensive screening for sexually transmitted infections (STIs) revealed an acute HIV infection, with a viral load of 3.41 million copies/mL and a CD4 T-cell count of 0.2 x 10^9/L.The patient was diagnosed with acute HIV infection and promptly initiated antiretroviral therapy (ART) with bictegravir/emtricitabine/tenofovir alafenamide.This case highlights that an atypical, generalized eruption, especially when preceded by constitutional symptoms such as fever and asthenia, should raise clinical suspicion for HIV-related dermatoses. It further underscores the importance of a thorough sexual history in the evaluation of unexplained cutaneous manifestations.

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Intralesional normal saline mesotherapy as a potential therapeutic strategy for steroid-induced cutaneous atrophy: A repot of two cases

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Steroid-induced cutaneous atrophy is a common iatrogenic complication of corticosteroid use, presenting with dermal thinning, hypopigmentation, and visible depressions. Effective and accessible treatments remain limited, prompting interest in simple modalities such as intralesional normal saline.
Patient 1: A 35-year-old male presented with a depressed, hypopigmented atrophic plaque over the lateral aspect of the left foot following prolonged topical corticosteroid use for hypertrophic lichen planus. The lesion showed surface wrinkling and reduced dermal thickness. He was treated with intralesional normal saline (0.9% sodium chloride) at 6-week intervals for three sittings, administered as multiple small-volume injections until slight elevation was achieved. Progressive improvement was noted, with restoration of dermal volume, improved texture, and reduction in the depth of atrophy. Patient satisfaction was high.
Patient 2: A 40-year-old male developed a localized atrophic depression over the medial aspect of the right foot after repeated intralesional corticosteroid injections for keloid. The lesion was well-defined, hypopigmented, and thinned. The same treatment protocol was followed. Gradual improvement was observed over successive sittings, with visible reduction in depression and improved skin quality. No adverse effects were noted.
Both patients demonstrated consistent clinical improvement with excellent tolerability. Intralesional normal saline appears to be a simple, safe, and cost-effective treatment for steroid-induced cutaneous atrophy, warranting further validation in larger studies.

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DRESS Syndrome with Ichthyosiform Scaling Induced by Trimethoprim–Sulfamethoxazole: A Case Report

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We report a case of drug reaction with eosinophilia and systemic symptoms (DRESS) syndrome with an atypical cutaneous presentation. DRESS is a severe delayed hypersensitivity reaction characterized by extensive skin involvement, hematologic abnormalities, and internal organ dysfunction.
A 40-year-old woman developed fever and a progressive generalized erythematous eruption 14 days after initiation of trimethoprim–sulfamethoxazole. Cutaneous examination revealed a diffuse morbilliform eruption with prominent ichthyosiform scaling and facial edema, without mucosal involvement.
Laboratory investigations demonstrated eosinophilia, elevated liver transaminases, and lymphadenopathy, consistent with systemic involvement. Infectious etiologies were excluded. The RegiSCAR score was 6, consistent with definite DRESS.
The offending drug was discontinued, and systemic corticosteroid therapy was initiated (prednisone 1 mg/kg/day orally), followed by gradual tapering, resulting in resolution of fever and cutaneous lesions within one week and progressive normalization of laboratory abnormalities. No relapse was observed during follow-up.
This case highlights ichthyosiform scaling as a rare and underrecognized manifestation of DRESS and underscores the importance of early recognition, prompt drug withdrawaland timely corticosteroid therapy to reduce morbidity and potential mortality.

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Squamous cell carcinoma (SCC) in situ and Syringocystadenocarcinoma papilliferum (SCACP) Overlap in an Asian Patient- A case report and review of the literature

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Syringocystadenocarcinoma papilliferum (SCACP) is a rare malignant adnexal tumor arising from apocrine glands and is considered the malignant counterpart of syringocystadenoma papilliferum. It most commonly arises from malignant transformation of pre-existing lesions but may also occur de novo. Clinically, SCACP often presents as an exophytic or

ulcerated plaque and may mimic other cutaneous malignancies, including squamous cell carcinoma (SCC).
We report a rare case of SCC in situ with underlying SCACP in a 73-year-old Chinese male with systemic lupus erythematosus and a history of cutaneous and pulmonary SCC. He presented with a persistent non-healing ulcer over the left forearm for one year. Incisional biopsy demonstrated SCC in situ with irregular acanthosis and near full-thickness squamous dysplasia, showing strong p40 positivity. Complete excision revealed severe squamous dysplasia compatible with Bowen disease overlying dermal SCACP characterized by invaginated epithelial nests extending into the dermis with papillary and micropapillary structures composed of markedly atypical cells. Immunohistochemistry demonstrated divergent staining patterns between the epidermal and dermal components, supporting two distinct but contiguous neoplastic processes.
Coexistence of SCACP with SCC is exceedingly rare, with only four cases previously reported. The presence of SCC may obscure the underlying adnexal malignancy, posing a diagnostic challenge in superficial biopsies. Recognition of this association is important as SCACP carries a risk of local recurrence and metastasis distinct from SCC alone. Complete surgical excision with long-term surveillance is recommended.

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Scleromyxedema Mimicking Systemic Sclerosis without Mnoclonal Gammopathy: A Rare Association with Rheumatoid Arthritis
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Scleromyxedema is a rare cutaneous mucinosis characterized by generalized waxy papular and sclerodermoid skin changes, typically associated with monoclonal gammopathy. Its propensity to clinically mimic systemic sclerosis may result in diagnostic delay, particularly in atypical presentations.
A 65-year-old man with poorly controlled diabetes mellitus presented with a 3-month history of progressive symmetric polyarthritis, followed by diffuse skin thickening and tightening over 2 months. Cutaneous examination revealed generalized woody induration with multiple waxy papules involving the face, neck, trunk, and extremities, along with characteristic doughnut and Shar-Pei signs. Associated features included pruritus, dysphagia, and bilateral hand paresthesia.
Laboratory evaluation demonstrated elevated rheumatoid factor and anti-cyclic citrullinated peptide antibodies, supporting concomitant rheumatoid arthritis, while antinuclear and systemic sclerosis-specific antibodies were negative. Serum protein electrophoresis revealed no evidence of monoclonal gammopathy. Nerve conduction studies confirmed severe bilateral carpal tunnel syndrome. Initial histopathology suggested scleroderma; however, repeat biopsy demonstrated dermal mucin deposition with fibroblast proliferation and thickened collagen bundles, establishing the diagnosis of scleromyxedema.
This case highlights an atypical presentation of scleromyxedema without monoclonal gammopathy, closely mimicking systemic sclerosis and coexisting with rheumatoid arthritis. Histopathological evaluation remains pivotal for accurate diagnosis. Early recognition of such variants is essential to prevent misdiagnosis and ensure appropriate management.

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Efficacy of a skincare regimen containing the patented MELAGEA™ depigmenting complex in Fitzpatrick IV–V skin: a 42-day prospective study conducted in Sri Lanka
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Hyperpigmentation disorders, including melasma and post-inflammatory hyperpigmentation (PIH), are particularly prevalent, persistent, and difficult to treat in skin of color, especially in Fitzpatrick phototypes IV–V. This study evaluated the efficacy of a combined skincare regimen specifically in this population. The regimen included a cleansing foam (14709), a day cream SPF50+ (14711), and a night cream (14712). The leave-on products contained the patented MELAGEA™ complex (ellagic acid and glabridin), targeting key pathways involved in melanogenesis. A total of 22 participants (15 females, 7 males; mean age 39.5 years), all with Fitzpatrick IV–V skin, completed the study conducted in Sri Lanka. Assessments were performed at baseline, Day 21, and Day 42. The cohort included melasma (n=10), PIH scars (n=4), and other hyperpigmentation (n=6). Evaluations included melanin index, erythema, corneometry, sebumetry, and mMASI. A progressive and consistent reduction in pigmentation was observed in this Fitzpatrick IV–V cohort. Melanin index decreased from 609.8 to 529.0 (−13.2%) by Day 42. mMASI showed a marked reduction from 7.05 to 3.58 (−50%), indicating clinically meaningful improvement in melasma-prone skin of color. Erythema decreased slightly (425.2 to 401.4), while hydration improved (57.1 to 62.8), confirming good skin tolerance and barrier compatibility. Sebum increased (15.3 to 28.4) and requires further evaluation. In conclusion, the regimen demonstrated significant and clinically relevant efficacy in reducing hyperpigmentation in Fitzpatrick IV–V skin. The results support the targeted use of the patented MELAGEA™ complex in skin of color, particularly in populations with high susceptibility to pigmentary disorders.

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Skin and Dermatoses in German Painting: An Iconodiagnostic Study Based on the International Society of Iconodiagnosis (ISI) Database
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Dermatological iconodiagnosis, defined as the systematic study of skin representations in art, constitutes a complementary tool for the historical understanding, epidemiology, and semiological teaching of skin diseases. This approach enables the systematic analysis of cutaneous conditions, develops visual literacy in dermatologists, and facilitates international comparisons of skin representations in art.
At the ESDR 2026 conference in Heidelberg, particular attention was given to German painting. Using the International Society

of Iconodiagnosis (ISI) database, which records artworks with dermatologically relevant features published in the scientific literature, we present German artworks dated from the 15th to the 19th century that exhibit iconodiagnostic potential.
Among 131 analyzed works, 4 (≈2.9%) are of German origin: *The Temptation of St. Anthony* by Matthias Grünewald (halo nevus), the *St. Elizabeth Diptych* by Hans Holbein the Younger (scabies and trichophytic sycosis), and *Head of a Young Man* by Hans Holbein the Younger (signs compatible with syphilis).
Although not strictly iconodiagnosis, the diptych *Adam and Eve* by Lucas Cranach the Elder and the Younger (after 1537, Royal Museums of Fine Arts of Belgium, Brussels) illustrates with exceptional precision the differential characteristics of male and female skin, providing a true lesson in cutaneous biology: thick skin and pronounced muscular tone for Adam, smooth, clear, and translucent skin for Eve, with veins visibly showing through her delicate skin.
In conclusion, these paintings will also be presented at the ESDR 2026 exhibition in Heidelberg. This presentation will raise participants' awareness of the field of dermatological iconodiagnosis and collect knowledge about other artworks exhibiting iconodiagnostic relevance.

605 [WITHDRAWN]

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Reactive Infectious Mucocutaneous Eruption (RIME) to Mycoplasma pneumoniae
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Reactive infectious mucocutaneous eruption (RIME) is an increasingly recognized entity characterized by prominent mucositis with minimal or absent cutaneous involvement, most commonly triggered by respiratory pathogens such as *Mycoplasma pneumoniae*. It is frequently misdiagnosed as herpes simplex infection or erythema multiforme, leading to delays in appropriate management.
A 19-year-old female presented with acute-onset painful erosions and haemorrhagic crusting of the lower lip following a recent febrile respiratory illness. The lesions rapidly progressed to form thick yellow–brown crusts with significant mucosal involvement and minimal cutaneous findings. Based on initial clinical suspicion of herpes labialis, oral acyclovir was initiated; however, there was no clinical improvement. Further evaluation with serological testing revealed negative HSV-1 and HSV-2 IgM and IgG antibodies, while *Mycoplasma pneumoniae* IgM antibodies were positive, confirming the diagnosis of RIME.
The patient was treated with oral azithromycin (500 mg once daily for 5 days), along with a short course of systemic corticosteroids and supportive mucosal care. This resulted in rapid symptomatic relief, progressive re-epithelialization, and complete resolution within two weeks. No recurrence was observed on follow-up.
This case highlights the importance of considering RIME in young patients presenting with predominant mucositis following respiratory illness, particularly when there is poor response to antiviral therapy. Early recognition and targeted therapy can significantly improve outcomes and prevent unnecessary treatments. Clinicians should maintain a high index of suspicion for atypical infectious triggers, especially *Mycoplasma pneumoniae*, in similar clinical scenarios.

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Rethinking IgA Vasculitis: A Chronic Steroid-Refractory Adult Case
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IgA vasculitis (IgAV), formerly Henoch–Schönlein purpura, is a small vessel vasculitis affecting the skin, gastrointestinal tract, joints and kidneys. It typically presents with palpable purpura on the lower limbs. Management in adults is poorly defined due to limited randomised controlled trial data. Although often self-limiting, severe disease may require treatment with corticosteroids or immunosuppressants such as azathioprine and rituximab. Evidence for methotrexate remains conflicting. We report a rare case of chronic, relapsing, steroid-refractory adult IgAV successfully managed with methotrexate and hydroxychloroquine.
A 46-year-old female with a 20-year history of IgAV presented with recurrent painful purpuric eruptions predominantly affecting the lower limbs. Triggers included prolonged standing, high heels and alcohol. Renal involvement remained stable; a 2015 renal biopsy showed IgA nephropathy without chronic damage. A 2018 skin biopsy confirmed leukocytoclastic vasculitis. Despite its typically self-limiting course, this patient had persistent relapsing disease with significant impact on quality of life. Hydroxychloroquine (200 mg daily) initiated in 2019 improved symptoms, with disease stability from 2020–2022. However, a flare following COVID-19 infection in late 2023 showed only partial response to corticosteroids.
Methotrexate was commenced in 2024 (7.5 mg weekly, escalated to 15 mg), resulting in improved control with only mild flares in 2025. By early 2026, the patient remained flare-free on methotrexate (15 mg weekly) and hydroxychloroquine (400 mg daily), with marked improvement in quality of life.
This case highlights a rare chronic, relapsing adult IgAV phenotype. While most cases resolve within months, persistent disease in adults is poorly understood. Steroid-refractory disease underscores limitations of corticosteroids in long-term control. Sustained response to methotrexate suggests a potential role for combination immunomodulatory therapy in refractory IgAV.

608 [WITHDRAWN]

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Early-Onset Calcinosis in Amyopathic Juvenile Dermatomyositis: Evidence for Cutaneous-Driven Dystrophic Mineralization
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Juvenile dermatomyositis (JDM) is an immune-mediated inflammatory disorder characterized by cutaneous and muscular involvement with prominent interferon-associated pathology. Amyopathic juvenile dermatomyositis (AJDM), defined by classic cutaneous features without clinical or laboratory evidence of myopathy, represents a rare phenotype. The mechanisms underlying early calcinosis in cutaneous-restricted disease remain incompletely understood.
A 7-year-old girl presented with a one-year history of progressive erythematous rash and eight months of painful subcutaneous

nodules over both lower limbs. Examination revealed heliotrope rash with periorbital edema, Gottron's papules and sign over metacarpophalangeal, elbow, and knee joints, shawl sign, photosensitivity, and multiple firm nodules consistent with calcinosis cutis. There was no proximal muscle weakness or systemic involvement; Gower's sign was negative. Serum muscle enzymes, nerve conduction studies, and electromyography were normal. Histopathology demonstrated interface dermatitis with basal vacuolar alteration and perivascular lymphocytic infiltrates. Radiography showed clustered subcutaneous calcifications. A diagnosis of amyopathic juvenile dermatomyositis with early calcinosis cutis was established.
Treatment with oral prednisolone (1 mg/kg/day) and weekly methotrexate (15 mg) resulted in marked clinical improvement within one month.
This case highlights a cutaneous-restricted inflammatory phenotype of JDM in which early dystrophic calcification developed independently of muscle involvement. These findings suggest that persistent cutaneous immune activation alone may be sufficient to drive ectopic mineral deposition, thereby expanding the pathogenic spectrum of JDM and underscoring the importance of early immunomodulatory therapy.

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Dermoscopic Features of Circinate Balanitis in Reactive Arthritis
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Circinate balanitis is a reactive inflammatory dermatosis of the glans penis, classically linked to reactive arthritis but also occurring independently. It presents as annular or polycyclic erythematous plaques with serpiginous margins and peripheral scaling. Dermoscopic features are not well described but may aid diagnosis and differentiation from psoriasis, erythroplasia of Queyrat, and fixed drug eruption.
A 28-year-old sexually active male presented with a 2-month history of asymptomatic penile lesions. There was no history of drug intake, genital ulceration, urethral discharge, or systemic features of reactive arthritis. Examination showed multiple well-defined annular and polycyclic erythematous plaques over the glans penis with serpiginous white-grey peripheral scaling and central clearing. Polarised dermoscopy revealed clustered dotted and glomerular vessels on an erythematous background, peripheral white-grey scaling in annular patterns, diffuse superficial erosions with a moist surface, focal haemorrhagic crusting, and central white structureless areas.
Differentials included genital psoriasis, erythroplasia of Queyrat, and fixed drug eruption. Based on typical clinical and dermoscopic findings, circinate balanitis was diagnosed; biopsy was deferred. The patient was treated with topical corticosteroids and hygiene measures, resulting in marked improvement.
This case highlights reproducible dermoscopic features of circinate balanitis—clustered dotted and glomerular vessels, serpiginous scaling, and superficial erosions—which aid diagnosis, reflect disease activity, and help distinguish it from mimickers, potentially avoiding invasive investigations.

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Clinical Suspicion Prevails Diagnostic Dilemma: A Case Report of Henoch Schönlein Purpura with Initially Absent Immunoglobulin A Deposition
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Henoch Schönlein Purpura (HSP) is a small vessel vasculitis. According to EULAR criteria, palpable purpura is the mandatory criterion, accompanied by at least one of the followings: abdominal pain, arthritis or arthralgia, renal involvement or histopathological evidence of leukocytoclastic vasculitis with IgA deposition on direct immunofluorescence(DIF). We present a 20-year-old woman with non-blanching palpable purpura on all extremities who had a six-month history of recurrent rash, episodic abdominal pain and arthralgia suggestive of HSP. Histopathology revealed fibrinoid necrosis and perivascular leukocyte infiltration consistent with vasculitis. However, DIF showed no IgA deposition. No proteinuria was detected initially. She was treated with methylprednisolone at a dose of 40 mg/day. Following discharge with a tapering schedule after regression of lesions, she presented several times with flare ups. A repeat DIF again revealed no IgA deposition. During flare-ups and treatment, she was refractory to methylprednisolone, colchicine, dapson however responded well to mycophenolate mofetil. On the fourth month of follow-up, upon proteinuria detection she was evaluated by nephrology. DIF was repeated for the third time and finally showed IgA deposition. IgA deposition on DIF is highly supportive for HSP, although it is not mandatory. Previous studies showed high sensitivity of DIF, also depending on lesion age, biopsy site,timing of sampling. In our case, biopsy was performed promptly from newly developed lesions; nevertheless, the first two DIF were negative, suggesting IgA deposition may vary between lesions or fluctuate during the disease course. Negative DIF results may therefore be misleading despite strong clinical clues for HSP. Since HSP may follow a relapsing course and involve systemic organs, particularly the kidneys, distinguishing it from other cutaneous vasculitides is essential for appropriate follow-up and management. To improve diagnostic accuracy, repeat DIF should be considered in persistent clinical suspicion, especially in the presence of recurrent lesions or emerging systemic involvement.

612 [WITHDRAWN]

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A case with sweat like-transudate leakage from the external auditory canal
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A 51-year-old woman had been experiencing transudate leakage from her external ear for the past two years. In a provocation test, leakage was observed from both external ear when she swallowed juice and chewed bread. Using the Minor test (iodine-starch reaction), 50 µl each of norepinephrine (NAd) and acetylcholine (Ach) were injected intradermally into each external ear. As a result, dot-shape black discoloration (approximately 10 µl total) was observed in the skin of the external ear with both NAd and Ach. The same tests on the two healthy individuals showed negative. Skin biopsies from the external ear and abdomen

revealed apocrine sweat glands (ASG) in the external ear, but no eccrine sweat glands (ESG) or signs of inflammation were found, and no antibody or complement deposition was observed on DIF. Only ESG was found in the biopsy from the abdomen. In this case, leakage was observed with intradermal NAd injection, suggesting proliferation of ASG, which are normally innervated by NAd. No ESGs were found in the tissue, suggesting ESG-induced sweating was unlikely. It is also possible that abnormal sweating occurred from the ASG due to Ach stimulation caused by abnormal cross-innervation, like Frey syndrome. Treatment with both oral Propantheline Bromide and topical Oxybutynin Hydrochloride reduced the amount of leakage. Amylase was not detected in the exudate, and possibility of saliva was ruled out. Dermcidin was not detected by ELISA. Normally, Dermcidin is present in sweat from ESGs but not in sweat from ASGs. In this case, ASGs possibly proliferated more than in healthy individuals, and there was possibly excessive sweating to the external ear in response to taste stimulation (usually only earwax-like). Furthermore, it was suggested that this gustatory abnormal sweating may be caused by ASG innervated by both the sympathetic and parasympathetic nervous systems, like Frey syndrome.

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Complex Management of a Generalized Pustular Psoriasis Exacerbation Triggered by Corticosteroid Withdrawal in an Oncologic Patient with Multiple Comorbidities

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We present the case of a 58-year-old female with a known history of plaque and pustular psoriasis, admitted for generalized pustular psoriasis triggered by systemic corticosteroid withdrawal. Her concurrent active cervical cancer significantly restricted her dermatological treatment options. The patient is a long-term heavy smoker (40 pack-year history) with a significant medical background, including Grade III hypertension and Stage IV (GOLD) chronic obstructive pulmonary disease. Pulmonary function tests revealed a severe obstructive pattern, with a FEV1 of 24% of predicted, complicated by chronic respiratory failure necessitating long-term oxygen therapy via a Portable Oxygen Concentrator (POC). Additionally, the patient has a history of cervical cancer treated with radiotherapy. Regarding her psoriasis, initial treatment with tildrakizumab and methotrexate was discontinued following the oncological diagnosis. Upon admission the patient presented with an eruption that consisted in a generalized pustular and erythematous-squamous eruption. The flare occurred upon tapering and discontinuation of methylprednisolone (Medrol), which had been administered for a recent respiratory exacerbation. Clinical evaluation revealed a GPPGA score of 3.33. Laboratory investigations showed marked systemic inflammation (elevated CRP and ESR) alongside normochromic normocytic anemia. Following oncological clearance, the dermatological management focused on continuing weekly methotrexate (15 mg), supplemented with folic acid 48 hours post-administration, alongside topical therapy. The clinical course was highly favorable; the pustules completely resolved, and the erythematous-squamous lesions significantly improved without compromising her complex cardiopulmonary or oncological status. Systemic corticosteroids used for concurrent acute illnesses can trigger severe rebound flares of pustular psoriasis upon withdrawal. This case demonstrates the crucial importance of multidisciplinary management in a critical patient with complex multiorgan pathology and concurrent therapies.

LATE-BREAKING ABSTRACTS

LB001

Migratory dendritic cell–activated Treg niches define severity-linked inflammation in atopic dermatitis

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Regulatory T cells (Tregs) maintain immune tolerance, but chronic inflammation persists in atopic dermatitis (AD) skin lesions where Tregs accumulate. This highlights a key uncertainty: whether lesional Tregs remain suppressive, become dysfunctional, or enter inflammatory tissue circuits.
We integrated single-cell RNA-seq from healthy, non-lesional and lesional AD skin, and utilized psoriasis as an inflammatory comparator, to map Treg remodeling in AD-skin immunity. We then tested whether the inferred Treg-linked circuit was spatially organized, reflected by secreted mediators, and related to severity and therapy response using spatial transcriptomics, blister-fluid proteomics and bulk lesional transcriptomes.
Alongside with AD severity a coordinated remodeling of migratory dendritic cells (MigDCs), Tregs and IL22/IL13 effector T cells was evident, indicating a connected lesional axis. Candidate MigDC-derived TNF-family and IL15/STAT5 signals were linked to activation-associated Treg target genes. Treg-state analysis identified an activated effector state that retained Treg hallmarks, yet expressing TNFR/non-canonical NF-kappaB and inflammatory activation programs. This state was enriched in AD compared with psoriasis and increased from healthy to lesional AD skin. Spatial profiling placed activated Treg, MigDC and IL22/IL13 effector T cell programs in shared lesional niches, and Xenium technique showed closer proximity between activated Tregs and MigDCs. The same axis was reduced after dupilumab treatment, with stronger reduction associated with better clinical improvement.
These data suggest that persistent AD inflammation is not explained by a lack of Tregs, but by a disease-linked Treg state embedded within MigDC-Th2/Th22 inflammatory niches. The MigDC-activated Treg-Th2/Th22 niche connects immune regulation, tissue organization, severity and treatment response, defining a clinically relevant module of chronic AD.

LB002

Fibrotic matrix mechanics amplifies TGF-β-driven fibroblast responses in a patient-informed in vitro 3D skin model

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Skin fibrosis is characterized by excessive extracellular matrix (ECM) deposition, persistent fibroblast activation, and progressive tissue stiffening. While transforming growth factor-β (TGF-β) is a central driver of fibrosis, how pathological matrix stiffness modulates fibroblast responses to profibrotic biochemical signalling remains poorly understood.
To address this, we developed mechanically tuneable 3D hydrogels that reproduce the stiffness of healthy and fibrotic human dermis. RGD-functionalized pectin hydrogels were engineered using ionic and Diels–Alder crosslinking to generate healthy-like

(G′ ≈ 1.9 ± 0.2 kPa) and fibrotic-like (G′ ≈ 5.0 ± 0.7 kPa) matrices. Primary human dermal fibroblasts were encapsulated (10 × 10^6 cells/mL) and cultured for 7 days under basal conditions or TGF-β1 stimulation (10 ng/mL). Proteomic profiling was performed on hydrogel-recovered proteins.
Proteomic analysis showed that TGF-β1 stimulation induced a shared profibrotic ECM-associated signature across healthy- and fibrotic-like matrices. In the absence of TGF-β1, matrix stiffness alone elicited only modest proteomic changes. However, under profibrotic stimulation, fibrotic-like matrices promoted a distinct proteomic signature enriched in metabolic adaptation, proteostasis and cellular stress pathways, indicating that pathological matrix stiffness modulates fibroblast responses beyond ECM remodelling.
These findings identify pathological matrix stiffness as a key modulator of TGF-β-driven fibroblast responses, highlighting the importance of incorporating disease-relevant tissue mechanics into human in vitro models of skin fibrosis.

LB003
Spatial transcriptomics of fixed drug eruption reveals an IFN-γ–driven keratinocyte–fibroblast–T cell circuit
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Fixed drug eruption (FDE) is a cutaneous adverse drug reaction characterized by well-demarcated lesions that reappear at the exact same sites after re-exposure to the culprit drug. Here, we used the Xenium in situ assay on FFPE samples of two recurring FDE lesions and healthy control skin to characterize immune cell subsets and spatial patterns of gene expression in FDE.
Compared to healthy skin, FDE lesions show compositional remodeling dominated by infiltrating immune cells (T cells, macrophages, monocytes, DCs and neutrophils) and activated fibroblasts. Specifically, effector CD8+ T cells, Th1/effector memory T cells, inflammatory monocytes, and MHC II+ macrophages are enriched in FDE, most prominently in the papillary dermis. Exhausted T cells are also increased in FDE within epidermis and papillary dermis. Spatial profiling showed cytotoxic effector molecules such as granzymes, perforin, and IFN-γ expressed by epidermal exhausted T cells and dermal CD8+ effector T cells. Comparing FDE and healthy skin, differentiated keratinocytes had the largest transcriptional shift, marked by an interferon-response signature and upregulation of *CXCL9* and *CXCL11*. Activated fibroblasts also upregulated *CXCL9*.
Our results suggest that MHC II+ macrophages present drug/hapten to resident T cells. Enrichment of exhausted T cells indicates repeated stimulation, and increased effector CD8+ and Th1/effector memory T cells aligns with the CD8-dominant nature of FDE, mediating keratinocyte injury. FDE lesions show IFN-γ–dominated keratinocyte activation with chemokine output by keratinocytes and fibroblasts. Thus, keratinocytes and fibroblasts might not only respond to damage but help sustain local T-cell recruitment. Influx of inflammatory monocytes and neutrophils indicates recruitment of innate cells, escalating tissue damage on top of a feed-forward cycle in which macrophages activate drug-specific cytotoxic T cells, leading to keratinocyte injury, chemokine release and further influx of T cells. These novel spatial insights identify a keratinocyte–fibroblast–T cell circuit with an IFN-γ–driven chemokine axis and previously unknown role of MHC II+ macrophages in FDE.

LB004
IL-34 regulates the expression of epidermal barrier proteins in terminally differentiated keratinocytes, a process disrupted by inflammatory signals in psoriasis and atopic dermatitis
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Interleukin (IL)-34 is a keratinocyte-derived cytokine with emerging roles in skin immunity. However, its regulation and contribution to epidermal barrier homeostasis remain poorly understood. Here, we characterize IL-34 expression, regulation, and function in terminally differentiated keratinocytes and in psoriasis and atopic dermatitis (AD), two skin diseases characterized by an altered epidermal barrier. We found that IL-34 expression increases *in vitro* in keratinocytes undergoing terminal differentiation and is negatively regulated by IL-22, IL-17, and IL-4/IL-13 cytokines. *In vivo*, immunostaining and transcriptomic analyses revealed that IL-34 is predominantly expressed in the upper epidermal layers of healthy skin, whereas its distribution is markedly altered in lesions of psoriasis and AD. Mechanistically, stimulation of 2D and 3D epidermal models with inflammatory cytokines, such as IL-22, or with psoriasis- and AD-associated T-cell supernatants decreased IL-34 expression, linking its reduction to impaired keratinocyte differentiation. Functionally, exogenous stimulation with IL-34 in terminally differentiated keratinocytes enhanced the expression of the tight junction protein claudin-4, whereas no evident effect was observed on occludin or ZO-1. Our data suggests that IL-34 may support epidermal barrier formation and that its disruption by inflammatory signals can contribute to the barrier defects commonly observed in psoriasis and AD.

LB005
Shared autoreactive T-cell responses across inflammatory skin diseases revealed by integrated humoral and cellular profiling
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Inflammatory skin diseases, including Pso, AD and LP are driven by dysregulated immune responses shaped by genetic and environmental factors. While these diseases are characterized by distinct immune polarization patterns, evidence suggests that autoreactive mechanisms contribute to their pathogenesis. T-cell responses against skin-derived autoantigens have been implicated across ISD, but their distribution and disease specificity remain unclear.
To systematically investigate autoreactivity, we analyzed humoral and cellular responses to a panel of skin autoantigens in patients with Pso (n=15), AD (n=9), LP (n=10), and healthy donors (n=8). Circulating autoantibodies were assessed by ELISA, while antigen-specific T-cell responses were evaluated by CFSE-based proliferation assays. Spatial transcriptomic data were examined to assess associations between autoantigen expression and disease-specific cytokine environments.
Humoral autoreactivity against classical structural skin antigens was largely absent in all groups. In contrast, antigen-specific T-cell proliferation was observed across all diseases and in healthy donors. The most frequent responses targeted KRT17 and ADAMTSL5, followed by BP180 and DSG3, with overlapping reactivity patterns across diseases. Patients in Pso and LP frequently exhibited responses to multiple autoantigens, whereas AD patients and healthy donors showed fewer responses. Neither the number of recognized autoantigens nor proliferation rates correlated with disease duration or clinical severity.
Spatial transcriptomic analyses revealed that candidate autoantigens are mainly expressed in epidermal compartments and their expression correlates with disease-specific cytokine signatures, suggesting that inflammatory microenvironments may enhance antigen availability and promote T-cell activation.
In conclusion, inflammatory skin diseases are not characterized by disease-specific humoral autoreactivity but rather by shared and overlapping T-cell responses against multiple skin autoantigens. These findings support a model of antigen spreading driven by chronic inflammation rather than distinct disease-defining autoantigens.

LB006
Epidermal organoids reveal inflammatory memory and disease-specific keratinocyte states
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Chronic inflammatory skin diseases such as psoriasis (Pso) and hidradenitis suppurativa (HS) impose a major disease burden despite current therapies. In Pso, lesions typically recur at prior sites after treatment withdrawal, pointing to local tissue "memory" attributed to tissue-resident T cells; yet immune-targeting therapies often fail to prevent relapse. Emerging evidence shows epithelial stem cells can also retain memory of prior inflammation, maintaining stress-responsive loci in a chromatin-accessible "poised" state. Whether such epithelial memory contributes to disease persistence in human skin, including in less-characterized diseases like HS, remains unclear.
We used a 3D primary human epidermal organoid (EpO) system to probe epithelial-intrinsic inflammatory memory and disease-associated keratinocyte alterations. Healthy-donor EpO exposed to IL-17A, a major driver of Pso, upregulated inflammatory chemokines and antimicrobial peptides. We next tested if organoids could be trained by inflammation to maintain higher reactivity to secondary triggers that lead to PsO plaque resurgence. we showed that inflammation-trained EpO had heightened CCL20/IL-8 responses upon IL-17A rechallenge. ATAC-seq revealed that a small but significant cohort of inflammatory and stress responsive loci-maintained accessibility. To the best of our knowledge, this is the first demonstration of inflammatory memory in human epithelial cells in culture.
Extending this platform to HS, patient-derived EpO showed differences in baseline keratinocyte differentiation and inflammatory response signatures compared with healthy controls. Ongoing work is expanding this cohort and investigating EpO responses to HS-relevant environmental cues, including bacterial- and microbiota-derived factors.

LB007
REVIVE - REstoration of oxidative balance and pigmentation in VItiligo skin by melanin precursors and nanoVEctors
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Vitiligo is a chronic autoimmune skin disorder characterized by melanocyte loss, in which oxidative stress contributes to disease onset and progression. This study evaluated the potential of phospholipid nanoformulations encapsulating the methyl ester of 5,6-dihydroxyindole-2-carboxylic acid (MeDHICA), a physiological melanin precursor, and DHICA-derived melanin to restore redox homeostasis, modulate inflammatory responses, and modulate melanogenesis in experimental models of vitiligo. Healthy (PIG1) and vitiligo-derived (PIG3) human melanocytes exposed to oxidative stress were treated with liposomal MeDHICA monomer or liposomal DHICA-derived melanin. Cell viability assessed by the Cell Counting Kit-8 assay confirmed good cytocompatibility of all formulations. Intracellular ROS, measured by DCFDA assay, were significantly reduced in PIG3 melanocytes following treatment with liposomal DHICA-derived melanin, which produced a greater reduction in ROS than both free DHICA-derived melanin and MeDHICA monomer formulations. This effect was associated with increased total superoxide dismutase (TSOD) activity, and a trend toward higher glutathione (GSH) levels, indicating enhanced antioxidant defenses. ELISA analysis of culture supernatants demonstrated reduced IL-6 and IL-8 secretion following treatment with liposomal DHICA-derived melanin. Western blot analysis revealed modulation of melanogenesis-related pathways, including increased tyrosinase expression, particularly in PIG1 melanocytes. Finally, the TBARS assay performed on *ex vivo* porcine skin demonstrated a significant reduction in malondialdehyde (MDA) levels, indicating protection against oxidative lipid peroxidation. Moreover, safety of the study products has been confirmed through patch test on 22 healthy volontiers. Collectively, these findings support the dual antioxidant and melanogenesis-modulating effect of liposomal DHICA-derived melanin. Together with its favorable safety profile, this nanoformulation may represent a promising therapeutic strategy for vitiligo.

LB008 [WITHDRAWN]

LB009

Neutrophils Reduction During Sensitization Drives a Cathepsin G-Dependent Shift Toward Th1 Immunity

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In the 2,4,6-trinitrochlorobenzene (TNCB)-mediated contact hypersensitivity (CHS) model, neutrophils are traditionally considered indispensable, as their total depletion before sensitization or challenge typically reduces the inflammatory response. However, the immunological consequences of partial neutrophil presence remain largely unexplored.
We utilized a mouse model of TNCB-mediated CHS to investigate the effects of partial neutrophil depletion (maintaining ~25% of baseline blood counts). Mice were partially depleted before sensitization, before challenge, or before both phases. We characterized the resulting inflammatory response, analyzed Th-cell subpopulations, and assessed antigen-presenting cell (APC) migration and maturation in draining lymph nodes (dLNs) via flow cytometry. The role of the neutrophil-derived protease Cathepsin G (CatG) was evaluated using topical inhibitors during the sensitization phase.
While partial depletion before the challenge phase inhibited the inflammatory response, partial depletion before sensitization paradoxically resulted in challenge effect compared to non-depleted control. Partial depletion before both phases reversed the inhibitory effect seen in the challenge-only group, indicating a bias toward a neutrophil-independent sensitization. This shift was driven by a Th1-response skew, evidenced by increased CD4+ Th1 cells and Ly6C-high monocytes. In the dLNs, partial depletion significantly increased cDC1 populations and decreased monocyte-derived DCs (Mo-DCs). Topical inhibition of CatG prior to sensitization successfully reproduced this APC skewing.
Our findings demonstrate that partial neutrophil depletion during sensitization promotes a less neutrophil-dependent immune memory. This transition is mediated by a reduction in CatG, which alters APC maturation and increases cDC1-mediated IL-12 production, ultimately favoring Th1-skewed CD4+ cell priming. Altogether, these results show that topical application of CatG modulators can effectively skew Th priming, offering a promising pharmacological target for the development of novel therapies in inflammatory skin diseases.

LB010

Chlormethine-induced changes in tumor microenvironment and skin barrier in early-stage mycosis fungoides

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Topical chlormethine is an established skin-directed therapy for early-stage mycosis fungoides (MF). However, clinical responses are heterogeneous and the mechanisms linking tumor clearance, inflammation, and barrier restoration remain incompletely defined. We investigated how response to chlormethine is shaped by coordinated remodeling of the tumor microenvironment (TME) and stratum corneum barrier lipid architecture.
In a prospective exploratory study, 21 patients with early-stage MF and 10 matched healthy controls were assessed. Patients applied chlormethine gel for 16 weeks, with paired lesional and non-lesional sampling at baseline and repeat lesional sampling after treatment. Immune profiling integrated suction blister fluid proteomics, flow cytometry, imaging mass cytometry, and TCRβ sequencing. Barrier function was assessed by transepidermal water loss, and stratum corneum ceramides were quantified by tape stripping and LC-MS.
At baseline, MF lesions exhibited barrier dysfunction, distinct ceramide remodeling, and a type-1-skewed inflammatory milieu. Chlormethine induced heterogeneous clinical responses. Responders with a mCAILS reduction >50% showed a pre-treatment immune-reactive contexture enriched for i) activated cytotoxic T cells, ii) treatment-associated immune activation, iii) dominant T-cell clonal contraction, and iv) reduction of aberrant and regulatory T-cell populations. In parallel, clinical responders demonstrated partial normalization of barrier function and stratum corneum ceramide composition, including reduced Cer[NS]:Cer[NP] ratio and increased ceramide chain length.
These findings identify chlormethine response in early-stage MF as a coordinated process involving direct tumor cytotoxicity, immune-mediated TME remodeling, and restoration of barrier lipid homeostasis. Baseline cytotoxic immune contexture and dynamic treatment-induced immune activation may stratify patients likely to respond, while persistent tumor-tolerant architecture may define resistance. Together, these data position the MF lesion as an integrated immune-barrier niche and provide a rationale for response-guided, immune-informed therapy.

LB011

Increased free cholesterol and cholesterol synthesis proteins in psoriatic epidermis

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Altered epidermal cholesterol metabolism has emerged as a potential contributor to psoriasis pathogenesis, but the spatial distribution of the cholesterol synthesis remains incompletely characterized. We investigated the localization of HMG-CoA reductase (HMGCR), squalene monooxygenase (SQLE), and free cholesterol in psoriasis. Paired lesional and non-lesional skin biopsies were obtained from eight patients with psoriasis vulgaris, matched with healthy controls. Immunohistochemistry for HMGCR and SQLE was quantified by digital whole-slide image analysis, while free cholesterol was visualized by filipin and quantified using standardized image analysis. HMGCR and SQLE localized predominantly to the upper nucleated epidermal layers and were expressed at significantly higher levels in lesional psoriatic skin than in non-lesional and healthy skin. Filipin staining demonstrated increased free cholesterol in lesional epidermis, with a distinct localization pattern in the upper epidermis compared with non-lesional and healthy skin. These findings provide spatial protein-level evidence that cholesterol synthesis and free cholesterol is upregulated in psoriatic epidermis, supporting dysregulated epidermal cholesterol metabolism as a feature of psoriasis and highlighting cholesterol synthesis as a potential target for topical therapeutic intervention.

LB012 [WITHDRAWN]

LB013
Differentially Expressed Circular RNAs in Psoriatic Skin
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Circular RNAs (circRNAs) are covalently closed, single-stranded non-coding RNAs that have emerged as important regulators of gene expression. Although circRNAs have been implicated in diverse biological processes, diseases, and treatment responses, their contribution to psoriasis pathobiology remains incompletely understood. To characterize circRNA expression in psoriasis, we performed RNA sequencing of lesional (PP; n = 71) and non-lesional (PN; n = 73) skin biopsies from individuals with psoriasis and skin biopsies from non-psoriatic controls (NN; n = 46). Sequencing reads were aligned to the human reference genome (GRCh38) using STAR, and circRNAs were identified using CIRCexplorer2 and DCC. circRNA abundance was quantified using two complementary metrics: back-splice junction reads per million mapped reads (BSJperMMR) and the proportion of back-splice junction reads relative to the total number of circular and linear splice junction reads (Fraction). Expression values were normalized using a rank-based inverse normal transformation, and differential expression analyses were conducted across PP, PN, and NN samples, adjusting for age, sex, and BMI. We identified 15 differentially expressed (DE) circRNAs ($|\log_2 FC| > 0.58$, FDR < 0.05) based on at least one expression metric. Six DE circRNAs were identified in the PP versus NN comparison, and 13 were identified in the PP versus PN comparison, whereas no statistically significant differences were observed between PN and NN samples. Based on FDR-adjusted p-values, circRNAs derived from *EPHX2* and *LDLR* exhibited the largest expression differences in both the PP versus NN and PP versus PN comparisons. *SBNO2* and *NEIL3* displayed the third largest differences in the PP versus NN and PP versus PN comparisons, respectively. These circRNAs represent promising candidates for future functional studies to elucidate disease mechanisms and evaluate their potential as biomarkers or therapeutic targets for psoriasis.

LB014
Sex hormones modulate epidermal differentiation in healthy skin and hidradenitis suppurativa
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Skin physiology and inflammatory skin diseases differ between females and males, but the mechanisms underlying these differences remain poorly understood. Sex hormones contribute to this dimorphism, yet their direct effects on human epidermal physiology and physiopathology are still largely unknown. This question is particularly relevant in hidradenitis suppurativa (HS), a chronic inflammatory skin disease that predominantly affects women and is altered during pregnancy. We therefore investigated how sex hormones regulate healthy and HS epidermal proliferation and differentiation. To do this, we used clonogenic assays and epidermal organoids (EpiO) from healthy donor and HS patient keratinocytes exposed to dihydrotestosterone (DHT), oestradiol (E2), progesterone (P4), or combined E2/P4 (pregnancy condition) to mimic distinct hormonal exposure. In healthy keratinocytes, P4 promotes expansion of colony-forming keratinocytes and DHT & P4 independently enlarged epidermal organoids without increasing Ki67 proliferating cells, indicating enhanced tissue expansion independent of proliferation. Instead, these hormones primarily modulate epithelial differentiation, reducing suprabasal DSG1 and cornified loricrin layers. In HS keratinocytes, sex hormones (E2, P4, E2/P4 & DHT) showed no effects on organoid growth but exhibited pronounced remodelling of epidermal differentiation, including reduced DSG1 organization. Notably, epidermal stress and activation markers, K17 was higher under P4 and DHT. S100A9 and K16 showed an increasing trend under E2 and P4. Together, these findings identify epidermal differentiation and activation, rather than proliferation, as the principal targets of sex hormone signalling in keratinocytes and reveal an altered hormonal responsiveness in HS epidermis.

LB015
Anti-IL1RAP treatment shifts hidradenitis suppurativa transcriptomes towards a non-lesional state in moderate-to-severe Patients
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Hidradenitis suppurativa (HS) is a chronic inflammatory skin disease characterized by immune dysregulation, tissue remodeling, and epidermal dysfunction. IL-1 and IL-36 signaling pathways are implicated in HS pathogenesis. LAD191 is an anti-IL-1RAP monoclonal antibody that inhibits IL-1RAP-dependent signaling of IL-1, IL-33, and IL-36 cytokines.
Five moderate-to-severe HS patients were enrolled in Part 3 of Phase I first-in-human study (NCT06488209) and received six weeks of treatment with LAD191 (n=3) or placebo (n=2). Paired lesional (LS) and non-lesional (NL) skin biopsies were obtained at baseline and after treatment and profiled by bulk RNA sequencing to characterize molecular changes associated with treatment.
At baseline, HS lesions exhibited inflammatory activation and epidermal dysfunction, with enrichment of cytokine signaling, immune recruitment, and tissue remodeling pathways. Following treatment with LAD191, broad transcriptomic remodeling was observed, characterized by downregulation of inflammatory pathways, including cytokine signaling, neutrophil chemotaxis, myeloid cell activation, and responses to bacterial stimuli, alongside upregulation of epidermal differentiation, keratinization, skin barrier development, and lipid metabolic pathways, consistent with restoration of epidermal homeostasis. Exploratory xCell analyses further suggested remodeling of the skin microenvironment, with reduced myeloid (macrophage & neutrophil) and stromal (fibroblast & endothelial) signatures.
IL-1RAP blockade with LAD191 in HS appears to be associated with attenuation of inflammatory pathways, enhancement of

epidermal differentiation and barrier-associated pathways, and transcriptomic shifts toward a non-lesional molecular phenotype. Although limited by the small sample size of this Phase I biomarker study, these findings support further investigation of LAD191 in larger studies with extended treatment duration.

LB016

Single-cell Multi-omics Analysis Reveals Transcriptional and Epigenetic Landscape in Irradiated Human Skin

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Adjuvant radiotherapy is essential for breast cancer management, but it frequently causes persistent skin complications that can last for years after treatment. However, the mechanisms that maintain skin cells in this dysfunctional state remain poorly understood. We performed single-cell multiome sequencing, including paired scRNA-seq and scATAC-seq, on three pairs of skin biopsies from breast cancer patients, comparing irradiated chest skin (RT+) with matched non-irradiated skin (RT−). Two additional paired samples were profiled using single-cell Nano-CUT&Tag for H3K27ac and H3K27me3. At the cellular composition level, RT+ skin showed an overall reduction in epidermal cells, with a marked loss of spinous keratinocytes, particularly the spinous-III subcluster. In the dermis, endothelial populations were remodeled after radiotherapy, with relative increases in both lymphatic and vascular endothelial cells. Keratinocytes exhibited the most profound radiation-associated molecular alterations. GSEA revealed significant enrichment of TNFα/NF-κB signaling and epithelial–mesenchymal transition (EMT) programs in RT+ keratinocytes. Motif enrichment analysis of RT+-accessible chromatin regions further revealed strong activation of AP-1 family transcription factors, including FOS, JUN, and JUNB, indicating an AP-1-dominated inflammatory regulatory program in irradiated epidermis. CellChat analysis revealed enhanced collagen-mediated signaling between keratinocytes and fibroblasts, suggesting a self-reinforcing fibrogenic loop. By integrating transcriptomic, chromatin accessibility, and histone modification profiles at single-cell resolution, our study demonstrates that radiotherapy establishes durable epigenetic memory in keratinocytes driven by NF-κB, AP-1, and EMT programs. These findings identify potential regulatory targets for mitigating radiation-induced skin complications.

LB017

Comprehensive Transcriptomic Profiling to Elucidate the Unique Pathophysiology of Hidradenitis Suppurativa

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Hidradenitis suppurativa (HS) is an intractable skin disease characterized by recurrent sterile pustules, fistula formation, severe skin sclerosis in the chronic phase, and intense pain. HS, along with pyoderma gangrenosum (PG) and generalized pustular psoriasis (GPP), is considered a neutrophilic inflammatory skin disease responsive to biologics. The purpose of this study was to compare these three diseases and normal skin to elucidate the unique pathogenesis of HS. Transcriptome analysis was performed using formalin-fixed paraffin-embedded samples of HS, PG, GPP (n=4 each) and normal skin (n=7). Principal component analysis (PCA) and enrichment analysis were conducted to evaluate gene expression changes, and the xCell2 algorithm was used to estimate cellular infiltration profiles. In PCA, the four groups formed independent clusters. Enrichment analysis revealed an upregulation of immune and inflammation-related pathways common to all three diseases. Upregulation of pathways associated with tissue remodeling, vascular permeability, and angiogenesis was specifically observed in HS. Analysis of cellular infiltration profiles demonstrated a high degree of neutrophil and macrophage infiltration common to the three diseases. Furthermore, a marked accumulation of fibroblasts, endothelial cells, and plasma cells was observed in HS, whereas infiltration of T-cell lineages and natural killer cells was prominent in PG. Additionally, genes for matrix metalloproteinase (MMP) subtypes that are rarely expressed in normal skin were highly expressed in HS, which may reflect the characteristic fibrosis and fistula formation specific to this disease. Upregulation of the Ras signaling pathway was also specifically observed in HS, which may be related to the background of HS as a precursor to carcinogenesis. These results demonstrate that although HS, PG, and GPP commonly present with neutrophilic inflammation, the underlying immunological mechanisms differ distinctly among each disease. In particular, plasma cell-predominant immune responses and tissue remodeling pathways are closely involved in HS.

LB018

Investigation of the effects of novel anti-diabetic and anti-obesity drugs on human sebocytes

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Several novel drugs (including glucagon-like peptide-1 receptor [GLP-1R] and glucose-dependent insulinotropic polypeptide receptor [GIPR] agonists, as well as sodium-glucose cotransporter [SGLT]-2 inhibitors) are available for the treatment of obesity and type 2 diabetes. Because cutaneous effects of these drugs are only scarcely studied, we investigated the effects of the GLP-1R agonist liraglutide (LIR), the GLP-1R/GIPR agonist tirzepatide (TIR), the pre-clinical triple (GLP-1R/GIPR/glucagon receptor) agonist retatrutide (RET), and the SGLT2 inhibitor dapagliflozin (DAP) on human SZ95 sebocytes.
First, by analyzing our previous RNA-Seq data, we determined that sebocytes most likely do not express GLP-1 and glucagon receptors but may express GIPR and SGLT2. Next, we demonstrated that LIR slightly but significantly reduced the MTT signal intensity (≥1 nM; 48 h), RET and DAP had no effect (48 h), whereas TIR increased it (≥10 nM; 24 h). Importantly, the levels of polar lipids (Nile Red) did not show significant changes, indicating that these alterations were most likely independent from changes in the cell count. While investigating the sebaceous lipid production (Nile Red), we found that TIR (10 μM; 48 h) slightly but significantly increased both spontaneous, homeostatic sebaceous lipid production and arachidonic acid (AA)-induced lipogenesis. DAP and LIR were ineffective (24 h), whereas RET proved to be moderately lipogenic (10 μM; 48 h). Finally, by monitoring the expression (interleukin [IL]-1α, IL-1β, IL-6, IL-8; RT-qPCR) and release (IL-6, IL-8; ELISA) of selected cytokines, we found that DAP did not influence the immune phenotype of human sebocytes.
Our results suggest that certain concentrations of the tested compounds may differentially influence the biological processes of human sebocytes.

LB019

Calcipotriol modulates inflammatory responses induced by *Staphylococcus aureus* or *Staphylococcus epidermidis* in human primary keratinocytes under Th2-skewed conditions

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Atopic dermatitis (AD) is a chronic inflammatory skin disease characterized by Th2-skewed immune responses. The pathogenic bacterium *Staphylococcus aureus* is a major contributor to AD pathogenesis and is associated with increased disease severity. In addition, commensal bacteria such as *Staphylococcus epidermidis* can modulate immune responses at lesional sites in patients with AD.
Vitamin D is a well-known regulator of immune function, and its analog, calcipotriol (MC903), has long been used in the treatment of psoriasis, another chronic inflammatory skin disease. Interestingly, calcipotriol is commonly used to induce AD-like phenotypes in mouse models of the disease. However, in humans, its effects on inflammatory responses are more complex and remain insufficiently understood.
The effects of calcipotriol on keratinocyte inflammatory responses under conditions combining bacterial stimulation and elevated Th2 cytokines, which are associated with severe AD, remain insufficiently understood. We used primary human proliferating keratinocytes cultured under 2D conditions to investigate how calcipotriol pre-treatment modulates keratinocyte inflammatory responses in the presence of elevated Th2 cytokines (IL-4 and IL-13) and infection with *S. aureus* or *S. epidermidis*.
Our preliminary results demonstrate that calcipotriol effectively modulates inflammatory responses in keratinocytes. A 24-hour pre-treatment with calcipotriol before *S. aureus* infection reduced the secretion of IL-6, IL-8, and IL-1β, particularly in the presence of Th2 cytokines (IL-4 and IL-13). Similar effects were observed following *S. epidermidis* infection for IL-6 and IL-1β, whereas calcipotriol had the opposite effect on IL-8 secretion. These findings suggest that the therapeutic potential of calcipotriol in severe AD warrants further investigation.

LB020

Zasocitinib demonstrates rapid and durable modulation of TYK2 biomarkers that is associated with clinical response in phase 3 LATITUDE-PsO-3001 and -3002 trials

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Zasocitinib is an investigational, highly selective, once-daily (QD) oral tyrosine kinase 2 (TYK2) inhibitor. In two phase 3 trials of zasocitinib in patients with moderate-to-severe plaque psoriasis (LATITUDE-PsO-3001 [3001; NCT06088043]; LATITUDE-PsO-3002 [3002; NCT06108544]), we assessed modulation of TYK2 biomarkers and association with clinical response.
Patients were randomized 3:1:1 or 2:1:1 to receive zasocitinib 30mg QD, twice-daily apremilast or placebo over 52 (3001) or 60 weeks (3002). Biomarker analyses included zasocitinib and placebo groups. We assessed changes from baseline (CfB) in serum interleukin (IL)-23/T-helper (Th)17 biomarkers (BD-2, IL-17A, IL-17C, IL-17F) and blood type I interferon (IFN) pathway gene expression at Week (W)4 and 16. Association of these biomarkers with CfB in Psoriasis Area and Severity Index (PASI; Spearman's coefficient [ρ]) and PASI90/100 responses stratified by baseline type I IFN gene expression (high vs low) was evaluated. CfB in IL-17A/F was assessed to W60 in 3002.
Zasocitinib reduced serum biomarkers and type I IFN gene signature vs placebo at W4 (p<0.001); reductions sustained through W16 (IL-17A, p<0.01; all others p<0.001). At W16, CfB in PASI positively correlated with CfB in BD-2 (ρ=0.652), IL-17A (ρ=0.404), IL-17C (ρ=0.560), IL-17F (ρ=0.556) and type I IFN (ρ=0.363); p<0.001. At W16, PASI90/100 responses were numerically higher in zasocitinib-treated patients with high vs low baseline expression of type I IFN-associated genes (78.9% vs 66.1%/57.9% vs 29.0%). Zasocitinib-induced reductions in IL-17A/-F were maintained to W60.
Zasocitinib demonstrated rapid and durable modulation of IL-23/Th17 biomarkers and type I IFN gene expression that was associated with PASI 90/100 response.

LB021 [WITHDRAWN]

LB022

Trained immunity phenotype in Psoriasis PBMCs is potentiated by Tri-DAP NOD1 ligand-driven reprogramming

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Trained immunity may contribute to chronic inflammatory diseases. This study investigated whether PBMCs from patients with moderate-to-severe psoriasis display a trained immunity-like phenotype after exposure to canonical or clinically relevant microbial-derived stimuli.
PBMCs were isolated from psoriatic patients, n 53, and matched surgical controls, n 37. Cells were primed for 24 h with β-glucan (fungal Dectin-1 ligand) or Tri-DAP (bacterial NOD1 ligand), rested, and restimulated on day 6 for 24 h with LPS (bacterial TLR4 ligand) or poly(I:C) (dsRNA, TLR3 ligand), respectively. The β-glucan/LPS sequence was used as canonical trained immunity paradigm, whereas Tri-DAP/poly(I:C) was designed to model combined bacterial and viral PAMP exposure. TNF-α and IL-6 release was quantified by ELISA as a readout of trained immunity. LDH release was assessed to exclude treatment-related cytotoxicity. Lactate production and residual glucose levels were assessed in culture supernatants as metabolic readouts of glycolytic remodeling.
Tri-DAP/poly stimulation induced a robust trained immunity-like response in PBMCs from psoriatic patients, with enhanced TNF-α and IL-6 production compared with controls. In contrast, the canonical β-glucan/LPS sequence, typically associated with trained immunity in isolated monocytes, induced a tolerogenic profile. The Tri-DAP/poly response was accompanied by

increased lactate production relative to residual glucose levels, supporting glycolytic metabolic remodeling during innate immune reprogramming.
These findings suggest that PBMCs from psoriatic patients exhibit a trained immunity-like phenotype that is preferentially revealed by combined bacterial and viral PAMP stimulation, through Tri-DAP and poly(I:C). By preserving multicellular immune interactions, the PBMC-based approach may uncover disease-associated innate immune reprogramming that is not fully captured by conventional monocyte-centered protocols. The Tri-DAP/poly(I:C) model may therefore provide a pathophysiologically relevant platform to investigate how infection-associated microbial and viral cues contribute to inflammatory amplification in psoriasis.

LB023
Ex vivo generation of functional Th2 cells from naïve and skin-derived memory CD4+ T cells: a tissue-relevant platform for modeling type 2 inflammation
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Type 2 immunity driven by T helper 2 (Th2) cells contribute to atopic dermatitis, asthma, and related allergic diseases. However, tractable human systems that yield functional Th2 cells for mechanistic and therapeutic studies remain limited. We sought to establish a flexible ex vivo platform to generate functional Th2 cells from both circulating and skin-derived CD4+ T cells, providing a tissue-relevant model of type 2 inflammation.
Two complementary approaches were developed. In the first, naïve CD45RA+CD45RO- CD4+ T cells isolated from leukopaks or PBMCs and cultured under Th2-polarizing conditions over 14 to 21 days. The resulting cells acquired a stable Th2 profile, with increased GATA3, elevated IL-4, IL-5, IL-10, and IL-13, and reduced IFNγ and TNFα, and retained lineage commitment after withdrawal of polarizing factors. These cells were functional in chemotaxis and protein half-life assays and could be cryopreserved, thawed, and re-cultured while retaining phenotype and function.
In the second approach, the platform was adapted to dissociated healthy human skin, with cultures initiated from bulk cells containing CD45RO+CD45RA- memory CD4+ T cells. After approximately two weeks under defined Th2-inducing conditions, cultures became highly enriched for CD3+ T cells (n = 10 donors or more donors). Within the CD3+CD4+ population, cells displayed a Th2-characteristic phenotype, with elevated GATA3 and minimal T-bet, RORγt, and Foxp3 (flow cytometry), and produced IL-4, IL-5, IL-13, and IL-31 (multiplex immunoassay and ELISA). CRISPR/Cas9-mediated GATA3 knockout markedly impaired these cytokine responses, supporting a GATA3-dependent functional identity.
Together, these approaches establish a single platform spanning from naïve CD4+ T cell differentiation to skin-derived memory CD4+ T cell polarization, providing a renewable (fresh or banked) cell source for therapeutic assay development.

LB024
The Seven Hallmarks of Sensitive Skin
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Sensitive skin is a multifactorial condition driven by an exaggerated response to exposome-related triggers, including UV radiation, pollution, microbes, stress and irritants. This work proposes a structured framework defining sensitive skin through seven interconnected hallmarks: exposome stressors, skin barrier, inflammatory cascades, neurogenic inflammation, immune cell recruitment and repair, neuro-immune communication and sebum overproduction initiated by T-cell activation.
The hallmarks were mapped from skin to cellular level, focusing on keratinocytes, sensory nerves, mast cells and sebaceous glands. Efficacy was assessed using complementary in vitro and ex vivo models, including transcriptomics, cytokine ELISAs, mast-cell degranulation, TRP-channel screening and skin explants, complemented by in vivo histamine prick and UV-erythema tests.
The hallmarks are linked by barrier impairment, inflammatory amplification, and neuro-immune dysregulation. Keratinocytes and Mast Cells (MCs) act as sentinel cells, releasing inflammatory mediators to provide the first line of defense. MCs and nerve interactions explain itch, stinging and burning. Here, substance P released by nerve endings and keratinocytes activates MC degranulation by binding to NK1 or MRGPRX2 receptors resulting in pERK and Ca2+ signaling. Upon degranulation, MCs secrete proteases, histamine and anti-bacterial enzymes. Those proteases again activate PAR-2 on sensory neurons, which interact with TRPA1, TRPV1 and TRPV4 to mediate itch, stinging and burning. The TSLP–IL-4/IL-13–sebum axis links sebum overproduction to inflammatory and barrier-related pathways. These intertwining processes contribute to the symptoms of sensitive skin, such as redness, discomfort, and impaired resilience.
Sensitive skin care should move beyond general soothing toward targeted modulation of each hallmark. A holistic strategy combining barrier support, anti-inflammatory activity, neuro-sensory calming, itch reduction and sebum rebalancing can provide both immediate comfort and long-term skin resilience. The “Seven Hallmarks of Sensitive Skin” framework offers a practical biological basis for developing next-generation cosmetic solutions for sensitive and reactive skin.

LB025 [WITHDRAWN]

LB026
Diagnostic Value of the Systemic Inflammation Response Index in Bullous Pemphigoid
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Bullous pemphigoid is a chronic autoimmune blistering disease predominantly affecting older adults, in which systemic inflammation may contribute to disease pathogenesis. This retrospective study aimed to investigate the diagnostic value of the systemic inflammation response index (SIRI) in patients with bullous pemphigoid and to compare its discriminatory performance with the neutrophil-to-lymphocyte ratio (NLR) and systemic immune-inflammation index (SII). Thirty-one patients with bullous pemphigoid and 30 age-matched controls without bullous pemphigoid, selected among individuals presenting for general medical examination, were included. Receiver operating characteristic analysis demonstrated significant discriminatory performance for all three inflammatory indices. SIRI showed the highest diagnostic performance, with an area under the curve

of 0.745 (95% CI, 0.619–0.870; p=0.001), followed by NLR (AUC=0.718; p=0.003) and SII (AUC=0.705; p=0.006). These findings suggest that SIRI may serve as a simple and accessible inflammatory biomarker for distinguishing patients with bullous pemphigoid from controls. Its superior discriminatory performance compared with NLR and SII warrants further validation in larger prospective cohorts.

LB027

TRKB-based Signature Identifies High-Risk Cutaneous Squamous Cell Carcinoma and TRKB Blockade Reprograms Tumor and Stromal Cells Toward Suppressive Phenotypes.

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Cutaneous squamous cell carcinoma (cSCC) is a common age-related malignancy, with a subset of patients highly prone to recurrence and metastasis. However, reliable biomarkers for high-risk cases are lacking. Based on our previous finding that age-driven alterations in the neurotrophin receptor tyrosine kinase-2 (TrkB) axis fuel skin tumorigenesis, we aimed to identify novel cSCC biomarkers and therapeutic targets.
We retrospectively analyzed TrkB and its downstream targets (E-cadherin, Yap1, Notch1) via immunohistochemistry in patient-derived *in situ* and invasive cSCC samples. Statistical and machine learning tools were then applied to evaluate subtype differentiation and patient risk stratification.
To explore therapeutic potential, we treated SCC cells, cancer-associated fibroblasts (CAFs), and 3D organotypic models with the TrkB inhibitor ANA-12. Protein expression levels correlated tightly with cSCC subtypes. Notably, the 'TrkB, E-cadherin, Yap1, Notch1' panel emerged as a robust signature for identifying high-risk cases and staging subtypes. Despite a modest sample size, our machine learning models achieved high predictive accuracy, which improved substantially when integrating stromal microenvironment markers.
Mechanistically, blocking TrkB with ANA-12 impaired key malignant traits in aggressive, TP53-mutant SCC cells—suppressing proliferation, EMT, invasion, and the IL-6/STAT3 signaling loop. Instead, it forced cells toward differentiation and senescence by modulating p63, Yap1, Notch1, and p21. ANA-12 also reprogrammed primary CAFs, reversing their inflammatory, fibrotic, and tumor-sustaining properties via -catenin, Yap1, and Notch1 suppression. These outcomes reflected a clear tumor-suppressive effect that restores tissue homeostasis, validated here in physiologically relevant 3D models.
Together, these insights deliver a practical, TrkB-based signature for high-risk patient stratification while offering a novel targeted strategy to overcome treatment resistance in advanced cSCC.

LB028

Epigenetic targeting of MHC-I-deficient melanoma to overcome immune resistance

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Immune checkpoint blockade (ICB) has transformed melanoma therapy, but resistance remains a major clinical challenge, frequently caused by impaired antigen presentation that disrupts CD8 T cell-mediated tumor recognition. To identify novel therapeutic strategies for MHC-I-deficient melanoma, we established an in vivo B2M-deficient melanoma model and performed a pooled in vivo CRISPR-Cas9 screen targeting 600 epigenetic regulators in immunocompetent and immunodeficient mice to identify genes involved in MHC-I-independent anti-tumor immunity. Tumor growth patterns differed markedly between immunocompetent and immunodeficient mice, indicating immune-mediated selection despite the absence of MHC-I-mediated antigen presentation. Guide RNAs targeting multiple epigenetic regulators were significantly depleted in tumors from immunocompetent mice, identifying candidate epigenetic regulators of immune resistance. Functional in vivo validation of the top candidate genes demonstrated reduced tumor growth and decreased competitive fitness following CRISPR-mediated gene knockout, supporting their contribution to immune resistance in MHC-I-deficient melanoma. These findings identify epigenetic regulators as potential therapeutic targets for overcoming immune resistance in MHC-I-deficient melanoma and provide a rationale for improving the efficacy of immune checkpoint blockade.

LB029 [WITHDRAWN]

LB030

Longitudinal Non-Invasive Melanoma Proteomics to Optimise Intratumoural Checkpoint Inhibitor Treatment

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Melanoma progression is a dynamic, non-linear process, yet it remains poorly defined, relying on only a few selected time points in animal models to map longitudinal changes in tumour biology. The aim of this study was to use skin stretching, a non-invasive method for directly sampling melanoma interstitial fluid, to identify critical transitions in the tumour microenvironment over time and determine if these changes could be used to optimise intratumoural checkpoint inhibitor treatment.
A B16F10 melanoma model, using 1 × 10^5 cells injected into the hind dorsal skin of C57BL/6 mice, was sampled daily from the tumour site using a skin-stretching device during tumour growth. The Orbitrap Astral Mass Spectrometer detected the proteins in the extraction fluid. A kernel-based maximum mean discrepancy (MMD) framework was applied to identify the critical transitions in the tumour biology over time. The therapeutic relevance of the critical transitions identified by the MMD framework was evaluated by intratumoural delivery of checkpoint inhibitors at the key tumour transition point.
Longitudinal ISF proteomics mapped well to published RNA data in the same model and revealed temporally distinct remodelling programs during melanoma progression. Inflammatory pathways were activated early in tumour growth, whereas extracellular matrix remodelling was activated in later tumour growth stages. MMD analysis identified a critical transition at day

10 and an early perturbation in the tumour biology at day 5 after tumour injection. The day 5 perturbation was characterised by transient immune activation that failed to develop into sustained host control. Intratumoural administration of a checkpoint inhibitor cocktail comprising anti-PD1 and CTLA-4 inhibitors on day 5 suppressed tumour growth almost completely, suggesting that days 1-5 represent an important treatment window in the B16F10, C57BL/6 mouse model.

LB031
Insights into the Molecular Landscape of Melanoma
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Melanoma is a biologically complex and heterogeneous tumor. Although BRAF and NRAS are the main MAPK pathway drivers and therapeutic targets, acquired resistance and limited responses highlight the need for additional treatment strategies. We retrospectively analyzed 878 melanoma patients who underwent surgery at IFO, IRCCS, between January 2017 and March 2025 using a 50-gene next-generation sequencing panel (CHPv2). The mutational landscape of the cohort was dominated by BRAF (47.8%) and NRAS (24.2%), acting as mutually exclusive primary drivers. Notably, only nine patients harbored concurrent mutations. $NRAS^{mut}$ tumors more frequently co-occurred with CDKN2A, APC, and ATM alterations, whereas $BRAF^{mut}$ tumors preferentially carried MET, PTEN, and IDH1 mutations. Among 255 samples of double wild-type tumors, KIT emerged as the main alternative driver, accounting for 19 of the 22 KIT^{mut} cases in the entire cohort, presenting a significant difference compared to $BRAF^{mut}$ and $NRAS^{mut}$ groups. Furthermore, in the double wild-type subgroup, TP53 became the most frequently altered gene (11.8%), suggesting that, in the absence of canonical MAPK drivers, melanoma progression relies on KIT activation and TP53-associated genomic instability in addition to CDKN2A alterations. Other genes, including KRAS, CTNNB1, PTEN, ERBB4, ATM, APC, MET, JAK3, and KIT, showed mutation frequencies between 1% and 5%. The remaining genes were only sporadically, as well as EZK2, KRAS, HRAS, PI3KA, FBXW7, PDGFRA, RB1, FGFR3, GNAQ, SMARCB1, STK11, FLT3, EGFR, SMAD4, RET, PTPN11, NOTCH, ABL1, TERT, ALK, FGFR1, CDH1, FGFR2, MAP2K1, MYC, SRC, GNAS or never altered, counting ERBB2, FGFR2, VHL, CCND1, K642, ROS1, JAK2, CDK4, RAD15. Despite their low prevalence, most are clinically actionable and may represent therapeutic opportunities in precision oncology, with available targeted therapies for all except FBXW7, SMAD4, MYC, CDH1, RB1, TP53, and CTNNB1. Overall, molecular profiling may therefore expand therapeutic options supporting precision management of melanoma.

LB032
Targeting NRIP1 Reprograms Senescent Dermal Fibroblasts for Skin Rejuvenation
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Skin rejuvenation requires the functional restoration of aged skin by repairing and reprogramming accumulated cellular damage. Dermal fibroblasts play a central role in this process by regulating extracellular matrix remodeling and metabolic programs essential for tissue homeostasis. However, the precise upstream regulators governing fibroblast senescence and metabolic reprogramming remain to be fully elucidated. In this study, we identify nuclear receptor interacting protein 1 (NRIP1) as a key regulator of senescence reprogramming in dermal fibroblasts. NRIP1 protein levels were markedly elevated in the dermis of aged human skin compared with young skin. Consistent with our previous finding that NRIP1 suppresses PPARα promoter activity, NRIP1 was upregulated whereas PPARα was downregulated in aged mouse skin fibroblasts.
To determine whether NRIP1 drives fibroblast senescence, we isolated senescent dermal fibroblasts from 22-month-old aged mice and suppressed *Nrip1* expression by siRNA-mediated knockdown. *Nrip1* suppression significantly reduced senescence-associated β-galactosidase activity and increased relative cell viability. At the transcriptional level, *Nrip1*knockdown downregulated senescence markers (*p16* and *p21*) and senescence-associated secretory phenotype (SASP) factors (*Mmp3, Mmp13, Cxcl1, Ccl2*), while upregulating fatty acid oxidation genes (*Ppara, Cpt1a, Slc25a20, Acadm, Acadl*) and procollagen. Consistently, at the protein level, *Nrip1* knockdown decreased NRIP1 and $p16^{INK4a}$ while increasing PPARα and procollagen. Collectively, our findings demonstrate that age-associated NRIP1 accumulation suppresses the PPARα-driven fatty acid oxidation axis and collagen production, thereby sustaining the senescent state and its deleterious secretory phenotype.*Nrip1* suppression reverses this senescence program to restore a youthful, functionally rejuvenated fibroblast state. These results establish NRIP1 as a critical upstream regulator of the PPARα–fatty acid oxidation axis in dermal fibroblast senescence and highlight it as a promising molecular target for skin rejuvenation strategies.

LB033
The Indigenous Skin Microbiome reveals Enrichment of Photoprotective Taxa
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The human skin microbiome plays an important role in barrier function and immune regulation, yet most current knowledge is derived from Western populations. We investigated how urbanization influences skin-associated microbial diversity and functional potential by characterizing the skin microbiome of Indigenous Yanomami individuals living along an urbanization

gradient in the Brazilian Amazon.
Skin samples were collected from 124 Yanomami individuals across four body sites (scalp, forehead, armpit, and hand) from 3 different Yanomami villages (Bicho-Açu, Curua, Xamakorona) and compared with an age- and sex-matched Belgian cohort (n= 662). Shotgun metagenomic sequencing was used to profile taxonomic composition and functional gene repertoires associated with increasing urban exposure.
A distinct skin microbiome composition was revealed across villages and body sites, with clear body site–specific differences between the Yanomami and Western cohorts. Increased diversity was observed in the de novo assembly–based approach: the Belgian cohort yielded 324 MAGs (260 species-level annotations; 80% annotation rate), whereas the Yanomami cohorts yielded 810 MAGs (398 species-level annotations; 49% annotation rate). Differential abundance analysis identified taxa enriched across the four Yanomami skin sites, most notably *Deinococcus radiodurans*–related taxa, a species with an extraordinary capacity to survive high levels of UV radiation. Consistent with a photoprotective role, carotenoid biosynthesis (*crt*) genes were enriched in the Yanomami population, and the deinoxanthin biosynthesis pathway—encoding a carotenoid with H2O2-scavenging activity exceeding that of β-carotene—was recovered in skin-associated *D. radiodurans* MAGs.
This study enables a high-resolution comparison of skin microbial diversity and functional capacity between minimally industrialized and Westernized populations. By leveraging one of the few remaining Indigenous cohorts with limited Western influence, this work provides insight into how urbanization alters the skin microbiome, with potential implications for photoprotection (and skin cancer), immune regulation and susceptibility to inflammatory skin disorders.

LB034
Age-associated transcriptomic signatures in human skin reveal molecular endpoints relevant to cosmetic anti-aging research
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Chronological skin aging is associated with progressive changes in immune homeostasis, epidermal barrier function, extracellular matrix organization, and cellular stress responses. These alterations contribute to visible signs of aging and represent important biological targets for the development and validation of anti-aging cosmetic formulations. Human-relevant models are therefore needed to better characterize aged skin biology, capture molecular features associated with biological age, and support mechanism-based efficacy evaluation.
In this study, bulk RNA sequencing was performed on healthy *ex vivo* human skin samples from two age-defined female donor groups: 5 young donors below 35 years of age and 6 aged donors above 55 years of age. Differential gene expression and pathway analyses were used to identify molecular signatures associated with age-related skin changes and to define endpoints relevant for cosmetic anti-aging research.
Aged skin showed a distinct transcriptomic profile, with 440 upregulated and 310 downregulated genes compared with young skin. The main aging-associated signature was inflammaging, reflected by activation of immune pathways, cytokine signaling, complement engagement, and leukocyte trafficking. Additional transcriptomic changes highlighted proteostasis decline, extracellular matrix remodeling, fibroblast senescence, altered chromatin and RNA homeostasis, and cytoskeletal disorganization. Epidermal barrier disruption was further supported by reduced FA2H expression, suggesting age-associated impairment of lipid-related barrier mechanisms.
Together, these results establish a human tissue-based transcriptomic profile of chronological skin aging and identify molecular features that may reflect biological age in human skin. By defining endpoints linked to inflammaging, barrier disruption, matrix integrity, proteostasis, and cellular homeostasis, this *ex vivo* human skin model combined with transcriptomic analysis provides a translational platform for evaluating anti-aging cosmetic ingredients and formulations in a human-relevant, non-animal testing context.

LB035
A MALDI FTICR mass spectrometry imaging workflow maps glucose and redox metabolites in human skin and skin equivalents
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Skin aging is accompanied by altered glucose- and redox metabolism. Glucose-6-phosphate dehydrogenase (G6PD) directs glucose into the pentose phosphate pathway, generating NADPH that sustains glutathione-dependent clearance of reactive oxygen species. Localizing where these metabolites reside within the skin requires spatially resolved analysis, yet small molecules remain challenging targets for mass spectrometry imaging (MSI).
We developed an N-(1-naphthyl)ethylenediamine dihydrochloride (NEDC) MALDI FTICR MSI workflow to map glucose and related metabolites in the epidermis. NEDC was applied by solvent-free sublimation to minimise analyte delocalisation, and fresh-frozen sections were measured on a 7T FTICR MSI instrument in negative-ion mode at 15 µm lateral resolution. Resulting ion annotations were supported by sub-ppm mass accuracy and database matching.
The workflow was validated on reconstructed skin equivalents (supplemented with glucose and/or the G6PD inhibitor DHEA) and then applied to human biopsies from a young (28 y) and an aged (59 y) donor. The chloride adduct of glucose ($[M+Cl]^-$, m/z 215.033) was consistently detected and localised predominantly to the epidermis, with additional signal in hair follicles. Glucose-6-phosphate, reduced glutathione, and cholesterol sulfate showed related epidermis-enriched distributions. Glucose-supplemented equivalents gave the strongest glucose signal, confirming sensitivity to glucose availability. In the donor pair, aged skin showed higher glucose and glucose-6-phosphate signals and slightly lower glutathione, consistent with reported age-associated decline in G6PD activity.
These results demonstrate that NEDC-assisted MALDI FTICR MSI enables spatial mapping of glucose-related and redox metabolites in native and engineered skin, providing a workflow to study epidermal metabolism in skin aging.

LB036

Progress in "neurocosmetics": Topical GABA exerts multi-level anti-aging properties in human skin *ex vivo*

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In the context of developing novel "neurocosmetics" targeting the skin-brain axis, the key neurotransmitter, gamma-aminobutyric acid (GABA), is of particular interest. Not only is GABA enzymatically synthesized by human dermal fibroblasts, but it can also stimulate their production of hyaluronic acid and elastin as well as elastic fiber formation *in vitro.*
In the current study, we have asked whether GABA can be topically applied and alters selected biomarkers of skin aging in organ-cultured human scalp skin under serum-free, "speed aging" conditions. 4 mm skin fragments from facelift surgeries of 6 aged male or female donors (age range: 50-71 y-o) were treated topically in e vehicle containing 40 % Ethanol, 20% Propylene glycol, 5% Glycerin and 35% water every other day for 6 days with 0.2%, 2%, or 4% GABA.
Histologically, topical GABA was well-tolerated by human scalp skin *ex vivo* at all tested doses. Quantitative immunohistomorphometry showed that topical 2% and 4% GABA significantly reduced mTORC1 activity (p-S6), while 0.2% and 4% GABA significantly increased perinuclear Lamin B1 expression in the epidermis compared with vehicle-treated skin. 0.2% GABA also significantly stimulated epidermal protein expression of the key driver of human skin rejuvenation, VEGF-A. In the dermis, 0.2% GABA significantly stimulated collagen III expression. This pilot study provides the first direct evidence that GABA is topically effective and improves key aging biomarkers in the ex vivo aged human epidermis and dermis. This positions topical GABA as a promising active in future "neurocosmetics" that target human skin aging.

LB037

Dermal progerin expression disrupts dermal-epidermal crosstalk and recapitulates skin aging features in a 3D full-thickness model

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Understanding fundamental processes of aging is essential to develop interventions to treat age-related skin pathologies and improve barrier function. Skin aging involves complex bidirectional interactions between the epidermis and dermis, yet the specific contribution of senescent dermal fibroblasts, remains incompletely understood. To investigate this, we developed 3D full-thickness skin models using wild-type keratinocytes combined with either wild-type fibroblasts, or fibroblasts expressing dox-inducible progerin. Progerin, a mutated form of the nuclear intermediate filament protein lamin A, causes premature senescence in the accelerated aging syndrome Hutchinson-Gilford progeria. We compared progerin expression with three control conditions: non-induced progerin, dox-treated wild-type, and untreated wild-type models. Histology (H&E) revealed marked changes, including keratinocyte infiltration into the dermis, reduced keratohyalin granules in the stratum granulosum, and mild epidermal thinning. Immunohistochemistry showed decreased dermal Procollagen 1, Procollagen 3, and FBN1; reduced epidermal FLG; and diminished ITGB1 at the dermal-epidermal junction (DEJ) following progerin expression. Remarkably, RNAscope demonstrated that senescence-associated *MMP9*, but not *MMP1* or *MMP3*, was strongly upregulated specifically in the infiltrated keratinocytes upon dermal progerin induction. Collectively, progerin expression in dermal fibroblasts drives aberrant dermal-epidermal crosstalk, leading to an impaired DEJ and dermal matrix deposition, as well as secondary epidermal defects, recapitulating features of aged human skin. Our progerin-based 3D model offers a competent platform to delineate how senescent fibroblasts may contribute to dermal and epidermal skin aging and to test potential rejuvenation strategies.

LB038

Understanding Dermal-Epidermal Crosstalk as a Key to Skin Aging

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Skin aging compromises barrier integrity and increases disease susceptibility. While aging involves reciprocal interactions between the epidermis and dermis, these tissue dynamics remain unclear. To investigate this crosstalk, we developed an *in vitro* 3D full-thickness skin model using primary donor-matched cells and a human extracellular matrix-based dermal equivalent. Compared to young models, aged skin models showed reduced epidermal thickness, impaired differentiation, and compromised dermal-epidermal junction (DEJ) integrity. Specifically, differentiation markers (K10, CDSN, FLG), stratum granulosum granules, and the DEJ component COL17 significantly decreased. Additionally, aged dermal fibroblasts expressed the senescence-associated secretory phenotype (SASP) marker *MMP1*, which was absent in young fibroblasts. To evaluate the causal roles of this cellular crosstalk, we generated heterotypic models by combining young dermis with old epidermis and vice versa. Young dermis rescued the old epidermal phenotype, restoring epidermal thickness, stratum granulosum granules, and the expression of K10, CDSN, FLG, and COL17. Concurrently, young epidermis suppressed *MMP1* expression in old dermal fibroblasts. Notably, the impact of old dermis on young epidermis depended on the culture duration of the dermal equivalent layer, whereas old epidermis did not induce *MMP1* in young fibroblasts. These reciprocal rescue effects reveal distinct regenerative capacities: young dermis restores aged epidermal differentiation and DEJ integrity, while young epidermis independently results in reduced *MMP1* expression in aged fibroblasts. Our findings position the epidermis and dermis as interdependent partners in aging and suggest that mapping these youthful tissue signatures could unlock novel therapeutic strategies for skin rejuvenation.

LB039 [WITHDRAWN]

LB040

In vitro Analysis of a Microalgae-Derived Cosmetic Ingredient: A Senotherapeutic Targeting Skin Aging.

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Beyond wrinkling and laxity, skin aging implies a functional decline, representing a key dermatological concern. Moreover, this deterioration relies on the senescence of the skin-resident cells. Aiming to target skin aging from a functional perspective and considering its cellular bases, this work addresses the potential of a biotechnological ingredient derived from 2 algae species (Senesblock®).

Under this aim, human primary fibroblasts, keratinocytes, and a model of H2O2-induced senescent fibroblasts were used as experimental systems. Colorimetric and immunofluorescence techniques assisted by microscopy were carried out for the detection of several biomarkers.

First, in keratinocytes, treatments with the ingredient increased the levels of the cell-cell adhesion proteins E-Cadherin, β-catenin, and Vinculin, which are reported to decay during aging, altering the epidermal development and structure. Then, in fibroblasts, we observed enhanced proliferation and reduction of metalloproteinase 1 (MMP-1). These results pointed to a plausible benefit of the ingredient in mitigating age-related skin functional decline, where impaired wound healing means one of the most relevant age-associated burdens. Interestingly, wound healing assays revealed marked benefits of the treatments, especially when senescent fibroblasts were used. Thus, further evaluation in induced-senescent fibroblasts assessed proliferation rate, Beta-Galactosidase (β-Gal), Mechanistic Target of Rapamycin (mTOR), Sirtuin 1 (SIRT1), Procollagen, and Advanced Glycation End Products (AGEs). Treatments with the ingredient reverted all senescence-associated biomarkers tested.

Hence, this microalgae-derived technology emerges as a novel senotherapeutic candidate, targeting not only the aesthetic manifestations of skin aging but, most importantly, the related functional decline, thus addressing key dermatological challenges and supporting its incorporation into innovative cosmetic formulations.

LB041 [WITHDRAWN]

LB042 [WITHDRAWN]

LB043

Comparative Proteomic Profiling of Human Burn Blister Fluid Across Age Groups, Gender, and Anatomical Skin Sites

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Burn blister fluid contains systemic and locally released proteins reflecting inflammatory, regenerative, and tissue remodelling processes after skin injury. This study characterised the proteomic profile of human second-degree burn blister fluid and examined associations with age, gender, and anatomical site.

Blister fluid from 83 patients with second-degree burns was analysed using mass spectrometry-based proteomics. Differential protein expression, principal component analysis, and pathway analysis identified signatures associated with patient characteristics and location.

Proteomic profiles showed substantial overlap between male and female patients, indicating that gender was not a major driver of variation. However, specific differences were identified, including increased sex hormone-binding globulin in females and PLEKHA7 in males, suggesting variation in hormonal regulation and epithelial junction remodelling.

Age-related analysis revealed distinct healing signatures. Paediatric and younger adult patients showed increased proteins associated with proliferation, keratinocyte activity, extracellular matrix synthesis, and regeneration, including PCNA and collagen-associated proteins. Older adults demonstrated increased inflammatory, complement, extracellular matrix remodelling, and protease-related proteins, suggesting a prolonged inflammatory and fibrotic wound environment.

Comparison of haired and non-haired sites showed that most of the blister proteome was shared, but local signatures reflected anatomical differences. Non-haired skin showed enrichment of barrier defense and extracellular matrix proteins, with increased dermcidin suggesting antimicrobial protection and ANGPTL7 indicating fibroblast-mediated remodelling. Haired skin showed enrichment of immune regulation, oxidative stress, and cytoskeletal proteins, with immunoglobulin fragments indicating local immune activity and GPX1 reflecting redox regulation.

These findings demonstrate that burn blister fluid provides insight into wound repair biology. Age and anatomical sites are key determinants of the blister proteome and may contribute to differences in healing responses.

LB044

Differential Effects of Calcium Alginate and Carboxymethylcellulose Wound Dressing Extracts on Human Sensory Neuron Regeneration and Secretome

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Peripheral nervous system is critical in wound healing, as cutaneous sensory nerve endings release neuropeptides that modulate key skin cell functions. Damage to these endings can impair healing and predispose to chronic wounds. Thus, selecting dressings that foster nerve repair offers a promising strategy for managing complex wounds. Calcium alginate dressings are of particular interest as they release calcium ions, activating vital processes for neuronal function.

This study explores the *in vitro* effects of three wound dressings on sensory neurons: a pure calcium alginate (Algostéril®), a calcium alginate combined with carboxymethylcellulose - CMC (Biatain® Alginate), and a pure CMC (Aquacel Extra™). Sensory neurons (iSN) from human neural progenitors were exposed to dressing extracts for five days. Viability was tested using a calcein assay. Neurite outgrowth was quantified in iSN neurospheres (NS) and their secreted factors were quantified on day 5 using proteome profiler arrays. Neurite extension was evaluated in compartmentalized chips where extracts were

restricted to the neurite compartment.
Only Algostéril was non-cytotoxic (>70% viability) at all concentrations. Algostéril significantly promoted neurite outgrowth in neurospheres, increasing total length and neurite count. In contrast, Biatain Alginate had a strong inhibitory effect, while Aquacel had no significant influence. These outcomes were consistent with the upregulation of 39 factors secreted in Algostéril-treated NS, including brain-derived neurotrophic factor and vascular endothelial growth factor, whereas Biatain Alginate and Aquacel downregulated secreted factors. In compartmentalized culture, observed trends suggested neurite elongation with Algostéril, regression with Biatain Alginate, and no effect with Aquacel.
This study, although limited to *in vitro* models, evaluates for the first time the effects of advanced wound dressings on neurons. Results support the potential therapeutic advantage of the pure calcium alginate dressing in promoting nerve repair and optimizing wound healing, relevant for neuropathic conditions such as diabetic wounds.

LB045
Optimizing Endpoint Selection in Chronic Wound Clinical Trials: Increasing Focus on Meaningful and Patient-Centered Outcomes
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Chronic wounds, including diabetic foot ulcers, venous leg ulcers, surgical wounds burns, and pressure injuries, are characterized by prolonged healing, recurrent infections, pain, and substantial impairment in the quality of life (QoL). Contemporary wound research supports a broader endpoint framework as explained in results.
To review clinically relevant endpoints used in chronic wound clinical trials and discuss strategies for selecting standardized, patient-centered, and technology- enabled outcome measures that improve trial quality and regulatory relevance.
We synthesized evidence from pivotal regulatory programs, phase studies, studies conducted under the Human Cell, Tissue, and Cellular and tissue-based products (HCT/P) pathways, medical devices, and peer-reviewed literature to identify endpoints that are clinically relevant, measurable, and useful for both regulatory and patient-centered decision-making.
Complete wound closure defined as full re-epithelialization without drainage or dressing dependence. it remains regulatory gold standard but is challenging for powering trials.
Long duration of follow-up: PAR at 40-50% at 4 weeks is a robust surrogate predictor of 12-week healing.
Time to healing captures the speed of response or recovery. It is one of the few non- closure outcomes with strong support for trial use.
Pain reduction and Infection-related endpoints**:** Reduction in wound infection is highly valued by patients because it alleviates immediate symptoms, decreases treatment burden, and improves overall well-being.
Health-related QoL, Digital wound measurement and Health economic outcomes are essential as it imposes substantial direct and indirect costs.
Modern chronic wound clinical trials should adopt multidimensional endpoint strategies that retain complete wound closure as the anchor endpoint and pair it with patient-centered and economic endpoints to better reflect real-world benefits. This multi-end approach improves scientific interpretability and supports regulatory relevance.

LB046
Early removal at 6 hours versus continued dressing use after facial surgery: A randomized controlled trial
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For clean wounds, current evidence fails to establish an optimal postoperative wound care strategy, leaving clinical practice largely subjective. In dermatology, the facial region presents unique challenges due to its sebaceous-rich microenvironment and high aesthetic demands. These factors may increase patient concern about cosmetic outcomes, yet no trial has compared dressing protocols for facial wounds. Therefore, this trial compared early dressing removal versus continued dressing use on wound healing and scar formation after facial surgery.
This randomized, assessor-blinded trial enrolled patients undergoing excision of benign facial lesions. Wounds were covered with sterile gauze and patients randomized 1:1 to early removal (dressing removed 6 hours post-surgery, followed by daily 0.5% iodophor cleansing) or continued use (dressing changed every 48 hours with 0.5% iodophor until postoperative day 7[POD7]). No other topical treatments were permitted. Primary outcomes were early wound reactions (edema, suppuration, erythema, tenderness, bleeding, ecchymosis) within 7 days. Secondary outcomes were quality of life (Skindex-16) at POD7, scar assessment (POSAS 2.0, mSBSES) and patient satisfaction at POD90.
Of 144 randomized patients, 73 were assigned to early removal and 71 to continued use. Baseline characteristics were comparable except for age and Fitzpatrick skin type. Early removal significantly reduced edema at POD7 (16.4% vs 32.4%, P=0.026). Multivariate analysis confirmed early removal as an independent protective factor against edema (aOR=0.40, 95% CI 0.18-0.92, P=0.032). No significant differences were observed in other complications or Skindex-16 scores (P>0.05). At POD90, scar assessments and patient satisfaction showed no differences (P>0.05; n=108).
In conclusion, early dressing removal after facial surgery significantly reduces early wound edema while maintaining comparable safety, scar outcomes, and satisfaction. Our findings suggest that prolonged dressing coverage may not be necessary for all patients with benign facial lesions, and early removal offers a practical strategy to simplify wound care.

LB047 [WITHDRAWN]

LB048
A GoF temperature-sensitive (TS) mutation in TRPM4 gene that causes progressive symmetric erythrokeratodermia (PSEK) impairs physiological function of sebaceous glands in mice
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Specific Gain-of-Function (GoF) mutations in the activation gate region of TRPM4 gene have been identified as causative in PSEK. Other GoF mutations in TRPM4 are responsible for progressive heart block type I (PHBI) without skin phenotypes. We generated TRPM4 (equivalent to human PSEKTRPM4) mutant mice, including heterozygous (HET) and homozygous (HOM)

mutants, and analyze sebaceous gland morphology by histology and sebaceous function by visual and quantitative assessment via water retention on skin and hair after water immersion. Skin lipidomics was performed on UPLC-MS/MS platform and analyzed with Innomics' bioinformatics pipeline. We assessed the temperature dependence of TRPM4 mutant (PSEK- and PHBI-causing) or WT transfected cells using patch clamp techniques. Histology revealed under-developed and fewer sebaceous glands in skin of TRPM4 compared to WT mice. Oil-red-O lipid staining showed larger lipid droplets in WT mice skin than that seen in HET or HOM mice. Our analysis showed decreased total lipids and major lipid classes, including triglycerides (TG), ceramide NS (Cer-NS), ceramide AS (Cer-AS), and other lipid species, in HET/HOM (vs. WT) mice. HOM- and HET-TRPM4 mutant mice displayed poor shedding of water after retrieval from water, resulting in mutant mice having water-logged fur up to 3.5~5 min after WT mice showed well-groomed appearance (average15 min). In stark contrast to WT and PHBI-mutant TRPM4-transected cells, PSEK TRPM4 mutant-transfected cells retained calcium-triggered channel activity at 30°C whereas the other variants lost channel activity at 30°C. In sum, sebum/lipid production, which is critical for normal shedding of water from hair, appears to be disrupted in GoF TRPM4 mutant mice. Since lipid abnormalities have been linked to genetic keratodermas, our data raises the possibility that PSEK patients may have reduced sebaceous gland activity that contributes to clinical phenotype. The TS nature of the TRPM4 mutant may explain the location of skin disease to the cooler acral surfaces (~30°C) of affected individuals.

LB049

Multi-Omic Analysis Identifies the Interferon Signaling Pathway as a Key Pathogenic Driver and Therapeutic Target in Hailey-Hailey Disease

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The objective of this study was to better understand the pathophysiology of Hailey-Hailey disease (HHD) to guide therapy using a multi-omic approach. HHD is a rare dominant genetic skin disease caused by loss-of-function pathogenic variants in *ATP2C1* leading to acantholysis of the epidermis. Patients with HHD undergo painful blistering and inflammatoty erosions in interginous regions which are often subject to secondary infections. The disease leads to significant disability and there is no effective therapy.

We applied a multiomics approach to define a high-resolution molecular and cellular landscape of HHD lesional skin. Integrated bulk transcriptomics and proteomics revealed a striking type I and type II interferon (IFN) signature in lesional skin biopsies compared with healthy controls and non-lesional samples. Single cell and spatial transcriptomics identified inflammatory niches composed of keratinocytes, immune cells, vascular and stromal cells which create an IFN-driven signaling network through multiple intercellular communications and sustain a self-amplifying loop.

Crucially, topical treatment with the JAK1/2 inhibitor ruxolitinib led to rapid and lasting healing with drastic reduction in pain, itching, oozing and a marked improvement in quality of life in ten patients with refractory HHD. These results support topical JAK inhibition as an effective mechanism-based therapy, redefining the standard of care for HHD.

*SC and HDMP contibuted equally.

LB050

Raman spectroscopy resolves a graded molecular progression toward squamous cell carcinoma in recessive dystrophic epidermolysis bullosa skin

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Recessive dystrophic epidermolysis bullosa (RDEB) is a severe genodermatosis caused by biallelic pathogenic variants in *COL7A1*, encoding type VII collagen. Its loss drives blistering, chronic wounds, scarring and early-onset squamous cell carcinomas (SCCs), currently detected visually and often needing multiple biopsies to confirm. Here, we use Raman spectroscopy, a non-destructive technique, to track the molecular changes accompanying malignant progression in RDEB, from non-lesional (NL) through peri-lesional (PL) skin to established carcinoma (SCC).

Raman microspectroscopy imaging of frozen sections from RDEB skin (NL n=9, PL n=6, SCC n=6) and healthy control skin (HC; n=8) quantified whole-section composition against a library of 14 tissue biomolecules. As epithelial and tumour tissue expanded and displaced dermis, composition shifted with disease progression: keratin (NL 13, PL 19, SCC 37, HC 16%), and total lipid (NL 23, PL 26, SCC 29, HC 20%) led by phosphatidylcholine signal (NL 6, PL 7, SCC 8, HC 3%) rose, while collagen (NL 31, PL 26, SCC 10, HC 29%) and elastin signal (NL 17, PL 14, SCC 6, HC 19%) fell. Spatial mapping localised these to hyperplastic epidermis, dermal fibrosis and tumour nests, with immunofluorescence corroborating ECM remodelling (type I collagen, elastin), altered epithelial organisation (cytokeratin 14, E-cadherin) and increased proliferation (Ki-67).

This signature (primarily reduced collagen and elevated lipids) was recovered in ex vivo bulk tissue with a handheld fibre-optic Raman probe, where a partial least squares-discriminant analysis classifier separated SCC from non-SCC spectra under leave-one-patient-out cross-validation, reaching 81% sensitivity, 76% specificity and 0.85 AUC (contact probe) and 88%, 79% and 0.88 AUC (non-contact configuration, suited to fragile skin).

Raman spectroscopy captures RDEB-associated SCC as a graded, molecularly detectable progression, apparent already in PL skin and readable in real time with a clinic-ready fibre-optic probe, supporting non-invasive bedside early detection, biopsy and margin guidance, and chronic-wound monitoring.

LB051

Autosomal Dominant Pure Hair and Nail Ectodermal Dysplasia Associated with KRT85 Variants

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Biallelic variants in *KRT85* are associated with a rare form of ectodermal dysplasia characterized by the combination of hypotrichosis and nail abnormalities, while other ectodermal structures are unaffected. In these cases, heterozygous carriers are asymptomatic. Here, we describe a novel, more severe phenotype of ectodermal dysplasia in patients carrying heterozygous *KRT85* variants.

Clinical and molecular data were collected from patients carrying a single pathogenic *KRT85* variant. Patients were recruited through French national rare disease reference centers.

9 European patients were identified. 7 patients belonged to 3 unrelated families, 2 were sporadic. All presented with hypotrichosis since birth, associated with hyponychia of 20 nails (n = 6), oligodontia (n = 2), amelogenesis imperfecta (n = 1). Targeted next-generation sequencing panels for hypotrichosis and ectodermal dysplasia genes and/or whole-genome sequencing identified the following heterozygous *KRT85* variants: c.919C>T (family 1), c.1255G>A (family 2), c.1288G>A (family 3, 2 sporadic cases). In each family, the variant segregated with the phenotype. All variants reside within the α-helical coil 2 domain of keratin 85, in the C-terminal region of the rod domain, which is critical for intermediate filament assembly. All variants were predicted to be deleterious by multiple prediction tools.

As reported for other ectodermal dysplasia genes, *KRT85* variants can cause an autosomal dominant ectodermal dysplasia. Notably, the clinical phenotype differs according to the mode of inheritance. Unlike the previously reported recessive forms, the variants identified here cluster within the C-terminal region of the α-helical coil 2 rod domain, required for intermediate filament assembly

This study expands the phenotypic spectrum associated with *KRT85* by identifying an autosomal dominant form of ectodermal dysplasia characterized by hypotrichosis since birth, nail abnormalities, and, in some patients, dental defects.

LB052

RNF20 Regulates Skin Stem Cells Development

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Epigenetic regulation plays important roles in controlling stem cell identity and tissue development. Among epigenetic mechanisms, histone mono-ubiquitination has emerged as an important post-transcriptional modification. Here, we uncover an essential role of heteromeric RNF20/40 complex – a key chromatin regulator that catalyzed histone H2B lysine 120 mono-ubiquitination (H2BK120ub) – in skin development. Utilizing epithelial-specific deletion of *Rnf20* (*Rnf20* cKO) in the developing murine skin, we show that RNF20 is essential for the proper development of skin epithelium. Epidermal stem cells lacking RNF20 exhibited dramatically reduced epidermal proliferation that resulted in reduced epidermal thickness, although apparently normal induction of epidermal differentiation markers and barrier functions were observed. Additional analysis probing deeper into changes in skin development uncovered that both embryonic and newborn mice lacking RNF20 show dramatically reduced Merkel cell numbers. Further analysis demonstrated no signs of apoptosis around hair placodes, where Merkel cells are normally formed. Interestingly, analysis of early and late Merkel cell differentiation markers showed the proper expression of those markers in the few residual Merkel cells that do form in the back skin of *Rnf20* cKO mice. These data suggest that the loss of RNF20 impairs Merkel cell formation rather than their differentiation or survival. Finally, analysis of *Rnf40* cKO mice demonstrated a similar reduction in the number of Merkel cells formed, indicating that RNF20 regulates the formation of Merkel cells as a part of the canonical RNF20/40 E3 ubiquitin ligase complex. Altogether, our studies identified the heteromeric RNF20/40 complex as an essential epigenetic regulator required for Merkel cells formation.

LB053

Cell Penetrating Peptides Enables Efficient Delivery of Genome Editing Ribonucleoproteins into Human Keratinocytes and Organotypic Skin Models

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Efficient and safe delivery of genome editing tools remains a major bottleneck for epithelial cell models and therapeutic development, particularly in human keratinocytes where gene editing is often inefficient, difficult and compromise cell viability. Here we introduce a delivery strategy in which amphipathic cell-penetrating peptides (CPPs) are used as tool to deliver SpCas9 ribonucleoprotein (RNP) and edit cells both in 2D and 3D. To quantify intracellular delivery and functional gene editing event in adherent N/TERT 1 keratinocytes and in 3D organotypic skin tissue, we establish an antibody-readable validated COSMC (C1GALT1C1) knockout reporter, revealing the Tn antigen exposure in both monolayered N/TERT 1 keratinocytes and 3D organotypic epidermal models. CPP-SpCas9-RNPdelivery yields robust on-target editing while supporting post-treatment recovery of the keratinocytes. As a proof of concept, this approach allowed to establish an *Epidermolysis bullosa simplex* (EBS) mimicry by introducing both a mono-allelic K14 KO and a complete K14 KO. Together, these results establish the CPP-SpCas9 as a strategy to deliver genome editors into difficult to transfect adherent cells and apply the method on a disease-relevant model.

LB054

Skin microbiome alterations in the predilection sites of Hailey-Hailey disease: a single-center study

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Hailey-Hailey disease (HHD) is a rare autosomal dominant non-syndromic epidermal differentiation disorder that affects the symmetric flexural areas, such as the axillary, submammary, and inguinal regions, with vesicles, erosions, and inflammatory plaques. The skin dysbiosis may play a role in the disease exacerbations in the predilection areas. Therefore, this study aimed to examine the clinical, genetic, and microbiome characteristics of patients with HHD. We analyzed the skin microbiome of lesional and non-lesional skin from three predilection sites in eight genetically and/or histologically confirmed HHD patients (F:M 7:1) and age- and sex-matched controls using 16S rRNA (V3-V4) sequencing followed by bioinformatics analysis. Skin microbiome alterations were observed between lesional and non-lesional skin of patients with HHD and healthy control skin. No significant differences in alpha diversity were detected between cohorts. However, beta diversity analysis showed significant differences in microbial composition between lesional and non-lesional skin ($p=0.01$) and healthy controls ($p=0.008$). The axillary region was characterized by *Staphylococcus aureus* and *Pseudomonas aeruginosa*; submammary samples showed increased abundance of *Finegoldia magna* and *Anaerococcus sp*., whereas the inguinal microbiome was dominated by anaerobic taxa. Microbial alterations were most prominent in lesional areas, and clinically more severe cases showed a pathogenic profile. In conclusion, the detected dysbiosis can potentially influence the HHD phenotype, exacerbation, and persistence of the lesions in predilection areas. These findings suggest that dysbiosis may play a role in HHD pathogenesis.

LB055

Identification of evolutionarily conserved skin appendage proteins

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The evolution of hair and other skin appendages is a major research topic in the skin biology of vertebrates. The structural proteins of hair, known as hair keratins, are homologous to keratins in claws of reptiles and clawed frogs, indicating that the gene family encoding hair keratins evolved in a common ancestor of all land-dwelling vertebrates. Here, we used comparative genome analysis, RNA-sequencing and mass spectrometry-based proteomics to investigate the expression of keratin intermediate filament proteins in *Xenopus* frogs and non-model species of amphibians. Genes encoding hair keratin homologs were identified in all main clades of amphibians except caecilians. Besides claws, these genes are expressed in the pigmented spines of nuptial pads of frogs, in cornified teeth of tadpoles and in the cornified beak of a neotenic salamander. mRNA *in situ* hybridization demonstrated the expression of hair keratin homologs in terminally differentiated epithelial cells. These results show that hair keratins contribute to the cornification of a wide variety of skin appendages in amphibians and suggest that an evolutionarily ancient cornification program was not only repurposed during the evolution of hair in mammals but also for the evolutionary diversification of non-mammalian skin.

LB056

An epidermolysis bullosa simplex case solved by long-range PCR of the KRT14 gene

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A female baby was born to non-consanguineous parents. There was no family history of blistering skin disease in the family. Blistering skin on fingers was recognized 2 days after birth. At 2-3 months of age, hands, toes, chin, chest, knees, gluteal and abdominal regions were affected by skin blistering. Oral mucosa (tongue) was also involved. Serous blisters, wounds and erosions with crust were noticeable. Scars and milia were not present and nails looked intact.

An apparently homozygous KRT14 c.364C>A, p.(Leu122Ile) variant was identified in the patient genomic DNA sample and surprisingly the KRT14 haplotype did not match that of the mother and was consistent with one allele of the father except the variant. After maternity and paternity were confirmed, we expected a complex case involving a structural variant. The karyotypes of the patient and parents were not explanatory. CGH array of the patient revealed an extra copy of a segment of chromosome 14 with no OMIM genes. We were looking for an intragenic deletion within the KRT14 gene and because of the small size of the gene, we decided to perform long-range PCR. By performing KRT14 long-range PCR we did not detect deletion in the DNA of the patient or parents.

By sequencing the long-range PCR products, we recognized a KRT14 c.525+70C>G benign variant in the KRT14 Intron 1 at the reverse primer binding site that was heterozygous in the patient DNA. By analyzing the long-range PCR products, we found that the controversy was caused by allelic dropout in the conventional PCR of the patient DNA. Finally, our data showed that the patient is heterozygous for the pathogenic variant *(de novo)*. Currently, we are investigating the allelic dropout by quantitative PCR. Our results have an importance in the diagnostics of epidermolysis bullosa simplex patients.

LB057

Polygenic risk scores identify distinct atopic disease associations in erythrodermic psoriasis

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Erythrodermic psoriasis (EP) is a severe variant of psoriasis affecting over 90% of the skin and is characterised by mixed Th17- and Th2-mediated inflammation. Despite its clinical and immunological differences from plaque psoriasis, it remains unclear whether EP is characterised by distinct inherited susceptibility to psoriasis, atopic diseases, or both. We investigated whether genetic liability to atopy, atopic diseases, or psoriasis is associated with EP.
We performed a case-case analysis comparing individuals with EP and psoriasis patients without EP. Genotype data from the Biomarkers and Stratification To Optimise outcomes in Psoriasis (BSTOP) study were linked with phenotype data originating from the British Association of Dermatologists Biologics and Immunomodulators Register (BADBIR). All participants had psoriasis and were considered to have EP if this was present at registration or developed during follow-up. Those with only historical EP were excluded. Polygenic risk scores (PRS) for general atopy (PRS_{AT}), atopic dermatitis (PRS_{AD}), asthma (PRS_{AS}), allergic rhinitis (PRS_{AR}) and psoriasis (PRS_{PS}) were generated using PRSice and independent reference genome-wide association study (GWAS) summary statistics. Associations with EP were evaluated using logistic regression, adjusting for genotyping batch and for population structure using the first 5 principal components.
Amongst 4,452 individuals with psoriasis, 286 had EP. EP was associated with PRS_{AD} (OR 1.14, 95% CI 1.01-1.28, P=0.026) and inversely associated with PRS_{AR} (0.82, 0.73-0.93, P=0.002). EP was not associated with PRS_{AT} (0.94, 0.83-1.06, P=0.28), PRS_{AS} (0.93, 0.83-1.05, P=0.26) or PRS_{PS} (1.08, 0.96-1.22, P=0.19).
Among individuals with psoriasis, erythrodermic psoriasis was associated with increased genetic liability to atopic dermatitis and decreased liability to allergic rhinitis, whereas genetic liability to asthma and psoriasis did not differ between individuals with and without EP. These findings suggest that genetic liability to atopic diseases differs between individuals with and without EP, warranting further investigation of atopic disease-related pathways in this phenotype.

LB058
Bacteria-specific T cells are enriched in lesional skin and exhibit altered functional responses in severe atopic dermatitis
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Atopic dermatitis (AD) is characterized by chronic skin inflammation, frequent *Staphylococcus aureus* colonization and extensive T-cell infiltration. While T cells are key drivers of disease pathology, the antigen specificity of skin-infiltrating T cells remains incompletely understood, particularly in response to commensal and pathogenic skin bacteria. This study aimed to characterize bacteria-specific CD4+ T-cell responses in AD, determine their functional phenotype, and investigate their presence within lesional skin.
PBMCs from patients with AD and healthy controls were stimulated with bacterial extracts of *S. aureus, S. epidermidis*, *S. cohnii* and *Streptococcus salivarius* to identify bacteria-specific CD4+ T cells by CD154 upregulation and intracellular cytokine staining. Bacteria-specific T cells were expand and their cytokine secretion profiles were assessed. To determine whether bacteria-specific T cells infiltrate lesional skin, T-cell receptor β-chain (TCRβ) CDR3 sequences from bacteria-specific T cells were compared with TCR repertoires obtained from autologous lesional skin biopsies.
Bacteria-specific CD4+ T cells were detected in both AD patients and healthy controls and displayed heterogeneous functional polarization. In most patients with AD, responses were dominated by Th1/Th17-associated cytokines, including IFN-γ, IL-17A, and IL-17F, comparable to healthy individuals. In contrast, a subgroup of patients with severe AD exhibited reduced bacteria-specific T-cell activation, diminished IFN-γ production, and a shift towards Th2 cytokine responses, with IL-13 production correlating with disease severity. TCR repertoire analysis demonstrated that bacteria-specific T-cell clones were enriched in lesional skin, accounting for up to 7.6% of clonally expanded T cells.
These findings demonstrate that bacteria-specific T cells infiltrate lesional AD skin and exhibit distinct functional profiles according to disease severity. While Th1/Th17 responses predominate in most patients, severe AD is associated with impaired IFN-γ responses and enhanced bacteria-specific Th2 polarization, suggesting a contribution to disease progression.

LB059
PPARa deficiency causes skin dysbiosis and triggers innate immunity in keratinocytes
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Peroxisome proliferator-activated receptor-a (PPARa) is a transcription factor expressed in the skin, where its physiological role remains largely unknown. In this work, we found decreased bacterial density and dysbiosis associated with reduced amounts of *Staphylococcus* in the skin of PPARa-deficient mice compared to littermate controls. The Nod2 pathway was enhanced, as shown by up-regulation of both Nod2 and its downstream targets, including the antimicrobial peptides mBD3 and mBD4. Moreover, the Th17 immune response was enhanced in keratinocytes and epidermal dendritic T cells. Topical treatment of PPARa-deficient mice with an antibacterial solution did not normalize the upregulation of the innate immunity in keratinocytes. However, the up-regulation of Flg and excessive oxidative stress detected in the epidermis of PPARa-deficient mice were markedly inhibited by topical antibacterial treatment. Thus, our study highlights the overarching role of PPARa in shaping skin immune system and microbiota, with the latter largely controlling Flg expression and inducing oxidative stress in keratinocytes.

LB060
***In vivo* evidence of the brain-gut-skin axis: restoring dysbiosis-associated skin imperfections with bitter orange bioactives**
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Skin and gut microbial ecosystems maintain balance with the host, supporting barrier integrity and immune defense. Dysbiosis in one system can trigger inflammation and dysfunction in the other — the gut-skin axis. Evidence implicates bitter taste receptors (TAS2Rs), expressed in the gut, skin, and brain, as sensors of microbial metabolites modulating immunity, forming a

functional triad.
To confirm this link, an *in vivo* study conducted on 23 Caucasian women with or without gut dysbiosis revealed associations between dysbiosis and increased stress, sedentary lifestyle, and skin imperfections. Microbiota profiling (16S rRNA) characterized shifts in bacterial community composition, while metaproteomic analysis provided insight into cutaneous consequences: heightened local inflammation, impaired barrier integrity, and opportunistic bacterial proliferation. Together, these findings confirmed the impact of quality of life on skin appearance.
A unique extract enriched with hesperetin and naringenin, derived from discarded immature oranges, was developed. Hesperetin selectively activates TAS2R10 and TAS2R14 in transfected cells and exhibits anti-inflammatory (−56%*** IL-8 at 0.1%) and pro-collagen (+51%*** at 0.1%) properties in fibroblasts. The extract also reduces inflammation triggered by bacterial secretome *ex vivo* (−77%** IL-8 at 1%).
A double-blind clinical study in volunteers with gut dysbiosis and stressful lifestyles confirmed that the bitter orange extract at 1% counteracts skin imperfections and improves global skin health and self-confidence. Metaproteomic profiling demonstrated a rebalancing of the dysbiotic signature toward a healthy profile.
This work provides the first *in vivo* characterization of the brain-gut-skin axis, revealing the intricate interplay between gut dysbiosis, skin condition, and quality of life. TAS2Rs, acting as molecular sentinels, represents a compelling cosmetic target for holistically restoring homeostasis.

LB061
Necrotic erythema nodosum leprosum revealing previously undiagnosed multibacillary Hansen disease: a diagnostic challenge
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Necrotic erythema nodosum leprosum (ENL) is a rare and severe type 2 lepra reaction that may present with extensive ulceronecrotic lesions, posing a diagnostic challenge because of its resemblance to infectious and vasculitic dermatoses. Delayed recognition of multibacillary Hansen disease may culminate in this destructive inflammatory complication. We report necrotic ENL as the presenting manifestation leading to the diagnosis of previously undiagnosed multibacillary Hansen disease. A patient presented with progressive painful pruritic ulceronecrotic lesions predominantly affecting both lower extremities, accompanied by constitutional symptoms and a seven-year history of generalized pruritus. Dermatological examination revealed leonine facies, diffuse xerosis, multiple necrotic crusted plaques on both upper extremities, and extensive ulceronecrotic lesions with black eschars involving both lower extremities. Cardinal signs of Hansen disease, including hypoesthetic skin lesions, enlargement of the great auricular and ulnar nerves, and a positive slit-skin smear, were identified. Laboratory investigations demonstrated microcytic anemia, an elevated erythrocyte sedimentation rate (57 mm/hour), and an increased neutrophil-to-lymphocyte ratio (3.83). Slit-skin smear demonstrated a bacteriological index of 1+ and a morphological index of 8%, confirming multibacillary disease. The combination of characteristic clinical findings, bacteriological confirmation, systemic inflammatory features, and favorable early response to systemic corticosteroids supported the diagnosis of necrotic ENL. Treatment with multidrug therapy, systemic corticosteroids, wound care, and topical antimicrobial therapy resulted in reduced inflammation and progressive drying of the ulcerative lesions. This case highlights that necrotic ENL may be the initial manifestation of previously undiagnosed multibacillary Hansen disease. Recognition of cardinal signs and prompt immunomodulatory treatment are essential to prevent irreversible tissue destruction and disability.

LB062
Dissecting Molecular and JAK/STAT Signaling Heterogeneity in Chronic Hand Eczema
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Chronic hand eczema (CHE) is a common and disabling inflammatory skin disease characterized by marked clinical heterogeneity and limited therapeutic options. Current clinical and histopathological classifications often fail to capture its underlying biological diversity, limiting patient stratification and precision treatment. The molecular mechanisms driving CHE, particularly the involvement of intracellular signaling pathways such as JAK/STAT remain poorly defined.
We performed an integrated molecular and signaling analysis of lesional and control skin biopsies from patients with CHE (n=16), atopic dermatitis (AD, n=6), psoriasis (PSO, n=7), and healthy donors (n=6). Targeted transcriptomic profiling of 100 immune-related genes was conducted using Nanostring technology. In parallel, phosphorylation of JAK1-3, TYK2, and STAT1-6 proteins was quantified by immunohistochemistry combined with AI-assisted image analysis. Differential expression, immune module scoring, unsupervised clustering, principal component, and correlation analyses were performed to link gene expression programs with intracellular signaling activity.
CHE lesions displayed a distinct molecular profile that partially overlapped with AD and PSO but remained clearly separable from both. Transcriptomic analyses revealed a predominant type-2 inflammatory signature, with substantial interpatient heterogeneity. Subgroup analyses identified additional activation of IFN-driven, IL-17/neutrophil-associated, or IL-36-related pathways. Phospho-protein profiling demonstrated heterogeneous but structured activation of the JAK/TYK2/STAT axis, with increased pTYK2, pSTAT1, pSTAT5, and pSTAT6 signals. Integration of transcriptomic and signaling data revealed coherent associations between immune modules and pathway activation states at the patient level.
These findings demonstrate that CHE comprises distinct molecular and signaling endotypes not captured by clinical classification alone. Integrated profiling of immune programs and JAK/STAT activity provides a framework for pathway-based stratification and supports the development of precision therapeutic approaches targeting dominant inflammatory axes in CHE.

LB063

Impaired ADAM17-dependent EGFR signaling drives IL-12B-mediated inflammation in neonatal inflammatory skin and bowel disease

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Neonatal inflammatory skin and bowel disease 1 (NISBD1) is an ultra-rare autosomal recessive disorder caused by homozygous or compound heterozygous mutations in the ADAM17 gene. The disease is characterized by severe epithelial barrier dysfunction, recurrent infections and chronic inflammation. The immunopathogenesis of NISBD1 was investigated, and a mechanism-based therapeutic approach was evaluated in a patient carrying a homozygous ADAM17 p.L407S mutation. Lesional skin was analyzed by quantitative RT-PCR, immunofluorescence and flow cytometry. Functional consequences of the ADAM17 variant were investigated using ADAM17-deficient HEK293 cells and CRISPR-engineered keratinocytes expressing the patient-specific mutation. EGFR ligand shedding, epidermal EGFR signaling and Treg populations were assessed. Cytokine-guided off-label treatment with ustekinumab was initiated, and longitudinal clinical and immunological responses were evaluated. The p.L407S variant markedly impaired ADAM17 shedding activity, resulting in reduced amphiregulin release and diminished EGFR signaling in the epidermis. Lesional skin exhibited selective upregulation of IL12B together with a profound reduction of cutaneous Tregs, suggesting impaired EGFR-dependent immune regulation. Based on this molecular profile, treatment with ustekinumab induced sustained improvement of erythroderma, scaling, pruritus and infection frequency, accompanied by reduced IL-12B expression and restoration of circulating skin-homing Tregs. These findings identify impaired ADAM17-dependent EGFR signaling as a driver of Treg deficiency and IL-12B-mediated inflammation in NISBD1 and support the use of comprehensive molecular profiling to guide precision therapy in this ultra-rare disorder.

LB064

Pretreatment multi-compartment profiling reveals non-type 2 programs linked to incomplete dupilumab response in atopic dermatitis

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Dupilumab is effective for moderate-to-severe atopic dermatitis, but baseline molecular features associated with incomplete response remain unclear. We investigated pretreatment multi-compartment programs associated with incomplete week-12 EASI-75 response. Twenty adults with moderate-to-severe atopic dermatitis received dupilumab for 12 weeks. Baseline lesional biopsies were profiled by single-cell RNA sequencing, and serum and tape-strip samples by Olink Explore proteomics. Patients were classified as EASI-75 achievers (n=16) or non-achievers (n=4). An 11-feature non-type 2 composite score was constructed from four serum proteins, three tape-strip proteins and four basal keratinocyte transcripts enriched in non-achievers. Baseline EASI, IgE, TARC and LDH did not clearly define response groups. The signature spanned serum IFNG/TNF/SPP1/COL9A1, tape-strip KLK4/MSMB/SPINK7 and basal keratinocyte KRT17/KRT6A/KRT6B/TNFRSF12A. Several proteomic features remained elevated at week 12 despite treatment. The score correlated negatively with EASI achievement (rho=-0.58, p=0.007) and was higher in non-achievers (Wilcoxon p=0.0029), while showing minimal correlations with baseline TARC, IgE and LDH. These findings identify a baseline non-type 2 multi-compartment signature associated with incomplete response to dupilumab and suggest that non-type 2 profiling may inform future stratification and therapeutic strategies for patients with incomplete response to IL-4/IL-13 blockade.

LB065

Predicting methotrexate discontinuation and stratifying specific failure trajectories in psoriasis within a real-life setting using explainable machine learning

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We are currently unable to predict psoriasis treatment response in individual patients. Unpredictable methotrexate (MTX) failure exposes psoriasis patients to unnecessary toxicity and prolonged poor disease control. We aimed to predict overall discontinuation and identify drivers of adverse events and ineffectiveness in a real-world setting. We analysed 1151 adult patients from the British Association of Dermatologists Biologics and Immunomodulators Register (BADBIR) (2007-2024). Our cohort was derived from 6589 MTX-exposed cases. Patients with prior MTX or concomitant therapies were excluded; baseline PASI and core laboratory data were required. We developed composite and competing-risk XGBoost survival models using all baseline features. Models were validated via 5-fold cross-validation, demonstrating robust discrimination (test C-indices: composite 0.62, AE 0.64, ineffectiveness 0.66). High-risk patients (>median score) failed 57% faster than low-risk cohorts (<median score) (hazard ratio 1.57, 95% CI 1.06-2.34, p=0.02; median survival 485 vs 947 days). One- and two-year survival probabilities were 59.7% and 42.7% (high-risk) compared to 79.3% and 54.8% (low-risk). Stratifying failure modes via SHAP isolated distinct mechanisms: adverse event predictions were driven by physical/metabolic markers (body mass index, platelet count), whereas ineffectiveness was driven by age, demographic factors (e.g. sex), and prior systemic exposure. Finally, these models were integrated into a clinical app providing individualized hazard scores for overall, AE, and ineffectiveness trajectories to guide prescribing.

LB066

PsoriAxis: Mapping a Continuous Transcriptomic Healing Axis in Psoriasis

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Targeted biologics transformed psoriasis treatment, yet clinical improvement often reflects inflammatory suppression rather than cure. Clinically resolved skin remains biologically distinct from healthy skin, consistent with residual disease and relapse. To address this gap, we hypothesized that healing is better modeled as a continuous molecular process than as discrete pre/post-treatment comparisons. We developed PsoriAxis, a computational framework that scores skin transcriptomes along a disease-to-resolution axis.
Public longitudinal transcriptomic datasets were integrated using a trajectory-alignment method developed in our laboratory to construct a shared healing-trajectory metric. Each sample received a continuous score, enabling comparison of molecular response depth beyond visit labels or clinical categories. Multiple independent datasets were projected onto the same axis to assess generalizability. Co-expression modules were grouped by kinetic behavior to identify resolved, residual, and tissue-restoration programs.
Across independent datasets and treatments, PsoriAxis recovered a shared ordering of baseline lesional, treated lesional, and non-lesional samples, showing molecular healing can be quantified along a common axis. Notably, even post-treatment PASI90 responder samples differed molecularly, spanning near-resolved and residual disease-proximal states. Module kinetics showed that healing was not simply a reversal of disease: known inflammatory programs resolved with different timing, residual programs persisted in intermediate states, and underexplored repair-associated programs emerged only with deeper resolution. Thus, PsoriAxis mapped inflammation suppression, residual disease, and active tissue restoration as ordered phases of molecular healing.
In summary, PsoriAxis provides a robust strategy to model resolution and identify both persistent disease programs and active healing programs that may be prioritized for durable disease control.

LB067

Application of Artificial Intelligence to Direct Immunofluorescence in the Diagnosis of Autoimmune Skin Diseases

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Direct immunofluorescence (DIF) is a fundamental tool in the diagnosis of autoimmune skin diseases, particularly in bullous skin diseases. However, the interpretation of DIF patterns is complex, time-consuming and operator-dependent, requiring specialized personnel. The main objective of this study is to develop and test an artificial intelligence (AI) algorithm for the automated classification of DIF patterns in autoimmune skin diseases, to enhance diagnostic accuracy, improve process efficiency and potentially expand the use of the technique beyond referral centers.
Immunofluorescence images from skin biopsies of patients suspected of having an autoimmune skin disease were collected. Skin tissue was obtained via a 4- or 5-mm punch biopsy and underwent standard processing for DIF. The images were classified by experienced Dermatologists into six patterns: linear basement membrane zone (BMZ), granular BMZ, granular in the dermal papillae, intercellular, intercellular plus linear BMZ and negative.
We exploited a transfer learning framework, specifically, we employed an ImageNet pre-trained convolutional neural network model (DenseNet201), fine-tuning it on our images. For the evaluation protocol, we assessed the generalization of the trained models through a stratified k-fold approach. Thus, we split our data into five folds, every time holding one fold out for test, while training on the remaining four.
The average overall test accuracy across the folds was (79.20 +- 3.30) %, while the balanced test accuracy was (77.7 +- 8.70) %. It is important to highlight that, in order to reflect real-life settings, our dataset was intentionally not curated, with data collected with different protocols and acquisition systems. A detailed evaluation of the potential impact of acquisition metadata and imbalance on the model's performance will be the object of future research.
In conclusion, our encouraging results support the feasibility of developing an accurate deep learning trained model that could help implement a standardized approach in the diagnosis of autoimmune cutaneous diseases, potentially improving accuracy and optimizing cost- and time- efficiency.

LB068

PD-1 receptor occupancy and soluble PD-1 kinetics after adjuvant anti-PD-1 in melanoma

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Adjuvant PD-1 antibodies improve recurrence-free survival in high-risk melanoma, yet about a quarter of recurrences occurred off therapy. Little is known about how PD-1 receptor occupancy (RO) on T cells and soluble PD-1 change after discontinuation of anti-PD-1 therapy, or whether these off-therapy kinetics are associated with drug type, treatment duration, or relapse.
We monitored 35 melanoma patients previously treated with adjuvant nivolumab or pembrolizumab from treatment discontinuation (T0p) up to 12 months post-therapy. PD-1 RO, unblocked PD-1 (CD279), bound drug, and PD-1 expression on CD4+ and CD8+ T cells were investigated by flow cytometry at 3 month intervals. Mixed-effects models with REML estimation and Sidak-adjusted post-hoc tests assessed longitudinal changes and explored associations with drug (nivolumab versus pembrolizumab), treatment duration (<6 versus ≥6 months), and relapse within one year. In a subset of non-relapsing patients (n=11), soluble PD-1 (PDCD1) was measured at T0p and T12Mp by Olink‘s Reveal assay.
PD-1 RO on CD4+ and CD8+ T cells declined after treatment, approaching near-zero levels after 12 months, while the proportion of unblocked CD279 increased. PD-1 expression showed a modest rise at 3 months and remained largely stable

thereafter. RO and unblocked-PD-1 kinetics differed significantly between nivolumab- and pembrolizumab-treated patients during the first 6 months post treatment, whereas PD-1 kinetics did not differ with respect to treatment duration or the occurrence of relapses. Soluble PD-1 levels were significantly lower after 12 months than at T0p in the non-relapsing subset, paralleling the reappearance of unblocked PD-1.
To our knowledge, these data provide a first integrated description of cell-bound and soluble PD-1 off-kinetics after discontinuation of adjuvant anti-PD-1 in a real-life setting. The combination of declining soluble PD-1, loss of cellular RO, and preserved PD-1 expression suggests a regulated reshaping of both, soluble and membrane-bound PD-1 after anti-PD-1 withdrawal; the mechanisms driving this redistribution remain unclear and warrant further study.

LB069
Precision imaging and automated analysis for epidermolysis bullosa: Advancing photometry, volumetry, and digital clinimetry
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Accurate assessment of epidermolysis bullosa (EB) lesions is essential for monitoring disease progression and guiding treatment, but conventional evaluation requires in-person visits, placing a substantial burden on patients. This proof-of-concept study aimed to improve EB lesion assessment through single-image analysis, supporting remote evaluation.
We analyzed 157 images from 29 patients with EB, captured with the Scarletred®Vision mobile application, a clinically validated software as a medical device platform providing consistent, high-quality image capture in real-world settings. A deep learning model (CNN) originally developed for psoriasis monitoring was fine-tuned on the EB images to segment lesions and delineate affected tissue. A second model, trained to distinguish intact from broken skin, identified open wounds requiring further attention. Image processing applied to the segmented areas detected and counted blisters, using augmented signal maps to enhance contrast between lesions and
surrounding tissue.
The platform also supports three-dimensional surface reconstruction from a single phone capture, enhancing blister and affected area topological characterization, such as height and volume measurement relative to the surrounding skin; this capability was tested on an EB staging model and offers a promising extension of the two-dimensional measures.
The segmentation model isolated EB lesions from healthy tissue with high accuracy, enabling targeted blister detection and counting. The wound detection model reliably identified regions of broken skin, providing further insight into lesion severity relevant to wound care. Together, lesion segmentation, blister counting, and open wound detection formed a comprehensive system for automated EB lesion assessment, reducing the need for in-person evaluation and supporting remote, continuous monitoring and treatment planning, with potential application to other rare, chronic skin conditions.

LB070
Decoding the Skin Spectrum: Automated Fitzpatrick classification from facial imagery
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Accurate skin type classification is essential in dermatology and cosmetology, as skin tone influences diagnostics, treatment, and product development. However, traditional Fitzpatrick assessment is often subjective and inconsistent across diverse populations. This study aimed to estimate the Fitzpatrick skin type automatically from facial images, combining deep facial segmentation with color analysis to improve objectivity and inclusivity.
Images spanning diverse ethnicities and Fitzpatrick types were acquired using Scarletred®Vision mobile app and a calibration skin patch, ensuring standardized lighting and color calibration. In the first step, facial landmarks detection was used in combination with multi class face segmentation to obtain skin, hair, iris, and eyebrow regions. Color features were extracted using the CIE-LAB color space, while the Individual Typology Angle (ITA) map was used to estimate the skin tone. To mitigate effects of temporary erythema in lighter skin phototypes (I–III), a redness correction was applied. Finally, a custom decision tree model was developed for automated Fitzpatrick classification. A phototype validation was conducted using portrait images from 128 subjects collected from a controlled clinical trial, independently assessed by physician for skin phototype and compared with the automated estimates. Agreement was quantified as exact match or one, two, and three or more level differences.
Using a clinically validated acquisition platform, we achieved reliable classification across all six Fitzpatrick types. The automated pipeline successfully segmented key facial regions and extracted the dominant colors, showing robustness to variations in lighting and background. Phototype measurements showed high agreement with physician assessments, with 85% of cases within one level of difference. This study demonstrates that Fitzpatrick type can be automatically estimated from facial images using region specific segmentation and advanced color analysis, supporting effective and equitable remote assessment across all skin types.

LB071
Translational Dermatology Research in Italy: Current Landscape, Challenges, and Future Directions from the SIDeMaST Study Group on Experimental Research and Bioengineering in Dermatology
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Translational research is a key driver of innovation in dermatology, bridging laboratory discoveries and clinical practice. However, the current organization of translational dermatology research in Italy has not been systematically evaluated.
Objectives: to assess the current landscape of translational dermatology research in Italy, including research activity, infrastructure, collaborations, scientific productivity, funding, training, perceived barriers, and future priorities.
A cross-sectional national survey was conducted among members of the SIDeMaST Study Group on Experimental Research and Bioengineering in Dermatology. An anonymous 27-item electronic questionnaire was distributed to all members (n=42). Results were analyzed using descriptive statistics.
Thirty-five members completed the survey (response rate 83.3%). Most respondents were actively engaged in translational research, although it accounted for a limited proportion of their professional activity. Multidisciplinary collaboration was the predominant research model, with generally good access to laboratory facilities and molecular technologies. Scientific

productivity was satisfactory despite limited funding. Major barriers included unequal access to specialized infrastructures, insufficient training opportunities, regulatory complexity, and limited involvement of early-career researchers. All respondents supported establishing a national translational dermatology research network.
Translational dermatology research in Italy is supported by an active collaborative community but is limited by organizational, educational, financial, and regulatory challenges. National initiatives promoting collaborative networks, shared infrastructures, structured training, and sustainable funding could strengthen its international competitiveness and clinical impact.

LB072 [WITHDRAWN]

LB073
Adjunctive Topical Tapinarof for Persistent Localized Psoriatic Lesions During IL-17A Inhibitor Therapy
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Biologics targeting the IL-17A axis have markedly improved psoriasis outcomes; however, some patients develop persistent lower-extremity plaques despite otherwise satisfactory disease control.
To investigate the molecular characteristics of persistent lesions during IL-17A inhibitor therapy, We enrolled patients with psoriasis who achieved PASI75 or greater after at least 52 weeks of IL-17A inhibitor therapy but had persistent lower-extremity lesions. Bulk RNA sequencing was performed on persistent lesions (PL), clinically resolved lower-extremity lesions (RL) from the same patients, and baseline lesions (BL) from treatment responders, followed by validation in an independent cohort. Principal component analysis demonstrated that PL clustered closely with BL, whereas RL separated distinctly from BL, indicating that persistent lesions failed to undergo treatment-associated transcriptomic remodeling. Compared with RL, PL retained higher expression of IL17A, IL17F, IL23A, and other IL-17 pathway–related genes, whereas CYP1A1 expression remained significantly lower. Notably, CYP1A1 downregulation was observed only in PL but not in RL relative to BL, suggesting that CYP1A1 dysregulation may represent a molecular feature of persistent localized lesions.
Because CYP1A1 is a canonical downstream target of the AhR, we investigated whether restoration of AhR-CYP1A1 signaling could improve persistent lesions. Five patients with persistent lower-extremity lesions despite stable IL-17A inhibitor therapy received adjunctive topical tapinarof while maintaining their biologic regimen. After 16 weeks, all patients demonstrated marked clinical improvement. Transcriptomic analysis before and after treatment demonstrated restoration of CYP1A1 expression accompanied by reduced expression of IL-17 pathway–related genes.
Impaired AhR-CYP1A1 signaling may represent a molecular feature of persistent localized psoriatic lesions during IL-17A inhibitor therapy. Adjunctive topical AhR-CYP1A1 agonist therapy while maintaining the original biologic regimen may offer a therapeutic option for managing persistent localized lesions in patients with otherwise well-controlled disease.

LB074
Relationship between Anisakis-specific IgE levels and basophil activation induced by Anisakis extract
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Anisakis allergy is expected to become increasingly clinically important worldwide due to the global expansion of raw seafood consumption and the increasing abundance of Anisakis larvae in marine fish. However, diagnosis remains challenging because oral food challenge testing is impractical and no standardized skin-test reagent is commercially available. While Anisakis-specific IgE detects sensitization, it does not necessarily reflect functional allergic reactivity. The purpose of this study was to investigate the relationship between Anisakis-specific IgE levels and BAT results and to explore mechanisms underlying discordant findings.
Thirty-six patients presenting with allergic reactions after seafood consumption were retrospectively analyzed. Anisakis-specific IgE was measured using ImmunoCAP, and basophil activation was evaluated by quantifying CD203c expression following stimulation with phosphate-buffered saline extracts of Anisakis. Mean basophil activation rates (mean ± SD) were 1.69 ± 2.80 in class 0 (n=9), 48.74 ± 62.31 in class 1 (n=3), 89.48 ± 33.56 in class 2 (n=8), 109.32 ± 13.49 in class 3 (n=7), 101.05 in class 4 (n=1), 101.97 ± 22.87 in class 5 (n=5), and 102.71 in class 6 (n=1). Two basophil non-responders were excluded. BAT positivity was observed in 0/9 class 0, 2/3 class 1, 7/8 class 2, and all class 3–6 patients. In one class 2 patient with negative BAT results, Western blot analysis demonstrated IgE binding to proteins from both crustacean and Anisakis extracts, suggesting cross-reactive IgE rather than functional sensitization.
These findings indicate that Anisakis-specific IgE positivity does not always correspond to functional basophil activation. BAT may provide complementary information regarding sensitization, particularly in low-level IgE-positive patients including class 1 cases. Combining allergen-specific IgE measurement with BAT may improve in vitro diagnosis by distinguishing functional sensitization from cross-reactive or clinically irrelevant IgE responses.

LB075
Proteomic Assessment of Povorcitinib in Patients With Vitiligo
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Vitiligo is an autoimmune disease in which melanocyte destruction causes skin depigmentation. Selective Janus kinase (JAK)1 inhibition promotes repigmentation, yet underlying molecular mechanisms remain unclear. Povorcitinib, an oral, selective JAK1 inhibitor, is under evaluation for treatment of autoimmune and inflammatory diseases including nonsegmental vitiligo (NSV). In a phase 2, randomized, dose-ranging study in adults with extensive NSV (NCT04818346), povorcitinib showed substantial repigmentation vs placebo (PBO) and was well tolerated at 15, 45, and 75 mg doses; no clear clinical dose response was observed.
Serum samples were collected at baseline and Weeks 4, 12, and 24 and analyzed using Olink Target 48 and Explore panels. Change from baseline and difference from PBO were assessed using mixed-effects repeated-measures models with Benjamini-Hochberg False Discovery Rate correction for multiple comparisons.
Povorcitinib produced dose-dependent changes in serum proteins (log2 fold-change of ≤−0.25; $P \leq 0.05$) at Week 24. In the Target 48 panel, proteins significantly reduced from baseline included none with PBO, 5 with 15 mg (2 vs PBO), 8 with 45 mg (7 vs PBO), and 12 with 75 mg (12 vs PBO) povorcitinib doses. In the Explore panel, proteins significantly reduced from

baseline included 2 with PBO, 109 with 15 mg (14 vs PBO), 85 with 45 mg (45 vs PBO), and 330 with 75 mg (57 vs PBO) povorcitinib doses.
Key downregulated proteins spanned T-cell activation (TNFRSF9, SLAMF1), cytotoxic effector molecules (granzyme A, granzyme B, FASLG [Explore, significant vs PBO across doses]), chemokines mediating T-cell trafficking and tissue recruitment (CCL19 [Target 48, significant vs PBO across doses], CCL21, XCL1, CXCL9, CXCL10), and proinflammatory cytokines (IL-12, TNF, LTA [Target 48, significant vs PBO across doses], IFNL1).
While further investigation is warranted, pathway analyses suggest broad suppression of inflammatory and cytokine signaling—consistent with disruption of the autoimmune cascade targeting melanocytes—and support clinical repigmentation in patients with NSV with povorcitinib treatment.

LB076
Clinical characteristics and treatment outcomes assessed by time to next treatment in 136 patients with mycosis fungoides : a single-center retrospective study
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Mycosis fungoides (MF) is the most common subtype of cutaneous T-cell lymphoma. Its variable stage and skin manifestations make treatment selection challenging and often dependent on physicians' experience. We retrospectively reviewed the clinical characteristics, treatment patterns, and outcomes of patients with MF at our institution by time to next treatment (TTNT).
We reviewed 136 patients with MF who visited our department between January 2015 and December 2024. Clinical data and treatment histories were obtained from medical records. For systemic therapies, TTNT was defined as the interval from treatment initiation to the next treatment or death. Patients without subsequent treatment were censored at last follow-up. Median TTNT was estimated by the Kaplan–Meier method.
The cohort included 82 men and 54 women, with a median age of 65 years. At first visit, 89 patients had stage I disease, 29 stage II, 14 stage III, and 4 stage IV; one-third had stage IIB or higher disease. Median TTNT was 24 months for bexarotene (n=38), 16 months for etretinate (n=27), 20 months for systemic interferon-γ (n=10), 26 months for brentuximab vedotin (n=4), 3.5 months for forodesine (n=4), 10 months for etoposide (n=3), and 1.5 months for denileukin diftitox (n=2). Median TTNT was not reached for mogamulizumab (n=7).
Among patients treated with bexarotene, median TTNT by T classification at treatment initiation was not reached for T1 (n=2), 18 months for T2 (n=3), 27 months for T3 (n=26), and 12 months for T4 (n=7). Bexarotene was used most frequently in T3 disease and continued for a substantial period despite tumor-stage lesions. Median TTNT was 27 months with bexarotene monotherapy and 24 months with bexarotene plus systemic interferon-γ, with no significant difference (log-rank p=0.54). Because interferon-γ was often added after disease worsening, comparable TTNT may suggest that add-on interferon-γ helped maintain bexarotene treatment in selected patients.
TTNT summarized real-world treatment continuation across systemic therapies for MF. The prolonged TTNT observed in some agents may partly reflect combination therapy.

LB077
Observational study on cutaneous manifestations of Chikungunya in pediatric patients
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The study aimed to evaluate cutaneous manifestations of chikungunya, identify early and late cutaneous manifestations in children to improve clinical diagnosis, and differentiate it from other viral diseases.
An observational cross-sectional study was done in children aged 0-14 years diagnosed according to clinical criteria or with a confirmed laboratory diagnosis, who have been attending the dermatology clinic at Lady Ridgeway Hospital for Children for 16 weeks since April 2025.
The data of 19 children were analyzed. Hyperpigmentation was present in all. Linear pigmentation was observed in 84.2 % while spotty pigmentation was observed in 57.9%, and chik sign in 10.5%. Other cutaneous manifestations were macular papular rash in 47.4%, blisters in 15.8 %, skin peeling in 47.4 %, mucositis in 31.6%, pedal edema in 26.3%, and nail changes in 5.3%. Rash was asymptomatic in 63.2%, itching in 21.1%, and burning in 15.8% of children. Fever was observed in 94.7%. Joint pain in 47.4%, and fits were observed in 15.8%. Only 36.8 % of children were able to do an antibody test, in which 85.7 % were positive for IgM antibody.
Cutaneous manifestations occur in almost all children affected with chikungunya. They occur in diverse patterns like hyperpigmentation, blisters, purpura, and macular papular rash. Cutaneous manifestations in children significantly help in clinical diagnosis, even with the limited resources to confirm it serologically.

LB078
Clinical and serological analysis of flounder roe allergy caused by cross-reactivity with alpha-Gal syndrome
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Alpha-Gal syndrome is a condition in which a person, following a tick bite, becomes sensitized to the sugar chain galactose-alpha-1,3-galactose (alpha-Gal) present in tick saliva, leading to the development of red meat allergy, cetuximab allergy, and other conditions. Our analysis has shown that, in Japan, patients with alpha-Gal syndrome are known to develop allergies to flounder roe due to cross-reactivity, but the details remain unclear. The aim of this study was to elucidate the clinical and serological characteristics of flounder egg allergy as part of alpha-Gal syndrome through detailed medical history interviews and the use of methods such as the CAP-FEIA assay and Western blot analysis. Of the 35 patients with flounder roe allergy, 25 developed an allergic reaction after consuming red meat. Of these patients, 21 had developed an allergy to red meat before developing an allergy to flounder roe. 14 developed anaphylactic shock after consuming flounder roe. Of the 36 allergic reactions for which details are known, in 19 cases, the allergic reaction occurred more than 3 hours after the consumption of flounder roe. All 35 patients were alpha-Gal-specific IgE-positive. Using the Western blot method, we found that IgE from these patients bound to soluble tick salivary proteins, soluble beef protein, insoluble flounder roe protein, and cetuximab. The results of the inhibition assay revealed that the binding of their IgE to soluble beef protein and insoluble flounder roe protein was

weakened or eliminated in a concentration-dependent manner by the soluble beef protein used as an inhibitor, indicating that their specific IgE cross-reacts with red meat and flounder roe. Allergies to flounder roe, which are part of alpha-gal syndrome, often manifest with a delayed onset and tend to be associated with a high incidence of anaphylactic shock. Given the global increase in the consumption of fish and fish roe, caution is needed regarding flounder roe allergies associated with alpha-Gal syndrome.

LB079

Trends in Cutaneous Malignancy Incidence, Immunosuppression Mediation, and Risk-Stratified Surveillance in United States Kidney Transplant Recipients

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Cutaneous malignancy is a leading cancer after kidney transplantation, yet demographic risk stratification for dermatologic surveillance remains incompletely defined. Temporal trends in cutaneous squamous cell carcinoma (SCC), basal cell carcinoma (BCC), and melanoma among United States adult kidney transplant recipients were characterized, and risk-stratified cumulative incidence estimates were derived. A retrospective cohort of 398,216 adult recipients transplanted 2000-2022 was drawn from the Organ Procurement and Transplantation Network Standard Transplant Analysis and Research file; three-year incidence was modeled by joinpoint regression, and Aalen-Johansen cumulative incidence was estimated within 16 demographic-by-donor strata. Within 3 years, 5,846 SCC, 3,571 BCC, and 628 melanoma cases were ascertained. SCC and BCC incidence rose steeply then inflected in 2011 (SCC pre-/post-joinpoint annual percent change +10.7%/−2.4%) and 2012 (BCC +9.6%/−3.0%); melanoma rose monotonically (+3.98%/year). Initial immunosuppression mediated 17.3% (95% CI, 13.0–21.8%) of the SCC year effect; approximately 80% remained unexplained. Five-year cumulative incidence of any cutaneous malignancy ranged from 14.4% (non-Hispanic White, ≥65 years, living donor; number needed to screen, 7) to 0.13% (non-Hispanic Black, living donor; 776), with 52–66% of the 15-year burden accruing by year 5 in high-risk strata. SCC/BCC incidence has plateaued while melanoma continues to rise, with absolute risk varying more than 100-fold across strata. These findings support further investigation into the proposed risk-stratified dermatologic surveillance: annual full-body examination through at least post-transplant year 10 for non-Hispanic White recipients aged ≥50 years (particularly living-donor); biennial for intermediate-risk strata; and risk-factor-based referral for low-risk groups, with attention to SCC predominance in non-Hispanic Black recipients. Accrual beyond year 10 argues against tapering high-risk surveillance; integration into dermatology pathways may improve detection.

LB080 [WITHDRAWN]

LB081

Corticosteroid-refractory Sweet syndrome in high-risk chronic lymphocytic leukemia responding to acalabrutinib

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Sweet Syndrome is an acute neutrophilic dermatosis that can occur in the setting of hematologic malignancies, but is rarely described in chronic lymphocytic leukemia. We report a 69-year-old woman, with a six-month history of recurrent fevers and fatigue, followed by abrupt development of tender, edematous, erythematous papules and plaques on the extremities, face and neck. Further examination revealed hepatosplenomegaly and generalized lymphadenopathy and blood counts showed persistent lymphocytosis. Immunophenotyping demonstrated a clonal B-cell population that was CD5+, CD19+, CD23+, while cytogenetics revealed trisomy 12 with 17p deletion and TP53 disruption, consistent with high-risk chronic lymphocytic leukemia. Skin biopsy showed dense dermal neutrophilic infiltrates without leukocytoclastic vasculitis, and immunohistochemistry identified mature neutrophils that were MPO+, CD34-, CD117-. Fluorescence in situ hybridization of the lesions was negative for the bone marrow abnormalities, excluding leukemia cutis, supporting the diagnosis of paraneoplastic Sweet syndrome. The lesions showed transient response to escalating corticotherapy, relapsing on tapering, while second-line therapies, including colchicine, dapsone, methotrexate and adalimumab failed to achieve clinical remission. The Bruton tyrosine kinase inhibitor (BTKi) acalabrutinib was then administered for the underlying leukemia. Within a month of treatment, a marked and sustained remission of the lesions was observed, which notably preceded the hematologic response. To our knowledge, this marks among the first reports of corticosteroid-refractory Sweet syndrome responding to acalabrutinib, with cutaneous lesions clearing before the leukemic response. This outcome suggests that BTK inhibition may act beyond tumor debulking and control of the underlying clone, and contribute to modulating the key pathophysiological effectors of Sweet syndrome, identifying BTKi as a candidate therapy in such cases.

LB082

Dermatology-Outpatient Prevalence and Clinical Profile of Hidradenitis Suppurativa in Mainland China: A Nationwide Multicenter Retrospective Study

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Hidradenitis suppurativa (HS) exhibits substantial heterogeneity, yet large-scale data on its prevalence and clinical profiles in the Chinese population are limited. This nationwide multicenter retrospective study aimed to estimate the prevalence of HS among dermatology outpatients in mainland China and to characterize its clinical spectrum, severity distribution, and risk factors for severe disease.
Fifty tertiary dermatology centers covering 31 administrative regions participated. Using all dermatology outpatient visits in 2025, we calculated the HS detection rate. Included were patients with clinician-confirmed HS or HS-related syndromes;

excluded were those with alternative diagnoses, single visits lacking evidence of chronicity, or missing critical data. Among 19,563,708 dermatology outpatient visits, 11,418 HS spectrum cases were identified, yielding rates of 58.36 and 37.26 per 100,000 for the overall HS spectrum and for HS/acne inversa alone, respectively. The male-to-female ratio was 4.45:1 (9,322 vs 2,096). The core cohort comprised 1,212 patients (81.2% male). Compared with Western cohorts, Chinese patients had lower BMI, less familial clustering (6.7%), and distinct lesion topography, with lower groin involvement (14.5%) but higher scalp (29.4%) and gluteal (22.5%) involvement. Sex-dimorphic distribution was observed. Median diagnostic delay was 2.0 years. Hurley stage III accounted for 44.7% of patients. Disease duration was the strongest predictor of severe disease (adjusted OR 8.67 for >10 years). Sex-stratified analysis revealed that male severity was driven by comorbidities and metabolic burden, whereas female severity was predominantly driven by diagnostic delay. Incision and drainage rates remained high across all stages, and fewer than half of stage III patients received biologics or radical excision.
Chinese patients with HS exhibit a clinical profile substantially different from that of Western populations. Severe disease is markedly undertreated, highlighting the need for improved awareness and standardized management in China.

LB083
The Heavy Toll of Grief and Poverty: A Case of Head-to-Toe Rupioid Psoriasis and Severe Malnutrition Following Bereavement
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A 46-year-old male presented with a devastating, generalized skin eruption covering him literally from head to toe. The lesions were classically rupioid-thick, yellowish-brown, and heavily crusted-yielding a Psoriasis Area and Severity Index (PASI) score of 21. The patient was diagnosed with plaque psoriasis 7 years ago but completely stopped all treatment 4 years ago after the death of his mother, which left him living entirely alone.
On examination, the patient was visibly shaking, cachectic with a 10 kg weight loss over the past year, and in severe pain. Blood work confirmed systemic depletion, revealing profound hypoalbuminemia, severe vitamin D deficiency, and critically low iron levels. To ensure absolute therapeutic safety, a rigorous diagnostic checklist was deployed: immediate skin scrapings and dermoscopy ruled out ectoparasites; serology excluded HIV and secondary syphilis; and a skin biopsy was performed. Histopathology definitively demonstrated the classic architecture of psoriasis vulgaris.
Management required a rapid approach. To address his systemic collapse,nutritional support and metabolic rehabilitation were initiated immediately. Topically, hyperkeratotic plaques were treated with keratolytics to facilitate de-scaling. Concurrently, systemic biologic therapy with adalimumab was safely initiated at a dose of 80 mg subcutaneously, followed by 40 mg every other week.
This case serves as a powerful reminder that psoriasis is a systemic disease deeply connected to a patient's mental health and social environment. In extreme, neglected variants, establishing a thorough diagnostic protocol to rule out infectious mimics like crusted scabies is a mandatory safeguard. Mistaking an active infestation for psoriasis can lead to catastrophic complications upon initiating anti-TNF therapy. Ultimately, healing this vulnerable patient required a compassionate, multidisciplinary approach combining targeted biologics with nutritional rehabilitation and social support.

LB084 [WITHDRAWN]

LB085
The Protective Effect of Tranexamic Acid on Post-Inflammatory Hyperpigmentation, Erythema, and Melasma in Acne Patients: A TriNetX Cohort Study
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Post-inflammatory hyperpigmentation (PIH) and erythema (PIE) frequently complicate acne vulgaris, often persisting beyond active lesions and worsening quality of life, while melasma remains a common comorbid pigmentary disorder in this population. Tranexamic acid (TXA), by inhibiting plasminogen-driven melanocyte activation and modulating vascular endothelial growth factor expression, has shown promise for pigmentary and vascular skin conditions. Still, its real-world effects on acne-associated PIH, PIE, and melasma remain poorly characterized.
We used TriNetX, a federated real-world EHR network, to compare pigmentary and vascular outcomes between propensity-matched acne patients with and without TXA exposure. Outcome analysis was performed on 60,111 patients after propensity matching for age, gender, race, ethnicity, and pre-existing comorbidities.
We found TXA reduces the risk of PIH ($P < 0.0001$), PIE ($P<0.05$), and other specified pigmentation disorders such as melasma ($P < 0.0001$). These findings suggest TXA may confer a broad protective effect against post-inflammatory pigmentary and vascular sequelae of acne, consistent with its known inhibition of plasminogen-driven melanogenesis and modulation of VEGF-mediated vascular reactivity. This real-world analysis extends prior trial evidence, largely limited to melasma, to a broader acne population with a substantially greater sample size.
TXA use in acne patients is associated with a reduced risk of developing PIH, PIE, and melasma, supporting its potential role as an adjunctive therapy for mitigating post-inflammatory pigmentary and vascular sequelae of acne. Prospective studies are warranted to confirm these associations, define optimal dosing and administration strategies, and establish TXA's role in standard acne management protocols.

LB086 [WITHDRAWN]

LB087 [WITHDRAWN]