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Experimental Dermatology

ABSTRACT

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Allergology

P001 | Hereditary alpha tryptasemia: A retrospective study at a comprehensive allergy center

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Introduction: The clinical significance of elevated baseline serum tryptase (BST) in the absence of mast cell disorders or allergic reactions has long remained unclear. Recently, a genetic variation of the TPSAB1 gene, which among other encodes for alpha tryptase, has been reported and named Hereditary Alpha Tryptasemia (HaT). HaT has been linked to various manifestations, including severe allergic reactions. However, clinical studies are limited. Here, we aim to determine HaT prevalence and characterise its clinical manifestations in patients at a specialised allergy center.
Methods: From January 2022 to December 2023, patients with at least once elevated BST were screened for HaT at the outpatient clinic. A control group included patients with a history of anaphylaxis undergoing specific hymenoptera immunotherapy. TPSAB1 copy numbers, BST levels, and clinical parameters were assessed and analysed.
Results: Of 47 patients with elevated BST (≥11.4 μg/L), 93% showed increased TPSAB1 copy numbers. Individuals diagnosed with HaT displayed a BST range between 9.6 μg/L to 28.4 μg/L, with 84.4% associated with TPSAB1 duplication and 15.6% with triplication. HaT predominated in females (86.7%) and was associated with thyroid disease (26.7%). Over half had a history of anaphylaxis (55.6%), mainly low-grade.
Discussion: In patients with elevated BST but no mastocytosis, the most likely cause of elevated BST is an increase in the copy number of the TPSAB1 gene. A heightened risk of anaphylaxis, especially hymenoptera venom allergy, should be considered. Further research is needed to explore the female predominance and the emerging link with thyroid disease.

P002 | Higher levels of MSCRAMM ligands in AD skin colonised with *Staphylococcus aureus*

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Patients with atopic dermatitis (AD) have a dysbiosis within the skin microbiome, often characterised by a large overgrowth of *Staphylococcus aureus*. While the importance of *S. aureus* as a driver of disease symptoms has been extensively studied, the factors underlying the favourable conditions for the pathogen are not fully understood.
This study aimed to quantify serum coagulation factors in the skin, several of which serve as ligands for *S. aureus* microbial surface components recognising adhesive matrix molecules (MSCRAMMs).
Tape stripping samples from the lesional skin of 87 patients with AD were analysed for fibrinogen, prothrombin,

antithrombin, factor XIII, fibronectin, plasminogen and its homologue apolipoprotein A1, and compared to samples from healthy controls and non- AD *S. aureus* skin infections by ELISA and bead-based immunoassays. Skin swabs from the same site were tested for *S. aureus*, and AD patients were subdivided depending its presence. The severity of AD was assessed by SCORAD as well as the local SCORAD of the lesion investigated.

Our study confirms previous studies reporting that presence of *S. aureus* is associated with higher SCORAD, which was also reflected by the local SCORAD. Correcting for disease severity, samples from AD lesional skin colonised with *S. aureus* expressed significantly higher levels of fibrinogen, antithrombin, plasminogen, prothrombin, and factor XIII compared to those without. Compared to healthy skin, these levels were increased as well (irrespective of *S. aureus* presence), but not significantly different from *S. aureus* infected non-AD skin. Notably, the levels of coagulation factors did not correlate with disease severity. Samples derived from patients treated with Dupilumab, which has been reported to have a beneficial effect on the skin microbiome diversity, showed lower levels of several factors tested.

In conclusion, our study demonstrates that AD skin harbours increased levels of coagulation factors, some of which may act as MSCRAMM ligands, thereby presumably facilitating *S. aureus* colonisation. While *S. aureus*-colonised skin lesions depict even higher levels, this appears to be reversable by Dupilumab therapy. MSCRAMM ligands are therefore a putative therapeutic target for inhibiting *S. aureus* overgrowth and thereby restoring a healthy skin microbiome.

P003 | Regulation of the AhR signalling pathway during skin allergy

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The aryl hydrocarbon receptor (AhR) has a well-known role in regulating skin barrier function and allergic reactions. Its activity is regulated by the AhR repressor (AhRR) and three cytochrome P450 (CYP) enzymes, CYP1A1, CYP1A2 and CYP1B1, that metabolise AhR ligands. We have recently shown that the AhRR is highly expressed in immune cells but not epidermal cells suggesting a different regulatory mechanism for AhR in skin cells. Therefore, the molecular mechanisms and beneficial or adverse consequences of dermal AhR activation are not entirely understood. Here we show how depletion of AhR regulatory pathways such as AhRR and CYP enzyme deficiency, affect onset and pathology of allergy models, such as contact hypersensitivity (CHS) and atopic dermatitis (AD). Mice lacking AhRR and CYP enzymes display different symptoms during CHS despite both being negative regulators of AhR activity. This is potentially caused by differences in the immune response in these mice. Thus, our findings will help to understand the mechanism of regulation of AhR activation by AhRR and CYP enzymes in the skin allowing future development of therapeutic agents for AD that target the AhR signalling pathway in the skin.

P004 (OP03/01) | Nickel uptake into bone marrow derived dendritic cells (BMDC) is an active, but TLR4-independent process

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Metal ions, such as nickel (Ni2+) are among the most frequent causes of allergic contact dermatitis (ACD). For initiation of an immune response resulting in allergic sensitization, antigen is normally taken up by antigen-presenting cells (APC), presented by MHC determinants together with an activation signal to prime T cells. For nickel, activation is achieved by nickel binding to the Toll like receptor 4 (TLR4). However, it is recently unclear, whether presentation of nickel ions in the context of MHC is preceded by nickel uptake as seen for other antigens, or whether immune recognition of nickel is facilitated by the formation of bonds directly with amino acids side chains of proteins, such as MHC. Therefore, we studied in vitro nickel uptake into bone marrow-derived dendritic cells (BMDC) derived from mice with different status for TLR4 expression.

To investigate the uptake process of nickel, we incubated BMDC with NiCl2 in vitro in the absence or presence of LPS as a maturation signal and analysed the content of intracellular nickel by staining with Newport Green. To clarify the impact of TLR4 to the intracellular uptake of nickel, BMDC derived from mice expressing none, the murine, or the human TLR4, respectively. To further study active vs. passive uptake mechanism, nickel incubation was allowed under normal cell culture conditions at 37°C as well as at 4°C.

In detail, we used B10.ScN mice lacking the Tlr4 gene ('knockout'). Further, B10.ScN mice transgenic for the murine Tlr4 (B10.ScN-mTlr4) correspond the normal murine expression ('wildtype') and B10.ScN mice transgenic for the human TLR4 (B10.ScNhuTLR4) express the human equivalent ('human'). Since mice expressing the murine Tlr4 do not have the respective Ni2+ binding sites in their receptor, these mice usually lack binding and activation of the TLR4 pathway after nickel application and therefore do not develop nickel sensitization.

Analysing nickel content by flow cytometric detection of NPG signal in CD11c+ MHCII+/++ BMDC clearly showed an increase in signal after 24 h of incubation with NiCl2. This increase was seen in BMCD from all mouse strains, B10.ScN 'knockout' controls, B10.ScN-mTlr4 'wildtype' controls, and B10.ScN-huTLR4 'human' equivalent, indicating that nickel uptake is not dependent on human or murine TLR4.

Further, the nickel signal was higher when BMDC were incubated with Medium +NiCl2 compared to LPS + NiCl2, which is in line, that rather immature to semi-mature BMDC than mature BMDC take up antigen. Concordantly, when dissecting the maturation status of BMDC by expression levels of MHCII, we found that MHCII+ expressing BMDC have higher NPG levels compared to MHCII++ expressing BMDC.

In another set of experiments, we incubated BMDC at 4°C instead of 37°C. Here, none or only a slight increase of NPG signal was detectable, which indicates to a rather active uptake mechanism of nickel into the cells. This was true for all types of BMDC, irrespective of expression status of TLR4.
Together, our results clearly show that nickel uptake is an active process, which is not dependent on TLR4. Nickel might use transporters such as the divalent metal transporter 1 (DMT1) or ZIP transporters, which are used for other heavy metal ions such as Zn2+, Fe2+, Cu2+. Further studies are needed to clarify whether nickel is taken up by such pathways and whether this uptake is a prerequisite for antigen presentation and therefore is obligatory during sensitization process.

P005 | Differential responsiveness of human skin mast cells to SCF and IL-33—Reduced reactivity to SCF but not to IL-33 in the post-mitotic phase

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Background: Skin mast cells (MCs) are crucial effector cells in acute allergic reactions, but they also contribute to chronic diseases like urticaria, atopic and contact dermatitis, psoriasis, rosacea and others. In the skin environment, MCs are long-lived. Following extraction from skin, MCs proliferate for a period of several weeks and then exit the cell cycle permanently, but still survive in the presence of SCF. IL-33 acts as a further growth factor of skin MCs, which does not induce proliferation on its own but increases mitogenesis by SCF. How early responses are differentially regulated by these two factors remains poorly defined. It is also unclear how MCs respond to SCF and IL-33 after a definitive exit from the cell cycle.
Methods: Primary human MCs were isolated from foreskin tissue and cultured in the presence of SCF and IL-4. Induction of immediate early genes and cytokines was assessed by RT-qPCR and ELISA. In selected experiments, actively cycling MCs (2–4 weeks) were compared with post-mitotic cells (≥6 weeks, plateau phase). Phosphorylation of signalling intermediates was determined by immunoblot.
Results: Among early response genes, SCF induced FOS, EGR1 and NR4A2 with greater potency than IL-33, while IL-33 was strikingly more efficient at stimulating JUN. Regarding cytokines, TNF, CCL1 and IL-13 were preferentially activated by IL-33, while SCF activated higher levels of LIF. We queried whether permanent exit from the cell cycle is accompanied by hyporesponsiveness to SCF. Indeed, the phosphorylation of KIT, ERK1/2, AKT, and STAT5 was overall decreased and/or of shorter duration in the plateau phase compared to actively cycling cells. Cytokine stimulation by SCF was still detectable in post-mitotic MCs, yet with somewhat lower potency. Responsiveness to IL-33 was unperturbed after exit from the cell cycle, and in fact even further increased for selected cytokines.
Conclusions: IL-33 and SCF differ in the repertoire of the early responses they elicit in human skin MCs. After chronic exposure to SCF, MCs become less responsive to the growth factor, but do not lose reactivity completely. Interestingly, reduced stimulability of post-mitotic MCs is limited to SCF, since cells simultaneously retain (or even further upregulate) sensitivity to IL-33 after exit from the cell cycle.

P006 | Positive outcome of the pivotal Phase III study with PQ Grass 27600 SU, a modified grass allergen subcutaneous immunotherapy (SCIT) product using MicroCrystalline Tyrosine (MCT) and monophosphoryl lipid A (MPL) as adjuvant system

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Rationale: PQ Grass 27600 SU is a modified broad spectrum grass allergen SCIT product using MCT & MPL as adjuvant system. During development phase of PQ Grass, the optimal cumulative dose (OCD) of 27600 SU was established in Phase II dose finding1 based on a highly statistically dose–response relationship with plateau formation. ED50 was identified at 2900 SU and the currently marketed formulation of 5100 SU demonstrated significant efficacy representing 81% of the theoretically possible effect (ED81). Interestingly, almost identical outcomes were observed for the birch product of this platform2 (OCD = 27 300 SU, ED50 = 2723 SU, ED83 = 5100 SU). Following a first explorative phase III field study 3, we report the results of primary & secondary outcome of the RESONATE study conducted to support a marketing authorisation application.
Methods: RESONATE was a Phase III randomised double-blind, placebo-controlled clinical study in US & EU to evaluate efficacy & safety of PQ Grass 27 600 SU in patients (18 to 65 years) with seasonal allergic rhinitis ascribed to grass pollen. Primary endpoint was the EAACI recommended combined symptom & medication score (CSMS4) during peak grass pollen season (P-GPS). Key secondary endpoints were symptom & medication score, the CSMS during the entire grass pollen season, Rhinitis quality of Life Questionnaire (RQLQ), IgG4 & safety.
Results: 555 pts. with allergic conjunctivitis and/or rhinitis were randomised to PQ Grass 27 600 SU; (278 patients) or placebo (277 patients) & 507 (91.4%) completed all 6 injections. CSMS over the P-GPS demonstrated a statistically significant

improvement of 20.3% ($p=0.0002$) compared to placebo. Consistently, the secondary endpoints demonstrated significant superiority of PQ Grass 27600 SU. Furthermore, a statistically significant improvement in RQLQ ($p=0.0003$) was observed, indicative of a clinically meaning improvement in quality of life. Similarly, immunogenicity of PQ Grass 27600 SU was shown by a ~6-fold increase in IgG4 compared to placebo ($p<0.0001$). PQ Grass 27600 SU was generally safe and well tolerated with no unexpected safety signals.

Conclusions: PQ Grass 27600 SU is an innovative short-course allergen-specific SCIT product with 6 injections per treatment year. RESONATE is the first state-of-the-art Phase III SCIT trial to reach both a clinically relevant and statistically significant primary outcome and beneficial secondary efficacy results for an AIT product under the TAO.

References

[1] Zielen, S., et al., Strong dose response after immunotherapy with PQ grass using conjunctival provocation testing. World Allergy Organ J, 2019. 12(11): p. 100075.

[2]. Worm, M., et al., Randomised controlled trials define shape of dose response for Pollinex Quattro Birch allergoid immunotherapy. Allergy. 2018 Sep;73(9):1812–1822.

[3]. de Kam PJ, Zielen S, Bernstein JA et al. Short-course subcutaneous treatment with PQ Grass strongly improves symptom and medication scores in grass allergy. Allergy. 2023 Oct;78(10):2756–2766. doi: 10.1111/all.15788. Epub 2023 Jun 27.

[4] Pfaar, O., et al., Recommendations for the standardisation of clinical outcomes used in allergen immunotherapy trials for allergic rhinoconjunctivitis: an EAACI Position Paper. Allergy, 2014. 69(7): p. 854–67.

P007 | Meta-analysis of primary endpoint of Grass MATA MPL field studies with optimised dose and treatment regimen of PQ Grass 27600 SU

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Rationale: PQ Grass 27600 SU is a modified broad spectrum grass allergen subcutaneous immunotherapy (SCIT) product using MicroCrystalline Tyrosine (MCT) and Monophosphoryl Lipid A (MPL) as adjuvant system. Two highly comparable randomised double-blind, placebo-controlled field studies with PQ Grass 27600 SU have been recently completed to support a marketing authorisation application. Here, we report on the integrated primary endpoint results using a meta-analysis approach.

Methods: A common effects meta-analysis1 was applied and justified due to the similarity of study designs, study populations, primary endpoint definitions (i.e., the EAACI recommended combined symptom and medication score (CSMS2)) and statistical analysis methods. These were the only 2 field studies performed with the same optimised cumulative dose of PQ Grass 27600 SU, both conducted simultaneously in the EU and US to evaluate efficacy and safety of PQ Grass 27600 SU in patients (18 to 65 years of age inclusive) with seasonal allergic rhinitis ascribed to grass pollen exposure.

Results: In total 674 subjects with allergic conjunctivitis and/or rhinitis were included in this meta-analysis. The homogeneity of the data for the meta-analysis approach was also statistically confirmed (heterogeneity test $p=0.21$). The results of this meta-analysis showed a clinically relevant and highly statistically significant treatment effect for the primary endpoint CSMS during the peak grass pollen season (P-GPS).

Conclusions: PQ Grass 27600 SU is an innovative short-course allergen-specific SCIT product applying a potent adjuvant system. The two field studies with PQ Grass 27600 SU included in the meta-analysis were highly comparable and demonstrated a clinically relevant and statistically significant integrated primary endpoint result, supporting the marketing authorisation application of PQ Grass 27600 SU.

References

[1] Bender R, Friede T, Koch A, Kuss O, Schlattmann P, Schwarzer G, et al. Methods for evidence synthesis in the case of very few studies. Res Synth Methods. 2018;9(3):382–92.

[2] Pfaar, O., et al., Recommendations for the standardisation of clinical outcomes used in allergen immunotherapy trials for allergic rhinoconjunctivitis: an EAACI Position Paper. Allergy, 2014. 69(7): p. 854–67.

P008 | TAPAS—A long-term non-interventional study evaluating MCT (MicroCrystalline Tyrosine)-associated allergoids shows non-inferiority of clinical effectiveness in children compared to adults during a 3-year course of allergen immunotherapy

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Background: The primary objective of TAPAS (Tyrosine Allergoid Paediatric and Adult Study) is to evaluate clinical effectiveness of allergen immunotherapy (AIT) in children compared to adults with allergic rhinitis, as an identical dosing regimen is used. Long-term effects after perennial treatment with subcutaneous glutaraldehyde-modified and microcrystalline tyrosine-associated allergoids (MATA) are investigated to support this practice.

Method: Patients aged 5 years and older who were treated with MATA for their allergy to grass or birch, alder and hazel pollen could be included in the study prior starting AIT. During the treatment phase (3 years) daily symptom scores and medication usage during the respective pollen seasons (PS) were collected over 4 weeks via an eDiary and quality of life (QoL) was examined using a standardised questionnaire. Furthermore, at start and during the study (outside the PS), patients retrospectively reported about severity and frequency of their allergic/asthma symptoms during the previous PS.

Results: 129 children/adolescents and 191 adults were included between November 2020 and May 2022. In this interim analysis, the primary endpoint, the combined symptom and medication score (CSMS), collected over 3 consecutive pollen seasons showed no significant difference between adults and children/adolescents. Comparison of the median CSMS between the 1st and the 3rd PS under treatment revealed a significant reduction by −14% ($p=0.03$) in the adult cohort (children/adolescents: −12%, $p=0.4$). Further, a significant decrease in the rhinoconjunctivitis score after 2 years of treatment compared to baseline could be shown for children/adolescents (−60%, $p<0.001$) and adults (−50%, $p<0.001$), as well as in the asthma score (children/adolescents: −33%, $p=0.001$; adults: −25%, $p<0.001$). QoL improved significantly in adults (median difference 1st vs. 3rd PS: −0.54 score points, $p=0.001$), but not in children/adolescents (−0.09, $p=0.5$). Overall, adverse drug reactions were reported in 21.7% of the paediatric cohort and in 13.1% of adults. Less systemic reactions were observed in children/adolescents (7.8%) than in adults (10%).

Conclusion: The results from the TAPAS study indicate non-inferiority between children and adults after 3 years of treatment with MATA supporting the common practice of using the identical dosing regimen in children and adults. Furthermore, the data support the excellent safety profile of subcutaneous AIT with MATA.

P009 (OP01/01) | Host-microbiome interplay in early life determines atopic dermatitis development in children

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Atopic dermatitis (AD) is a chronic inflammatory skin disease affecting up to 20% of children and 5% of adults in industrialised countries. AD is frequently observed in early infancy and although its pathogenesis is much better understood, the factors triggering and driving AD development remain unknown. This cohort from the Munich Atopy Prediction Study (MAPS) aims to define the host and environmental factors associated with early AD onset and development. To this end, 480 pregnant mothers were recruited and a broad sampling of biomaterial skin swabs, stool and blood were collected from their infants at regular visits from birth to the age of 4 years. Here, we explored the cutaneous microbiome dynamics in the first 2 years of life using 16S rRNA-gene ribotyping and assessed the impact of key ecological factors including skin pH, TEWL, cutometry and corneometry. Also, nasal swabs from AD and control infants in addition to skin swabs from respective mothers were analysed to trace the transfer of protective or detrimental microbes to infants' skin. Moreover, skin microbiopsies were collected for transcriptome analysis to pinpoint the role of host-microbiota interactions in AD aetiology. The obtained results revealed a delayed cutaneous microbiome maturation in infants with AD within the first year of life exhibiting also significant correlations with changes of functional skin parameters. Additionally, we detected a reduced diversity and high *S. aureus* loads in the nasal cavity of AD affected infants compared to controls and observed that mothers and early infant skin microbiomes may influence AD development in children. Finally, we detected a significant increase of signatures representing epidermal repair, immune response and autophagy pathways in AD skin with marked correlations between key regulated-genes and dysbiotic taxa. Being a longitudinal study with a large set of samples and clinical records taken prior to, during and following AD development this cohort provides an in depth understanding of the early life microbiome and its connection to AD development and exacerbation.

P010 | Setae of the oak processionary moth induce severe skin inflammation

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Thaumetopoea processionea (Oak Processionary Moth (OPM)) is a species that infests oak trees in almost all European countries. Although the moth is harmless, the OPM larval stages contain toxic and allergenic hairs called setae, which pose a serious health risk to humans and animals. Upon contact to the skin, setae can cause a severe skin reaction known as 'caterpillar dermatitis'. Inhalation of setae may provoke airway inflammation, which can lead to the development of severe allergic asthma. Due to global warming, OPM-related health complications have increased exponentially in Germany among forest workers, hunters, farmers, and walkers, causing a significant socio-economic problem in recent years. However, the pathophysiological mechanisms of how setae cause severe skin or allergic reactions remain enigmatic. To understand how setae induce skin inflammation, the ear of Balb/c mice were sensitised with setae. The topical setae application induced immediate vasodilation and ear swelling (ear thickness; setae: 0.068 0.021 mm; PBS control: 0.0220.07 mm; $n=4$). The ear inflammation gradually increased 4 days after single setae application and resolved thereafter. Setae induced massive immune cell infiltration at the setae application site and increased the thickness of the epidermis, as shown in histology and flow cytometry. Setae did not alter the frequency of immune cells in peripheral blood. However, topical setae application increased eosinophils count in the lungs, analysed by broncho-alveolar lavage (BAL). Our study shows that setae or setae toxins and/or allergens induce severe skin inflammation.

P011 | Comparison of the clinical performance of the RIDA qLine Allergy Test System in identifying food and aeroallergen-specific IgE with the gold standard

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In the diagnostic workup of unclear type 1 allergies, multiparameter testing using immunoblot techniques is increasingly being used as an alternative to multiple individual IgE measurements. The aim of this study was to determine whether the performance of the more cost-effective immunoblot method, the RIDA qLine®Allergy Test System (R-Biopharm AG), is comparable to the current gold standard.

Three study-specific panels comprising a total of 57 common individual allergens (food and aeroallergens) were measured in serum samples from 200 patients with signs and symptoms of IgE-mediated allergies using both the RIDA qLine®Allergy Test System and the primary reference method, the ImmunoCAP⇔ Specific IgE Test (Thermo Fisher Scientific). The resulting CAP classes were evaluated on an allergen-specific basis. In case of discrepant results, the corresponding allergens were reassayed using a secondary reference method, the 3gAllergy⇔ Specific IgE Universal Kit (Siemens).

In the quantitative evaluation, the mean overall agreement of all food and aeroallergens with the reference method was 94.9%. In the qualitative evaluation, an average negative percentage agreement of 98.9% and a positive percentage agreement of 75.1% was observed after testing with both reference methods for all individual allergens.

In conclusion, the comparative analysis of the RIDA qLine Allergy Test System against established reference methods for specific IgE detection demonstrated a strong correlation between measured serum IgE levels and clinical presentations. Broad allergen-specific IgE profiling proves particularly valuable in the diagnostic workup of polysensitized patients, yet the results also emphasise the importance of careful interpretation within the clinical context to ensure diagnostic accuracy.

P012 | Assessment of skin inflammatory biomarkers in dermal interstitial fluid collected with hollow microneedles in response to skin provocation

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Introduction: Dermal interstitial fluid (dISF) is an information-rich bodily fluid with the potential for biomarker detection. Vacuum-assisted dISF extraction using hollow microneedles is a novel technology that enables minimally invasive and rapid sampling of adequate volumes of interstitial fluid. This study aims to measure tryptase, histamine, and inflammatory factors in both dISF and blood, before and after skin provocation with the MRGPRX2 agonist codeine.

Methods: Serum and dISF were collected before, 15 min after as well as 4 h after intradermal codeine injection. Samples from 10 healthy volunteers were analysed for histamine (using HTRF), tryptase (via ELISA), and inflammatory biomarkers (PEA; Olink Target 96 Inflammation). The procedure was repeated on a second day independently to assess the intra-individual heterogeneity as well as the overall variability in the response.

Results: An increase of histamine and tryptase levels was observed in the dISF obtained immediately after provocation. Additionally, levels of selected inflammatory mediators were significantly increased in the dISF following provocation, whereas no changes were detected in serum. Virtually the same results were obtained from samples obtained on the second day, confirming specificity of the response.

Conclusion: Vacuum-assisted dISF extraction using hollow microneedles is a novel and promising tool to monitor inflammatory biomarkers. Further studies will decipher, whether individual patterns of inflammatory markers can be identified in response to different stimuli such as IgE-dependent triggers. Assessment of dISF thus may help to distinguish different subtypes of CSU.

P013 | CRISPR/Cas9 engineering iPSC: A novel cell-based model for mastocytosis

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Introduction: Mastocytosis is a disorder caused by an excessive accumulation of mast cells (MCs) in various organs. Symptoms mainly result from the release of mediators by the degranulation of MC. Indolent systemic mastocytosis (ISM) is the most common clinical subtype of mastocytosis, which is usually associated with an acquired somatic gain-of-function KIT-D816V mutation. Unlike in the advanced subtypes, in patients with ISM additional mutations other that KIT-D816V are rarely found. However, the existing MC models either burden several additional leukaemia-associated factors or exhibit limitations in IgE-dependent MC activation. This creates obstacles for

research on ISM and the mechanism of KIT-D816V mutation. To overcome this challenge, we developed a new KIT-D816V cell model of mastocytosis.

Methods: We generated a new mast cell model (hiPSC-MC KIT-D816V) derived from mutant KIT-D816V induced pluripotent stem cells using CRISPR/Cas9 gene editing. To characterise the new cell model, we performed DNAseq and whole genomic sequencing (WGS) and investigated immunophenotype, survival, proliferation, degranulation, KIT signalling and the response to various tyrosine kinase inhibitors (TKIs).

Results: The hiPSC-MC WT and hiPSC-MC KIT-D816V are tryptase-chymase-postive cells. They showed typical MC surface markers and activation via IgE and MRGPRX2- dependent signalling pathways. The SCF-independent survival and imatinib resistance have been found in hiPSC-MC KIT-D816V, but not in hiPSC-MC WT. In addition, the autophosphorylation of KIT and downstream signalling was confirmed by western blotting in hiPSC-MC KIT-D816V.

Conclusions: The novel hiPSC-MC KIT-D816V is a functional MC model for preclinical research on mastocytosis.

P014 | Characterising the antiviral function of the antimicrobial protein RNase 7 in the context of atopic dermatitis and eczema herpeticum

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Atopic dermatitis (AD) is a chronic inflammatory skin disorder characterised by a compromised skin barrier, immune dysregulation and an imbalance of skin microbiota, increasing the risk of infections such as eczema herpeticum (EH), mainly caused by herpes simplex virus type 1 (HSV-1). RNase 7 (R7), an antimicrobial protein (AMP) produced by keratinocytes, exhibits a broad-spectrum of antibacterial and immunomodulatory activities.

We have previously shown that R7, in the presence of self-DNA, promotes an immune response that protects keratinocytes from HSV-1 infection. Moreover, at higher but still physiological concentrations, R7 inhibits HSV-1 infection even without the addition of self-DNA and independently of the induction of interferon-stimulated genes.

To analyse the direct antiviral effects of R7 in keratinocytes, we infected R7 knockdown and control cells with different doses of an HSV-1 reporter virus and analysed viral gene expression by automated microscopy. While addition of recombinant R7 reduced HSV-1 gene expression, the knockdown of R7 did not increase HSV-1 infection. We are currently optimising the detection of R7 by immunofluorescence microscopy to address whether recombinant R7 is taken up by cells and colocalizes with incoming HSV-1 particles.

We also collected skin rinses from AD patients with (ADEH+) or without (ADEH−) a history of EH, as well as from healthy donors, and measured AMP levels by ELISA. Consistent with previous studies, the concentrations of R7, psoriasin, and human beta-defensin-2 (hBD-2) were higher in AD lesions than in healthy control and non-lesional AD skin. The AD lesions of ADEH+ patients also showed higher concentrations of these AMPs than those of non-lesional skin and healthy controls. Herpetic lesions from ADEH+ patients tended to secrete more R7 than their AD lesions and skin from healthy donors. The local SCORAD of AD lesions correlated significantly with the hBD-2 but not with the R7 or psoriasin concentrations in the skin rinses. The time since the patient's last shower was also associated with R7 concentrations in the lesions, with a longer time interval resulting in higher R7 levels. We will extend the analysis of skin rinses to measure RNase inhibitor and self-DNA, both of which modulate the activity of R7, as well as samples from herpes zoster patients.

In conclusion, our data suggest that R7 may play an important role in the antiviral response against HSV-1 in AD patients, and further analysis will enhance our understanding of the regulatory mechanisms governing R7 in skin immunity.

P015 | Investigation of novel allergen components in IgE cross-reactivity reveals continued dominance of PR-10 proteins

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Background: Allergologic diagnoses in numerous countries, including the United States, largely depend on specific IgE measurements (sIgE) derived from whole allergen extracts. Various molecular components, such as profilins, pathogenesis-related (PR)-10 proteins, and cross-reactive carbohydrate determinants (CCDs), have been identified as potential causes in the significant cross-reactivity observed among sIgE measurements for distinct allergens. Despite the emerging description of novel allergen components that might cross-react, the clinical relevance of these interactions remains largely undefined.

Methods: In this single-center, cross-sectional study, we analysed the sera of 200 patients known to have at least one IgE-mediated allergy. To perform this analysis, we used 60 distinct single-plex sIgE assays (ImmunoCAP, Thermo Fisher, Waltham, MA, USA), investigating a variety of allergen extracts, including food ($n=30$), aeroallergens ($n=27$), insect venoms ($n=2$), and latex ($n=1$). Additionally, we measured sIgE against a number of cross-reactive allergen components in all sera: Pru p 3 (lipid-transfer protein, LTP), Bet v 1 (PR-10 protein), Phl p 12 (profilin), Phl p 7 (polcalcin), MUXF3 (CCD), Der p 10 (tropomyosin), Pru p 7 (gibberellin-regulated protein, GRP), and Ara h 18 (cyclophilin). To identify positive sIgE results, we set the cutoff at 0.1 kU/L.

Results: We detected sIgE against PR-10 proteins (60%), profilins (16%), LTPs (15%), polcalcins (4.5%), CCDs (17%), tropomyosins (2.5%), GRPs (4.5%), and cyclophilins (8.5%). Mean sIgE against PR-10 protein (12.87 kU/L) and profilin (0.39 kU/L) were higher than mean sIgE against LTPs (0.23 kU/L), polcalcins (0.23 kU/L), CCDs (0.20 kU/L), tropomyosins (0.01 kU/L), GRPs (0.02 kU/L), and cyclophilins (0.14 kU/L). As expected, correlation analyses and subsequent hierarchical clustering revealed

significant sIgE cross-reactivity between allergens such as tree pollen, stone fruit, hazelnut, and PR-10 proteins, with additional cross-reactivity noted between different types of grass pollen and profilin. No such clustering was observed for cyclophilins, LTPs, and polcalcin. CCDs, GRPs, and tropomyosins correlated and clustered with insect venoms and foods such as seafood, nuts (walnut, cashew nut and almond) among others.
Conclusion: The vast majority of cross-reacting sIgE uncovered in our study can be traced back to PR-10 protein epitopes, such as Bet v 1. We observed that the sIgE cross-reactivity instigated by GRPs, CCDs, and tropomyosins impacts similar families of allergens. Although sera occasionally display sIgE targeting cyclophilins and polcalcins, these markers show only weak correlations when compared to other allergens, with PR-10 proteins and profilins presenting a much stronger association. Hence, our findings highlight the dominant role of PR-10 proteins in IgE cross-reactivity in a German population.

P016 | Characterisation of pathomechanisms of acute and chronic spontaneous urticaria

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Introduction: Acute spontaneous urticaria (AU) is characterised by the occurrence of wheals and/or angioedema for up to 6 weeks. The progression of AU to chronic spontaneous urticaria (CSU) and the role of autoimmunity remain elusive.
Methods: Clinical data and blood samples were collected from AU patients presenting to the emergency department (V1) at UCARE Berlin (2018–2022). A second data collection and blood sampling were performed after ≥6 weeks (V2). Total IgE serum levels as well as autoantibodies IgG anti-IgE, IgG anti-FcεRI and IgG anti-TPO were determined, and a basophil histamine release assay (BHRA) was performed.
Results: Of 81 AU patients at V1,74% no longer had urticaria at V2 (AU/noCSU;65% female, 39 years [mean], BMI 24.9), while 26% were diagnosed with CSU at V2 (AU/CSU; 52% female, 40.2 years [mean], BMI 26.6). At V1, AU/noCSU and AU/CSU groups showed no differences in their total IgE levels (224 vs. 209 kU/L, $p=0.86$) and distribution of patients with isolated wheals (65% vs. 62%), wheals and angioedema (33.3% vs. 33.3%), and standalone angioedema (1.67% vs. 4.76%). At V1, auto-IgG anti-IgE was detected in 7% of AU/noCSU but not in AU/CSU patients and at V2, in 5% of AU/noCSU and in 6% of AU/CSU patients. At V1, auto-IgG anti-FcεRI was detected in 93% of AU/noCSU and 90% of AU/CSU patients and at V2 in 12% of AU/noCSU but not in AU->CSU patients. At V1, four AU/noCSU but no AU/CSU patients were BHRA+. At V2, one patient from the AU/noCSU but no patient from the AU/CSU group turned BHRA+. IgG-anti-TPO were detected in two AU/noCSU and in two AU/CSU patients at V1, while only one patient of each group remained positive at V2.
Conclusion: The results of this pilot suggest that the presence or absence of autoantibodies does not define a CSU patient, and that a prolonged production of auto-IgG anti-FcεRI may only arise during the course of CSU.

P017 | Does elevated tryptase indicate disease impact in chronic spontaneous urticaria? – Preliminary data

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Introduction: Hereditary Alpha-Tryptasemia (HαT) is an autosomal dominant disorder that affects approximately 4–6% of the Western population. It is caused by an increased copy number of the TPSAB1 gene, leading to persistently elevated serum tryptase levels (≥8 ng/mL). Symptoms are often non-specific, commonly including mast cell activation manifestations like urticaria, angioedema and flushing. However, studies have not consistently linked HαT to specific mast cell-mediated symptoms, indicating a need for further research. This study aims to investigate the prevalence of elevated tryptase levels in patients with chronic spontaneous urticaria (CSU) and determine if these levels are more common in Type I (autoallergic) or Type IIb (autoimmune). Additionally, it will assess how elevated tryptase levels may influence disease control, symptoms, progression and quality of life.
Methods: Tryptase values from 693 patients at the Institute of Allergology, Charité Berlin, were analysed. Patients with elevated tryptase levels (≥8 ng/mL) were compared to an age- and sex-matched control group with normal levels (< 8 ng/mL). The comparison focused on disease symptoms, activity, and quality of life. Recorded parameters included urticaria symptoms (wheals and/or angioedema), use of antihistamines or omalizumab, patient-reported outcome measures (PROMs) like the Urticaria Control Test (UCT) and Chronic Urticaria Quality of Life questionnaire (CU-QoL), and specific lab markers (CRP, anti-TPO, total IgE) for classifying the urticaria type.
Results: Out of the 693 samples analysed, 95 patients had elevated tryptase levels. Data from 74 of these patients were recorded and compared to a control group of 68 patients with normal tryptase levels. The average tryptase level was 13.5 ng/mL in the patient group and 4.4 ng/mL in the control group. There was no significant difference in gender distribution, but the patient group was older (49 years vs. 43 years). Symptoms like wheals and angioedema were not significantly more frequent in either group. The need for antihistamines and omalizumab was similar, though a higher tendency for omalizumab use was observed in the patient group (35.1% vs. 29.4%). Patients with elevated tryptase levels had lower UCT values (5.7 vs. 6.3) and higher CU-QoL values (44.0 vs. 40.6), indicating poorer disease control and quality of life, though not statistically significant. No significant differences were found in laboratory parameters

(total IgE, CRP, anti-TPO), but tryptase levels correlated positively with total IgE, CRP and anti-TPO-AK, suggesting possible inflammatory or allergic processes.

Outlook: The data presented are initial findings from an ongoing study that may indicate a high prevalence of elevated tryptase levels (13.7%), and potentially a high frequency of HαT, in CSU patients as compared to the general population. Our preliminary results suggest that elevated tryptase levels in these patients may be linked to increased disease severity and reduced quality of life, as indicated by greater omalizumab use, lower CU-QoL and UCT scores. To further validate these associations, continued data collection with additional clinical and laboratory parameters in a larger patient cohort is needed. A larger sample will clarify whether elevated tryptase levels are significantly linked to more frequent symptoms, poorer symptom control, reduced quality of life, and a higher prevalence of Type I or Type IIb urticaria.

P018 | Anti-KIT monoclonal antibody barzolvolimab does not induce apoptosis but reduces the activation of KIT D816V-mutated mast cells

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Background: Indolent systemic mastocytosis (ISM) is a heterogeneous disorder characterised by proliferation and accumulation of mast cells (MCs) in different organs, with KIT D816V activating mutation in the tyrosine kinase receptor c-KIT (KIT) being the major driver of the disease. The anti-KIT monoclonal antibody barzolvolimab recently was reported to potently reduce the number of MCs in healthy volunteers and urticaria patients. The aim of this study was to assess effects of barzolvolimab on MCs harbouring wild-type vs. KIT D816V.

Methods: Primary MCs from healthy donors, HMC-1.2 human mast cell line harbouring KIT D816V and KIT G560V mutations, and MCs derived from wild-type and KIT D816V mutated human induced pluripotent stem cells (iPSC-MC WT and iPSCMC KIT D816V) were incubated with barzolvolimab in absence or presence of the KIT ligand stem cell factor (SCF). Cell viability was assessed by flow cytometry. MC activation was assessed by release of ß-hexosaminidase. Western blot was performed to show the effect of barzolvolimab on KIT signalling pathways.

Results: Healthy donor primary MCs and iPSC-MCs WT showed SCF-dependent survival, in contrast, HMC-1.2 and iPSC-MCs KIT D816V could survive in the absence of SCF. After a 10-day treatment, barzolvolimab induced apoptosis of healthy primary MCs and iPSC-MCs WT in a dose-dependent manner (dose-range from 1 nM to 1 µM), but did not affect survival of HMC-1.2 and iPSC-MCs KIT D816V. Western blot analysis demonstrated that barzolvolimab inhibited KIT and downstream AKT phosphorylation in healthy MCs and iPSC-MCs WT, but not in iPSC-MCs KIT D816V and HMC-1.2. Interestingly, MC activation assays showed that barzolvolimab inhibited SCF-dependent IgE-mediated activation of healthy primary MCs, iPSC-MCs WT as well as iPSC-MCs KIT D816V in a dose-dependent manner.

Conclusion: Barzolvolimab does not induce apoptosis of KIT D816V mutated MCs, however, it may inhibit their activation in vivo. Thus, the efficacy of barzolvolimab as a potential treatment for patients with ISM needs to be further investigated.

P019 (OP04/03) | Adaptive immune responses to *Staphylococcus aureus* serine protease-like protein B in atopic dermatitis

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Staphylococcus aureus is a common coloniser of atopic dermatitis (AD) skin where it produces a variety of virulence factors including Staphylococcal serine protease-like proteins (Spls). While Spls are known to elicit a dominant type-2 immune response in patients with allergic asthma, their role in AD remains unexplored.

In this study, we investigated the adaptive immune response to Spls in AD. Serum IgE levels specific to Spls were quantified and T cell responses were investigates using peripheral blood mononuclear cells (PBMCs) from AD patients and healthy individuals. SplB-specific T cells were identified and characterised by MHC-tetramer staining, proliferation assays, cytokine secretion and analysis of surface markers (CD154, CCR4, CCR6, CCR10, CXCR3) following in vitro stimulation with SplB. The presence of SplB-specific T cells in lesional AD skin was assessed by comparing the TCRB CDR3 sequence with those from SplB expanded T cell lines.

Elevated levels of Spl-specific serum IgE were observed in AD patients, while SplBspecific CD4+ T cells were detected in both AD patients and healthy individuals, exhibiting a mixed Th1/Th2 dominated response. Two HLA-DRB1*15:01 and HLADRB1* 11:01 restricted immunodominant epitopes of SplB were identified using in silico prediction and in vitro validation. SplB-epitope-specific T cells were capable of secreting IFN-γ and IL-13. Furthermore, TCRB sequencing revealed clonally expanded SplB-specific T cells in lesional AD skin.

In conclusion, the SplB protease induces Th1/Th2 dominated responses in both healthy individuals and AD patients. The presence of SplB-specific T cells in AD lesional skin, along with the cytokine response and the elevated serum IgE levels, suggests that SplB contributes to AD skin inflammation.

P020 | Drug Reaction With Eosinophilia and Systemic Symptoms (DRESS): Deciphering the diversity of systemic immune profiles

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Drug Reaction with Eosinophilia and Systemic Symptoms (DRESS) syndrome is a rare, severe delayed-type drug hypersensitivity reaction. It can range from mild reactions with little/no organ involvement to severe, potentially life-threatening forms. It is unclear whether these distinct clinical phenotypes are associated with different immune signatures, and, ultimately, distinct pathomechanisms.

The aim of this study was to explore systemic immune profiles of DRESS patients. Our hypothesis was that different grades of DRESS severity would be associated with distinct inflammatory profiles.

In this multicentric study, we included a total of 26 DRESS patients from Switzerland and South Africa. Serum samples were obtained at the time of diagnosis. 6 healthy controls (HC) were included. Serum levels of inflammation−/immune response-associated proteins were measured by two panels [180 proteins] of a targeted highthroughput proteomics assay (OLINK).

Our targeted proteomics findings from serum show a immune activation in DRESS type 2-/eosinophil-axis associated proteins (e.g., IL4, IL5 and IL13) were increased in all DRESS patients compared to HC. In addition, we saw an upregulation of proinflammatory mediators like IL6, IL10, CXCL9, and IFN-gamma and of immune regulatory proteins such as PSIP1, ADA, and SH2D1A.

Regarding disease severity, all grades showed overlap in upregulated proteins involved in core immune responses, including chemokines (CXCL9, CXCL10), interferon signalling (IFN-gamma), immune checkpoint molecules (LAG3, PD-L1).

Strikingly, there was a heterogeneous expression of immune proteins in DRESS patients regardless of the severity grade. We identified three immune clusters based on unsupervised clustering. Group 1 was characterised by the upregulation of IL7, and milder immune activation while they are older with shorter drug latency and longer hospitalisation stay. Group 2 displayed a pronounced type 3 immune response, marked by the innate response marker IRAK4 and increased levels of Th17-related cytokines and proteins involved in Toll-like receptor signalling, contributing to a more inflammatory profile. This group was also associated with the negative regulator of immune cell activation, SPRY2, and proteins involved in viral defence mechanisms, and had a significantly higher rate of HIV coinfection. Group 3 was defined by elevated levels of key chemokines like MCP-4 (CCL13), which is highly effective at recruiting eosinophils, along with high eosinophil counts and a predominant type 2 cytokine response, suggesting a strong inclination towards allergic reactions and eosinophilic inflammation.

Our targeted serum proteomics findings suggest a systemic immune activation, particularly of the type 2−/eosinophil-axis. Our findings of different DRESS immune profiles could, if further validated, pave the way to patient stratification and more targeted treatment approaches.

P021 | Cold urticaria: Exploring the presence of soluble cold-induced antigens in dermal interstitial fluid

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Introduction: Cold urticaria (ColdU) is a frequent form of chronic inducible urticaria and characterised by the development of wheals, angioedema or both in response to cold exposure. IgE-dependent mast cell activation in transferrable ColdU is believed to be an important pathomechanisms, however antigens are not identified.

Methods: Peripheral CD34+ stem cell-derived mast cells (PSCMCs) were sensitised with serum from ColdU patients ($n = 4$) or healthy controls ($n = 3$) in the presence or absence of anti IgE, Omalizumab, and activated with dermal interstitial fluid (dISF) extracted by hollow microneedles from healthy volunteers after cold provocation. In addition, PSCMCs were sensitised with purified IgE from one patient and activated with dISF after cold provocation. Mast cell surface markers (CD63 and CD107a) of activation and degranulation were assessed by flow cytometry.

Results: Collected dISF after cold provocation from healthy individuals activates PSCMCs sensitised by patient serum to varying degrees that can be blocked by omalizumab, while control sera from healthy individuals do not induce mast cell activation. In addition, PSCMCs upregulate surface CD63 when sensitised with purified IgE from ColdU patient and incubated with dISF obtained after cold provocation. IgE-depleted serum lost this activity and Omalizumab can block the activation.

Conclusion: Cold-induced soluble antigens are generated in the skin which activate and induce degranulation of mast cells. After exposure to cold stimulation, the skin interstitial fluid of healthy individuals can activate and degranulate PSCMCs sensitised with the IgE purified from the serum of cold urticaria patients. This can be blocked by Omalizumab, suggesting an IgE-dependent mechanism.

P022 | Exploring the potential of telemedicine in care of allergy patients

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Introduction: The increasing prevalence of allergies, coupled with a shortage of allergy specialists, poses significant challenges in delivering timely and comprehensive care to patients with allergic diseases. Telemedicine offers an innovative solution to address these challenges by providing rapid and efficient medical care. While telemedicine is well-established in other medical fields, its application in allergy care, especially for large patient populations with diverse allergic diseases, remains underexplored.
Methods: To evaluate patient preferences and experiences with telemedicine, we conducted a 27-question survey. We developed a novel questionnaire and handed it out to patients over 18 years old with various allergic conditions, prior to their consultations at our outpatient clinic at the Division of Allergy at the University Hospital Basel.
Results: In an interim analysis conducted between May 29, 2024, and September 25, 2024, 102 patients completed the questionnaire. Among the 87 participants who answered the relevant question, 78 (89.66%) viewed telemedicine as beneficial for managing common allergies, such as drug and insect venom allergies. Additionally, participants acknowledged its potential for addressing less common allergic conditions, including mastocytosis (8 participants, 9.20%) and angioedema (12 participants, 13.79%). Furthermore, of the 94 participants who responded to another question, 42 (44.68%) reported prior experience with telemedicine, with 14 (33.33%) specifically utilising teleallergy services.
Discussion: Our interim findings indicate that allergy care by telemedicine could be valuable for managing various allergic conditions. Participants of our survey could well imagine using telemedicine for care of allergic diseases, indicating its potential for broader adoption. Ongoing data collection will provide further insights needed to refine teleallergy services for specific allergic conditions. We will particularly focus on exploring how these services can be effectively combined with in-person consultations to enhance long-term patient care.

P023 | *Staphylococcus aureus* correlation with severity of atopic dermatitis as function of clinical and environmental factors

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Background: The severity of Atopic dermatitis (AD) is known to be associated with the relative abundance of *Staphylococcus aureus* (aureus) on patients' lesional skin. However, the linear correlation of AD severity score (SCORAD) with aureus abundance is not very strong ($R < 0.5$), in particular with a significant fraction of patients having high SCORAD but low aureus abundance (severe-outliers) or having moderate SCORAD with high aureus abundance (moderate-outliers). Here we used the data from the large ProRaD cohort of AD patients to better understand the correlation and its outliers as function of various clinical and environmental factors.
Methods: Lesional and non-lesional skin swabs were sampled cross-sectionally from 355 adult patients with mild to severe AD. Microbiome relative abundance was estimated using next-generation sequencing (NGS, 16S V1-V3). An iterative bootstrap algorithm was used to define a Hill function model that best fits the SCORAD-aureus correlation for the majority of patients and the outliers to the model.
Results: Most (80%) patients show a good correlation ($R = 0.8$) of SCORAD with aureus abundance when using a Hill model instead of a linear correlation. We identified 23 (6%) patients as moderate-outliers and 48 (13%) patients as severeoutliers. Severe-outliers were significantly associated with older age (30% of severe AD with aureus <5% are of age >70, as comparted to 4% in the cohort in general). Furthermore, severe-outliers with low aureus had significantly higher abundance of other Staphylococcus species, but not of C. acnes, as compared to mild–moderate patients with same aureus abundance. Moderate-outliers were significantly associated with a samples taken in summer or spring season, and were not found in samples taken in winter and autumn.
The skin location of the lesion, age, gender, time from AD onset, and manifestation of other atopic disease were not associated with patients being outliers to the SCORAD-SA correlation.
Conclusions: These findings suggest that AD severity is in general strongly correlated with *Staphylococcus aureus*, and that the outliers to these correlation are associated either with older age, high abundance of other bacteria or with seasonal effects.

P024 | Comparison of dermal interstitial fluid and plasma reveals tissue-specific soluble biomarker profiles

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Introduction: Histomorphometric analyses of skin biopsies combined with the analysis of blood, serum and skin microdialysis samples are current gold-standard tests in dermatology research. However, inflammatory mediators which are released by dermal (immune) cells, become highly diluted, interact with other plasma proteins or have a very short half-life in blood or do not spill over into the circulation. Thus, analysis of serum may provide only limited knowledge about locally-produced soluble biomarker.
Methods: Microneedle chips (5th generation) containing 130 sharp hollow microneedles of 450 µm length were used to extract dermal ISF from the forearm of healthy volunteers. At the same time, fingertip peripheral blood was collected. The ISF was analysed by proximity extension assay (PEA) for inflammatory biomarkers and compared to plasma from non-matching controls.
Results: By using a sequential procedure for skin penetration, on average 15 µL ISF could be extracted from the volar forearm. There was no differences between male and female volunteers. Dermal ISF contained unique, tissue-specific cytokines which could be found only in ISF but not in plasma.
Conclusion: Dermal interstitial fluid (ISF) is an information rich body fluid which can be used to monitor biomarker with the potential to improve diagnostic options and facilitate biomarker discovery. Hollow microneedles are easily accessible, do not damage blood vessels, are painless when applied and have a low risk of infection and thus represent a ideal tool for dISF collection.

Angiology

P025 | Monocyte-mediated bystander T cell activation in varicose veins

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Varicose veins, caused by reduced venous blood flow, occur in every third woman and every fifth man, and even though they can be removed via surgical or endovenous methods, it is very likely to recur. Varicose veins are considered one of the common clinical signs of chronic venous disease (CVD), which is a progressing disease that can eventually, in its severe stages, present a risk factor for deep vein thrombosis and lead to ulceration through a persisting cycle of chronic inflammation. T cells play a central role in perpetuating chronic inflammatory reactions. However, their role and interaction with myeloid cells in case of sterile inflammation, as in varicose vein, is not well studied.
Our results have demonstrated that superficial varicose veins contain approximately 30-fold more immune cells compared to healthy saphenous veins. T cell infiltrate of varicose veins is mainly composed of effector-memory T cells and large portion of these cells are activated. Bulk RNA sequencing revealed monocytic cells, located both at inner and outer part of varicose veins, express several cytokines known to activate T cells. To investigate the crosstalk between the T cells and monocytic cells in varicose veins, we analysed the response of isolated effector-memory T cells to the cytokines of varicose vein monocytic cells regarding their ability to trigger cytokine production and activation in effector-memory T cells. These findings provide a more detailed understanding of innate and adaptive immune cell interactions in varicose veins and possible contribution of antigen-independent bystander T cell activation to the inflammation.

Cellular Biology

P026 | Adaptation of keratinocyte and fibroblast scratch wound models to investigate delayed wound healing conditions

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Introduction: Wound healing is a complex, highly regulated process entailing diverse epidermal and dermal cells among white blood cells involved in inflammatory reactions, which are controlled by various cytokines and growth factors. Chronic, non-healing wounds represent a major challenge in hospitals and health care settings. Models of keratinocyte and fibroblast scratches help to elucidate effects of various interventions on epithelial and dermal healing. However, these scratch wound models are mostly performed under optimal cell environments, which is not the case in wound healing disorders. In accordance, we have recently begun to investigate how non-optimal settings, like infection, chronic TH1 or TH2 inflammation and glucocorticoid presence affect cell layer regeneration.
Methods: Healing progression of keratinocyte and fibroblast scratches under these non-optimal conditions was investigated. Gene expression analysis for cytokines (IL1A, IL6, CXCL8), growth (TGFB1, PDGFC) and transcription factors (NFKB1, TP53), heat shock proteins (HSP90AA1, HSPA1A, HSPD1) and keratinocyte desmogleins (DSG1, DSG3) and fibroblast collagen (COL1A1, COL3A1) was performed.
Results: Keratinocyte and fibroblast wound healing under non-optimal conditions was found to be distinctly reduced in vitro. Especially the inflammatory response in infected keratinocytes as well as under chronic TH1 inflammatory conditions was critically augmented and decreased wound healing progression. In contrast, in the TH2 setting or after glucocorticoid

administration, correct initiation of an acute inflammatory response necessary after injury to stimulate the regenerative processes and advance scratch closure was inhibited.
Conclusions: The scratch wound model can easily be adapted to investigate how non-optimal cellular conditions affect wound healing as well as be used to analyse the influence of various wound therapies under non-healing conditions.

P027 | Comparison of different human keratinocyte cell lines for modelling skin aging in two-dimensional cell cultures and organotypic skin equivalents

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The accumulation of senescent cells is associated with several age-related pathologies and represents a primary driver of skin aging. Senescent cells cause tissue damage by secreting pro-inflammatory cytokines and extracellular matrix remodelling factors. This study aims to develop and refine existing methods based on stress-induced cellular senescence for modelling skin aging in 2D and 3D cell cultures.
We exposed primary human dermal fibroblasts, primary human keratinocytes, and different immortalised keratinocyte cell lines to repeated doses of doxorubicin and tBHP to induce cellular senescence. To validate the senescent phenotype, we used a panel of molecular and morphological markers and compared senescent to proliferating, quiescent, and acutely stressed cells. We found that the different cell strains showed diverse responses to the stressors and required individual dose optimization for optimal senescence induction. Furthermore, we generated organotypic human skin equivalents containing 20% senescent fibroblasts in the dermal compartment and different keratinocyte cell strains in the epidermis.
Our work will promote a better understanding of the contribution of cellular senescence to skin aging and provide novel skin models for compound testing in the cosmetic and pharmaceutic industries.

P028 | DEC205, the prototypic antigen-uptake receptor in Langerhans cells, is also expressed by endothelial cells of the nervous system and regulates inflammation in the Experimental Autoimmune Encephalomyelitis (EAE) model

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The surface receptor DEC205 (alias CD205, NLDC145, Ly75) has for long been characterised as marker for murine dendritic cells and Langerhans cells and functions as a receptor to facilitate antigen uptake. We revisited older immunohistology data (Witmer-Pack MD et al.; doi: 10.1006/cimm.1995.1110), showing that blood endothelial cells in the brain, in addition to dendritic cells, react with anti-DEC205 antibodies (Dec: Abs). By flow cytometry and immunofluorescence microscopy, we verified expression of DEC205 by the brain endothelial cell line bEnd.3. Functional investigations further revealed that DEC205 mediates endocytosis. It guides Dec:Abs to a non-recycling (i.e., transferrin receptor negative) and only slowly degrading (i.e., endocytosed Dec:Abs remain intracellular for 48 h) compartment. To verify expression of DEC by endothelial cells in vivo, double labeling of sections of brain, spinal cord, eye, lymph node and skin with anti-DEC and anti-CD31 (a marker for endothelial cells) antibodies was performed. Here we show, that DEC was selectively expressed by endothelial cells in brain, spinal cord and eye. In other tissues endothelial cells were devoid of DEC205, indicating that exclusively endothelial cells derived from the neural tube express DEC205. This specific expression pattern of DEC205 by brain endothelial cell prompted us to test whether Dec:Abs would specifically target to the brain. Therefore, we injected purified Dec:Abs i.v., sacrificed the mice 8 min later, and stained sections of the brains with fluorescently labelled anti-rat secondary antibodies. Only blood endothelium of the brain, the spinal cord and the eye reacted with secondary antibodies, indicating a specific targeting of Dec:Abs to the respective DEC205-expressing endothelium. As the blood endothelium acts as gate keeper for leukocytes, regulating their recruitment and the following inflammatory reaction, we further investigated the effects of DEC:Abs on the inflammatory brain disease EAE, which serves as model for multiple sclerosis. Mice were induced for EAE according to standard protocols and some groups were treated with Dec:Abs alongside. DEC205 deficient (DEC−/−) mice served as further controls. Analysis revealed a drastic increase and prolongation of EAE symptoms in DEC−/− and Dec:Abs injected animals, as well as an increased infiltration of the brains by CD4+ T cells and macrophages. This indicates that blockade of DEC on endothelial cells by Dec:Abs injection and/or its absence (i.e., DEC205−/− animals) loosens the brain blood barrier, making the endothelium permissible for leukocyte infiltration. This conclusion was further substantiated by in vitro experiments, showing that treatment of monolayers of DEC205+ bEnd cells in Boyden chambers with Dec:Abs enhanced the trans endothelial migration of isolated CD4+ T cells towards an CXCL21 gradient. In summary, these data verify for the first time in 30 years that the DC-specific receptor DEC205 is also expressed by brain endothelial cells and beyond its function as endocytic receptor in DCs, it may be involved in regulating the blood brain barrier in endothelial cells.

P029 | Sodium butyrate accelerates diabetic wound healing through epigenetic modifications of dysfunctional macrophages

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Non-healing wound disorders, including diabetic ulcers, chronic venous leg ulcers, and pressure ulcers, remain major unmet medical needs and pose a difficult challenge to healthcare systems worldwide. The persistent accumulation of dysfunctional pro-inflammatory macrophages has previously been identified as a fundamental cause of chronic wounds, however, the molecular details underlying their dysfunction remains elusive. Here we report that histone H3K27 acetylation, an epigenetic mark that regulates macrophage gene transcription, is significantly suppressed under closely resembling that of diabetes. We identify palmitate, a saturated fatty acid that occurs at high concentrations in diabetic patients and db/db mice, to be the responsible culprit. In a series of in vitro and in vivo experiments (RNA-seq analysis, transposase-accessible chromatin assay, in vivo wound monitoring, immunostaining, and shRNA silencing), we found that palmitate supplementation—via activation of HDAC-dependent histone deacetylation pathways—significantly suppressed histone acetylation. Furthermore, a shift in transcriptional control from STAT1 to JUN was observed in LPS/IFNIFNϒ stimulated macrophages exposed to palmitate. The histone deacetylation inhibitor sodium butyrate profoundly promoted cutaneous wound healing in diabetic mice. Butyrate induced restoration of the histone H3K27 acetylation-dependent transcriptional signature and corresponding pathways in wound-associated macrophages and in consequence, reestablished pro-regenerative STAT1 signalling. Furthermore, we found that butyrate-mediated inhibition of HDAC preserves the morphological features and, more importantly, improves the phagocytic activity and migration of macrophages even under the palmitate-imprinted inflammatory conditions mimicking that in diabetes. These findings are clinically relevant as butyrate concentrations are severely reduced in diabetic patients and mice. Since butyrate is a metabolic product of gut bacteria, its decrease also indicates a dysfunctional gut microbiome in diabetic mice and humans. Our study highlights a novel pathological mechanism controlling the dysfunctional macrophages, which could be exploited therapeutically to improve or even cure chronic wounds dominated by macrophage dysregulation in diabetes and possibly other chronic wound healing disorders.

P030 | Oxidation-sensitive cysteines drive IL-38 amyloid formation

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Cytokines of the interleukin (IL)-1 family are widely expressed in epithelial surfaces, including the epidermis, where they play key roles in the maintenance of barrier integrity and host defence. A recent report associated the IL-1 family member IL-33 with stress granules (SGs) in epithelial cells. Formation of SGs is promoted by the aggregation of proteins harbouring low complexity regions (LCRs). In this study, using computational analyses, we predicted the presence of LCRs in six of the 11 IL-1 family members. Among these, IL-38 contained a long LCR and localised to Ras GTPase-activating protein binding protein 1 (G3BP1)-positive SGs, as well as to G3BP1-negative intracellular protein condensates in keratinocytes exposed to oxidative stress (OS). In addition, we identified two highly aggregation-prone amyloid core (AC) motifs in the IL-38 LCR and detected the formation of amyloid IL-38 aggregates in response to OS in cells and in vitro. Disulfide bond mapping, in silico modelling and the analysis of specific cysteine mutants supported a model in which specific oxidation-sensitive cysteines act as redox switches to modify the conformation of IL-38 and thus the surface exposure of its ACs, shuttling IL-38 from a soluble state into biomolecular condensates. Finally, the presence of IL-38 granules in human epidermal layers highly exposed to environmental OS suggests that oxidation-induced formation of amyloid aggregates is a previously unrecognised intrinsic biological property of IL-38, and may be physiologically relevant at this epithelial barrier.

P031 | Dissecting aging mechanisms in ribosomes using skin fibroblast cells

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In previous studies, our lab identified disturbed ribosomal biogenesis in premature aging diseases like Cockayne syndrome and trichothiodystrophy. This pathomechanism might contribute to the neurodegeneration observed in these diseases. Disturbed ribosomal biogenesis results in higher translational infidelity which causes a loss of protein homeostasis (Alupei et al. 2018, Phan et al. 2021, Khalid et al. 2023). A loss of protein homeostasis characterises most neurodegenerative diseases of the aging body. Comparing skin fibroblasts from healthy young and old donors, we find a decreased error rate of the ribosomes with aging, which contrasts our initial hypothesis. We could identify endoplasmatic reticulum (ER) stress as a regulator to increase the accuracy of protein translation at the cost of inhibition of protein synthesis. Countering ER stress reverses that effect and leads to a higher error rate in translation. This mechanism is most likely humanspecific because fibroblasts isolated from old and young mouse ears do not show a difference in translational error rate. Furthermore, mouse fibroblasts do not adapt their translation process (protein synthesis and error rate) upon ER stress induction. We now hypothesize that healthy aging might depend on the sustained accuracy of protein synthesis by the ribosome. Regulation of translation by ER stress could be a critical event in aging, any disturbances in this mechanism could contribute to neurodegenerative diseases such as Alzheimer's and Parkinson's disease. Cellular compensation mechanisms that balance the proteome are decreasing with aging, therefor a misregulated translational error rate might disturb the homeostasis mechanisms of cells and organs.

P032 | Oxidation-driven processes in human keratinocytes analysed by mass spectrometry

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Actinic keratoses (AK) is a widespread carcinoma in situ caused by chronic ultraviolet (UV) exposure and having a high risk of progressing to squamous cell carcinomas. Due to its cumulative nature, the prevalence of AK increases with age. The underlying pathogenesis is complex, and besides genetic factors, it involves oxidative damage to DNA and proteins, resulting in alterations in keratinocyte proliferation, local inflammation, and immune suppression. Accordingly, therapeutic interventions such as imiquimod or photodynamic therapy (PDT) aim to normalise cell proliferation and immune response. Similar to PDT, gas plasma technology provides a local and tunable mix of reactive species that can target cancerous cells. First case studies suggest the effectiveness of gas plasma in AK, yet the biomedical mechanisms need further clarification. To study the impact of gas plasma in AK, a simplified in vitro model using human keratinocytes (HaCaT) and a UV-compromised variant cultured for several passages with intermitting low-intensity UVB exposure (HaCaT-UV) was established. The impact of a clinically approved argon plasma jet (kINPen) was tested using mass spectrometry to infer protein expression levels (nLCHRMS2, DIA mode), oxidative modification patterns (nLC-HRMS2, DDA mode), and lipide/lipoxidation profile (UHPLC-HRMS2). The results were correlated with cell viability, migration, and cell morphology (high-content imaging) data, identifying oxidative-stress-related expression patterns. Gene-ontology enrichment results of the differential proteins showed significant upregulation of biological processes like cell division, protein translation, and cell proliferation, and down regulation of apoptotic process, type I IFN-mediated signalling pathway. In proteins, a higher level of oxidative modifications indicative of oxidation stress was observed in HaCaT-UV.

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P033 | Oxidised skin cancer cell line lysates modify the inflammatory profile in myeloid cells in vitro

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Antitumor immunity plays a major role in cancer treatment. In these processes, inflammation is a critical process associated with the generation of reactive oxygen species (ROS) capable of inducing both oxidative stress in tumour and immune cells as well as oxidatively modifying proteins. Oxidative protein modifications have been associated with altered antigen presentation and immune recognition. However, it is unclear which types and quantities of ROS and oxidative protein modifications are associated with altered inflammatory responses. To this end, we here time attempt to generate an oxidative modification map with the aim of correlating against immune cell responses for identifying critical modification determinants in a multistep process. First, gas plasma technology, a partially ionised gas operated at body temperature, was used to produce various ROS types and concentrations. Intriguingly, these ROS mixtures can be modified by altering the plasma feed gas compositions to enhance and attenuate some ROS types over others selectively. More than 30 different conditions were mapped in terms of gas phase ROS output using optical emission spectroscopy, and selected ROS types were also quantified in liquids. In the second step, four human melanoma cell lines (MNT-1, SK-Mel-28, A375, MaMel-86a) were lysed, and lysates were exposed to each of the gas plasma conditions, effectively producing oxidative melanoma cell line protein modifications with unmatched diversity. Third, the lysates were added to two myeloid cell lines (THP-1, HL-60), and the cells' viability, metabolic activity, proliferation, inflammatory surface marker expression, and cytokine and secretion profiles were analysed. Finally, the results were correlated against gas and liquid phase profiles to associate distinct reactive species to specific surface markers and secretion profiles. In the future, all data sets will be correlated by detailed high-resolution mass spectrometry analysis of all oxidised protein lysates to identify distinct oxidative protein modifications qualitatively and quantitatively and associate these with cellular responses to such modifications in vitro. In this way, besides creating the first structural map assorting oxidative modifications

to myeloid immune cell responses, it is explored how this may be of relevance in human skin cancer.

P034 | Research on Alzheimer's skin fibroblast model to investigate neurological degeneration:CRISPR-Cas9-mediated manipulation of Presenilin1 and APP leads to elevated endoplasmatic reticulum stress and affects cell translation

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In former work with skin cells, we demonstrated that children suffering from premature aging (Cockayne syndrome and Trichothyodystrophy) experience a loss of proteostasis driven by dysfunctional ribosomes. Asking if this pathomechanism is also active in age-related diseases, we are currently studying Alzheimer's disease (AD) using skin fibroblasts. Alzheimer's disease (AD) is a complex neurodegenerative disorder marked by the accumulation of amyloidbeta plaques and neurofibrillary tangles in the brain. Mutations in the Presenilin1 (PSEN1) and Amyloid-beta precursor protein (APP) lead to familial forms of AD. In this study, we employed CRISPR-Cas9 technology to knockdown PSEN1 and APP expression in human fibroblasts and HEK 293T cells, creating a cellular model of AD. Our findings reveal a significant change in ER stress markers and unfolded protein response (UPR) levels in these modified cells, accompanied by changes in the accuracy of protein synthesis by the ribosome. These molecular alterations impacted on cellular translation dynamics. These results provide insights into the potential role of PSEN1 and APP in the quality of protein synthesis, shedding light on the molecular mechanisms underpinning AD pathogenesis. Understanding these processes may pave the way for the development of novel therapeutic strategies to combat this disease.

P035 | Skin mast cell derived exosomes can act as a communication system in the skin

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Background: Skin mast cells (MCs) contribute to inflammatory dermatoses by communicating with neighbouring cells. MicroRNAs regulate protein expression within the producing cells, or they can be shuttled to other cells, mainly encapsulated in exosomes. We set out to determine how MC-derived exosomes may act in the micromilieu of the skin.
Methods: MCs were purified from human foreskin tissue and cultured for 2–4 weeks in the presence SCF and IL-4. MCs were stimulated by FcepsilonRI cross-linking for 2.5 h and supernatants were subjected to exosome isolation with anti-tetraspanin-beads. Exosomes were characterised by immunoblot and transmission electron microscopy. miRNAs were quantified by RT-qPCR. Exosomes and exosome-free supernatants were presented to HaCaT keratinocytes and dermal fibroblasts; Jurkat cells served as malignantly transformed cells. The impact on growth was studied by MTT assay, and ATP assay.
Results: Skin MC derived exosomes were ≅50 nm in diameter and of round shape. By immunoblot, strong syntenin-1 positivity and absence of calnexin were characteristic features. Flotillin, Rab27a and CD9 were likewise detectable, whereas beta-actin and alpha-actinin were absent. Conversely, the corresponding lysates contained all of the above proteins except CD9, and additionally strong betaactin and alpha-actinin signals. The pattern was comparable between stimulated and unstimulated MCs. miRNA-718 (found in the serum of food allergic patients after oral food challenge) was primarily localised in exosomes and upregulated by stimulation, whereas miRNA-132 was mainly cell-associated. In HaCaT cells, MC-derived exosomes led to a reduction in cell growth, while a stimulatory effect was noted in Jurkat cells; a minor effect was noted in fibroblasts. Conversely, the exosomefree soluble fraction had opposite effects, interfering with Jurkat proliferation, but stimulating HaCaT (and fibroblast) growth.
Conclusion: Skin MC-derived exosomes are identified as a novel system of cell–cell communication within the skin microenvironment. Exosomes and the exosome-free fractions can exert opposite effects on the same cell subset. Since exosomes are able to act more remotely, MCs may have distinct effects on recipient cells depending on their distance. Noteworthy, malignant cells apparently respond differently to MC-derived biomolecules compared to normal cells. Skin MC exosomes are rich in selective miRNAs which are expected to modulate gene expression in recipients.

P036 | Molecular monitoring of transcriptional changes in male volunteers with commencing hair loss following treatment with *Quassia Amara* wood extract containing serum

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Androgenetic alopecia (AGA) is the most common type of hair loss driven by genetic predisposition and hormones. Men suffering from AGA experience an impact on their quality of life; technologies to prevent hair loss are highly appreciated. Reliable in vivo screening methods are essential to identifying those technologies.
Previously our group presented the beneficial effects of a *Quassia Amara* Wood Extract (QAW) on the extracellular matrix targeting protection, synthesis, processing, and organisation of essential skin-compartments. QAW further stimulates intracellular antioxidant systems. Initial lab experiments revealed promising effects on hair follicle cells, namely, induction of KI67, Versican, Catalase and hair-keratins.

In the present study, 91 male participants with commencing hair loss were assigned to 4 test groups to investigate the effects of a QAW treatment vs. placebo and other technologies. RNA-Seq was performed on plucked hair follicle samples before, after 4-day and after 6 weeks of treatment.
Following QAW treatment, 1895 and 1461 genes showed differential expression ($p < 0.05$) at 4-day and 6-week-treatment, respectively. Further analysis showed the involvement of 11 hair-growth-associated pathways, e.g. FGF- and EGF-pathways. The strongest upregulation compared to placebo was observed for GREM1, DKK3-3; KRT33A1, KAP4-4 and POSTN; while downregulation was shown for CALML5, DAPK3 and CDK2.
Using different molecular-biological tools to depict the effects of QAW on the hair follicle provides a deeper understanding of active principles, helping to develop effective products against hair loss in men with commencing hair loss. However, further studies are needed to prove the relevance of the shown gene modulations regarding hair loss prevention in vivo.

P037 | ABCB5+ MSCs from old adults fail to elicit multilayered microbicidal functions to control gram negative bacteria

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Previously, we showed that skin-derived ABCB5+ mesenchymal stem cells (MSCs) upon exposure to infection mimicking lipopolysaccharide (LPS) fundamentally shift their transcriptome with high expression and the release of neutrophil activating chemokines. This adaptive response also resulted in a significant increase in neutrophil expulsed DNA traps (NETs) and proteolytic enzymes which guarantees the defence from bacterial attack. With age the propensity for severe skin infections dramatically increases. We here set out to address the question whether MSCs from old healthy donors (>60 years) unlike young healthy donors (<30 years) may change their adaptive response upon LPS exposure towards a reduced microbicidal response. We found that co-cultures of LPS primed MSCs from old donors with activated neutrophils revealed a significant reduction in NET formation, phagocytosis of FITC labelled *E. coli* and a severely reduced killing ability (3 log phases) of either *E. coli*, *P. aeruginosa* or Staph. aureus when compared to young MSCs. To explore the underlying mechanisms, we subjected young and old donor MSCs non-primed or LPS primed MSCs to bulk RNA seq analysis. Enrichment analysis showed that NF-κB and Wnt signalling, among others, are highly upregulated, while innate immune signalling and genes encoding antimicrobial peptides like CAMP (cathelicidin/LL-37) were suppressed in LPS primed old MSCs. In fact, NF kappa B activation as depicted by Western blots and immunostaining with enhanced phosphorylation and nuclear translocation of p65 occurred in LPS primed old MSCs vs. lesser NF-κB activation in LPS primed young MSCs. IL-6, a major target gene of NF-κB, was significantly induced with a prolonged high IL-6 release from old MSCs compared to young MSCs. By contrast to the high release of cathelicidin from young MSCs, very low cathelicidin concentrations were released from old MSCs. As IL-6 suppresses cathelicidin, we studied whether silencing of the CAMP/cathelicidin gene in young MSCs may affect the microbicidal neutrophil functions in co-culture. In fact, NET formation was significantly reduced in CAMP/cathelicidin silenced young MSCs compared to non-silenced or scramble RNA treated young MSCs. The degree of cathelicidin inhibition by CAMP silencing in LPS primed young MSCs now reflects the low cathelicidin concentrations occurring in LPS primed old MSCs. Of note, cathelicidin degradation in supernatants from LPS primed young MSCs cocultured with neutrophils abrogates the strong microbicidal effects. We furthermore employed full thickness skin samples with standardised epidermal and dermal wounds (Genoskin) which were infected with a standardised load of *E. coli* in the absence and presence of supernatants from MSCs from old adults and young adults co-cultured with neutrophils. Our preliminary data show a reduced transcriptomic antibacterial defence gene signature in *E. coli* infected human skin wounds when exposed to supernatants from young adults and neutrophils as opposed to a much stronger skin response of supernatants from old MSCs and neutrophil co-cultures. These data confirm our in vitro data in human skin in situ. Collectively, we here uncovered a previously unreported, dysregulated anti-bacterial adaptive response in LPS-primed MSCs from old individuals with an impressively reduced killing ability of gram-negative bacteria. This is likely clinically relevant and highlight that aging dysregulate the adaptive response of MSCs. This may contribute to the higher susceptibility for severe local and systemic infections in elderly.

P038 | Caspase-8 influences NLRP1 signalling in human keratinocytes

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The NLRP1 inflammasome is the most important inflammasome in human keratinocytes with a critical role in regulating innate immunity in the skin. NLRP1 is formed upon activation by pathogens or stress signals, leading to caspase-1 activation. Active caspase-1 cleaves its substrates IL-1β and IL-18 and the poreforming protein gasdermin D (GSDMD). The N-terminal fragment of GSDMD oligomerizes in the cytoplasmic membrane, forming pores through which IL-1β and IL-18 are released. Caspase-8 is an initiator caspase with a critical role in the control of the apoptotic cell death signalling. Additionally to its pro-apoptotic function caspase-8 was shown to be involved in the control of the NLRP3 signalling in immune cells.
Here we present a potential role of caspase-8 in the NLRP1 inflammasome pathway in primary keratinocytes and the immortalised keratinocyte cell line N/TERT-1 upon stimulation with the dipeptidyl peptidase (DPP) 8/DPP9 inhibitor talabostat. We demonstrate that talabostat treatment results in a robust inflammasome activation in both PKs and N/TERT-1 cells

shown by activation of caspase-1 and processing and secretion of IL-1β. Moreover, cell death analyses demonstrated that talabostat treatment increased the sensitivity of keratinocytes to cell death. When caspase-1 was blocked by the specific inhibitor VX765 a robust activation of the initiator proapoptotic caspase-8 and consecutively of the executioner caspase-3 were observed. The lack of active caspase-1 resulted in inhibited but not completely blocked cleavage of both caspase-1 substrates IL-1β and GSDMD. Furthermore, GSDME was also cleaved in conditions of blocked caspase-1, confirming the activation of caspase-3. Interestingly, despite the partial activation of both pore-forming molecules GSDMD and GSDME, no IL-1β secretion was detected. Moreover, the activation of the pro-apoptotic caspase-8, did not result in increased cell death, as determined by PI staining and FACS analysis.
Taken together our data demonstrate an impact of the pro-apoptotic caspase-8 on the regulation of talabostat-induced NLRP1 inflammasome activation in human keratinocytes and suggest an interplay between signalling molecules from both apoptotic and inflammasome pathways.

P039 | Spatial transcriptomics exposes differences in the gene expression program of meibomian glands compared to sebaceous glands

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Meibomian glands (MG) are specialised exocrine glands embedded within the tarsal plates of the eyelids that continuously produce and excrete meibum, a lipid-rich material via a ductal system directly onto the ocular surface. By forming the tear film lipid layer, meibum is crucial for maintaining ocular health, and quantitative or qualitative changes in meibum may result in various ocular pathologies including dry eye disease. In spite of its importance for ocular health, the progressive transcriptional changes that lead to meibum production by MG acinar cells (meibocytes) have not been characterised in a systematic way so far. Furthermore, while it is known that meibum and sebum, the product of skin sebaceous glands (SG), show differences in their chemical composition, the transcriptional program of meibocytes in comparison to that of sebocytes remains poorly characterised. In the present study, we assessed the human eyelid with untargeted spatial transcriptomics (ST) to reveal its cellular composition and in particular to assess the pathways governing MG homeostasis and differentiation. Combined ST, pseudotime, RNA velocity, and functional enrichment analysis enabled us to map the landscape of meibocyte differentiation in space and time. In addition, the availability of published ST data for the human skin SG and the presence of hair follicle-associated SG in the analysed eyelid sample allowed a direct comparison between the sebaceous and meibomian transcriptome.
ST resolves about 18 distinct regions of the eyelid dominated by different cell types matching different structures such as the epidermis, conjunctiva, sweat glands of Moll, hair-associated SG, and MG. The latter includes three cell states, representing the basal, differentiating and terminally differentiated meibocytes (in part mixed with duct cells), thus allowing us to investigate the molecular mechanisms of meibocyte differentiation. The transcriptomic landscape of meibocyte differentiation showed a great overlap with that of sebocytes, but important differences were detected.
In conclusion, our report, accompanied by a freely browsable online tool, delivers the first high-resolution spatial portrait of the MG transcriptional landscape. Our data enhances our understanding of MG physiology and indicate novel candidate molecules for regulating MG and SG homeostasis in health and disease.

P040 | Generation and characterisation of SZ95 sebocyte spheroids: A novel three dimensional model

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Classical two-dimensional (2D) cell cultures, also known as monolayer cultures, are widely used in biological and medical research for disease modelling and drug screening. However, they exhibit in some cases several limitations, such as altered cell morphology, function, and the absence of proper cell–cell and cellmatrix interactions, making them less reflective of in vivo conditions. In this study, SZ95 sebocyte spheroids, a three-dimensional (3D) cell culture system, were generated using vessels with non-adhesive properties to create three-dimensional cell aggregates. Linoleic acid and staurosporine were used to induce lipogenesis and apoptosis, respectively. These processes were analysed using Nile Red staining under fluorescence microscopy and Annexin V/PI through flow cytometry (FACS). Protein expression levels were examined via Western blot, revealing significant differences in the expression of autophagy and apoptosis-related proteins, including ATG12, Bcl-2, BAX, P53, and phospho-p53, between 2D monolayer cultures and 3D spheroids. The results demonstrate that SZ95 sebocyte spheroids show higher basal levels of apoptosis and are responsive to induction of lipogenesis. Additionally, 3D cultures exhibited increased apoptotic cell populations compared to 2D cultures. These findings suggest that 3D sebocyte spheroids offer a more physiologically relevant model for studying cellular processes, providing valuable insights into sebaceous lipogenesis and apoptosis.

P041 | Tyrothricin strengthens cutaneous innate defence

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Tyrothricin is an antimicrobial peptide (AMP) mix of tyrocidines and gramicidins produced by the bacterium *Bacillus brevis*.

The primary therapeutic application is the topical treatment of *Staphylococcus aureus* (SA) infected skin wounds. The aim of this study was to gain more insight into the capability of tyrothricin to enhance cutaneous defence against SA.

Since SA shows increasing resistance against various antimicrobial agents, we first investigated if clinical relevant SA strains are susceptible towards tyrothricin. We found that 20 SA strains derived from lesional skin of atopic dermatitis patients and 10 wound-derived strains were efficiently killed by tyrothricin in vitro. This suggests that natural existing resistance of SA against tyrothricin is rare.

The ability of tyrothricin to combat SA skin infections was further demonstrated by in vitro and ex vivo studies. In these experiments, the application of tyrothricin to primary keratinocytes and ex vivo wounded skin explants infected with SA efficiently reduced SA growth.

Previously, we observed that the application of tyrothricin to cultured primary keratinocytes induced the expression of RNase 7, an AMP with high activity against SA. We speculated that the skin microbiota may enhance these effects. To address this hypothesis, we stimulated primary keratinocytes with tyrothricin together with a bacterial supernatant mix derived from commensals of healthy skin. This revealed a significantly higher induction of RNase 7 as compared to stimulation with either tyrothricin alone or the commensal supernatant mix alone. These data suggest that tyrothricin induces cutaneous defence against SA in the presence of commensals in a synergistic way.

In conclusion, the cutaneous application of tyrothricin may strengthen skin defence against SA in two ways: (I) Tyrothricin is able to reduce the growth of SA on the skin and on skin wounds. (II) Tyrothricin induces the skin expression of the SA-killing AMP RNase 7. These observations further highlight the potential of tyrothricin as an efficient drug to treat cutaneous infections caused by SA.

P042 | A new melanocyte specific mouse model for elucidating the role of LRIG1 in cutaneous melanoma

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Recreational exposure to the sun and a history of sunburn are often connected to an increased risk for developing malignant melanoma and the incidence of melanoma is still rising worldwide. The mortality rate has slightly decreased in the past few years as a result of the development of novel therapeutics. However, there is still a great need for further research to understand the tumorigenesis and biology of melanoma in order to respond to the rather rapid emergence of resistance to these therapeutics.

The members of the ERBB receptor family, which belong to the receptor tyrosine kinases (RTKs), are highly expressed in the epidermis. They are important regulators of various homeostatic processes, such as cell proliferation, apoptosis, and migration. Dysregulation of these receptors often leads to malignant cell transformation. Increased epidermal growth factor receptor (EGFR, ERBB1, HER1) and ERBB3 (HER3) expression is associated with a higher metastatic risk in melanoma. Leucine rich repeats and immunoglobulin like domain (LRIG) proteins are ERBB receptor regulators which act by mediating either negative or positive feedback loops. LRIG1 is known to promote stem cell quiescence and to negatively regulate the EGFR in the skin. Therefore, manipulation of the LRIG transmembrane proteins could be an interesting option for improving therapeutic outcomes during melanoma treatment. LRIG1 usually acts as a tumour suppressor and was shown to be a conserved regulator of the RTK-RASRAF- MEK–ERK signalling pathway. Human melanoma samples displayed a high LRIG1 immunoreactivity in immunohistochemistry and LRIG1 was found to be downregulated during the development of resistance to a serine/threonine-protein kinase B-raf (BRAF) inhibitor. Interestingly, treatment with a recombinant LRIG1 ectodomain successfully suppressed the proliferation of BRAF inhibitor resistant cells. However, in previous studies of our group keratinocyte-specific LRIG1 overexpression (pTRE-LRIG1;KRT5-tTA) led to the formation of heavily pigmented naevi in a two-stage skin carcinogenesis model. In this model the keratinocyte derived LRIG1 ectodomain appears to exert a paracrine function on the melanocytes of the interfollicular epidermis, resulting in increased melan-A expression, which is a diagnostic marker for cutaneous melanoma.

To further investigate the controversial tumour suppressive and oncogenic effects of LRIG1, we established a new mouse model in which a tetracycline trans-activator is expressed under the control of the endogenous tyrosinase promoter (Tyr-tTA), allowing conditional overexpression of target genes specifically in melanocytes. In this study we characterise transgenic mice expressing LRIG1 under the control of a tetracycline-responsive promoter element (pTRE-LRIG1;Tyr-tTA) to analyse the consequences of LRIG1 overexpression in melanocytes.

P043 | Identification of autophagy-regulated proteins by proteomic analysis of hair shafts and tape-stripped stratum corneum

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Autophagy is a major mechanism for the degradation of cell components during homeostasis, stress and differentiation. Dysfunctions of autophagy are implicated in the aetiology of skin diseases such as psoriasis and alopecia areata. Therefore, methods and markers for the detection of impairments of autophagy are required to validate the clinical significance of autophagy. Here, we investigated whether the suppression of autophagy in keratinocytes in vivo can be detected by the proteomic analysis of the end products of their differentiation, i.e. stratum corneum and cornified hair shafts. As a model, we utilised normal mice in comparison to mice in which the essential autophagy gene Atg7 is deleted specifically in keratinocytes. Stratum corneum was sampled by tape-stripping from the soles and hair was cut from the back. Proteins were extracted and identified by mass spectrometry-based label-free proteomics. Ten proteins were significantly elevated in the stratum corneum, whereas more than

400 proteins were increased in abundance in hair shafts when autophagy was suppressed. Pyruvate kinase, lamin A/C and filaggrin were upregulated in autophagy-deficient in the stratum corneum. The subunits of the CCT chaperonin and proteasomes were the top markers of defective autophagy in hair shafts. The results of this study demonstrate that the impairment of keratinocyte autophagy manifests in alterations of the molecular composition of the stratum corneum and hair. The proteomic analysis of hair is the most promising approach for detecting aberrations of autophagy in the epithelial compartment of the skin.

P044 | Talabostat enhances the development of AK23-induced acantholysis

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Pemphigus vulgaris (PV) is a life-threatening autoimmune skin disease characterised by flaccid bullae and persistent production of autoantibodies to desmoglein 3 (Dsg3) and, to a lesser extent desmoglein 1 (Dsg1). Previous studies and our findings showed that acantholysis induced by AK23, can be enhanced by caspase 8/3 activation. The NLRP1 inflammasome is the major inflammasome in the skin with an important role in the host response to microbial pathogens and contributing to skin immunity. Upon activation by stress signals, NLRP1 inflammasome is formed, resulting in caspase-1 activation, and IL-1β processing and secretion.

Here we aim to investigate a possible role of NLRP1 inflammasome signalling in the development of PV using primary human keratinocytes (PKs) based in vitro assays. Dipeptidyl peptidase (DPP) 8/DPP9 inhibitor talabostat was used for activation of the NLRP1 inflammasome in PKs and dispase-based keratinocyte dissociation assay (DDA) was used for analysis of antibody-induced acantholysis in PV in vitro.

Our data demonstrate that sublethal concentrations of talabostat significantly enhance AK23-induced acantholysis. Both, the pan-caspase inhibitor zVAD-fmk and the specific caspase-1 inhibitor VX765 partially suppressed this effect, indicating that talabostat-induced caspase activation is partially responsible for enhanced AK23- induced acantholysis, but further talabostat-dependent singling pathways might also be involved.

It is known that AK23 leads to translocation of Dsg3 into lipid rafts with following endocytosis. This results in decreasing levels of full-length Dsg3 in Triton soluble fraction (TSF) and in parallel increasing levels of Dsg3 in the Triton insoluble fraction (TIF). As a consequence, Dsg3 is targeted to lysosomal degradation, resulting, at the later time points, in reduced levels of Dsg3 in TIF. Here we show that combined treatment with AK23 and talabostat leads to stronger reduction of Dsg3 in both TSF and TIF, indicating a possible talabostat-mediated increase of Dsg3 lysosomal degradation. Furthermore, we demonstrate that AK23-induced acantholysis can be completely blocked by p38 inhibitor SB203580, but not in the presence of talabostat. Since p38 activation is relevant for NLRP1 activation these data might suggest that the NLRP1 inflammasome influences acantholysis.

Altogether these data suggest that talabostat might have an augmentative effect on acantholysis, which can be partially mediated by caspase activation. These findings warrant future studies to dissect the role of alternative mechanisms which are relevant for the development of antibody mediated acantholysis.

P045 | Brown seaweed extract has antimicrobial effects against *S. aureus* and shows promising effects in a 2D in vitro AD model

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Brown seaweeds contain a broad variety of unique bioactive compounds due to evolutionary adaption to the complex environment in the ocean. Some of these secondary metabolites are known to have strong antioxidant and anti-inflammatory effects, which makes them interesting as ingredients for the therapy of inflammatory skin diseases such as atopic dermatitis (AD). AD is characterised by chronic inflammation and skin barrier defects, often accompanied by colonisation with the pathogenic bacterium *Staphylococcus* (*S.*) *aureus*. While the treatment of severe AD has improved by the use of biologicals in the past years, there is still a need for more effective topical treatment options for patients with mild to moderate AD.

In our study, we examined the effects of a brown seaweed extract in an inflammatory 2D in vitro AD model. Therefore, we first checked if the extract had an impact on the viability of normal human epidermal keratinocytes (NHEKs) using a resazurin viability assay. Then, we analysed if the extract could improve the pathological gene expression pattern in the AD model. Furthermore, we examined the antibacterial effects of the extract against a *S. aureus* strain isolated from lesional AD skin in an antimicrobial assay.

We could show, that the extract had no impact on the viability of NHEKs. Stimulation with the seaweed extract inhibited the gene induction of the inflammation marker carbonic anhydrase 2 (CA2), CC-chemokine-ligand (CCL) 26 and tumour necrosis factor (TNF) alpha in the 2D in vitro AD model. Furthermore, the downregulated skin barrier molecules filaggrin and loricrin were normalised to a healthy expression level when the AD model was stimulated with the extract. Interestingly, the seaweed extract even induced the expression of antimicrobial peptides and it also had direct antimicrobial activity by significantly reducing the bacteria growth of *S. aureus* in the antimicrobial assay.

In conclusion, these first results show the promising potential of the brown seaweed extract for the topical therapy of AD. Further experiments in more complex models are needed to verify the results. In future, it would be interesting to determine which compound(s) of the extract are responsible for the observed effects.

P046 (OP06/02) | Altered Ca2+ homeostasis in keratinocytes of darier disease patients contributes to Th17-dominated skin infiltrate

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Darier disease (DD) is a rare genodermatosis that is caused by mutations in ATP2A2, encoding for sarco/endoplasmic reticulum calcium ATPase 2 (SERCA2). Patients present greasy papules and often malodorous plaques on seborrheic body areas, which significantly affect the quality of life of the individuals. Although some research has been conducted on the pathophysiological mechanism of the disease and its onset, neither the exact mechanism of DD, nor the pathways that are affected by the heterozygous mutation in SERCA2 in different cell populations, are known. We were able to detect a never-before-observed Th17- skewed cytokine profile in the lesional skin of DD patients. Therefore, we initiated the treatment with approved antibodies targeting IL-17A and IL-23A, resulting in marked clinical improvement of skin manifestation and relief of pruritus and malodor.

In the current project we aim to find out whether the T cells, the microbial infiltrate due to barrier disruption or the keratinocytes-T cell interaction is responsible for the Th17-dominated skin phenotype of DD patients. We used keratinocytes CRISPRed to express heterozygously mutated ATP2A2, we analysed proliferation (live cell imaging), differentiation (IHC) behaviour, Ca2+ homeostasis and gene expression (qPCR) of the engineered DD keratinocytes.

We found that DD keratinocytes showed decreased proliferation and impaired differentiation. Using Ca2+ imaging with Fura-2 we saw altered Ca2+ fluxes upon store depletion and extracellular Ca2+ concentration change. Using qPCR we found that DD keratinocytes express increased levels of IL-23A as compared to parental cells, pointing towards an intrinsic Th17-promoting function of keratinocytes harbouring an ATP2A2 mutation.

In further experiments we aim to analyse how T cell behaviour changes when cocultured with normal as compared to engineered DD keratinocytes.

P047 (OP06/04) | Human memory CD4+T cells enhance mast cell activation

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Introduction: During inflammation, infiltrating immune cells engage in close crosstalk with mast cells (MCs) via surface receptor-ligand interactions or soluble mediators, leading to bidirectional activation that may drive the inflammatory process. Skin-infiltrating or tissue-resident T cells, along with their secreted cytokines, play key roles in MC recruitment (e.g., IL-9), activation (e.g., IL-4, IL-5, IL-13, IFN-gamma), and survival (e.g., IL-3). Thus, T cells can regulate MC functions and may contribute to chronic MC-mediated disorders. The functional outcomes of MC-T cell interactions, particularly involving primary human MCs and T cells, have not yet been studied in detail.

Objective: This study aimed to assess the functional consequences of interactions between primary human skin MCs and memory CD4+ T cells (Tm).

Methods: Anti-CD3/CD28-stimulated Tm (act-Tm) and their corresponding supernatants (Tm-sup) were cultured with IgE-sensitised MCs for 18 h. Following incubation, MCs were challenged with different concentrations of anti-IgE. MC degranulation was measured by assessing CD63 surface levels via flow cytometry. In addition, the cytokine profile of Tm-sup was analysed. To further elucidate the potential mediators involved, MCs were primed overnight with cytokines, and cytokine-blocking antibodies were applied to investigate the contribution of T cell cytokines to MC activation.

Results: In the presence of act-Tm ($p = 0.0011$) and Tm-sup ($p = 0.0056$), MC degranulation was significantly increased on anti-IgE-challenged MCs, even when treated with sub-degranulating concentrations of anti-IgE (0.01 µg/mL, enhanced by 14%), while act-Tm and Tm-sup alone didn't cause MC degranulation. Priming of MCs with recombinant IFN-gamma and IL-4 enhanced degranulation levels on FcεRIactivated MCs, from 43% to 58%, while only IFN-gamma blocking diminished the effects of Tm-sup on hsMCs by 32%.

Conclusion: Activated T cells and/or IFN-gamma promote a hyperresponsive MC phenotype, characterised by enhanced degranulation even in response to low antigen concentrations. Disrupting physical interactions between MCs and T cells, or inhibiting T cell-derived mediators such as IFN-gamma, could represent a targeted approach to mitigate MC activation in chronic MC-driven diseases.

P048 | Pro-fibrotic activation profile of normal and cancer-associated fibroblasts from squamous cell carcinoma after modulation of the actin-binding protein filamin B

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Despite recent therapeutic advances, the treatment of advanced squamous cell carcinoma (SCC) remains challenging due to contraindications to immunotherapy as well as high toxicity and low response rates to alternative therapeutics. The tumour microenvironment and in particular cancer-associated fibroblasts (CAFs) affect SCC progression through remodelling and stiffening of the tumour matrix, resulting in impaired therapeutic susceptibility. Deciphering the underlying mechanisms responsible for ECM remodelling, such as actin cytoskeleton and adhesion

receptor modifications, may reveal novel "anti-fibrotic" targets that counteract matrix stiffness and thereby enhance therapeutic responsiveness.

First, CAFs from different SCC donors were isolated and defined regarding their profile on focal adhesions, actin cytoskeleton and associated cell motility compared to adult dermal fibroblasts from healthy donors. CAF motility was significantly reduced compared to adult fibroblasts exhibiting decreased accumulated distances over a 10 h period. Focal adhesion (FA) analysis revealed a 3-fold higher number of FAs per cell in CAFs and an increased appearance of actin stress fibres compared to adult fibroblasts. Gene expression of collagen IA1 and adhesion molecule integrin beta 1 and actin binding protein filamin B (FLNB) was increased in CAFs, as shown in our previous analyses. Similarly, immunohistochemical stainings of SCC tumours revealed elevated expression of FLNB compared to healthy skin. Functional analysis using anti-FLNB siRNA revealed reduced cell motility and increased contractility in dermal BJ fibroblasts as well as increased FA number and F-actin signal intensity per cell. Similar trends were observed in FLNB-suppressed CAFs with a pro-fibrotic expression profile and slightly reduced cell motility.

Taken together, FLNB downregulation induced a phenotype shift of normal fibroblasts towards a profibrotic (CAF-like) state and tended to further enhance CAF activation profile. Surprisingly, FLNB suppression did not impair but rather promoted pro-fibrotic activation cascades to different levels; whether these are primary or compensatory effects will be defined in follow-up studies. In addition, reciprocal communication mechanisms of CAFs with tumour keratinocytes and the impact of FLNB are currently being investigated in 3D skin tumour models.

Chemokines/Cytokines

P049 (OP01/02) | A transcriptome atlas of inflammatory skin diseases allows disease stratification based on cytokine signatures

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Inflammatory skin diseases are the most common dermatologic conditions with a high burden on quality of life of patients. Previous studies compared these conditions to healthy individuals to identify dysregulated pathways that ultimately led to identification of targeted treatment options. However, comparing the underlying signatures of inflammatory skin diseases with each other has not been performed on a big scale. Here, we performed bulk-RNA sequencing from skin biopsies of 753 different patients to build a transcriptome atlas to identify dysregulated pathways as well as cytokine and chemokine signatures across 40 inflammatory skin diseases. Notably, Cytokine gene expressions were able to split cases versus controls in most diseases. Importantly, using specific response signatures in keratinocytes, to IL-4, IL-13, type I and II interferons, IL-36, IL-17A, TNFα, and IL-17A+TNF, we stratify diseases across their cytokine signature enrichment. Furthermore, we used these cytokine signatures to train a prediction model (Naive Bayes machine learning algorithm) to classify every disease against all the other conditions. Despite unique and shared cytokine responses across many diseases, 39 of 40 diseases showed high areas under the receiver operating characteristic curve (AUC >89%), indicating that multidimensional cytokine response signatures can stratify inflammatory skin diseases from another. In summary, we have created a transcriptome atlas of inflammatory skin diseases that can be used for clinical diagnosis, management and decision making for targeted treatment of dermatological diseases based on cytokine signatures.

P050 | Dichotomous STAT5 and STAT6 activation in T cells reflects cytokine shifts between blood and skin in atopic dermatitis

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Background: Atopic dermatitis (AD) is a chronic, Th2-driven inflammatory skin disease that can be treated through JAK–STAT inhibition. However, STAT activation of T cells in peripheral blood in comparison to skin has not been investigated.

Methods: Blood immunophenotyping of AD patients ($n = 22$) with active skin lesions receiving no systemic therapy was performed by assessing immune cell subsets, T cell activation and STAT activation compared to healthy controls (HC, $n = 20$) via flow cytometry. Disease severity was measured by the Eczema Area and Severity Index (EASI) and selected patients were assessed before and after systemic treatment. ScRNA sequencing data were analysed to assess STAT signatures in T cells from lesional and nonlesional AD skin compared to HC.

Results: We observed significantly higher frequency of activated peripheral T cells and pSTAT5+ T cells in AD while pSTAT3+ and pSTAT6+ T cells were within range of HC. pSTAT5+ T cells correlated with disease activity and were induced by IL-2 and IL-4. Notably, pSTAT5+ T cells diminished with systemic treatment. By contrast, T cells in the skin of AD harboured a STAT6 enriched signature and strong pSTAT6 staining compared to HC.

Conclusion: Our study identifies a STAT5 signature in peripheral blood T cells from patients with AD, reflective of common γ chain receptor cytokine signalling. In lesional AD skin, we found STAT6, but not STAT5, to be dominant in T cells,

indicating a cytokine switch in T cells entering the skin. This finding of dichotomous STAT signatures in T cells will broaden our understanding of immune activation in AD.

P051 | Serum cytokine profiles in neutrophilic dermatoses

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Introduction: Neutrophilic dermatoses are a heterogenous group of inflammatory skin diseases characterised by the accumulation of neutrophils in the skin in the absence of clinical infection. In most cases, neutrophilic dermatoses are associated with systemic disorders including haematological, autoimmune or autoinflammatory diseases. Patients present with polymorphic skin lesions including wheals, plaques, papules, nodules, pustules, abscesses, bullae and ulcers. Neutrophilic dermatoses are primarily mediated by the innate immune system, but the exact pathophysiology is largely unclear.
Objective: Characterisation of serum cytokine profiles in neutrophilic dermatoses.
Methods: Patients with the following neutrophilic dermatoses were included: pustular psoriasis ($n=21$), hidradenitis suppurativa ($n=21$), nodulocystic acne ($n=12$), pyoderma gangraenosum ($n=13$), Behcet's disease ($n=8$), adult onset Still's disease ($n=15$), Schnitzler syndrome ($n=12$) and healthy controls ($n=22$). Sera were obtained and a multiplex assay including 45 secreted mediators was performed. The mediators were categorised into Th1/Th2, Th9/Th17/Th22/Treg cytokines, inflammatory cytokines, chemokines, and growth factors.
Results: Of the 45 mediators analysed, 27 were significantly upregulated across all diseases. Hidradenitis suppurativa showed the most upregulated mediators ($n=16$), followed by pustular psoriasis ($n=14$), Schnitzler syndrome ($n=13$), pyoderma gangraenosum and Still's disease ($n=12$ each), acne ($n=10$), and Behcet's disease ($n=5$). The Th1/Th2 cytokines IL-1 beta ($p<0.0001$ to 0.014) and IL-2 ($p<0.0001$ to 0.005) were consistently upregulated in all conditions. Except for Behcet's disease, IL-5, IL-9, IL-23, IL-31, TNF-beta, and VEGF-D were also significantly elevated across all diseases. Disease-specific upregulation was observed for IL-6 and MCP-1 in hidradenitis suppurativa IL-21 in Schnitzler syndrome, GM-CSF in pyoderma gangraenosum, IL-1alpha in acne, IL-18, IFN-gamma, and MIP-1beta in Still's disease, and IL-10 and GRO-alpha in pustular psoriasis. In Behcet's disease, IFNalpha and Eotaxin were significantly elevated.
Conclusion: The obtained disease-specific cytokine profiles offer valuable insights into the inflammatory environment of neutrophilic dermatoses. Our findings highlight the roles of individual mediators and their interactions in disease pathology. These findings will contribute to better patient stratification and support the development of targeted therapies.

P052 (OP01/03) | Immune complex induced migration of Slan+ non-classical monocytes and its implications in systemic lupus erythematosus

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The deposition of immune complexes (IC) in tissues plays a key role in the immunopathogenesis of systemic lupus erythematosus (SLE), with cutaneous manifestations occurring in 75% of individuals over the course of the disease. Previously, we have shown that immobilised IC capture slan+ non-classical monocytes (slanMo) from blood flow and induce a haptokinetic response. This project now investigates a novel, IC-induced and gradient-directed migration mechanism in slanMo in the context of SLE. We utilised a transmigration assay to assess the chemotactic response of slanMo to different IC concentrations. Blocking experiments using antibodies to different Fcγ receptors (FcγRs), pertussis toxin to block chemokine-dependent migration, and a Syk-inhibitor to block Fcγ receptors collectively demonstrated that the induced migration is CD16 (FcγRIII) dependent. Furthermore, we compared the migration behaviour of slanMo with classical monocytes and other CD16-expressing cells, i.e., NK cells and neutrophils, in response to different IC concentrations. Our findings show that IC induce a significant chemotactic effect on slanMo, comparable to known chemotactic agents such as Fractalkine and C5a. This transmigration is specific to slanMo and is dependent on the concentration of IC. In contrast, classical monocytes and other CD16-expressing cells, including NK cells and neutrophils, did not exhibit an ICdependent transmigration response. Further transcriptomic analysis and gene set enrichment analysis revealed a highly significant enrichment of a systemic lupus erythematosus gene set in slanMo responding to IC. The study concludes that slanMo exhibit a unique CD16-dependent migratory response to IC, distinguishing them from classical monocytes and other CD16-expressing cells. These findings highlight the specific role of slanMo in IC-mediated immune responses and support their role in the pathogenesis of SLE.

P053 | Drivers of inflammation in pyoderma gangrenosum: The role of IL-1 family cytokines

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Pyoderma gangrenosum (PG) is a rare, neutrophilic dermatosis characterised by rapidly developing skin ulcers with peripheral erythema and undermined borders. PG is commonly associated with immune-mediated disorders, such as inflammatory bowel disease or rheumatoid arthritis. Despite being considered an autoinflammatory disease, the precise pathogenic mechanisms remain unclear. This study investigates the involvement of key

cytokines, including members of the IL-1 family, in driving PGassociated inflammation.

Quantitative real-time PCR (qRT-PCR) was conducted to assess the expression of IL-1 and IL-36 cytokines in patients with PG ($n=20$) and other skin diseases where neutrophils are typically present, namely psoriasis vulgaris (PSO, $n=15$), and pustular psoriasis (PP, $n=10$). Immunohistochemistry (IHC) was performed to evaluate the presence of T cells (CD3+, CD4+, CD8+) and neutrophils (MPO+) in tissue samples from these patients.

The qRT-PCR analysis showed elevated levels of IL-1A, IL-1B, IL-33, and IL-8 in PG patients, with IL-1B demonstrating the highest expression. IL-36R expression was highest in PG, although differences in other IL-36 family members, such as IL-36A and IL-36G, were less pronounced across the diseases. Notably, IL-1RA and IL-36RN expression were also elevated in PG and PP patients, indicating potential regulatory mechanisms. IL-6, IL-17A, and IL-23 were significantly expressed in PG, supporting the role of Th17 cells in PG pathology. The IHC data revealed moderate infiltration of CD3+ and CD4+ T cells in PG lesions, with a low presence of CD8+ T cells. MPO staining indicated a dense infiltration of neutrophils in PG, contrasting with lower levels observed in psoriasis patients.

The combined cytokine and IHC data suggest that PG is driven by a complex interplay between elevated IL-1 family cytokines (IL-1B, IL-33, IL-8) and immune cell infiltration, particularly neutrophils and CD4+ T cells. IL-1B, IL-33, and IL-8 appear to be key drivers of the inflammatory response, while IL-36's role is less clear but warrants further investigation. The dense presence of neutrophils in PG supports its classification as a prototypical neutrophilic dermatosis and suggests potential therapeutic targets for modulating both cytokine activity and immune cell infiltration.

Clinical Research

P054 | Baseline pathological liver function tests in patients with psoriasis support the indication for systemic therapy rather than being a reason against it: A realworld analysis

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Background: Patients with psoriasis have a higher risk of liver disorders such as metabolic dysfunction-associated steatotic liver disease (MASLD, formerly known as non-alcoholic fatty liver disease (NAFLD)), drug-induced hepatitis and alcoholic liver disease (ALD) than the general population. Th17 cells and IL-17 are linked to hepatic steatosis and a proinflammatory response in MASLD. In addition, phosphodiesterase (PDE) enzymes are suggested to play a major role in fibrogenesis of the liver as they degrade cyclic adenosine monophosphate (cAMP). Newer cytokine blockers and apremilast are considered safe with regard to adverse effects on the liver and in light of the latest research in pathophysiology, might even have beneficial effects on the liver. However, dermatologists are often reluctant to start biologics in patients with elevated liver function tests (LFTs) or known hepatic disease due to concerns that their liver condition may worsen.

Objective: The aim of this study was to provide real-world evidence to address this issue.

Methods: This study retrospectively analysed the treatment courses of $N=278$ patients with psoriasis who received systemic anti-psoriatic therapy with secukinumab, ixekizumab, adalimumab, or apremilast in clinical routine. The cohort was divided into two subgroups based on normal or elevated LFTs prior to the start of therapy. AST, ALT, and GGT levels as well as the Fibrosis-4 Index for Liver Fibrosis (FIB-4) were measured at baseline, after 3 months, and after 6 months of therapy.

Results: The subgroup of patients with elevated LFTs had a higher mean Psoriasis Area and Severity Index (PASI), was more likely to be male and had a higher prevalence of metabolic syndrome comorbidities compared to the subgroup with normal LFTs. During the follow-up period, there were no significant changes in LFTs for the subgroup with normal LFTs at baseline. In the group of patients with initially elevated LFTs, all LFTs decreased significantly over time. Drug survival, discontinuation rates and PASI-75 response did not significantly differ between subgroups. In total, $N=4$ patients (1.4%) discontinued treatment due to liver-specific side effects. All of these patients had elevated LFTs at baseline. At the time of treatment discontinuation, no worsening of the FIB-4 was observed, nor was there a parallel increase in bilirubin, decrease in albumin, or decrease in blood coagulation parameters.

Discussion: We saw amelioration of LFTs in patients with initially elevated LFTs and no adverse effects on LFTs for the subgroup with normal LFTs at baseline. However, our retrospective approach does not allow for an interpretation of the mechanisms underlying the observed effects. The improved liver function in patients with initially elevated LFTs might result from a reduction in systemic inflammation due to the systemic anti-psoriatic therapy also leading to dampened inflammatory reactions in the liver.

Conclusion: This study provides real world evidence that a systemic therapy with secukinumab, ixekizumab, adalimumab, or apremilast does not present a general risk, but rather an opportunity for patients with psoriasis and elevated LFTs at baseline. Our findings will inform future studies, which are necessary to improve the level of evidence and to further disentangle the mechanisms underlying our observations.

P055 | Patterns of skin lesions in bullous pemphigoid suggest control of inflammation by local skin tissue

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In bullous pemphigoid (BP), autoantibodies target hemidesmosomal structural proteins located between the epidermis and the basement membrane. Clinically, this autoimmune response leads to scattered skin manifestations including erythema and blisters.
Other systemic immune-mediated inflammatory conditions, including rheumatoid arthritis, often exhibit a predilection for specific body regions. Furthermore, relapses frequently occur at previously affected sites, potentially due to local precipitation of inflammation by tissue-resident cells, such as fibroblasts, a phenomenon referred to as inflammatory tissue priming.
This retrospective study aimed to determine whether skin lesions in BP follow a similar pattern of localization.
To identify potential predilection sites, we analysed photographs of 119 BP patients (49 females (41%), 70 males (59%); mean age: 75 years) using the Bullous Pemphigoid Disease Area Index (BPDAI) to evaluate blisters/erosions, erythema/urticaria and mucosal lesions. Differences between body regions were assessed using a non-parametric Friedman test. Our analysis revealed that blisters/erosions were most frequently observed on the arms (77%) and legs (77%), followed by the back/buttocks, feet and hands. Similarly, erythema/urticaria were most often found on the arms (82%) and legs (76%), followed by the back/buttocks, abdomen and chest. Higher BPDAI scores, indicating more severe disease activity, were predominantly seen on the arms and legs. The majority of patients (92%) did not exhibit mucosal lesions with all examined mucosal sites being affected at similar frequencies.
To investigate whether inflammation tends to recur in previously affected skin areas, we examined 15 pairs of photographs from 9 BP patients, with each pair consisting of an image of the relapse and one of a prior occurrence. The percentage of affected skin across predefined skin areas at both timepoints was measured by Photoshop software. The overlapping area was then identified to calculate the proportion of skin involved during both the relapse and the earlier episode. A Wilcoxon signed rank test comparing the observed and expected overlap proportions indicated that the percentage of overlapping area was statistically significantly larger than would be expected by chance ($p = 0.0039$).
Our findings indicate a heterogeneous distribution of BP lesions across the skin, with preferentially affected regions and a tendency for relapses to occur in areas previously affected by inflammation. This raises the possibility that local inflammatory tissue priming may influence the localization of recurrent skin inflammation in BP. Further mechanistical studies are needed to explore these findings, as they could serve as a potential target for alternative local therapeutic approaches.

P056 | Rifampicin versus clindamycin-rifampicin in HS: Same effect or measurable differences?

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Hidradenitis suppurativa (HS) is a chronic inflammatory skin disease of the sebaceous gland unit. Treatment of HS includes beside biological still various antibiotics as doxycycline, tetracycline, rifampicin and clindamycin. So far, the mechanism of action of antibiotics in HS has not been fully elucidated, but therapy with rifampicin shows a notable improvement in the disease.
Thus, in this clinical study we aimed to determine the inflammatory pattern in blood and biopsies of HS patients before and after treatment with clindamycin in combination with rifampicin or rifampicin as a monotherapy for 12 weeks by immunohistochemically staining and protein profiling array. Corresponding samples were labelled by fluorescence and then analysed for various surface markers and cytokines/chemokines using several scioCD antibody microarrays (Sciomics).
Fifteen patients were included in the study. According to the clinical routine, 10 participants received 600 mg rifampicin and five patients received clindamycinrifampicin, 300 mg and 600 mg respectively, daily. In both groups, the IHS4 score was significantly reduced, and PRO's were improved. Treatment with rifampicin alone or clindamycin-rifampicin leads to a decrease in HS-associated cytokines measured by protein array. While the combination therapy appears not to affect neutrophils, monotherapy tends to reduce IL-8 plasma levels, which is important for the chemotaxis of the neutrophils. The number of Myeloperoxidase(+) cells in the biopsies also tends to be lower after rifampicin treatment. Regarding patientpersonalized therapy, monotherapy with rifampicin could be more successful in patients with a high number of neutrophils in their lesions.

P057 | Impact of an emollient-induced skin pH reduction on the microbial balance in atopic dermatitis

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Introduction & Objectives: Atopic dermatitis (AD) is an inflammatory skin disorder characterised by disrupted skin barrier, increased skin pH and *Staphylococcus aureus* colonisation. The complex interplay between the skin microenvironment and microbiome is not yet fully understood. The objective of this study was to investigate the ability of a verumemollient to reduce the pH and remodel the microbiome of the skin.
Methods: A double-blind, placebo-controlled study was performed with 29 individuals suffering from AD flares on contralateral bodysides. Flares were treated locally with corticosteroids until a SCORAD <15 was reached. Afterwards, participants applied the verum and a placebo emollient twice daily for 6 weeks on opposite bodysides, followed by verum application for 4 weeks. In total, six visits took place before, during and after the emollient-comparison phase. At each visit, clinical, skin physiological and skin microbiome data was collected. Furthermore, *S. aureus* isolates were cultivated in buffered medium in a pH range from 5.5 to 8.0 in vitro.
Results: Skin pH values ranged from 4.68 to 6.77 with an inverse correlation between skin pH and hydration at baseline. Both emollients successfully increased skin hydration compared to baseline. Interestingly, the verum significantly decreased the skin pH compared to baseline and more efficiently compared to placebo. While in vitro, the pH significantly influenced *S. aureus* growth, there was no clear association in vivo between skin pH and *S. aureus* relative abundance on the baseline of the study. Furthermore, the verum-induced reduction of skin pH had no significant influence on *S. aureus* and the microbiome composition during the emollient-comparison phase.
Conclusion: While the emollient positively modulated the skin barrier, the pH reduction via the verum did not alter the microbiome compared to placebo, suggesting that solely the change in microenvironment is insufficient in re-balancing the skin microbiome in AD.

P058 | Addressing the significance of the time interval between primary melanoma excision and sentinal lymph node biopsy

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Melanoma of the skin is characterised by high rates of metastasis, and although new therapeutic strategies have greatly improved the prognosis of patients in recent years, the vast majority of all deaths from skin tumours are still attributable to melanomas. Metastatic spread mostly occurs via the regional lymph nodes, and in patients with median tumour thickness of 1–4 mm, positive sentinel lymph nodes are found in 15–20%. The lymph node status therefore represents an important and additional prognostic factor. In agreement with the guidelines of the American Joint Committee on Cancer, sentinel lymph node biopsy (SLNB) is routinely performed for tumours of 1.0 mm thickness and above as well as for tumours of 0.75–1.0 mm, when there are additional risk factors as ulceration, increased mitotic rates or younger age. In patients with positive sentinel lymph nodes, there is an indication for adjuvant therapy with immune checkpoint inhibitors or targeted drugs.
However, the significance of the time interval between primary tumour excision and SLNB remains an open question, and most current national guidelines do not provide clear recommendations for the appropriate or maximum time interval. Often, SLNB is carried out together with tumour re-excision. For other patients, the time interval may be extended for various reasons. Thus, waiting times may vary in different clinics, or certain patient factors as comorbidity may lead to an extended interval. Additionally, delays have been reported due to the Covid-19 pandemic. Current data on the impact of the time of SLNB on patient survival are still conflicting. While some studies suggest that the early implementation may reduce the risk of distant metastases, a delay could also support the immune response against the tumour.
The primary goal of the present study is to determine whether a delay between diagnosis and SLNB may have an impact on the positivity rate for the sentinel lymph node. In the Charité tumour registry, 2935 melanoma patients were documented, which received SLNB between 2000 and 2024. Of these patients, 459 showed a positive sentinel lymph node, resulting in an overall positivity rate of 15.6%, which is in good agreement with data published by other tumour centers. As expected, the positivity rate increased with tumour thickness. While only 6.0% (26/430) of patients with tumours of < 1 mm were positive, 15.0% (273/1826) of patients with tumours of 1–4 mm were positive. In contrast, 28.3% (122/431) of patients with thick tumours (> 4 mm) were positive. The median tumour thickness of men appeared as slightly increased (1.7 mm) as compared to women (1.5 mm). In particular, the numbers of thick tumours (> 4 mm) were higher in men (15.9%; 250/1572) than in women (11.4%; 152/1339). Possibly related with this fact, also the positivity rate of sentinel lymph nodes was slightly increased in men (16.6%; 261/1572) as compared to women (14.8%; 198/1339).
To further address the question of the significance of the time interval, different subgroups of patients are presently compared. Thus, patients, which received SLNB before 4/6/8/12 weeks are compared to patients, which received SLNB after 4/6/8/12 weeks. Secondary evaluation parameters shall include Breslow thickness, age, gender, ulceration as well as location of the primary tumour. Answering these questions shall help to provide greater certainty for the decision when to perform SLNB in melanoma patients.

P059 | Link between system xCT/Slc7a11 and ferroptosis in systemic sclerosis

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Systemic sclerosis (SSc) is a chronic disease, characterised by the hardening, tightening of the skin and connective tissues, and often extending to internal organ failure. It is associated with vascular abnormalities, immune system activation, and increased collagen production, resulting in fibrosis development. Fibroblasts from SSc have been found to contain elevated levels of reactive oxidative species (ROS) compared to healthy individuals. The system xCT/Slc7a11 plays a crucial role in antioxidant defence by importing cystine in exchange of glutamate to synthesise glutathione (GSH). Further, glutathione peroxidase 4 (GPx4) will be activated due to GSH and this inhibits ferroptosis, an iron dependent form of programmed cell death.

RNA bulk sequencing of CD45- SSc skin cells showed a pronounced increase of solute carrier 7 a 11 (Slc7a11)/system xCT expression compared to healthy donor (HD) skin. To functionally analyse this antiporter in fibrosis development, we subjected xCT knockout (KO) mice to chronic oxidative stress through daily injections of hypochlorous acid (HOCl), which led to dermal collagen accumulation. Notably, PET/MRT imaging of a [68Ga] labelled fibroblast activation protein inhibitor (FAPI) showed less tracer binding to FAP+ cells in HOCl skin of xCT KO mice compared to WT mice. These results were reflected by a significant reduced collagen accumulation and myofibroblast activation in xCT KO animals. Gene expression profiling using Nanostring's nCounter panels verified a decrease of fibrotic genes in xCT KO mice. Furthermore, fibroblasts derived from xCT KO mice could not survive in vitro without adding ß-mercaptoethanol (ROS scavenger).

We hypothesize, that fibroblasts may undergo ferroptotic cell death due to a deficiency in xCT and therefore a lack of GPx4 activity. Importantly, we detected enhanced lipid peroxidation levels in SSc fibroblasts upon xCT-blockade. Additionally, co-injection of xCT-inhibitors imidazole ketone erastin (IKE) or sulfasalazine (SAS) and HOCl, led to a decreased dermal fibrosis in vivo. IKE and SAS are known to be ferroptosis inducer. Further, we additionally used Fra-2tg mice, that spontaneously develops a multi-organ fibrotic phenotype due to AP-1 overexpression under Hk2b-promoter control. SAS treatment in Fra-2tg mice also diminished the cutaneous fibrotic phenotype.

In summary, absence or blockade of xCT prevents skin fibrosis development, indicating its potential as a novel therapeutic approach for SSc.

P060 | Gas plasma technology optimization for enhanced drug-resistant bacteria targeting in wounds

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Microbial drug resistance (AMR) exacerbates the failure of antimicrobial wound therapies, and hard-to-heal and chronic wounds are challenging for patients and healthcare systems. An evolutionary ancient but therapeutically understudied antimicrobial tool is reactive oxygen and nitrogen species (ROS). The treatment of chronic injuries and pathogen-related skin diseases with ROS generating gas plasma technology has been shown to effectively reduce bacterial load as well as to promote wound healing and tissue regeneration. Gas plasma is a partially ionised gas, and varying the gas altered the antimicrobial effects against drug-sensitive and drugresistant bacteria in vitro. The marketed and certified gas plasma jet kINPen was used with novel ROS output chemistry to investigate cell toxicity and genotoxicity in human keratinocytes. No increase in apoptosis, micronuclei formation, or changes in the cell cycle was found with novel gas composition when compared to standard gas plasma. Exudates of human chronic wounds were evaluated for the identification of bacterial genes important for metabolic activity, cell stress, and drug resistance by customised NanoString technology. Furthermore, elevated levels of pro-inflammatory cytokines and an increased number of monocytes were found in plasma-treated wounds when exudates were analysed by flow cytometry and multiplex cytokine assay. Together, it is expected that the project's holistic approach will help improve clinical wound AMR management in the future.

P061 | The Hawthorne effect in prospective dermatological studies: Changes in perception and behaviour in a remote non-interventional study in adults and children suffering from AD

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Background: Basic emollient therapy represents a cornerstone in long-term management of atopic dermatitis (AD) aiming to reduce exacerbations and therefore the need for a pharmacological intervention (e.g., corticosteroids, calcineurin inhibitors). While pharmacological therapies typically involve defined dosage regimes, emollient use is subject to individual factors often resulting in application of inadequate amounts. A previously reported remote 12-week prospective observational study demonstrated improved, but still insufficient efficacy of individually applied skin care regimes regarding itch sensation and eczema control in AD patients. The Hawthorne effect, 'bias of being observed', is suggested to underlie the improved efficacy in surveilled real-world settings, warranting further investigation on its impact/relevance in dermatological studies.

Objectives: Data from a previously reported remote 12-week prospective observational study was subjected to secondary analyses aiming to deduce improvements in subjective AD-related outcome measures by evaluation and quantification of the Hawthorne effect.

Methods: Participants reported their individual skin care regimes and provided data regarding subjective AD symptom burden monthly over 12 weeks (Jan–Apr/2023) in an online survey. Peak pruritus intensity was rated by Numerical rating scale (NRS)-11. Eczema control was captured by the Recap of atopic eczema (RECAP) questionnaire. Data was analysed descriptively and differences were compared before and after 12 weeks for RECAP and NRS-11 scores (two-sided paired ttest/Wilcoxon signed-rank test). Correspondingly, the frequency of use of different skincare product classes (body lotion, intensive

care product, hand cream, face cream) was reported by participants at the beginning, every 4 weeks during und at the end of the study. To identify if the improvements in subjective outcome measures originate from changes in perception or behaviour, respectively, the collected data were further analysed by subgroups categorising participants based on changes in frequency of skin care use during the study period.

Results: The population comprised a total of 304 subjects, including 159 adults (mean age 34.6 ± 11.4) and 145 children (mean age 5.5 ± 3.5). Even though over 90% of the included study population reported using any form of emollient for the management of AD, a relevant subjective disease burden (mean ± SEM: RECAP sum score: 12.2 ± 0.3; NRS-11: 5.4 ± 0.1) was found at baseline. During the 12-week study period, peak pruritus (mean NRS-11 score) decreased significantly ($p \leq 0.001$) by 0.9 points (17%) with 40% of participants experiencing a minimal clinically important improvement of at least 2 points. Experience of eczema control measured by RECAP (mean sum score) significantly ($p \leq 0.001$) improved by 2.4 points (20%) with 44% of participants classifying as RECAP responders (improvement by a least on banding category). Despite the non-interventional study design, merely 30% of participants maintained their skin care frequency during the entire observation period, while 34% were found to have increased and 9% to have decreased the frequency of skin care use at least once (residual 27% with ambiguous changes were disregarded). In the two subgroups characterised by an unchanged or decreased frequency of skin care use, respectively, the improvements in mean peak pruritus NRS-11 scores (−0.7 points/−14%; −1.4 points/−26%) as well as mean RECAP sum scores (−2.0 points/−18%; −4.4 points/−35%) were still significant ($p \leq 0.05$). In particular, among participants who did not change their skin care frequency 42% were classified as responders for RECAP and 38% for peak pruritus. These response rates indicate, that a mere change in perception of the symptom burden has a more prominent role in the improvements over time than an adaption in behaviour.

Conclusion: Despite the purely observational character of the study, both itch sensation and eczema control improved significantly in AD patients during the 12-week period. The improvements appear to be subject to an observation bias (Hawthorne effect), since subgroup analyses indicate that predominantly changes in perception underlie the observed decrease in subjective disease burden. The results highlight, that the Hawthorne effect may substantially impact outcomes in dermatological studies and must be considered when interpreting clinical results, especially in the field of AD or other inflammatory skin diseases.

P062 | The role of the tumour microenvironment in primary cutaneous B-cell lymphoma: A retrospective study from the lymphoma unit at Charité

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Primary cutaneous B-cell lymphomas (CBCL) encompass the following main diseases: Follicle center lymphoma (FCL), marginal zone lymphoproliferative disorder (MZLPD) and diffuse large B-cell lymphoma, leg type (DLBCL-LT).

Currently, evidence about the tumour microenvironment (TME) and its prognostic value for (relapse-free) survival (RFS) is lacking. Insight into the TME and its potential prognostic value is particularly relevant for CBCL, as patients frequently relapse during their disease course.

Aims of this study were to evaluate histological characteristics of the TME in CBCL and to identify prognostic factors.

In this monocentric cohort study, data from all available biopsies from patients with confirmed CBCL were retrospectively analysed. Statistical analyses including univariate analysis about cell histology and immunohistochemistry were run.

Totally, 216 biopsies from 91 patients with CBCL were identified. 108/216 (50%), 89/216 (41%) and 19/216 (9%) biopsies were taken from patients with FCL, MZLPD and DLBCL-LT, respectively.

In FCL, a mix of small and large cells was found in 48/108 (44%) biopsies; atypical cells were identified in 24/108 (22%) biopsies; a dense cell infiltrate was found in 53/108 (49%) biopsies; no light chain restriction was found in 26/108 (24%) biopsies and 12/108 (11%) biopsies had a monoclonal B-cell population.

In MZLPD, a mix of small and large cells was found in 43/89 (48%) biopsies; atypical cells were identified in 22/89 (25%) biopsies; a dense cell infiltrate was found in 40/89 (45%) biopsies; a light chain restriction to kappa and lambda was identified in both 20/89 (22%) and 20/89 (22%) biopsies, respectively. In 9/89 (10%) biopsies, a monoclonal B-cell population was detected.

In DLBCL-LT, large cells were found in 11/19 (58%) biopsies; atypical cells were identified in 5/19 (26%) biopsies; a diffuse cell infiltrate was found in 7/19 (37%) biopsies; no light chain restriction was identified in 3/19 (16%) biopsies and 1/19 (5%) biopsy had a polyclonal B-cell population.

In 155 of 216 biopsies, immunohistochemical staining was performed:

In FCL, expression of BCL-2 and BCL-6 was observed in 31/85 (36%) biopsies and 39/85 (46%) biopsies, respectively. Ki-67 expression between 0 and 24% was most often found in FCL biopsies and was detected in 17/85 (20%) biopsies.

In MZLPD, expression of BCL-2 and BCL-6 was observed in 17/55 (31%) biopsies and 13/55 (24%) biopsies, respectively. Ki-67 expression between 0 and 24% was most often found in MZLPD biopsies and was detected in 7/55 (13%) biopsies.

In DLBCL-LT, expression of BCL-2 and BCL-6 was observed in 8/15 (53%) biopsies and 4/15 (27%) biopsies, respectively. Ki-67 expression between 75 and 100% was most often found in DLBCL-LT biopsies and was detected in 7/15 (47%) biopsies.

In all 216 biopsies, univariate analysis showed that 5-year RFS was 86%, 82% and 23% in biopsies with small, mixed (small and large) and large lymphocytic cell infiltrate, respectively (Kaplan Meier curve, $p = 0.009$). Another univariate analysis showed that 5-year RFS was 94% and 80% in biopsies with a diffuse and dense cell infiltrate, respectively (Kaplan Meier curve, $p = 0.016$).

In 108 FCL biopsies, univariate analysis showed that 5-year RFS was 86% and 76% in biopsies with typical cell infiltrate and atypical cell infiltrate, respectively (Kaplan Meier curve, $p = 0.009$).

This study revealed recent histological insights into the TME of CBCL and identified factors which might be of prognostic value for patient survival in a cohort with large sample size. Multivariate analyses and analyses of changes in marker

expression of patients who have relapsed at least once have yet to be performed.

P063 | Reprogramming of human monocytes by glucocorticoids: Enhanced survival and functional benefits

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Glucocorticoids (GC) are still the drugs of choice for the treatment of many chronic inflammatory diseases. Our previous studies have shown that GC treatment does not suppress monocyte functions but induces a distinct anti-inflammatory phenotype in these cells in vitro. Also the treatment of LPS-stimulated monocytes with GC leads to re-programming of the cells towards a specific population involved in resolution of inflammation. Here we further analysed GC-induced effects in monocytes from human individuals ex vivo. They received high doses of GC (at least 1 mg/kg body weight prednisolone) for 3 consecutive days. Blood was taken before and 24 h post-administration of GC- therapy. We revealed a significant upregulation of anti-inflammatory molecules like CD163 and IL-1R2 on protein level as well as gene expression after GC-administration. On the other hand, genes known to be negatively regulated by GCs, like CD36 and CXCL9/10 were significantly downregulated after oral administration of GC. Since we have already shown an increased survival in in vitro monocytes treated with GC depending on the stimulation of the adenosine receptor 3 (ADORA3), we could confirm enhanced ADORA3 expression promoting survival during staurosporine-induced apoptosis in our ex vivo monocytes from GC-treated individuals. Analysing functional properties of these ex vivo monocytes revealed a better spontaneous migration as well as an enhanced ability to phagocytosis compared to ex vivo monocytes before GC-administration. Thus we could confirm our in vitro findings of a GC-induced anti-inflammatory reprogramming of monocytes also ex vivo. Our findings underscore the ability of glucocorticoids to induce a distinct anti-inflammatory phenotype in human monocytes both in vitro and ex vivo, highlighting their role in promoting cell survival, enhancing functional capabilities, and facilitating the resolution of inflammation.

P064 | *S. aureus* as a potential predictor of therapy response to baricitinib—Skin microbiome response of adult patients with moderate-to-severe atopic dermatitis under therapy with baricitinib over 12 weeks in a real world setting

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Atopic Dermatitis (AD) is a chronic inflammatory skin disease going along with microbial dysbalance and increased *Staphylococcus aureus* abundance in lesional skin. The approval of biologics and Janus kinase inhibitors (JAKi) as new targeted therapies has revolutionised the therapeutic options in the last years. However, therapy response is heterogeneous. The search on predictors for best therapy response in distinct patient subgroups is ongoing to enable precision medicine. Baricitinib is an oral selective JAK1/2 inhibitor approved for moderate-to-severe AD with indication for systemic therapy. To date, the effects of baricitinib or other JAKi on the skin microbiome in AD are largely unknown.

This study aims at evaluating the skin microbiome in AD patients under therapy with baricitinib (i) for a better pathophysiological understanding of distinct responses and (ii) its value as a potential predictive biomarker of treatment response over 12 weeks.

34 adult patients with moderate to severe AD and indication for systemic therapy received baricitinib plus topical therapy as needed in a real world setting at the University Hospital of Bonn. Clinical and microbiome response over 12 weeks were assessed by clinician- and patient reported outcomes and microbial sampling. At baseline and week 12, optionally also at week 4 and 8, 2 skin swabs from the antecubital fossa and lesional skin were taken. Microbial taxonomy was analysed by Next-Generation Sequencing of the V1–V3 region of the 16S rRNA.

32 patients completed the study. At week 12, 15 (46.9%) achieved an 75% improvement of the Eczema Area and Severity Index, 12 (37.5%) an (almost) clear skin (validated Investigator Global Assessment) 0–1. Mean clinical severity significantly improved by week 4 and 8, followed by a slight attenuation of response by week 12. Simultaneously, mean *S. aureus* relative abundance decreased between week 0 and 8 and increased between week 8 and 12. Strikingly, patients with a vIgA 0–1 response to baricitinib at week 12 presented a lower average *S. aureus* relative abundance at all timepoints compared to patients with lower response. Patients with a high *S. aureus* relative abundance at

baseline were less likely to reach a clinical vIGA 0–1 response than patients with low *S. aureus* relative abundance at baseline. Our study supports the association of *S. aureus* load and eczema severity and demonstrates the persistence of this connection under systemic therapy with baricitinib. Our study hints a potential predictive value of *S. aureus* relative abundance at baseline for therapy nonresponse to baricitinib. However, this might be a bystander phenomenon of the association of *S. aureus* and clinical severity and needs to be studied in a larger collective.

P065 | Development of a classifier for diagnosis of early mycosis fungoides

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Mycosis Fungoides (MF) is a potentially life-threatening disease that is frequently misdiagnosed in its early stages as eczema and/or psoriasis due to the limitations of conventional diagnostic methods, such as clinical observation and histopathology. While T-cell receptor (TCR) clonality analysis may assist in diagnosis, its reliability is compromised by the occurrence of polyclonality in MF and monoclonality in benign conditions such as lichen planus. Misdiagnosis of MF, followed by inappropriate treatment with biologics designed for eczema and psoriasis, can trigger or exacerbate MF. Furthermore, late diagnosis typically limits treatment options to palliative care.

Accurate early diagnosis of MF remains an urgent unmet clinical need. To address this, we developed and validated machine learning-based predictors using bulk RNA sequencing of lesional and non-lesional skin samples from 19 MF, 105 eczema, and 112 psoriasis patients. We employed a novel biomarker identification method that combines univariate and multivariate feature selection techniques, leading to the identification of eight genes differentially expressed in MF and eczema and/or psoriasis. These markers were further validated using RT-PCR on 39 formalinfixed and paraffin embedded (FFPE) samples from MF patients and 70 FFPE samples from eczema/psoriasis patients. A gene expression-based classifier was constructed using logistic regression with repeated ($n = 10$) stacked stratified cross-validation (4-fold), achieving a mean sensitivity of 90.3% and specificity of 93.5%. The discriminative power of the selected genes, validated through RT-PCR, underscores the robustness of this approach.

In conclusion, this gene expression-based classifier represents a promising tool for early and accurate diagnosis of MF in standard FFPE tissue specimens, enabling appropriate treatment at earlier stages of the disease with potential for curative outcomes.

P066 | An open-label, uncontrolled, multicenter phase II study to evaluate the safety, local tolerability, systemic exposure, and efficacy of 1% GPB cream in adolescents with severe primary axillary hyperhidrosis

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Primary axillary hyperhidrosis (PAHH), characterised by excessive sweating due to the dysregulation of the sympathetic nervous system and overactivation of cholinergic signalling, typically begins at puberty and influences 2–16% of population. PAHH impacts patients' quality of life similarly to psoriasis and other chronic diseases. In 2022, the EU approved a cream containing 1% glycopyrronium bromide (GPB) for treating severe PAHH in adults. This study examines the safety and efficacy of 1% GPB cream in adolescents.

The objective of this open-label, uncontrolled, multicenter Phase II study was to evaluate the safety, local tolerability, systemic exposure and effectivity of 1% GPB cream in adolescents with severe PAHH. The study enrolled 42 subjects aged 12 to 17, including 23 males and 19 females. The treatment involved applying the cream once daily for the first 4 weeks (until Day 29), followed by a flexible dosing scheme (application at least twice a week up to once daily, as needed) for the next 4 weeks (until Day 57, end of treatment [EoT]). The frequency of application was recorded in a subject diary. The primary endpoints included the frequency of adverse drug reactions (ADRs), local tolerability and in a subgroup of 22 patients, plasma concentration of GPB was determined at BL, Day 8, and Day 15 to evaluate safetyrelevant systemic exposure based on the pharmacokinetic profile. Efficacy, measured by the reduction in sweat production, was assessed using gravimetric measurement (GM) at Baseline (BL), Day 29, and Day 57.

1% GPB cream was well tolerated, with no local skin reactions. Adverse drug reactions included dry eyes and increased bilirubin (each in 1 patient, 2.4%). No serious treatment-emergent adverse events (TEAEs) were reported.

Significant reductions in sweat production were observed from Baseline (296.4374.0 mg) to Day 29 (71.1 90.2 mg) and Day 57 (65.5107.8 mg) ($p = 0.0004$ and $p < 0.0001$, respectively). Improvements in the Children Dermatology Life Quality Index (CDLQI) and patient-rated hyperhidrosis severity score were also significant ($p < 0.0001$). Plasma levels of glycopyrronium bromide (GPB) were similar to those in adults, with mean values of 21.5 pg/mL at Day 8 and 28.5 pg/mL at Day 15. In line with previous findings in adults, the 1% GPB cream demonstrated excellent safety, local tolerability, and efficacy in adolescents. It significantly reduced sweat production and enhanced quality of life. Therefore, the 1% GPB cream is a safe and effective treatment option for both adolescents and adults with severe PAHH.

P067 | A proteomics-based approach for the classification of patients with systemic sclerosis

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Systemic sclerosis (SSc) is a very heterogeneous systemic disease, characterised by the development of fibrosis in multiple organs. The disease undergoes various phases depending on the activity of the inflammatory and/or fibrotic reaction. Therapeutic decisions have to be based on disease progression in individual patients. Therefore, identification of biomarkers reflecting disease activity, and the specific subset are required. Whereas analysis of biopsies requires an invasive procedure, serum samples are obtained more easily. There has been a rapid development in applying state of the art proteomics to the analysis of many different diseases to obtain an unbiased approach.

We used serum samples from well characterised patients with limited (lcSSc; 11 patients) and diffuse SSc (dcSSc; 8 patients) as well as controls. Mass-spectrometry based proteomics were used with and without previous depletion of the most abundant serum proteins.

First, it is possible to cluster serum samples of controls vs. diffuse SSc vs. limited SSc patients. The significantly altered serum proteins in limited SSc patients vs. controls belong to functional protein families involved in platelet aggregation, extracellular matrix, cytoskeleton formation and important intracellular domains such as nucleus and endoplasmic reticulum. The significantly altered serum proteins in diffuse SSc patients vs. controls belong to functional protein families involved in cytosol, nucleus, membrane components and focal adhesion as well as RNAbinding. However, there are only a few signatures that distinguish between limited vs. diffuse SSc. They were linked to fibrinogen complex, extracellular matrix and acute-phase-proteins. The clustering of all serum proteins differentiates 2 subsets. However, these subsets did not represent the traditional classification of diffuse or limited SSc. Clinical characteristics revealed similar clustering of upregulated serum proteins in patients with high vs. low mRSS. This clustering also correlated with lung involvement (TLCO). Based on the significantly altered proteins the data suggests a more refined grouping not only considering mRSS and pulmonary affection but also presence of other auto-antibodies or other internal involvement.

In conclusion, we here present data from a preliminary study using a limited number of scleroderma patient serum samples, which allow a clear distinction between SSc patients and controls. The data also indicate more heterogeneity of patients in the group of the limited and diffuse subset, depending on the severity of organ manifestation (especially mRSS and pulmonary affection). Further studies are required to analyse whether state of the art proteomic studies might be helpful to reconsider the current classification of SSc.

P068 | Assessing the influence of psoriasis and anti-inflammatory therapy on the progression of malignant melanoma

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Background: Psoriasis is a chronic inflammatory dermatological condition characterised by an accelerated proliferation of keratinocytes and an immune system that is dysregulated. The interleukin (IL)-23/IL-17 axis is a pivotal factor in the pathogenesis. IL-23 has been demonstrated to stimulate the differentiation of T helper 17 (TH17) cells, which in turn secrete the pro-inflammatory cytokine IL-17. This process serves to maintain the inflammatory response. A more profound comprehension of these immunological processes has facilitated the creation of biologics that impede IL-17 and diminish the inflammatory response in psoriasis. It is also well established that patients with psoriasis are at an elevated risk of developing cancer, which is likely attributable to systemic inflammation. The results of animal studies have yielded the initial evidence indicating that the therapeutic inhibition of IL-17 may exert a detrimental impact on the growth and therapeutic efficacy of malignant melanoma. It is difficult to make treatment decisions for patients with psoriasis and malignant melanoma due to a lack of evidence. Further data are required to elucidate the effects of IL-17 inhibition on melanoma progression. The objective of this planned study is to utilise mouse models to examine the influence of chronic inflammatory skin disease and antiinflammatory therapy on the growth and development of malignant melanoma.

We hypothesize that in the context of psoriatic inflammation and malignant melanoma, the inhibition of IL-17 may reduce the risk of tumour progression in the mouse model. This is based on the premise that anti-inflammatory therapies, particularly IL-17 blockade, have been associated with a reduced risk of cancer in previous studies. Conversely, previous studies indicate that IL-17 inhibition in conjunction with anti-CTLA4 and anti-PD1 therapies may also facilitate tumour growth. This is because low IL-17 levels have been linked to suppressed T-cell activity, which could potentially compromise the efficacy of immunotherapy.

Methods: The initial experiment will utilise the Card14DeltaE138 psoriasis mouse model, which is distinguished by a gain-of-function mutation of the caspase recruitment domain-containing protein 14 (CARD14) gene. In order to analyse the growth and progression of malignant melanoma in the context of systemic inflammation, the mice will be injected with Yale University Mouse Melanoma (YUMM) cell lines. Furthermore, the model will include anti-CTLA4 and anti-PD1 antibodies in conjunction with therapeutic IL-17 inhibition, with the objective of determining the potential impact of immune checkpoint blockade and IL-17 blockade, respectively, on the therapeutic outcome in malignant melanoma.

In a subsequent experiment, we will utilise a xenotransplantation mouse model with human psoriatic skin, with the objective of overcoming the limitations of a purely murine psoriasis model. The objective is to transplant human skin xenografts from psoriasis patients in immunodeficient NXG mice. Once

the transplants have undergone complete revascularisation, treatment with autologous PBMC (peripheral blood monocular cells) will commence. In order to ascertain the impact of the humanised psoriasis model on the efficacy of malignant melanoma therapy, human melanoma PDXs (patient-derived xenografts) that have demonstrated responsiveness to immunotherapies will also be transplanted. Subsequently, the impact of IL-17 inhibition in conjunction with and independently of anti-CTLA4 or anti- PD1 therapy will be evaluated.

P069 | Analysis of sex-specific differences in the outcome of first-line immunotherapy or targeted therapy in patients with metastatic stage IV melanoma—An ADO/DeCOG study in 2032 patients from the prospective, multicenter skin cancer registry ADOREG

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Introduction: The reactivity of the immune system, as well as tumour progression and metabolism, differ between men and women. This leads to the hypothesis that the success of tumour therapy differs between men and women, depending on the type of therapy used. The aim of our study was to examine these potential sex-specific differences in a large real-world patient cohort.

Methods: Patients with metastatic melanoma (stage IV, AJCCv8) of the skin or unknown primary were selected from the ADOREG, a prospective multicenter skin cancer registry, who had received first-line PD-1-based immune checkpoint inhibition (ICI) or BRAF/MEK-targeted therapy (TT). Endpoints included best overall response (BOR), progression-free survival (PFS) and overall survival (OS). The survival endpoints were evaluated using Kaplan–Meier analyses. Multivariate analyses were performed using Cox regression.

Results: A total of 2032 patients, m = 1274 (62.7%), f = 758 (37.3%), were included who received ICI ($n = 1484$) or TT ($n = 548$) between May 2010 and December 2020. The mean age was 66 years in the ICI and 61 years in the TT cohort. There were no sex-specific differences in BOR (G: $p = 0.77$; ICI: $p = 0.83$; TT: $p = 0.5$ 2), PFS (G: $p = 0.86$; ICI: $p = 0.46$; TT: $p = 0.21$) and OS (G: $p = 0.60$; ICI: $p = 0.20$; TT: $p = 0.30$). Multivariate analyses of PFS and OS also showed no sex-specific differences. LDH and ECOG could be confirmed as independent factors for PFS, as well as LDH and ECOG for OS. Subgroup analyses of patients treated with ICI showed an advantage for men in terms of PFS ($p = 0.041$) and OS ($p = 0.072$) with PD1-mono ICI ($n = 456$), but not with PD1 + CTLA4-combination ICI ($n = 872$). A sub-group analysis according to serum LDH (normal: $p = 0.41$; increased: $p = 0.39$) or tumour BRAF status (wt: $p = 0.15$; mut: $p = 0.82$) did not provide any relevant sex-specific differences in treatment response.

Conclusions: In the multicenter real-world cohort of patients with advanced metastatic melanoma under first-line therapy, men showed a trend for a PFS advantage over women under PD1-monoimmunotherapy. For PD1 + CTLA4 combined immunotherapy and BRAF-directed targeted therapy, however, no sex-specific differences in therapeutic success could be found.

P070 | Robust prediction of long-term disease course in atopic dermatitis employing machine learning

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The progression of atopic dermatitis is marked by fluctuating disease activity, leading to patients often not adhering to recommended therapies during remission. This reduced adherence has

serious negative effects on treatment success. Reliable longterm outcome predictions could help clinicians tailor therapy and provide patients with a clear prognostic outlook, thus improving adherence. To address this, we employed a retrospective cohort of 40 atopic dermatitis patients and control subjects with available multi-omics data, while 18 patient datasets were supplemented with 10-year follow-up records. The primary goal was to identify skin transcriptome features that can predict 10-year disease outcome parameters. By utilising supervised machine learning, a robust prediction of clinically relevant parameters, including oSCORAD, EASI, itch, and flare frequency could be achieved. To facilitate future clinical implementation, the number of predictive features was minimised. These initial skin biopsy-based findings could be translated into blood sample-based predictions for EASI and itch. Obtained predictions were accurate regardless of initial parameters such as therapy type and severity. Subsequent eQTL analysis was employed to further quantify a potential genetic contribution to these predictive features. This robust prediction of long-term outcomes in atopic dermatitis supports guided therapy and may improve patient adherence, bridging the gap between shortterm perspectives and lifelong disease management.

P071 | Adjuvant treatment decisions of patients with Stage IIB/C and Stage III melanoma: Real-word data from a large skin cancer center in Berlin

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Background: The introduction of immune checkpoint inhibitors (ICI; nivolumab, pembrolizumab) and BRAF/MEK inhibitors (dabrafenib/trametinib) for adjuvant therapy of stage III melanoma marked a paradigm shift, as these treatments almost halve the risk of recurrence. The change of paradigm extended to stage IIB/C with the approval of pembrolizumab in June 2022 and nivolumab in August 2023. However, benefits of modern adjuvants have to be weighed against the risk of potentially severe and irreversible adverse events (AE), and patient-specific factors including age, comorbidities and individual preferences play an essential role during shared decision-making. Despite tumour board recommendations for adjuvant therapy, patients may opt for different treatment paths, emphasising the complexity of patientcentered care and the need to understand influencing factors.
Objective: Our retrospective study aimed to examine tumour board recommendations and adjuvant treatment decisions of patients with stage IIB/C and stage IIIA-D melanoma from the Vivantes Skin Cancer Center in Berlin, to identify impact factors on these decisions and to assess treatment outcomes.
Methods: Tumour board recommendations, patients' treatment decisions, reasons for declining adjuvant therapy, and treatment outcomes were analysed in all patients with stage IIB/C and stage IIIA-D melanoma treated at the Vivantes Skin Cancer Center with three departments in Berlin between 01.01.2019 and 31.12.2022 (stage III) or 01.01.2019 and 30.09.2023 (stage IIB/C). Data were analysed with descriptive statistics, multivariate regression and Kaplan–Meier analyses.
Results: A total of 341 patients and 356 situations with adjuvant treatment decisions were considered. The tumour board recommended adjuvant therapy for 76.3% of the total cohort and 95.2% of the patients with the option of approved modern adjuvant therapies. In stage IIB/C, adjuvant treatment recommendations increased significantly from 35.5% in the era of interferon to 90.2% after approval of pembrolizumab in these stages ($p < 0.001$). Recommendation rates for modern adjuvant therapy in stage IIB/IIC and stage III were similar (90.2% vs. 96.4%; $p = 0.064$). Modern adjuvant therapy was accepted by 73.8% of the patients, however, with considerably higher opt-in rates in stage III than in stage IIB/C (81.7% vs. 43.1%; $p < 0.001$). Decisions in favour of modern adjuvants correlated with younger age (Odds Ratio (OR) 0.91, $p = 0.003$) and higher tumour stage. The major reason for opting against modern adjuvants was fear of AE, followed by advanced age and comorbidities. 185 patients received adjuvant therapy, among them 91.4% PD-1 inhibitors, 13.5% dabrafenib/trametinib and 1.1% interferon. Modern adjuvant therapy was associated with a trend towards improved recurrence-free survival (RFS) in stage IIC ($p = 0.067$) and significantly improved RFS in stage III ($p = 0.016$).
Conclusion: Modern adjuvant therapies are well established in routine care, but better accepted in stage III than in stage IIB/C. Our findings highlight the need for thorough patient education about the considerable risk of recurrence and the need for adjuvant therapy in these stages.

P072 | Liver metastases are associated with a short post-progression survival in a real-world cohort of melanoma patients treated with checkpoint inhibitors

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Introduction: The transition from the era of chemotherapy to the era of immunotherapy (IT) has significantly im-proved the survival of patients with metastatic melanoma. However, most patients still die due to progressive disease. The presence of metastases in immune-privileged organs such as the liver and the brain has been associated with increased therapy resistance. Therefore, we hypothesized that the era of IT alters the pattern of metastatic progression with an increased presence of metastases in liver and brain.
Materials and methods: We retrospectively compared cohorts of melanoma patients from the era of chemotherapy (2005–2008) and the era of IT (2015–2018) regarding treatment efficacy and the distribution of metastases in a real-world setting.
Results: This real-world study confirms that the transition from the era of chemotherapy to the era of IT has increased the survival of patients with metastatic melanoma. Contrary to our hypothesis, patients receiving IT did not show an increased proportion of brain or liver metastases compared to patients treated

with chemotherapy. Whereas post-progression survival was improved in patients receiving IT compared to chemotherapy for lymph node, lung and brain metastases, liver metastases were associated with a short post-progression survival also in patients treated with IT.

Discussion: In conclusion, our results challenge the concept of an immune privilege of the brain, and suggest that liver metastases are the target site limiting survival of patients with advanced melanoma receiving IT.

P073 | The role of the tumour microenvironment in primary cutaneous B-cell lymphoma: A retrospective study from the Lymphoma Unit at Charité

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Primary cutaneous B-cell lymphomas (CBCL) encompass the following main diseases: Follicle center lymphoma (FCL), marginal zone lymphoproliferative disorder (MZLPD) and diffuse large B-cell lymphoma, leg type (DLBCL-LT).

Currently, evidence about the tumour microenvironment (TME) and its prognostic value for (relapse-free) survival (RFS) is lacking. Insight into the TME and its potential prognostic value is particularly relevant for the CBCL, as patients frequently relapse during their disease course.

Aims of this study were to evaluate histological characteristics of the TME in CBCL and to identify prognostic factors.

In this monocentric cohort study, data from all available biopsies from patients with confirmed CBCL were retrospectively analysed. Statistical analyses about cell histology and immunohistochemistry were run.

Totally, 216 biopsies from 91 patients with CBCL were identified. 108/216 (50%), 89/216 (41%) and 19/216 (9%) biopsies were taken from patients with FCL, MZLPD and DLBCL-LT, respectively.

In FCL, a mix of small and large cells were found in 48/108 (44%) biopsies; atypical cells were identified in 24/108 (22%) biopsies; a dense cell infiltrate was found in 53/108 (49%) biopsies; no light chain restriction was found in 26/108 (24%) biopsies and 12/108 (11%) biopsies had a monoclonal B-cell population.

In MZLPD, a mix of small and large cells were found in 43/89 (48%) biopsies; atypical cells were identified in 22/89 (25%) biopsies; a dense cell infiltrate was found in 40/89 (45%) biopsies; a light chain restriction to kappa and lambda was identified in both 20/89 (22%) and 20/89 (22%) biopsies, respectively. In 9/89 (10%) biopsies, a monoclonal B-cell population was detected.

In DLBCL-LT, large cells were found in 11/19 (58%) biopsies; atypical cells were identified in 5/19 (26%) biopsies; a diffuse cell infiltrate was found in 7/19 (37%) biopsies; no light chain restriction was identified in 3/19 (16%) biopsies and 1/19 (5%) biopsy had a polyclonal B-cell population.

In 155/216 biopsies, immunohistochemical staining was performed: In FCL, expression of BCL-2 and BCL-6 was observed in 31/85 (36%) biopsies and 39/85 (46%) biopsies, respectively. Ki-67 expression between 0 and 24% was mostly found in 17/85 (20%) FCL biopsies.

In MZLPD, expression of BCL-2 and BCL-6 was observed in 17/55 (31%) biopsies and 13/55 (24%) biopsies, respectively. Ki-67 expression between 0 and 24% was mostly found in 7/55 (13%) MZLPD biopsies.

In DLBCL-LT, expression of BCL-2 and BCL-6 was observed in 8/15 (53%) biopsies and 4/15 (27%) biopsies, respectively. Ki-67 expression between 75 and 100% was mostly found in 7/15 (47%) DLBCL-LT biopsies.

In all 216 biopsies, univariate analysis as a Kaplan Meier curve showed that 5-year RFS was 86%, 82% and 23% in biopsies with small, mixed (small and large) and large lymphocytic cell infiltrate, respectively ($p = 0.009$). Another univariate analysis showed that 5-year RFS was 94% and 80% in biopsies with a diffuse and dense cell infiltrate, respectively ($p = 0.016$).

In 108 FCL biopsies, univariate analysis as a Kaplan Meier curve showed that 5- year RFS was 86% and 76% in biopsies with typical cell infiltrate and atypical cell infiltrate, respectively ($p = 0.009$).

This study revealed recent histological insights into the TME in CBCL and identified factors which might be of prognostic value for the RFS in a cohort with a large sample size. Multivariate analyses and analyses of changes of marker expression in patients who have relapsed at least once have yet to be performed.

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P074 | D-dimers as prognostic markers in melanoma patients under immune checkpoint inhibition—A two-center study

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Background: Immune checkpoint inhibition (ICI) has become the gold standard in the treatment of high-risk or metastatic malignant melanoma. It is known that venous and arterial thromboembolic events (TEE) occur more frequently in cancer patients and have a negative impact on overall survival. While an increased risk of these thromboembolic events has already been demonstrated in melanoma patients undergoing ICI, biomarkers to quantify the risk of TEE in this patient population are still lacking. D-dimers are a particularly sensitive plasma marker for systemic coagulation activity.

Methods: In this cohort analysis, 363 stage III/IV melanoma patients treated with ICI between April 2013 and July 2024 at the University Skin Cancer Center Hamburg and the University Medical Center Mannheim were included. D-dimers and differential blood counts were determined prospectively before the start of treatment and recorded sequentially every 3–6 weeks during treatment. TEEs were recorded and classified as thrombosis (e.g., deep vein thrombosis), apoplexy, pulmonary embolism or transient ischaemic attack.

Results: Patients with an elevated D-dimer concentration in the blood (> 0.4 mg/L) before the first cycle of ICI had a significantly

higher risk of developing thromboembolic events during the course of treatment. At the same time, patients with an elevated D-dimer concentration in the blood showed a reduced overall survival (OS) and a reduced progression-free survival (PFS). In addition, D-dimer levels were assessed during the course of ICI. **Discussion:** Patients with increased pro-coagulatory activity have a higher risk of thromboembolic events during treatment with ICI, and at the same time this activity is associated with a poorer prognosis. Markers of coagulation activity, such as the concentration of D-dimers in the blood, could be used as promising biomarkers for the occurrence of TEE as well as for the prognosis under ICI therapy.

Dermato-Endocrinology

P075 | Endothelial cells are target cells for the antifibrotic potential of the α7 nicotinic acetylcholine receptor in scleroderma

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Systemic sclerosis (SSc) is a prototypical chronic fibrotic skin disease and represents one of the biggest challenges for dermatology. Endothelial to mesenchymal transition (EndoMT) is considered to be a crucial event in the pathogenesis of SSc and describes the transformation of functional endothelial cells to collagen producing myofibroblasts. We previously showed that the α7 nicotinic acetylcholine receptor (α7nAChR) has antifibrotic potential in human dermal fibroblasts and in various models of experimentally induced skin fibrosis. Here, we investigated the role of the α7nAChR on EndoMT in human dermal microvascular endothelial cells (HDMEC). The α7nAChR expression was disclosed in normal HDMEC and also HDMEC from patients with SSc at RNA and protein level. In situ, in healthy human skin we detected a specific α7nAChR staining in endothelial cells whereas in SSc skin this staining was less prominent. Immunofluorescence analysis of normal HDMEC revealed cell membrane-associated immunoreactivity of the α7nAChR. Moreover, the α7nAChR was found to be functional in HDMEC due to calcium influx using α7nAChR agonists and antagonist. Regarding the imitation of EndoMT in vitro our functional experiments confirmed a suppression of endothelial cell markers (CD31, van Willebrand factor and VE-cadherin) in HDMEC after stimulation with transforming growth factor (TGF)-β1, tumour necrosis factor (TNF)-α, interleukin (IL)-β1 and interferon (IFN)-γ as shown by quantitative RT-PCR and Western immunoblotting. In contrast, these factors upregulated expression of mesenchymal cell markers such as collagen type I, alpha smooth muscle actin and fibronectin 1 in HDMEC. However, in whole skin organ cultures we detected a consistent specific CD31 expression in endothelial cells in situ with no alterations between the different treatments. Interestingly, in normal HDMEC TGF-β1, TNF-α and IL-1β lead to a suppression of the α7nAChR mRNA expression itself. Our ongoing investigations will clarify wether activation of the α7nAChR by specific agonists can modulate these effects in HDMEC. Intensified future research on the dermal endothelial cell system is currently being conducted to precisely dissect the role of the α7nAChR in fibrotic EndoMT.

P076 | Umbrella review on the relationship between vitamin D levels and cancer

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Background: Cancer is a growing public health problem and cancer is linked to vitamin D via several mechanisms. Recent umbrella reviews on the extra-skeletal effects of vitamin D did not turn their attention to cancer. Accordingly, an overview of the current state of research is needed.
Materials and methods: An umbrella review was conducted to provide an overview of systematic reviews on the association between vitamin D and incidence or mortality of breast cancer, colorectal cancer, lung cancer, pancreatic cancer, and prostate cancer.
Results: Inverse correlations were found between the vitamin D level (measured by circulating 25(OH)D) and mortality for all five types of cancer. For breast cancer, colorectal cancer, lung cancer, and pancreatic cancer, there are also hints of a lower incidence due to higher 25(OH)D levels.
Conclusion: As most reviews include observational studies, conclusions on causality cannot be made. Methodological differences between the included reviews and different study designs in the individual studies lead to methodological problems. Despite these problems, the review shows inverse correlations between 25(OH)D levels and mortality, and mostly inverse correlations between 25(OH)D levels and incidence.

P077 | Autoantibodies against pituitary proteins in melanoma patients with checkpoint inhibitor induced hypophysitis

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Background: Immune checkpoint blockade (ICB) therapy has revolutionised cancer treatment, particularly for melanoma patients. However, ICB can lead to immune-related adverse events (irAEs), including endocrine toxicities such as hypophysitis, hypothyroidism, and diabetes. These irAEs often result in permanent tissue damage and may require lifelong hormone replacement therapy. Specifically, hypophysitis can lead to life-threatening hormonal deficiencies, making early detection crucial for effective management. Identifying autoantibodies against pituitary-associated proteins in melanoma patients with ICB-induced hypophysitis may serve as predictive biomarkers for early diagnosis and risk assessment, guiding personalised treatment.
Methods: A multicenter study was conducted to identify potential autoantigens targeted by autoantibodies in ir-hypophysitis. Human pituitary gland lysates were immunoprecipitated using patient sera, and target antigens were identified through mass spectrometry. Enzyme-linked immunosorbent assays (ELISA) were used to validate the presence of autoantibodies against pituitary-associated proteins. Statistical analyses, including Receiver Operating Characteristic (ROC) curves, were performed to determine the diagnostic thresholds for the ELISA-based biomarkers.
Results: Autoantibodies against pituitary-associated proteins were detected in melanoma patients with ICB-induced hypophysitis, both in the discovery and multicenter confirmation cohorts. ELISA assays demonstrated that elevated autoantibody levels were significantly higher in ir-hypophysitis patients compared to controls. Using combined thresholds, the sensitivity and specificity for identifying hypophysitis in the discovery cohort were 70% and 66.7%, respectively. Validation in the multicenter cohort yielded a sensitivity of 69.8% and specificity of 63.2%. Preliminary data suggest that autoantibodies may be detectable at baseline, offering potential predictive value for ICB-induced hypophysitis.
Conclusion: The presence of autoantibodies against pituitary-associated proteins in melanoma patients undergoing ICB therapy indicates a potential biomarker for early diagnosis and risk stratification of ir-hypophysitis. Early detection may guide personalised treatment plans and timely interventions, particularly in cases where ICB is not mandatory. Further prospective studies are needed to assess the predictive utility of such autoantibodies for clinical application.

P078 | Acne and hidradenitis suppurativa lesions cycle

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Introduction & Objectives: The invisible microcomedone of the follicular canal in acne and hidradenitis suppurativa (HS) patients may spontaneously disappear, if it is not overstimulated by acne and/or HS lesion-inducing factors to further develop into a visible inflammatory lesion. While the hair follicle undergoes cycles of growth, the faith of the sebaceous and apocrine sweat glands during hair follicle cycling has not been studied. Indeed, an increase of the sebaceous gland volume has been observed in the telogene and catagene phases of the hair follicle. The asynchronous cycling of human pilosebaceous units may result into a continuous fluctuation of inflammatory follicular lesions. This spontaneous procedure might explain the high placebo rates that have occurred in both acne and HS treatment studies.
Materials & Methods: The left cheek of a 18 year-old male patient with untreated facial papulopustular acne was documented by light photography at the same time of the day on 4 consecutive days. The study protocol was approved by the Berlin Medical Association Ethics Committee. Topographical light photography detection of skin lesions was performed by a 3-D image analysis system (PRIMOS, GFMesstechnik GmbH, Berlin) able to capture the same lesion areas at different time points with a matching of approximately 100%. Subsequently, the four individual photographs (one photograph per each day) were processed to a 3 s video film sequence. The inflammatory skin lesions in the identical skin rectangle assessed over 4 days were as percentage of total skin surface by ImageJ. On the other hand, 20 physicians from different countries used the IHS4 outcome measure to evaluate five untreated HS patients in two consecutive days at the EADV School 2020 in Athens. The prospective study was approved by the ethics committee of the University of Athens.
Results: The video sequence provided the visual proof for the suspected daily fluctuation of inflammatory follicular lesions in acne. The inflammatory lesion skin area involvement in the identical assessment rectangle spontaneously declined from 29.4% to 24.5% into 4 days; the individual inflammatory skin lesions increased from 26 to 35 (Table 1). The inflammation area increased in 8 lesions ($p = 0.008$), decreased in another 8 lesions ($p = 0.008$) and remained unchanged in the last five lesions. The lesions whose size decreased with time covered 77.9% of the inflamed area on day 0 and decreased their surface to up to 57% into 4 days. In HS, the 0 to 22 detected inflammatory nodules and abscesses varied between 0 and 100% ($p = 0.00008$) in the two consecutive days, while the 0 to 6 draining tunnels only varied between 0 and 20% (Figure 1).
Conclusion: Our data corroborate a vivid, daily fluctuation of inflammatory follicular lesions in both acne and HS, providing evidence of the requirement for objective evaluation of individual lesions over time in clinical studies of pilosebaceous unit diseases.

P079 | Melatonin and BRAF/MEK inhibitors: An effective suppressive combination for targeted therapy of human melanoma

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Melanoma is a leading cause of cancer deaths worldwide. Although targeted therapy and immunotherapy have improved the outcome of patients with metastatic disease or unwanted side effects remain a problem.
Melatonin (MEL), 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, and immunomodulatory capacities.
Herein, by using human melanoma cell lines in vitro, we explore that MEL enhances anti-tumour activity of commonly used BRAF/MEK inhibitors, vemurafenib (VF) and cobimetinib (CB), respectively. Our results have demonstrated that compared to VF/CB alone, melatonin significantly reduced proliferation (scratch or drop assay) and induced apoptosis (cleaved Casp-3, PARP) in melanoma cells. Concurrently, VF/CB+MEL decreased melanoma invasiveness-related protein (E-cadherin), inducible nitric oxide synthase (iNOS), epithelial cell adhesion molecule (EpCAM), and proliferating cell nuclear antigen (PCNA); important players in melanoma tumorgenesis, tumour growth, and metastasis. In addition, we also found that the combined treatment caused significant mechanistic changes in cellular bioenergetics by (i) uncoupling of oxidative phosphorylation (OXPHOS), (ii) attenuation of glycolysis (Seahorse assessment), (iii) dissipation of mitochondrial transmembrane potential (mt$\Delta\Psi$) (FACS), (iv) changes in mitochondrial morphology (TEM), and (v) supported by a xenograft model in vivo using zebrafish embryos. Our results extend previously published data and they provide new perspectives and evidence for introduction of melatonin as an add-on complementary therapy in future treatment of melanomaaffected patients.

P080 | Metabolic regulation of neonatal epidermis composition and skin barrier formation

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The epidermis is one of the most rapidly proliferating tissues in the body with high demands for ATP but also cellular building blocks. Here we show that, in order to meet these requirements, keratinocytes constitutively express hypoxia-inducible factor-1α (HIF-1α), even in the presence of oxygen levels sufficient for HIF-1α hydroxylation. We previously reported that mice with severe epidermal mitochondrial dysfunction actually showed a hyperproliferative epidermis, but rapidly died of systemic lactic acidosis and hypoglycemia, indicating excessive glycolysis. In the present work, we interrogated HIF-1α function in glycolysis by its epidermal ablation combined with mitochondrial dysfunction, which resulted in decreased proliferation but even earlier lethality due to a severe barrier defect. Our data demonstrate that HIF-1α is indispensable for maintaining a high aerobic glycolytic flux necessary for supplying energy, but also for synthetizing cellular building blocks like lipids, which are both essential for proliferation as well as barrier formation. HIF-1α is stabilised in keratinocytes in the presence of oxygen by high levels of HIF-1α transcripts, low levels of prolyl-4-hydroxylases (PHD2 and 3) and a low cellular α-ketoglutarate/lactate ratio, likely inhibiting PHD activity. Our data suggest a key role for constitutive HIF-1α expression allowing a Warburg-like metabolism in healthy, highly proliferative keratinocytes, similar to tumour cells.
Additionally, we decipher how neonatal epidermal differentiation is mainly orchestrated by mitochondrial metabolism whereas proliferation of epidermal keratinocytes is mainly orchestrated by glycolysis.
These findings can now be applied to further improve understanding of skin homeostasis but also cutaneous pathologies such as autoimmune differentiation disorders, like in Filaggrin-mutated atopic dermatitis patients or hyperproliferative skin diseases like psoriasis.

P081 | Does the alpha-MSH-MC1R axis modulate UVA-mediated cellular responses crucially involved in dermal photoaging?

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Alpha-melanocyte-stimulating hormone (alpha-MSH)-mediated signalling plays a key role in the tanning response of the skin. Moreover, alpha-MSH directly antagonises genotoxic stress induced by UVB irradiation in both melanocytes and keratinocytes by reducing DNA damage and thus apoptosis. These biological effects of alpha- MSH are mediated by functional coupling of the melancortin-1 receptor (MC1R) with canonical cAMP signalling. Interestingly, loss of function mutations of the MC1R have been associated with increased dermal photoaging, the latter mainly mediated by UVA. Using adult human dermal fibroblasts (aHDFs) as an initial in vitro 2D model we wondered if alpha-MSH can counteract UVA-mediated cellular responses crucially involved in dermal photoaging. All primary cell lines were screened for the most common MC1R single nucleotide polymorphisms (SNPs) using a TaqMan SNP genotyping assay. Only aHDFs with wild-type MC1Rs were used for subsequent studies. Compared with the antioxidant vitamin C pretreatment (or even cotreatment) of aHDF ($n>8$ donors) with alpha-MSH did not reduce UVA-mediated accumulation of intracellular oxidative stress as measued by FACS analysis. Interestingly, pharmacological activation of canonical cAMP signalling likewise did not reduce UVA-mediated generation of oxidative stress in these cells. Indeed, aHDFs showed functional coupling of the expressed MC1R. Gene expression analysis and Western blot analysis of sirtuin-1, p21, IL-6, IL-8, matrix metalloprotease-1/3 and heme oxygenase-1 confirmed the lack of a suppressive effect of alpha-MSH or artificial pharmacological stimulation of the cAMP pathway suggesting that the alpha-MSH-MC1R axis is not protective against dermal photoaging. Studies using

spheroids and skin organ cultures are currently underway to support our findings.

Dermatopathology

P082 | Stromal mucin expression in basal cell carcinoma predicts progression under hedgehog inhibition

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Background: Basal cell carcinoma (BCC) is the most common skin cancer. The EADO published a classification in 2023 based on clinical experience including a difficult-to-treat score. This classification does not include histological criteria, although BCCs present with great morphological heterogeneity. One of the histological features of BCC are clefts between BCC tumour nests and stroma. As shown recently, these clefts are not an artefact of tissue fixation but are filled with the glycoprotein mucin. In other tumour entities, e.g. colorectal or gastric cancer, mucin is a well-known negative prognostic marker.
Aims: We set out to systematically assess morphological parameters, stromal characteristics and mucin distribution in BCCs to define histological patterns and evaluate their prognostic and predictive impact.
Patients and Methods: First, we defined an evaluation matrix including 6 categories with 3–5 parameters each. Then we analysed 75 formalin-fixed paraffin-embedded BCCs that were stained with haematoxylin/eosin and alcian blue. Our cohort consisted of 28 regular BCCs (EADO stage I) and 47 advanced BCCs (EADO stages IIB-IV). All of the latter were treated systemically.
Results: The most common histological subtypes were nodular (nBCC; $n=36$, 48.0%) and a newly defined subtype, which we termed "pleomorphic" (pBCC; $n=22$, 29.3%); 4 samples were characterised as sclerodermiform BCC (sBCC; 5.3%). Mucin was detected in 89.3% of the samples ($n=67$), predominantly within the tumour ($n=31$, 41.3%) or diffusely distributed in the stroma ($n=25$, 33.3%). An immune cell infiltrate was present in 54.7% of samples ($n=41$).
BCCs stage I/IIB presented predominantly as nBCC (50.0–53.8%) with intratumoral mucin expression (85.7–76.9%) and mild to dense immune cell infiltration (78.6–53.9%). In contrast, advanced BCCs (stage III/IV) were associated with pleomorphic or sclerodermiform subtype (48.3–60.0%), predominant stromal mucin expression (55.2–40.0%) and less frequent immune cell presence (41.4–0.0%).
In the advanced BCC cohort, 24 samples were collected before or during hedgehog inhibition (HHI). In this subset, "stromal mucin" was identified as a significant negative prognostic factor for progression-free survival in a multivariate analysis (HR 6.49, $p=0.038$) as well as in Kaplan–Meier regression ($p=0.015$).
Conclusion: Our data suggest a prognostic correlation between clinical BCC stage and (1) histological subtype, (2) immune cell infiltrate and (3) mucin distribution. In addition, stromal mucin is significantly associated with early progression under HHI treatment and may serve as a predictive marker. Further studies are planned to elucidate the mechanisms behind these findings.

P083 | Lesion localization in autoimmune blistering dermatoses is independent of site-specific antigen expression

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Autoimmune blistering diseases (AIBD) are characterised by autoantibodies targeting proteins within the epidermal/epithelial desmosome (pemphigus) or the cutaneous basement membrane zone (pemphigoid). Despite the widespread distribution of AIBD antigens across the skin and mucosa, the clinical manifestations exhibit a scattered pattern of anatomical involvement. This study describes the frequency and severity of AIBD lesions in various parts of the body and investigates whether differential expression of AIBD antigens contributes to specific predilection sites. The most commonly affected sites of blisters/erosions, erythema/urticarial lesions, and mucosal lesions were systematically analysed in cohorts of patients with bullous pemphigoid (BP), pemphigus vulgaris (PV), and pemphigus foliaceus (PF). To assess antigen expression, we performed indirect immunofluorescence (IF) staining of 11 key AIBD antigens in skin and mucosal tissues from 13 different anatomical sites of 10 body donors without AIBD. In BP, blisters/erosions and erythema/urticarial lesions predominantly affected the arms and legs. The buccal mucosa and back were found to be the most frequently involved mucosal and cutaneous sites in PV and PF patients, respectively. IF staining revealed significant regional differences in BP180, BP230, and integrin-beta4 expression, although these variations did not correlate with a higher frequency or severity of lesions. Other antigens showed consistent expression across all regions. This study suggests that the predilection sites for BP and PV/PF are largely unaffected by regional variations in antigen expression. Instead, additional factors, such as microbiota, mechanical stress, sunlight exposure, local immunity, or genetics may contribute to lesion localization in BP and pemphigus.

P084 | Exploring ribosomal biogenesis and function in ALS: The role of FUS depletion and mutation

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In skin cells of progeroid children suffering from CS and TTD, we could identify disturbances in ribosomal biogenesis and function followed by a loss of proteostasis. As loss of proteostasis, characterises aging-associated neurodegeneration, we are now investigating if disturbances in ribosomal biogenesis and function contribute to the development of these diseases. ALS(Amyotrophic Lateral Sclerosis) is a severe neurodegenerative disease associated with aging, characterised by loss of motor neuron cells, muscle atrophy and progressive paralysis. FUS accounts for about 4% of fALS (familial ALS) mutations and 1% of sALS (sporatic ALS) mutations, involved in many aspects of RNA metabolism, including transcription, splicing and translation.

In our work, we first choose HEK 293T and SHSY-5Y cells with knockout or ALS mutation in FUS as our research models to investigate ribosomal biogenesis under physiological and stress conditions. We can show that FUS depletion and FUS mutation have a significant impact on the accuracy of protein synthesis, translational fidelity and ribosomal biogenesis. In future work, ER stress and proteome stability that may be affected by FUS depletion/mutation will also be investigated. In addition, we are also planning to analyse changes in RNA metabolism in mouse cells with FUS mutation in this project.

P085 | Serration pattern analysis in the diagnosis of pemphigoid diseases

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The pemphigoid diseases are increasingly recognised as autoimmune skin disorders of the elderly with a significant morbidity and mortality rate. To enable adequate treatment for distinct clinical variants of this diseases, the correct immune serological classification is critical. High resolution direct immunofluorescence (DIF) microscopy of skin has been proposed as a sensitive measure to differentiate bullous pemphigoid (BP) from epidermolysis bullosa acquisita (EBA). The concept of serration pattern analysis of tissue-bound IgG deposits at the basal membrane zone proposes a userrated pattern as indicative for BP while a u-serrated pattern is characteristic of EBA. Recent studies show that after training, the serration pattern can be accessed and be used in routine diagnostics. The aim of our work was to evaluate the use of serration pattern in routine diagnostics of BP and EBA. In a retrospective bi-centric study, we identified and reanalyzed the DIF of a total of patients with BP ($n=45$) and EBA ($n=2$). After optimising our routine protocol, we analysed the serration pattern of tissue-bound IgG and C3 of each DIF sample using a confocal microscope. Our results show that it is possible to use the serration pattern to further increase our diagnostic capacities to distinguish BP from EBA. This was, however, accomplished after major adjustment of our standard DIF staining protocol and only upon use of a confocal microscope, which is generally not available for routine diagnostics.

P086 | Development of an immunologically active fibrosis model using hiPSC-derived skin organoids

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Skin fibrosis can lead to reduced mobility and painful skin changes. It can also lead to aesthetic changes and impaired wound healing. To better understand the underlying mechanisms and develop new effective treatments, it is important to establish skin models that mimic human skin as closely as possible. Accurately reproducing human skin models, with its complex structures such as hair, has long been a challenge. Mouse models have traditionally been used to study skin diseases. However, these models have limitations in faithfully recapitulating human skin physiology and pathology. Mouse skin differs significantly from human skin in structure and immune response, making translational relevance difficult. In addition, reliance on animal models raises ethical concerns and may not be fully consistent with the 3Rs principles of animal testing. In response to these challenges, researchers have increasingly turned to in vitro models. These models allow a better understanding of both the physiology and pathology of the skin.

However, conventional models often lack the 3D complexity and dynamic interactions found in native skin. Recognising this limitation, our work uses human induced pluripotent stem cells (hiPSCs) to create 3D skin organoids (SO) that mirror native skin architecture with higher accuracy. Therefore, we use an optimised protocol published by Lee et al. Within these hiPSC-derived SO, we were able to characterise a spectrum of skin-specific cell types, including keratinocytes (CK5), dermal cells (vimentin), adipocytes (nile red), and discerned complex hair development, indicated by the expression patterns of CK5 and CK17.

Despite the high complexity of our skin organoids, important components, like blood vessels or immune cells, are still missing to replicate the role of the human immune system. To bridge this gap, our study focus on integrating hiPSC-derived macrophages (RFP-tagged), which were developed in the research group led by Nico Lachmann, Fraunhofer ITEM. Macrophages were integrated into SO at different points in time and characterised by detection of RFP and CD86 (M1) and CD163 (M2), respectively. The macrophages integrated into the SO were successfully cultured for up to 50 days.

In parallel, our team has developed a skin fibrosis model within the SO using transforming growth factor-beta-1 (TGFß-1) and

cultured the organoids for 7 days. Using this immune-active fibrosis model, we aim to investigate the role of macrophages in the pathology of skin fibrosis. Characterisation of our fibrosis model was performed using PCR and immunofluorescence staining. We were able to identify classic fibrosis markers such as ACTA2 (smooth muscle marker that identifies myofibroblastic cells in tissue), FAP (fibroblast activation marker), α-smooth muscle actin (α-SMA, marker for myofibroblasts used to identify activation of fibrotic cells in tissue), Ki67 (marker for cell proliferation, identifies active cells in the cell cycle) and collagen 1. In addition, a change in hair growth was observed: the TGF-β treated skin organoids had few to no visible hair follicles.
In the upcoming step, we want to optimise our in vitro skin fibrosis model by cultivating an air-liquid interface (ALI-SO). ALI-SO have the advantage of a more physiological representation due to the air cultivation compared to the established SO's which are inside-out oriented. Our study will monitor changes in tissue morphology, extracellular matrix deposition, and inflammatory responses in the presence of fibrotic stimuli. In conclusion, our research highlights the importance of hiPSC-derived skin organoids as platform technology for understanding immunerelated skin disorders, offering a more translational alternative to animal models.

P087 | 3D segmentation and visualisation of skin vasculature using line-field confocal optical coherence tomography

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Background: The three-dimensional (3D) visualisation of vascular structures is crucial for understanding tumour angiogenesis, the process by which new blood vessels form. This process plays a key role in tumour growth, metastasis, and malignancy. Vascular morphology, especially in malignant skin tumours such as melanoma, squamous cell carcinoma, and basal cell carcinoma, reveals patterns that offer insights into tumour behaviour. Conventional two-dimensional (2D) imaging techniques, such as dermoscopy, lack the depth and resolution to fully capture these complex vascular networks. In contrast, Line-Field Confocal Optical Coherence Tomography (LC-OCT) provides high-resolution, real-time 3D images of the skin, making it a promising tool for studying vascular morphology in skin cancer.
Objective: This study aims to assess LC-OCT's effectiveness in providing detailed 3D visualisations of skin vasculature in malignant skin tumours. We analyse the morphological characteristics, spatial distribution, and blood flow patterns of vessels, exploring how these relate to tumour progression, metastasis potential, and therapeutic response.
Methods: LC-OCT was used to capture image stacks of various skin tumours, which were processed to enhance vascular visibility. The original image stacks were converted into negative images to improve contrast, followed by manual tracing of vessels using the Simple Neurite Tracer (SNT) plugin in ImageJ. Binary masks were created and smoothed to generate clear 3D models. This methodology was applied to melanoma, squamous cell carcinoma, and basal cell carcinoma, allowing comparative analysis of their vascular structures.
Results: The 3D reconstructions revealed diverse vascular morphologies, including serpiginous, corkscrew-like vessels in melanoma. These irregular, non-uniform patterns often extended across tissue layers, reflecting the tumour's aggressive nature. In squamous cell carcinoma, the vessels were disorganised and convoluted, while basal cell carcinoma showed elongated, sinuous vessels. The visualisations provided insights into blood flow patterns, which varied across tumour types. LC-OCT allowed for a detailed analysis of these features, offering a new perspective on tumour development and progression.
Conclusion: LC-OCT is a valuable tool for 3D visualisation of skin vasculature, particularly in malignant tumours. Its ability to reveal intricate vascular details improves diagnostic accuracy and opens new avenues for research into tumour biology and angiogenesis. The 3D reconstructions offer insights into how vascular networks support tumour growth and metastasis, highlighting their therapeutic potential. LCOCT has the potential to enhance skin cancer diagnostics and treatment.

P088 | AI-assisted evaluation of CD4/CD8-ratio in mycosis fungoides

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Introduction: Mycosis fungoides (MF) is the most frequently diagnosed cutaneous T-cell lymphoma. Early detection of the disease can impact prognosis and survival but remains a diagnostic challenge due to the great similarity of early MF to common benign inflammatory dermatoses (BID). One of the most relevant histological criteria for differentiation between the two entities is the ratio of CD4- and CD8-positive lymphocytes which is subject to inter and inner observer variability. We sought to examine whether an AI-assisted workflow could provide a similarly accurate measurement to that of human pathologists, thus improving the objectivity of the assessment.
Methods: The analysis was performed on digitalized immunohistochemical CD4 and CD8 stains from FFPE sections of 18 skin samples from MF patients. The slides were then analysed using a semi-automated AI-assisted workflow in QuPath that consisted of manual annotation of the regions of interest and automated cell segmentation using StarDist. Cell positivity was determined using an object classifier trained within QuPath.
Results: The workflow achieved mostly accurate cell segmentation and separation of positive from negative cells, showing a strong positive correlation between the average manual and AI-assisted measurements (Pearson-R 0.89). As expected, we observed a high interobserver variability and a poor agreement between the results of the workflow as compared to a single pathologist (Krippendorph's alpha 0.42).
Discussion: Despite its limitations, such as imperfect cell detection, this pilot workflow shows promise considering its excellent performance in the test scenario. We observed that the

averaged manual measurements are in much closer agreement with those obtained using the algorithms. We currently perform further analysis on a larger number of skin samples form MF and BID patients to examine the utility of the workflow in differentiating MF from BID. More research will be necessary to test interlaboratory reproducibility.

P089 | CCR4 expression in rare CTCL subtypes

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Introduction: Rare subtypes of primary cutaneous T-cell-lymphoma (CTCL) often present diagnostic and therapeutic challenges. The lack of approved treatments reflects our poor understanding of these diseases. The chemokine receptor CCR4 is physiologically expressed on effector memory (Th2) T cells and regulatory T cells (Treg), but also on tumour cells of the most common subtypes of CTCL, mycosis fungoides (MF) and Sézary syndrome (SS). The proportion of CCR4-expressing cells increases with tumour stage and correlates with a poor prognosis. The CCR4- targeting drug mogamulizumab, a defucosylated monoclonal antibody, is an effective and approved treatment option for MF and SS. Rare CTCL subtypes have not been insufficiently studied for their CCR4-expression, which could be relevant for response to targeted therapies and may serve as a potential prognostic marker. Our objective was to determine the immunohistochemical expression of CCR4 in rare CTCL subtypes and its prognostic relevance.
Material and methods: We retrospectively performed immunhistochemical stainings for CCR4 on excess FFPE tissue from skin lesions of 14 patients with rare CTCL subtypes. We included three patients with peripheral cutaneous gamma-delta T-cell lymphoma (PCGDTCL), three patients with primary cutaneous peripheral T-cell lymphoma NOS, two patients with subcutaneous panniculitis-like T-cell lymphoma and six patients with CD4+ small- to medium-sized T-cell lymphoproliferation. Slides were evaluated by a board-certified dermatopathologist and CCR4 expression was estimated as the percentage of positive cells in the lymphoid infiltrate.
Results: CCR4 expression in the analysed samples ranged between 0% to 50%. CCR4- expression was detected in all six SMPTCL (10–30%) and in one case of PTCL-NOS (50%). All other samples showed a CCR4-expression in less of <5% of the infiltrate.
Discussion: We observed a low or absent expression of CCR4 in most aggressive subtypes of CTCL with the exception of one PTCL-NOS. CCR4 expression was detectable and similarly strong within all SMPTCL samples. These findings indicate that CCR4 expression is heterogenous in rare CTCL and frequently low or absent in aggressive variants. Due to the limited treatment options in aggressive CTCL, screening for CCR4 expression could be a helpful diagnostic measure to provide a rationale for the treatment with an CCR4-antibody.

P090 | Architecture of peripheral sensory and sympathetic neurons in melanoma

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The immune and the nervous system coordinate a variety of critical responses to external danger. Together, both systems communicate through multiple neurohormonal and neuroanatomical routes to defend the host against biological and extrinsic threats. The possibility of whether immune-neuron circuits also cooperate against cancer is only beginning to be explored. We hypothesize that peripheral neurons engage in dynamic reciprocal interactions with immune cells in the tumour microenvironment.
As a basis for our work, we initially sought to characterise the neuronal architecture and determine the subsets of neurons that innervate melanomas in mice. To investigate the presence of different neuronal subtypes in melanomas in detail, we used genetically modified reporter mice that exhibit fluorescence in reporter genes for peripheral sensory (Nav1.8-Cre x R26-tdTomato) and sympathetic (TH-Cre x R26-tdTomato) neurons. We implanted TagBFP-labelled HCmel12 mouse melanoma cells or B16 melanoma cells into these mice, and harvested the melanomas once they established. Subsequently, we used optical tissue clearing and 3D imaging by light sheet microscopy in order to acquire a complete overview of the neuronal architecture in these melanomas. Both sensory and sympathetic nerves were predominantly visible in the tumour invasive margin area, and not often observed within the centers of the tumours.
Additionally, two-photon microscopy as well as confocal microscopy of cryosections of these melanomas were used to closely identify neuronal subtypes and observe potential neuro-immune interactions. Again, both sympathetic and sensory neuron subtypes were mostly visible in the tumour margin area, with the number of Nav1.8+ neurons exceeding that of TH+ neurons. Furthermore, we observed co-localization of CD11c+immune cells and neurons at the tumour edge area suggested neuro-immune interactions were occurring.
To provide more relevance to these observations, we then examined antibodystained cryosections of primary melanomas from Hgf-Cdk4 mice, in which melanomas spontaneously arise. We again observed the presence of nerves only at the melanoma border, and rarely within the center.
Taken together, these findings provide a foundation for understanding the neuronal architecture of melanomas and provide models to dissect neuro-immune interactions within the tumour environment. Currently we are establishing neuron-immunemelanoma co-cultures to better understand the cellular and molecular mechanisms between these cell types.

P091 | Cardiometabolic and psychiatric comorbidity alleviated by GLP-1RA in patients with psoriasis: A large-scale cohort study

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Introduction: Psoriasis is a chronic inflammatory skin disease linked to significant cardiovascular and metabolic, but also autoimmune and psychiatric comorbidities. Glucagon-like peptide-1 receptor agonists (GLP-1RA), like Ozempic, used to treat obesity and type 2 diabetes, may help mitigate cardiometabolic risks in psoriasis patients and were recently shown to also impact autoimmunity and neuropsychiatric conditions. This study explores mortality, cardiovascular, autoimmune and psychiatric outcomes, as well as adverse events in psoriasis patients treated with GLP-1RA.

Methods: We conducted a US-based retrospective cohort study with data from the TriNetX database, comparing psoriasis patients treated with GLP-1RA for at least 3 months to those receiving other systemic antidiabetic or obesity medications. Propensity-score matching balanced risk factors and socioeconomic variables, resulting in two cohorts of 6305 patients each, followed for 3 years. Primary outcomes included cardiometabolic, autoimmune and psychiatric complications, allcause mortality, and adverse events. Sensitivity analyses validated the results by varying the follow-up period and excluding patients with pustular psoriasis.

Results: GLP-1RA treatment was associated with significantly reduced all-cause mortality (HR 0.530, 95% CI 0.425–0.659, $p < 0.0001$), and lower risks of major adverse cardiac events (HR 0.802, 95% CI 0.696–0.925, $p = 0.016$), depression (HR 0.797, CI 0.699–0.910, $p = 0.004$), and substance abuse (HR 0.703, CI 0.584–0.848, $p = 0.001$) compared to the control group. Adverse drug events were not more frequent in the GLP-1RA group, and findings were consistent across sensitivity analyses.

Conclusion: GLP-1RA treatment is safe and associated with lower risks of cardiovascular and psychiatric comorbidities and reduced mortality in psoriasis patients. It should be given particular consideration for psoriasis patients with obesity or type 2 diabetes.

P092 | Dupilumab shows no elevated risk for maternal adverse pregnancy outcomes: A large-scale, propensity-score-matched retrospective cohort study

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Type 2 chronic inflammatory diseases (T2IDs) are common among women of reproductive age. Dupilumab, a monoclonal antibody, is increasingly used to treat T2IDs. While its use is not officially approved during pregnancy, smaller studies suggest no increased risk of adverse pregnancy outcomes (APOs). However, further data are needed to evaluate the safety of dupilumab during pregnancy. Therefore, this study aimed to retrospectively examine the risk of APOs in pregnant women treated with dupilumab using a large real-world dataset.

Data were extracted from the US-Collaborative Network of TriNetX, including pregnant women with T2ID who received dupilumab and who did not receive treatment as a control group. Propensity-score-matching was applied to balance demographics, diagnoses, medications, and potential APO risk factors. The study analysed outcomes as various maternal complications, using Kaplan–Meier survival analysis, log-rank tests for outcome differences, and Cox-regression models for hazard ratios (HR).

A total of 293 women were analysed for treatment with dupilumab during pregnancy. Additionally, 243 women were treated with dupilumab 6 months before pregnancy, and 300 women were analysed for postpartum outcomes. After propensity-scorematching, no increased risk for APOs was observed in the dupilumab-treated groups. Interestingly, the group with dupilumab treatment during pregnancy had reduced risks of preterm labor (HR: 0.11, confidence interval (CI): 0.03–0.45, $p = 0.0002$) and "any APO" (HR: 0.53, CI: 0.33–0.84, $p = 0.0067$). No differences in APO risks were found between dupilumab-treated and untreated women up to 6 months before pregnancy or during the postpartum period. This large, propensity-score-matched retrospective study suggests that dupilumab has a favourable safety profile during pregnancy. Given the difficulties of prospective studies during pregnancy, it provides valuable insights though further studies are needed to confirm these results and explore potential causal relationships.

P093 | FLCN knockdown increases DNA damage susceptibility and global hypomethylation in human hair follicles ex vivo

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Birt-Hogg-Dubé (BHD) syndrome is a rare genodermatosis caused by heterozygous pathogenic variants in the FLCN gene. The phenotype of this syndrome is defined by cutaneous fibrofolliculomas and trichodiscomas, lung cysts, and an attendant risk of pneumothorax and renal cell carcinoma. Fibrofolliculoma and trichodiscomas are follicular-sebaceous tumours which develop after the twentieth decade, prompting the hypothesis that susceptibility to DNA damage on sun-exposed sites may contribute to tumorigenesis. The pathogenesis of these tumours remains unexplained; however, accumulating evidence implicates epigenetic modifications in the development of certain dermatoses. The central epigenetic mark, 5-methylcytosine (5-mC), indicates DNA methylation and gene expression silencing. Conversely, DNA demethylation, facilitated by Ten-Eleven Translocation (TET) proteins, converts 5- mC to 5-hydroxymethylcytosine (5-hmC), potentially reactivating silenced genes. Moreover, 5-hmC together with γH2Ax can accumulate rapidly at sites of endogenous DNA double-strand breaks, serving as early DNA damage markers. Here, we investigate whether folliculin (FLCN) can regulate epigenetic changes in human scalp hair follicles ex vivo. First, we knocked down FLCN in organ-cultured human anagen HFs from 3 donors with FLCN siRNA for 6 days. Quantitative real-time PCR (qRTPCR) and quantitative immunohistomorphometry (qIHM) confirmed successful FLCN silencing at the mRNA and protein level compared to non-targeting oligonucleotides (NTOs). Moreover, qIHM revealed a significant decrease in expression of 5-mC and an increase in 5-hmC and γ-H2A.x expression in hair matrix keratinocytes. Our preliminary data demonstrate that FLCN knockdown in HFs leads to elevated levels of 5-hmC expression and decreased levels of 5-mC, which represents prominent DNA hypomethylation. These data suggest potential upregulation of gene expression through TET protein-mediated demethylation, which we plan to explore in future studies. Separately, elevated levels of γ-H2A.x and 5-hmC expression indicate increased DNA damage, which in the absence of TET may induce mitotic abnormality and contribute to tumour development. This epigenetic regulation pathway may mediate fibrofolliculoma and trichodiscoma development following intrafollicular FLCN inactivation.

P094 | Novel germline KIT variant, KIT F508C, with homozygous occurrence in a consanguineous kindred with familial mastocytosis

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Background: Mastocytosis, characterised by abnormal accumulation of mast cells in various tissues, usually arises sporadically, but rarely familial occurrence is also observed. Familial mastocytosis is in part associated with familial gastrointestinal stromal tumours (GIST) and pigmentation disorders. Most patients with sporadic mastocytosis carry an activating mutation in exon 17 of the KIT gene, KIT D816V. Familial occurrence is often also associated with KIT mutations, but then with germline KIT variants that mostly affect other regions of KIT, particularly exons 8, 9, 10, 11 and 13.
Objective: We report a kindred with familial mastocytosis carrying a novel germline KIT variant that can also occur in a homozygous state.
Case report: A 20-month-old girl presented with pruritus, orange-brown pigmentation of the skin, pronounced dermographism, and blistering. Symptoms had started at the age of 6 months. A skin biopsy showed increased numbers of mast cells in the upper dermis, and her serum tryptase level was elevated at 70 µg/L. She was diagnosed with diffuse cutaneous mastocytosis and treated with high-dose H1 antihistamines. At follow-up after 3 years, the family also presented their second child, a boy aged 25 months, who also suffered from pigmentation, dermographism, and blistering, indicating familial occurrence of mastocytosis. A more detailed family history revealed that also the father and two paternal cousins frequently suffer from pruritus and whealing, one of them also from skin pigmentation and blisters. Tryptase levels in the father and the two adult cousins ranged between 6.4 and 11.8 µg/L. The consanguineous family originated from a Kurdish village in Southeastern Turkey. Buccal swabs from the two children and peripheral blood from the father and cousins were collected for genetic analysis upon written informed consent. Sanger sequencing was conducted on exons 1 to 21 of the KIT gene, including 10 bp of flanking introns. Here, we identified a novel germline missense variant in exon 9, KIT F508C (c.1523T>G; p.Phe508Cys; OMIM 154800), present in all five affected family members. Interestingly, this variant was homozygous in the two index children and in one cousin, while the father and the other cousin were heterozygous carriers. No other KIT variants besides KIT F508C were detected in the five family members.
Summary: We report a novel germline KIT variant in exon 9, KIT F508C, in a consanguineous kindred with familial mastocytosis, thus expanding the spectrum of described KIT variants in mastocytosis. Furthermore, we show that this KIT mutation can also occur as homozygous variant. To our knowledge, this

is the first report of a KIT variant in a homozygous state in mastocytosis.

Health Services Research

P095 | Enhanced follicular delivery of isopropanol using hesperetin nanocrystals for deep and prolonged antisepsis

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Surgical site infections (SSIs) are among the most common healthcare-associated infections, affecting more than 800 000 patients in Germany each year, and costing hospitals an average of EUR1,500 per patient. SSIs can occur as a result of prolonged surgical procedures and can significantly compromise wound healing, leading to post-operative complications such as chronic inflammation, extended hospital stays, the need for additional surgeries, and even death. Analysis of SSI specimen has shown that endogenous microorganisms are the main cause of these infections. In fact, current antiseptic treatments are effective on the skin's surface, but do not penetrate into the hair follicles, leaving approximately 25% of these microorganisms intact. This creates a risk of recolonization during prolonged surgeries, which increases significantly every 15 min.

To address this limitation, we investigated the use of hesperetin nanocrystals as a novel delivery system for isopropanol, to enhance follicular antisepsis and reduce the risk of SSIs. The nanocrystals are designed to penetrate deeper into the hair follicle by leveraging the "ratchet effect", a mechanism in which the asymmetry and oscillatory motion of the hair during massage drives the nanocrystals and the formulation they're suspended in deeper into the follicle.

In our ex vivo model, porcine ear skin was used to apply two different formulations, the isopropanol-based hesperetin nanocrystal formulation and an isopropanol-based particle-free control formulation, while one skin area remained untreated and served as our baseline. Intrafollicular concentration and penetration depth were assessed in all samples using rhodamine 6G, a fluorescent marker included in each formulation. Quantitative fluorescence spectroscopy was used to determine concentration levels, while laser scanning microscopy was employed to measure penetration depth.

Our results demonstrated a significant increase in both the intrafollicular concentration (>85% higher) and penetration depth (~40% deeper) of the nanocrystal-delivered antiseptic formulation compared to the control. These findings suggest that nanocrystal-based formulations hold promise for reducing the risk of SSIs by achieving deeper follicular antisepsis, potentially preventing recolonization by endogenous bacteria during extended surgeries.

A follow-up in vivo human study is planned for the end of 2024. This study will implement our formulation into a two-step disinfection protocol, combining the nanocrystal formulation with a traditional isopropanol treatment, to thoroughly disinfect first the hair follicles and then the skin surface. This two-step process will allow us to compare our results to the current standard of care while also evaluating the efficacy of our formulation in reducing skin recolonization over time and preventing SSIs.

P096 | Data protection and its implementation in the CRUSE® mobile health application for chronic spontaneous urticaria according to the General Data Protection Regulation (GDPR)

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Background: Chronic spontaneous urticaria (CSU) is an inflammatory skin disorder characterised by the sudden appearance of intensely itchy wheals and/or angioedema in the absence of a definite trigger for more than 6 weeks and may severely impact the quality of life of affected patients. To ensure optimal treatment for CSU patients, continuous monitoring and documentation of disease activity is essential. Facing the high medical need for a convenient, user-friendly platform for CSU patients, the CRUSE® (Chronic Urticaria Self Evaluation) Control Urticaria mobile health app was developed by an international steering committee of urticaria specialists from the UCARE (Urticaria Centers of Reference and Excellence) team on the basis of patient-reported outcome measures (PROMs) and visual analogue scales (VAS) that assess overall urticaria symptoms, health and their impact on work or school productivity. CRUSE® allows users to document their symptoms and share results gathered in a medical report with their treating physician via email or QR code. CRUSE® is ad-free, free to download and use and available for Android and iOS systems. **Objective & Methods:** The use of CRUSE® implies the acceptance of a general information document (Terms & Conditions; T&C), which informs users about the purpose and operation of the application, their rights and the use of the data collected, as well as a data protection declaration (Privacy Policy; PP). As CRUSE is subject to German jurisdiction, these technical documents were created to comply with the new European legislation based on the General Data Protection Regulation (GDPR) law. **Results:** In collaboration with national and international lawyers, an easily accessible and understandable version respecting all main principles of personal data protection ('lawfulness, fairness and

transparency'; 'purpose limitation'; 'data minimisation'; 'accuracy'; 'storage limitation'; 'integrity and confidentiality', 'accountability') was implemeted in these technical documents and translated into national language. **Conclusion:** CRUSE® is a unique mHealth app to help chronic urticaria patients track and manage their condition. Patients' personal data provided upon acceptance of the CRUSE® T&C and PP documents are protected in accordance with the European Union's General Data Protection Regulation (GDPR).

P097 | Participation and disability in ichthyosis: A qualitative study on impairment and care experiences

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Background: Inherited ichthyoses are clinically and genetically heterogeneous keratinisation disorders, characterised by scaling, hyperkeratosis, inflammation, and sometimes blistering. Hypohidrosis and pruritus are common symptoms. Ichthyosis impairs quality of life and is associated with substantial personal, financial, and time burden [1]. The study analyzes patients' experiences regarding impairment and disability, as well as with medical and psychosocial care. The aim of the qualitative preliminary study is to identify key topics and patient-relevant outcome dimensions for questionnaire development, thereby improving its content validity [2].
Method: In a qualitative study, semi-structured in-depth interviews were conducted at two German dermatology departments (Muenster, Berlin) with affected individuals and their relatives. Two men and seven women (18 to 62 years) with different types of ichthyosis (Netherton Syndrome, ARCI, epidermolytic ichthyosis) were interviewed (mean duration 56 min); results were analysed using thematic analysis [3].
Results: The most significant effects of ichthyosis on daily activities, employment, and social participation stem from the severity of the skin condition, hypohidrosis, time-consuming care routines, and the experience of stigmatisation.
Limitations in social participation arise from feelings of exclusion from peer-related activities, such as travel, sports, and other social engagements, often accompanied by a sense of loneliness and isolation.
Participation in working life may be hindered by experiences of rejection and discrimination during job searches and within everyday work environments. Occupational choices may be restricted, potentially influencing an individual's overall life trajectory.
Disability is most frequently discussed in the context of social medical services. Legal recognition of disabilities and the assumption of costs for ongoing treatments are challenging due to the scarcity of qualified assessors to determine entitlement. Moreover, receiving a high level of care can negatively impact the recognition of disability status, thereby limiting access to social benefits.
Furthermore, the out-of-pocket expenses associated with the acquisition of essential items required for participation in daily life are frequently regarded as a significant financial burden.
Conclusion: Limitations in participation constitute an entitlement to compensation for disability [4]. Cost coverage for required topical treatments can reduce burden. Guidelinebased clinical care paths ensure access to specialised outpatient clinics and social medicine counselling. Dermatologists can support the recognition of disability by documenting and confirming the medical and social significance of the condition, as well as the associated participation restrictions. The question arises as to whether people with skin diseases are adequately considered in the context of inclusion and compensation for disadvantages. An empirical data basis is necessary. Therefore, a valid instrument for measuring impairment, disability and participation is crucial for deriving care needs and potential social law entitlements.
Due to a recruitment from two specialised sites there might be a selection bias with rather closely monitored patients. The real impairment in ichthyoses in the overall patient group in Germany and Europe could be even underestimated in this study.

References

1. Cortés, H., et al. (2023). High levels of anxiety, depression, risk of suicide, and implications for treatment in patients with lamellar ichthyosis. Healthcare, 11(14), 2071. https://doi.org/10.3390/healthcare11142071
2. Terwee, C., et al. (2018). COSMIN methodology for assessing the content validity of PROMs-user manual. Amsterdam: VU University Medical Center.
3. Clarke, V., & Braun, V. (2017). Thematic analysis. The Journal of Positive Psychology, 12(3), 297–298. https://doi.org/10.1080/17439760.2016.1262613
4. United Nations. (2006). Convention on the Rights of Persons with Disabilities. Treaty Series, 2515, 3. https://www.un.org/development/desa/disabilities/

P098 | A novel patient education tool of the CRUSE® mobile health application to empower chronic spontaneous urticaria patients

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Background: Chronic spontaneous urticaria is chronic inflammatory skin disease associated with the sudden appearance of intensely itchy wheals and/or angioedema in the absence of a definite trigger for more than 6 weeks that may lead to substantial impairment of the quality of life. These symptoms have a fleeting nature and are often barely recognisable or not recognisable at all at the time of consultation. Consequently, self-evaluation of disease activity is tremendously important.

Moreover, as urticaria symptoms often change in intensity, it is essential that patients document their symptoms continuously. The CRUSE®(Chronic Urticaria Self Evaluation) Control Urticaria mobile health app is a UCARE tool for patients with CSU enabling documention of signs and symptoms, medication use and sharing of results in a medical report with the treating physician prior to consultation. Objective. Clinical studies show, that better informed patients have better disease outcomes. To empower CRUSE® users, we intended to develop a tool with educating, engaging and motivating function providing comprehensible health information all about urticaria.

Results: The novel patient information platform 'Urticaria information' was launched in the CRUSE® app in March 2024 and is now available in 22 languages worldwide. According to the results of a paper-based in-house survey with a limited number of urticaria patients, we catogorised the educational content into groups: 'Urticaria', 'Therapy', 'Tools', 'CRUSE', and 'News' providing free access to health information. Health information topics were based on clinical experience and scientific approaches. Selected content was linguistically processed for each language and implemented as separate interactive articles. 'Urticaria Information' is subject to continuous updates and facilitates sharing of individual articles through various social media routes. First results of the analysis of the implemented feedback function showed preferential interest of CRUSE® users in therapeutic options. Conclusion. The patient information platform 'Urticaria Information' is a novel CRUSE® feature that may not only help patients better understand their disease, but also to emphasise the importance of continuous daily documentation of signs and symptoms which is a prerequisite for optimal disease management and has the potential to improve the quality of life of affected patients.

Immunology

P099 | Breadth of CD8+ T-cell response in patients with melanoma—Predictive marker, functional relevance and novel therapeutic perspectives

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Introduction: In the last decade, immune checkpoint inhibitors (ICI) have significantly improved the treatment and survival of patients with melanoma. However, the role of antigens recognised by T cells remains elusive.

Objective: Our aim is to decipher the role of T-cell specificities targeting tumorspecific antigens (TSAs) and tumour-associated antigens (TAAs) in patients with advanced melanoma undergoing ICI treatment.

Methods: Cryopreserved peripheral blood mononuclear cells (PBMCs) of 23 melanoma patients harbouring NRAS (10) or BRAF (13) mutations underwent stimulations with peptide pools covering TSAs and TAAs. 15 patients were classified responders, 8 non-responders according RECIST 1.1. TSAs included peptides of the mutated NRAS or BRAF protein synthesised according to patient's specific mutation and HLA-type. The cancer testis antigens (CTAs) MAGE-A1, −3, −4, NY-ESO-1 and the melanocyte differentiation antigens (MDAs) gp-100, MART-1, TRP2 and Tyrosinase were used as TAAs. T-cell reactivity was determined by TNFalpha and IFNgamma secretion in flow cytometry. Furthermore, melanoma cell line UACC257 expressing HLA-A*02:01, gp-100, MART-1 and MAGE-A3 was co-cultured with healthy PBMCs and treated with self-designed Immune mobilising monoclonal T-cell receptors against cancer (ImmTACs) targeting the expressed antigens. The effect of PBMCs with and without ImmTACs was measured according to LDH release.

Results: All eight CTAs and MDAs were able to lead to pronounced immune responses. Strikingly, patients showed significantly higher T-cell activation parameters when comparing mutated (neoepitope sequence) to wildtype peptide ($p=0.03$). However, the magnitude of T-cell response against these different antigens was not associated with treatment response.

Instead, the breadth of CD8+ T-cell activity emerged as a potential predictive indicator for the outcome of ICI therapy. Moreover, in vitro studies demonstrated that ImmTACs targeting gp-100, MART-1, and MAGE-A3 enhanced the killing of melanoma cells by PBMCs. This finding corroborated our T-cell stimulation data and emphasised the functional significance of broad T-cell responsiveness in melanoma patients.

Conclusions: A broad immune response is an important mechanism for therapy success in melanoma patients receiving ICI treatment. The use of different T-cell engagers targeting several antigens expressed on melanoma cells has the potential to synergize with the patients' immune cell tumour killing.

P100 | *Staphylococcus epidermidis* reduces *Staphylococcus aureus* skin colonisation by shaping skin immune responses

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Introduction: *Staphylococcus aureus* (*S. aureus*) is the most common cause of bacterial skin infections but is usually absent from healthy human skin. We previously showed that in healthy skin, members of the skin commensals such as *S. epidermidis* protect the skin from *S. aureus* colonisation. However, the mechanism behind this is not yet understood. Here we aim to investigate the mechanism behind this protective effect by analysing the induction of immune responses in keratinocytes treated with conditioned medium of *S. epidermidis*.

Objectives: In this work, we investigated the influence of bacterial conditioned medium (BCM) of *S. epidermidis* on primary human keratinocytes (PHKs), human skin explants and 3D human skin reconstructs by analysing the induction of immune responses. Furthermore, we analysed the mechanism of the

BCM *S. epidermidis*mediated reduced *S. aureus* colonisation and how pre-treatment with BCM *S. epidermidis* affects the immune response of the skin against *S. aureus* infection.

Materials and Methods: We conducted a comprehensive investigation of the immune responses elicited by BCM *S. epidermidis* in PHKs, human skin explants, and 3D human skin reconstructs employing a range of techniques including LEGENDplex analysis, RT2 Profiler PCR arrays and western blotting to examine involved signalling pathways. To further elucidate the mechanisms by which BCM *S. epidermidis* pretreatment reduces *S. aureus* skin colonisation, we conducted inhibitor studies targeting especially the aryl hydrocarbon receptor (AHR) signalling pathway, across these models.

Results: We show that BCM *S. epidermidis* induces a protective immune response in the skin and promotes an anti-inflammatory environment within the skin. Furthermore, treatment of the skin with BCM *S. epidermidis* enhances the skin's antimicrobial response and upregulates barrier function genes, thereby strengthening the skin's defence mechanisms. Moreover, BCM *S. epidermidis* pre-treatment effectively reduces *S. aureus* skin colonisation and diminishes *S. aureus*-induced immune responses. These protective effects are partly mediated through AHR signalling pathways.

Conclusion: These data indicate that soluble factors from *S. epidermidis* effectively protects the skin from *S. aureus* colonisation by establishing a robust antiinflammatory and antimicrobial environment partially through AHR signalling.

P101 (OP02/02) | FXa and GPIbα-mediated monocyte-platelet interaction drives systemic fibrosis

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"Platelets are there to close wounds". This was the opinion of many scientists for a long time. It is now known that these anucleated blood components have important modulating and initiating functions in the immune system. Be it in the secretion of cytokines such as profibrotic TGF-β or the activation of monocytes in the bloodstream. In this project, we aim to investigate expression patterns in SSc skin cells that are related to coagulation processes and to investigate the functional role of platelets and coagulation proteases in patients with SSc and in SSc mouse models.

CD45pos SSc skin cells reveal overexpression of coagulation proteins such as f13a1 (F13), f2r (PAR-1) and platelet-derived growth factors (PDGF), promoting cellular growth and infiltration. Interestingly, CD45neg cells (fibroblasts, keratinocytes) showed marked f13a1 expression, which matches chronic tissue regeneration responsible for extracellular matrix formation in SSc skin cells. To address coagulation processes in fibrosis functionally, we used Fos-related antigen 2 transgenic (Fra-2tg) mice. Fra-2tg mice develop spontaneous vasculopathy and multiorgan fibrosis.

Early vasculopathy and inflammation in Fra-2tg mice associates with an increased number of (CD11b+Ly6C+MHC+) inflammatory circulating monocytes, an iNOS+ to CD301b+cellular shift in tissue phagocytes, and an increase in platelet-monocyte clusters (PMC; CD11b+CD41+). PMC formation correlated with disease severity and fibrosis progression. To address if lack of PLT would affect fibrosis outcome we depleted PLTs in Fra-2tg mice (week 5 to week 12) and found that CD301b+cell polarisation, α-SMA+ cell counts in the dermis, and skin thickness were reduced.

The GPIbα protein binds the leukocyte integrin CD11b/Mac-1 (macrophage-1 antigen) on myeloid cells such as monocytes and might contribute to pre-fibrotic monocyte activation in SSc. Interestingly, IL-4R/GPIbα-Tg mice, which lack the extracellular GPIbα domain and whose platelets therefore cannot interact with coagulation factors, endothelium, and monocytes, showed significantly reduced cutaneous collagen accumulation (skin thickness) in response to fibrosis-inducing hypochlorous (HOCl) injections. As f2r was overexpressed in human SSc skin, we addressed PAR-1 activating coagulation factor 10a (FXa) in fibrotic mice by drugmediated blockade through rivaroxaban diet and analysed a myeloid-specific KO of FXa in the HOCl fibrosis model. Both FXa-targeted approaches reduced fibrosis in vivo.

Our study highlights coagulation proteins such as GPIbα and/or FXa as promising targets in systemic sclerosis and fibrosis in general.

P102 | Liposome-encapsulated antigens are superior in inducing T cell proliferation after subcutaneous injection

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Extracellular vehicles (EVs) are membrane-encapsulated structures that originate from the endosomal system or plasma membranes. Due to their content of immunoregulatory molecules, EVs play significant roles in regulating the biological pathways in recipient cells. However, naturally occurring extracellular vesicles have a variable composition and their content is not scalable. So, we set out to synthesise liposome-based artificial extracellular vesicles to encapsulate and deliver substances that can regulate immune responses. Depending on their surface charge during production, liposomes can be classified as cationic, neutral, or anionic.

In our study, we generated liposomes with different surface charges encapsulating ovalbumin (OVA) alone or co-encapsulating OVA and rhodamine. We first evaluated the impact of liposome surface charge on uptake by dendritic cells (DCs). Using bone marrow-derived dendritic cells (BMDCs), we observed significant uptake of cationic liposomes by DCs, as demonstrated by microscopy and fluorescence imaging. In contrast, neutral and anionic liposomes showed minimal or no uptake by BMDCs in vitro.

We further assessed the preferential uptake of liposomes by immune cells, including B cells, T cells, and DCs isolated from the spleen or ear skin. Our results confirmed a strong preferential uptake of cationic liposomes by antigen-presenting cells, with limited uptake by T cells.

To explore the functional impact of liposome-encapsulated OVA on antigen presentation, we co-cultured BMDCs pulsed with liposomes and CD4+ T cells from OTII.2 mice. Cationic liposome-encapsulated OVA induced robust T cell proliferation in vitro. To assess whether this effect extended to in vivo models, we subcutaneously injected liposome-encapsulated OVA or free OVA into C57BL/6 mice which had intravenously received proliferation dye-labelled CD4+ T cells from OTII mice. After 3 days, we observed significantly higher T cell proliferation in mice injected with liposome-encapsulated OVA compared to those injected with free OVA.

Finally, we compared OVA uptake by mature and immature BMDCs. Initially, a mixture of mature and immature DCs showed preferential uptake of cationic liposomes by mature DCs, whereas free OVA was equally taken up by both populations. Later on, when all DCs matured, a substantial increase in uptake of liposome-encapsulated OVA was observed.

In conclusion, cationic liposomes are preferentially taken up by dendritic cells compared to neutral and anionic liposomes, and encapsulated antigens are more efficiently presented to T cells than free antigens. These findings highlight the potential of cationic liposomes as superior antigen delivery vehicles in immune therapies and vaccines in the future.

P103 | The role of the complement system in pustular dermatoses

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Background: Pustular psoriasis (PP) is a group of autoinflammatory skin diseases characterised by the infiltration of neutrophils into the epidermis, resulting in clinically visible pustules on an erythematous background. This group of serious skin diseases can be divided into systemic and localised manifestations. Generalised pustular psoriasis (GPP) mainly presents with pustular eruptions on non-acral skin and acute systemic involvement, whereas chronic localised variants affect either the palms and soles (palmoplantar pustular psoriasis, PPP) or the tips of the fingers and toes (acrodermatitis continua of Hallopeau, ACH). Despite the frequent cooccurrence of pustular psoriasis and psoriasis vulgaris, pustular dermatoses are a distinct entity from the traditional understanding of psoriasis vulgaris. Apart from a few identified genetic defects (IL36RN, CARD14, AP1S3, TNIP1, SERPINA3, MPO), the pathomechanisms underlying these skin conditions have been insufficiently investigated. In particular, the complement system, one of the key systems of the early innate humoral immune response for defence against endogenous and exogenous threat molecules, has hardly been addressed in the context of pustular dermatoses.

Objective: The aim of this study is to focus on the involvement of the complement system in the disease mechanisms of pustular dermatoses at systemic and local level.

Methods: Blood samples and punch biopsies of lesional skin were collected from patients with pustular psoriasis after written informed consent (ethical approval 57/22, Ethics Committee, Ulm University). Levels of complement cleavage products (C3a, C5a, C3d) and circulating immune complexes containing C3 activation fragments (CIC + C3d) were determined in EDTA plasma by ELISA. In addition, plasma C4 concentrations were measured by immunoturbidimetry. Serum samples were used to test the activatability of the complement system by performing a classical pathway hemolysis assay. The ability to deposit C4 fragments on sheep erythrocytes after classical pathway activation was assessed by flow cytometry. Complement split products (C3d, C4d) and cellular components (MPO, CD3) were analysed in frozen sections of lesional skin biopsies by indirect immunofluorescence staining.

Results: Regarding systemic complement activation, no altered levels of complement components and split products (C3a, C5a, C3d, CIC + C3d, C4) were detected in plasma samples compared to healthy controls. However, some patients showed significantly reduced hemolytic activity of the classical pathway. In line with this, deposition of C4 fragments following classical pathway activation was diminished compared to corresponding controls. In summary, it was possible to establish a significant correlation between the reduction in hemolytic activity and the limited ability to deposit C4 fragments. At the local level, in some cases, indirect immunofluorescence of skin biopsies revealed C3d and C4d deposits in a granular pattern along the dermoepidermal junction. Disease-specific neutrophil infiltration (MPO) and lymphocyte accumulation (CD3) were also confirmed by immunohistochemistry.

Conclusion: Overall, our data provide evidence for an involvement of the complement system in the skin of patients with pustular psoriasis. To date, the precise connection between local complement activation and pustular dermatoses is poorly understood. Consequently, the triggers for locally activated complement and its exact contribution to the pathogenesis of pustular psoriasis must be the focus of future research.

P104 | IL-18 activates human Th2 cells in atopic dermatitis

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Background: T helper 2 (Th2) cells are crucial contributors to the pathogenesis of atopic dermatitis (AD) by secreting high levels of interleukin (IL-)13 and IL-22. Yet, the upstream regulators that activate Th2 cells in AD skin remain unclear. IL-18 is a putative upstream regulator of Th2 cells as it is implicated in AD pathogenesis and has the capacity to activate T cells.
Objective: To decipher the role of IL-18 in Th2 responses in blood and skin of AD patients.
Results: IL-18R+ Th2 cells were enriched in blood and lesional skin of AD patients. Of all the cytokines for which Th2 cells express the receptor, only IL-9 was able to induce IL-18R via an IL-9R-JAK1/3-STAT1 signalling pathway. Functionally, stimulation of circulating Th2 cells with IL-18 induced secretion of IL-13 and IL-22, an effect that was enhanced by co-stimulation with IL-9. Mechanistically, IL-18 induced Th2 cytokines via activation of both NF-κB and AP-1 signalling in Th2 cells, and neutralisation of IL-18 inhibited these cytokines in cultured explants of AD skin lesions. Finally, IL-18 protein levels correlated positively with disease severity in lesional AD skin.
Conclusion: Our data identify a novel IL-9-IL-18 axis that drives Th2 cell responses in AD and demonstrate a critical role of IL-9-mediated upregulation of the IL-18R via an IL-9R-JAK1/3-STAT1 signalling cascade. Our findings suggest that both IL-9 and IL-18 could represent upstream targets for future treatment of AD.

P105 | AhR agonism by tapinarof regulates TH2 and TH17 cell function in human skin by inhibition of fatty acid oxidation

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Background: The aryl hydrocarbon receptor (AhR) is a transcription factor for skin homeostasis and barrier function. Tapinarof, a topical AhR agonist, has shown impressive clinical efficacy in psoriasis (PSO) and atopic dermatitis (AD), inducing long-lasting remissions. However, tapinarof's anti-inflammatory mechanism remains unclear. We aimed to investigate tapinarof's effects on T cells in AD and PSO.
Methods: Using a short-term human skin explant model, we cultured skin biopsies from PSO and AD with tapinarof for 24 h.
Results: We observed elevated cytokine levels in disease-driving populations of tissue-resident T cells (TRM) (IL-13+CD4+ TRM in AD and IL-17A+CD8+ TRM in PSO), validating our model. Tapinarof significantly reduced IL-13 and IL-17A in the respective diseases and populations.
By single-cell RNA-sequencing of T cells isolated from tapinarof-treated AD and PSO biopsies, we found that these cells displayed significant metabolic impairments. Transcriptomic analysis on tapinarof-treated memory CD4+ T cells showed reduced CPT1A and SLC25A20, metabolic enzymes associated with beta oxidation of longchain fatty acids, and a reduction of IL13 and IL17A. Mechanistic studies confirmed that glycolysis and oxidative phosphorylation were reduced in resting and activated memory T cells after tapinarof treatment. Strikingly, basal respiration was significantly impaired in both resting and activated memory T cells, whereas basal glycolysis was only affected after activation. Additionally, memory CD4+ T cells showed a reduced energy availability after tapinarof treatment, indicated by the phosphorylation of AMPK.
Conclusion: Our ex vivo model demonstrated the impact of tapinarof on skin T cells of AD and PSO patients with significant reduction in disease-relevant cytokines. Lastly, tapinarof directly affected T cells, impaired oxidative phosphorylation, and the energy availability, revealing a previously unknown mechanism of action.

P106 | The immunoregulatory role of IRE1 in the progression of skin disease

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Psoriasis, a chronic inflammatory disease, has emerged as a global health burden. The disease is driven and sustained by the interplay between innate and adaptive immunity, with the IL-23/IL-17A pathway playing a pivotal role. The unfolded protein response (UPR) signalling, comprises three branches – ATF6, IRE1α, and PERK. It aims to maintain cellular homeostasis. However, growing evidence suggests that the UPR is also actively involved in inflammation. Notably, IRE1α, the only known endonuclease that splices the Xbp1 mRNA, plays a crucial role in IL-23 production by dendritic cells. Here, we explore the potential of IRE1α as a therapeutic target in psoriasis.
In our in vitro studies, IRE1α inhibitors significantly reduced pro-inflammatory cytokine transcription in bone marrow-derived dendritic cells. Building on these findings, we assessed the effects of the kinase inhibitor Kira6 in vivo using an Imiquimod (IMQ)-induced psoriatic-like dermatitis model. Kira6 partially alleviated inflammation, yet flow cytometry revealed divergent results. The kinase inhibitor Kira6 significantly inhibited γδ T cell activation, a key source of IL-17 in psoriasis progression. Additionally, Kira6 reduced macrophage numbers in the spleen while increasing neutrophil numbers across most tissues, except at the inflamed site.
Moving forward, we aim to address several key questions: (1) Given that the inhibitor was administered via intraperitoneal (I.P.) injection, would topical application yield different results? (2) How does Kira6 modulate γδ T cells, macrophages, and neutrophils in IMQ-induced dermatitis? Could these effects be due to off-target actions? To answer these questions, we plan to: (1) Validate the inhibitors' specificity in vivo by testing XBP1 splicing in treated tissues. (2) Conduct in vitro experiments using isolated and MACS-purified γδ T cells, neutrophils, and macrophages treated with inhibitors. (3) Generate IRE1α knockout cell lines to further investigate underlying mechanisms. (4) Perform single-cell RNA sequencing to assess gene expression across different cell types in mouse skin following IMQ and Kira6 treatment.

P107 | In naïve CD4+ T cells, a highly proliferative CD73+ early effector subset excels at infiltrating the skin during inflammation and subsequently developing into memory T cells

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CD73 is a membrane-bound extracellular 5′ ectonucleotidase that converts adenosine diphosphate (ADP) and adenosine monophosphate (AMP) to adenosine. Due to the immunosuppressive functions of adenosine, functional analyses of CD73 have mainly focused on immune regulatory cells, such as CD4+CD25+ regulatory T cells. Initial data from the 1970s indicate that CD73 may function as a T cell costimulatory molecule and/or as an adhesion molecule, enabling the adherence and extravasation of T cells through the endothelium. However, these data are mostly older than two decades and have not been followed up recently.

We set out to investigate the possible functions of CD73 in non-suppressive cells by analysing its expression in CD4+CD25-Foxp3- sorted T cells. We found that nearly 50% of all naïve CD4+ T cells expressed CD73 (CD73+). When cultivating CD73+ and CD73− subpopulations separately, we found that the CD4+CD73+ T cells exhibited superior proliferation and were prone to develop into a "Th1-like" cell type, expressing prototypic cytokines (IFN-γ, TNF-α) and specific transcription factors (i.e., Tbx21). Following in vitro culture, these cells produced TGF-β and downregulated the expression of Fas, which prevented activation-induced cell death and led to prolonged survival compared to their CD4+CD73−counterparts.

Comparable results were obtained in vivo. Upon transfer into lymphopenic hosts (RAG2−/− mice), CD73+CD4+ cells exhibited increased proliferation and survival and were the first cells to accumulate in inflammatory skin, developing a CD44+CD62Leffector memory phenotype. In conclusion, CD73 in naïve CD4+ T cells acts as a promoter of survival and proliferation and as a marker for their further differentiation into effector T cells.

Although the exact mechanisms by which CD73 mediates these differential functions are not yet clear, it is important to note that current and future anti-tumour therapies may use anti-CD73 antibodies as checkpoint inhibitors. The potential adverse effects of depleting CD73+ effector cells and impacting the development of immunologic memory must be carefully examined to establish the beneficial effects of anti-CD73 antibody treatment regimens in tumour therapies.

P108 | Differences in T-cell-signalling during sensitization of hapten-induced immune responses of the skin: Sensitization versus tolerance

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Contact Dermatitis is a well-known occupational disease, causing relevant suffering of patients as well as socio-economic damages. Therefore, induction of tolerance against allergens would be a choice therapy. In mice the DNTB-application is an established model system for inducing directed tolerance against the well-defined contact allergen DNFB. Although a lot of mechanistic details about toleranceinduction against DNFB have been published there is not much knowledge about T-cell-signalling during the sensitization-phase of hapten-induced tolerance against sensitization. In this experimental study we wanted to analyse T-cell-signalling in lymph nodes of sensitised versus tolerized mice. Therefore, we extracted mRNA of bulk lymph node cells in DNTB-tolerized and DNFB-sensitised mice. Initially, 94 different key-molecules of T-cell-signalling were analysed via qRT-PCR. Molecules that showed promising differences at mRNA expression were further analysed on protein-level by quantitative Western-blotting.

As a result, molecules that showed suppressed expression in tolerized T-cells as compared to non-tolerized T-cells were CD28, Grb2, IRf4, MAP2K2 and Skap1. Also, these molecules showed more expression in non-tolerized T-cells during sensitization as compared to control T-cells that were neither treated with DNTB not DNFB. All these molecules are key molecules of important pro-proliferative signalling pathways: namely PI3K/Akt/mTOR- and MAPK/Erk-pathways. In addition, it was also possible to show effects on Fas/FasL-Apoptosis-System by qRT-PCR. Tolerized T-cells expressed more Fas/FasL-mRNA in comparison to non-tolerized T-cells in lymph nodes. These results were confirmed via FACS-analysis of the T-cells. We could demonstrate that there is more Fas/FasL-expression on the protein-level of T-cells of tolerized mice as compared of T-cells of non-tolerized mice. FITC Annexin V staining showed that more Fas/FasL expression on tolerized T-cells also results in more apoptosis in comparison to non-tolerized T-cells.

We conclude that tolerance is not only attained by suppression of pro-proliferative signalling-pathways PI3K/Akt/mTOR and MAPK/Erk, but also through induction of apoptosis of T-cells.

P109 | Bullous pemphigoid develops independently of DAP12

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The signalling adaptor molecule DNAX-activating protein of 12 kDa (DAP12) is expressed in a variety of cells participating in innate immune response, yet its role in autoimmunity is ambiguous due to its pleiotropic functions in positive and negative regulation of leukocyte responses. Herein, we investigated the impact of the DAP12 pathway in the pathogenesis of bullous pemphigoid, the most frequent autoimmune blistering disease, using a mouse model induced by transfer of IgG targeting the murine type XVII collagen (Col17) non-collagenous domains 14–1. Following repeated injections of anti-Col17 IgG over 12 days, disease activity in mice deficient for DAP12 was comparable to wildtype littermates, including the (i) affected body surface area, (ii) skin histological changes, including subepidermal cleavage and skin inflammation with a predominantly neutrophilic infiltrate as well as (iii) IgG and (iv) complement C3 deposition along the cutaneous basement membrane. Flow

cytometry and indirect immunofluorescence analysis of lesional skin biopsies further demonstrated a significant downregulation of the DAP12 associated triggering receptor Trem-1 (pro-inflammatory) in DAP12-deficient vs. wildtype skin. In contrast, Trem-2 (anti-inflammatory) positive lymphocytes were comparable among both anti- Col17 IgG-treated wildtype and DAP12 knock-out groups, but were significantly reduced when compared to healthy control skin. Additional flow cytometry analysis also showed significant changes in the inflammatory infiltrate composition, including markedly lower frequencies of Siglec-f+ eosinophils in DAP12-deficient vs. wildtype lesional skin. Taken together, this study provides evidence that the DAP12-axis, including its associating receptors Trem-1 and Trem-2, does not modulate disease activity in experimental bullous pemphigoid. A tissue-specific targeting of DAP12 function as a therapeutic option for bullous pemphigoid is thus unpromising.

P110 | Pretreatment with the foreign protein ovalbumin emulsified in the adjuvant combination TiterMax/Alum prevents pathogenesis in the murine immunisation-induced model for epidermolysis bullosa acquisita

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Epidermolysis bullosa acquisita (EBA) is an autoimmune disorder characterised by subepidermal blistering driven by autoantibodies targeting type VII collagen at the dermal-epidermal junction (DEJ). In an established murine model, the disease is induced by immunising mice with murine type VII collagen (mCol7c) emulsified in the pro-inflammatory adjuvant TiterMax, leading to the break of tolerance, the appearance of autoreactive T and B cells and the formation of germinal centers (GCs). Previous research has shown that pre-treatment with a xenogenic antigen ovalbumin (OVA) emulsified in the adjuvant combination TiterMax/Alum, completely prevents disease development. Conversely, the control group, which received TiterMax/Alum and vehicle only, developed characteristic EBA symptoms.
Given the strong contrast between the asymptomatic OVA group and the severely symptomatic control group, we aimed to identify factors that discriminate between non-disease and disease and that could serve as potential therapeutic targets. Interestingly, comparative RNA-seq analysis of whole draining popliteal and inguinal lymph nodes revealed barely significant transcriptomic differences between the healthy and diseased groups. Minor changes were observed, such as reduced IFNγ expression in the lymph nodes of the OVA group. Levels of Col7c-specific IgG subclasses showed no variation in serum or at the DEJ, and no differences in serum Col7c-specific IgG Fc glycosylation patterns were observed between the OVA and control groups. The most notable difference was that total (non-specific) serum IgG antibodies exhibited significantly higher IgG Fc sialylation levels in the OVA group compared to the EBA group. In addition, T cell receptor sequences of T follicular helper (Tfh) cells in GCs showed a slightly greater diversity in the OVA group.
This data shows that xenogeneic protein immunisation with appropriate adjuvants may offer both prophylactic and therapeutic potential by modulating the microenvironment. Mechanistically, the enhancement of total IgG Fc sialylation could act like the sialylated subfraction of IVIg (intravenous immunoglobulin) from healthy donors used in high doses to treat inflammatory (auto)immune patients to reduce the expression of activating and increase the expression of inhibitory immune receptors. In addition, xenogeneic immunisation may increase the expansion of highly abundant, non-specific Tfh cell clonotypes in GCs, thereby contributing to the establishment of a less inflammatory milieu.

P111 | Immune profiling of desmoglein 3-reactive T helper cells in pemphigus vulgaris

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Pemphigus vulgaris (PV) is an autoimmune disease characterised by autoantibodymediated blisters of skin and mucous membranes. T helper cells play a crucial role in disease development and progression by secreting cytokines and assisting B cells in autoantibody production. This study aimed at detecting and characterising diseasepromoting Dsg3-reactive T helper cells in PV patients utilising an easy-to-perform ex vivo assay.
Immunodominant peptides of Dsg3 were co-cultured with CFSE-stained PBMC from PV patients or healthy controls (HC) to determine the relative frequency of Dsg3 peptide-specific T cells. Cytokine secretion of activated T cells was measured in culture supernatants utilising cytometric bead array and ELISA. Dsg3-reactive T helper cells specific for five defined epitopes were identified by dye dilution ex vivo in the majority of PV patients with active disease and, to a lesser extent, in remittent PV. PBMC from HLA class II matched HC also showed proliferative T cell responses to the same Dsg3 peptides. Cytokine secretion in response to Dsg3 peptides varied between PV patients and HC. Dsg3 peptide-reactive T helper cells from patients with active PV secreted Th1 and Th2 cytokines, while Th2 cytokines were predominant in remittent disease. In a subset of patients, Dsg3-reactive T cells with Th17 signature were also identified. In contrast, PBMC from HC secretes predominantly the immunosuppressive cytokine, IL-10. In summary, this modified CFSE assay has for the first time allowed to identify Dsg3 peptide reactive T cells with distinct cytokine profiles. This tool has major potential for the monitoring of autoreactive T cells in PV.

P112 (OP02/04) | Multi-omics skin disease atlas for accurate and rapid AI-driven clinical decision making

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A quarter of the world's population suffers from chronic inflammatory skin diseases (ISDs). ISDs encompass a broad spectrum of heterogenous entities and a wide range of different diagnoses. As a consequence, they are particularly challenging to diagnose accurately and timely, which is a prerequisite for the effective treatment of the underlying disease. Omic technologies in combination with artificial intelligence hold the promise to contribute substantially to clinical decision making and patient care. Here, we performed mass spectrometry (MS)-based proteomics with the latest generation mass spectrometer (Orbitrap Astral) in combination with transcriptomics to generate a comprehensive multi-omic atlas of all major ISDs. To this end, we have so far collected retrospective FFPE skin tissue biopsies and associated clinical metadata from 459 patients. To achieve best reproducibility, we utilised a semiautomated workflow and integrated normalisation strategies, with the ultimate goal to enable this workflow at other locations in the near future. This robust setup yielded >10000 proteins—a very deep proteome—across all diseases measured, spanning five orders of magnitude and contained many low-abundant cytokines. For our transcriptomic analysis we confidently identified around 20000 protein coding transcripts overall. With our in-depth omic dataset in hand, we evaluated the capability of our newly developed atlas to enhance clinical decision-making for erythroderma—a severe, life-threatening skin condition characterised by extensive erythema and scaling—in a study involving 115 patients measured across two independent cohorts. Erythroderma can result from various dermatological disorders, including psoriasis (Pso), atopic dermatitis (AD), pityriasis rubra pilaris (PRP), advanced-staged cutaneous T-cell lymphoma (CTCL) and drug reactions. Principal component analysis of both proteomics and transcriptomics data clustered AD with MF and drug-induced erythroderma, while PRP clustered with Pso. The most differentially expressed transcripts identified by analysis of variance included IL17C, CCL20, and IL1A in PRP, CXCL2 in Pso and IL32, as well as TOX (Thymocyte Selection-Associated High Mobility Group Box Protein), and TCF7 (Transcription factor 7) in MF—both involved in T-cell development and differentiation. Especially for PRP we identified the induction of the IL17C/IL17RE axis as a novel and disease specific biological process, confirmed on both transcriptomic and proteomic levels. Furthermore, through integration of machine learning (random forest algorithm), our dataset effectively distinguished between the erythrodermic diseases with high accuracy and an AUC of up to 0.92, using up to 50 proteins/transcripts. Feature selection was performed across the two independent cohorts by bootstrapping and permutation testing with 5-fold cross-validation. This study highlights the potential of omic technologies for AI-driven disease stratification and clinical decision making in cutaneous inflammatory disorders in general and for erythroderma in particular.

P113 | Skin-infiltrating T and B cells in the pathogenesis of pemphigoid diseases

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Introduction: Autoantibodies typically result from germinal center reactions in the secondary lymphoid organs through interactions between follicular T helper (Tfh) cells and B cells. However, also in non-lymphoid inflamed tissues, the recently discovered population of peripheral T helper (Tph) cells can deliver essential signals to B cells, leading to their differentiation into antibody-secreting cells or memory B cells. These tissue-resident B cells frequently express the transcription factor T-bet. This study investigates the involvement of Tfh, Tph cells, and T-bet+ B cells in the pathogenesis of bullous pemphigoid (BP), an autoimmune blistering skin disorder.
Methods: Peripheral blood cells and skin biopsies of BP patients were compared to those from healthy controls. Spectral flow cytometry with up to 27 parameters was applied to determine surface receptors, transcription factors, and cytokines, with a special focus on homing receptors that guide lymphocytes into inflamed tissues. The analysis aimed to compare the abundance of Tfh and Tph cells, activation markers, and the presence of T-bet+ B cells in patients and controls.
Results: In BP patients, increased frequencies of PD-1+ CXCR5- Tph cells and T cells expressing chemokine receptors for tissue homing were observed compared to healthy controls. These cells also exhibited high levels of CD40L expression, indicating their capacity to provide assistance to B cells.Tph cells expressing CLA, CCR2, and CCR5, were also found in the skin. The frequencies of IgD- CD27- T-bet+ B cells were higher in patients than in the controls.
Conclusion: In BP, PD-1+ T helper cells and T-bet+ B cells may play a key role in the disease's pathogenesis and could be a promising target for future therapeutic interventions.

P114 (OP06/03) | Exploring the role of human primary keratinocytes in candida auris infection: Antimicrobial defence and immune signalling

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Introduction: Candida auris (*C. auris*) is an emerging fungal pathogen of significant concern in hospitals and healthcare settings due to its multidrug resistance, persistence on human skin, and capacity to cause severe, often fatal infections and outbreaks. Developing effective treatments for *C. auris* infections necessitates a thorough understanding of the host's immunological response. In this study, we utilised human primary keratinocytes (pKCs) to examine the dynamic interactions between *C. auris* and human skin cells.

Methods: Flow cytometry analysis was performed on pKCs infected with GFP-tagged *C. auris* to assess whether the pathogen associated intra- or extracellularly with host cells. To investigate the impact of pKC-secreted factors on *C. auris* viability, the fungus was incubated in culture medium from infected pKCs (KC-conditioned medium), with growth monitored over time by measuring optical density and colonyforming units (CFUs) on YPD plates. Additionally, RNA was extracted from pKCs ($n=4$ biological replicates) after 12 h of infection and analysed using bulk RNA sequencing to identify transcripts upregulated in response to *C. auris* exposure.

Results: Flow cytometry demonstrated that pKCs do not phagocytose Candida cells. However, the two cell types exhibit a time- and dose-dependent extracellular interaction, which is abolished when the pathogen is heat-killed prior to infection. Analysis of Candida growth in KC-conditioned medium revealed that pKCs release soluble factors—likely antimicrobial compounds—that prevent further yeast proliferation. Gene set enrichment analysis of RNA sequencing data revealed upregulation of immune response pathways in infected pKCs compared to uninfected ones. Key upregulated genes within these pathways included interleukin (IL)-1 alpha, IL-1 beta, IL-6, CXCL-8, and TNF-alpha. These findings strongly suggest that KCs are involved in recruiting both innate and adaptive immune cells during *C. auris* infection. Additionally, the identification of antimicrobial genes such as thymic stromal lymphopoietin (TSLP), ribonuclease 7 (RNase7), and ribonuclease 5 (angiogenin), indicates that, during infection, KCs produce factors that directly target fungal cells. These genes are being analysed by PCR and protein assays, and potential antimicrobial peptides are being tested for their ability to inhibit Candida growth in vitro.

Conclusion: Our findings provide new insights into the cellular mechanisms of the skin's response to *C. auris*. The results suggest that KCs act as early responders by secreting molecules that both directly combat the fungus and recruit additional immune cells to the infection site. This study underscores the potential of targeting KC-derived factors for therapeutic development against *C. auris*.

P115 | Anti-tumour potential of alpha-melanocyte-stimulating hormone by reducing in vitro generated myeloid-derived suppressor cell numbers

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In 2020, the incidence of skin cancer (malignant melanoma or non-melanoma skin cancer (NMSC) like basal cell carcinoma (BCC) and squamous cell carcinoma (SCC)) in the European Union was 26 and 20 per 100 000 for men and women, respectively. Various risk factors contribute to skin cancer development like genetic predisposition or ultraviolet (UV) radiation. Furthermore, dysregulation or suppression of the immune system can promote skin cancer progression or worsen cancer outcome. In particular, myeloid-derived suppressor cells (MDSC) are known for their pro-tumour characteristics and are able to create an immunosuppressive tumour microenvironment in which skin cancer can perpetuate or metastasize. MDSC have different mechanisms to inhibit anti-tumour immunity such as blocking the proliferation and activation of cytotoxic CD8+ T cells (CTL) and enhancing regulatory T cell (Treg) expansion and induction. Consequently, inhibiting or reducing MDSC development and thereby, improving anti-tumour immunity represents a more targeted cancer therapy. UV radiation, a well-known risk factor for skin cancer, induces the generation of the neuropeptide alpha-melanocyte-stimulating hormone (alpha-MSH) in the skin, which acts as a regulator of melanogenesis and pigmentation. However, alpha- MSH has also been shown to have potent immunomodulatory and anti-inflammatory effects in mouse models and patient samples of different skin diseases including psoriasis or even melanoma.

In previous work, we observed that alpha-MSH was able to increase the expression of cytolytic molecules and upregulated the cytotoxic potential in peripheral blood mononuclear cells (PBMC) from patients with malignant melanoma. Since the underlying mechanism is not completely understood, we investigated the effect of alpha-MSH on MDSC with the aim to assess whether alpha-MSH might be able to modulate MDSC generation and expansion or function. Interestingly, alpha-MSH treatment significantly reduced the generation of total MDSC in sorted PBMC from healthy donors stimulated with granulocyte-macrophage colony-stimulating factor (GM-CSF) and Interleukin-6 (IL-6). Next, we analysed the impact of alpha-MSH treatment on the immunosuppressive capacity of MDSC and therefore, performed T cell proliferation assays. For this purpose, CD8+ T cells were labelled with carboxyfluorescein succinimidyl ester (CFSE), co-cultured with MDSC and T cell proliferation was quantified by CFSE dilution. As expected, MDSC suppressed T cell proliferation while alpha-MSH did not markedly alter the suppressive capacity of MDSC. To further characterise the impact of alpha-MSH on MDSC generation and function, multiplex cytokine quantification assays, gene expression studies (qPCR) and metabolic analyses will be performed. Moreover, we will investigate the direct effect of alpha-MSH on the suppressive activity of MDSC and on the conversion of myeloid progenitor cells into MDSC in samples from individuals with NMSC or melanoma. Taken together, we could show that alpha-MSH significantly downregulates MDSC numbers, which could lead to a general improvement of anti-tumour immunity. Thus, alpha-MSH might in the future be suitable as a local adjuvant treatment in skin cancer therapy and could be a promising target for further development.

P116 | Anti-inflammatory potential of novel peripheral kappa-opioid receptor agonists by altering mitochondrial activity and cell metabolism

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In inflammatory and pruritic skin diseases, including atopic dermatitis or psoriasis, the perception of itch is partially regulated by the endogenous opioid system. Recent studies suggest, that an imbalanced kappa-opioid receptor (KOR)/mu-opioid receptor (MOR) signalling in either the skin or the nervous system leads to the induction of itch. In this regard, the activation of MOR is discussed to intensify itch, whereas the activation of KOR seems to be associated with itch suppression. However, the antipruritic effects of KOR agonists (KORA) were assumed to be linked to their binding to peripheral KOR. Interestingly, an upregulated KOR expression is found on peripheral immune cells, like T cells or antigen presenting cells. During T cell activation, KORA significantly reduced the production of proinflammatory cytokines including IFN-y and IL-17 and moreover, inhibited T cell proliferation, thus finally resulting in their anti-inflammatory properties. Hence, peripheral KORA are of particular interest, since besides their anti-inflammatory and anti-pruritic properties, they in contrast to agonists of other opioid receptors, are not associated with visceral side effects. Therefore, we newly developed different peripherally restricted KORA based on a perhydroquinoline backbone with different substituents in 4- or 5- position, which helped to induce a high polarity and thus, a decreased capacity to enter the central nervous system (CNS).
In order to better understand the relevance of KOR signalling in the pathology of inflammatory or pruritic disorders, we first focused on CD8+ and CD4+ T cell responses following KOR activation with the novel KORA that we generated. In particular, we investigated the effects of peripherally restricted KORA on the phenotype, function and migratory behaviour of TH1 and TH17 cells, cytotoxic and regulatory T cells (Tregs). For this purpose, sorted and stimulated cells were phenotypically characterised by flow cytometry. To functionally analyse the T cell subsets, cytokine quantification and metabolic analyses were performed clearly showing that our novel KORA had potent anti-inflammatory properties, which among others was demonstrated by a down-regulated expression of TH1- or TH17-specific transcription factors, cytokines or cytolytic molecules in CD4+ or CD8+ T cells, respectively. Moreover, mitochondrial analyses of T cell subsets using MitoSOX green and TMRM revealed that the peripherally restricted KORA increased the production of mitochondrial reactive oxygen species as well as the mitochondrial membrane potential (mitochondrial activity) particularly in TH1 and CD8+ cytotoxic T cells, whereas in TH17 cells the amount of mitochondrial reactive oxygen species and the mitochondrial membrane potential seemed to be reduced in the presence of our novel KORA. To further investigate the metabolic alterations induced in effector T cell subsets by peripherally restricted KORA, Seahorse analyses to assess ATP consumption, ROS production or glycolytic activity will be performed. Moreover, RNA sequencing will be used to characterise gene expression profiles and to analyse signalling pathways altered upon stimulation with our peripherally restricted KORA. Taken together, treatment with peripherally restricted KORA might be promising in ongoing skin inflammation and itch, thus potentially suggesting these compounds as potential candidates for further development.

P117 (OP04/02) | Topical C5aR1 inhibition reduced clinical lesions in a preclinical model of mucous membrane pemphigoid

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Mucous membrane pemphigoid (MMP) is an autoimmune blistering disease primarily affecting surface-close mucosal tissues such as the oral cavity and conjunctivae. Linear deposits of IgG, IgA and/or C3 at the basal membrane zone are diagnostic characteristics seen by direct immunofluorescence microscopy of a perilesional biopsy. Notably, C3 Binding is present in 85% of MMP patients, suggesting a pathophysiological role of the complement system in this disease. Current treatments for MMP are often ineffective and associated with severe adverse events. In particular, conjunctival involvement is a therapeutic challenge and regularly leads to scarring and blindness.
This study aimed to evaluate the therapeutic effects of DF3966A, a selective C5a receptor 1 (C5aR1) inhibitor, on clinical lesions in a mouse model of MMP.
MMP was induced in adult C57BL/6J mice by repetitive subcutaneous injections of anti-murine laminin alpha3 IgG over 10 days. Mice were treated with either 3 eyedrops/eye of 0.05% DF3966A or vehicle twice daily for 12 and 28 days, respectively ($n = 14$/group/time point, equal sex distribution). Daily treatment with methylprednisolone (MP) by i.p. injection of 20 mg/kg was used as a control ($n = 14$/time point, equal sex distribution). Therapeutic efficacy was assessed by evaluating conjunctival/oral/skin lesions, semi-quantifying inflammatory infiltration of conjunctiva/buccal mucosa/skin biopsies, measuring body weight, and determining levels of circulating total mouse IgG levels after 12 and 28 days.
Topical treatment with DF3966A reduced conjunctival split formation by more than 50% on days 12 ($p = 0.0016$) and 28 ($p = 0.0414$). Histological analysis confirmed a reduced inflammatory infiltrate in conjunctival biopsies of DF3966A-treated mice compared to the vehicle group on day 28 ($p = 0.0188$). In addition, DF3966A reduced the severity of oral lesions as assessed by endoscopy at both time points (day 12 $p = 0.0479$, and day 28 $p = 0.050$). In contrast, the reference control group showed significantly fewer skin lesions with little effect on conjunctival pathology. Notably, DF3966A treatment was well- tolerated and did not result in body weight loss, unlike MP treatment.
Our findings suggest that pharmacological inhibition of C5aR1 may be a promising therapeutic approach for MMP, particularly in patients with ocular involvement, and underlines the impact of complement activation in MMP pathology.

P118 (OP05/04) | A new mouse model of classical/mechanobullous EBA induced by a pathogenic autoantibody generated by AIBD-specific B cells under treg deficiency

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Previous studies have demonstrated that regulatory T cell (Treg)-deficient scurfy mice develop several autoimmune diseases including autoimmune blistering disease (AIBD), associated with the production of high levels of autoantibodies targeting important basement membrane proteins.
To elucidate the cellular mechanisms of autoimmunity leading to the development of AIBD, we characterised the B cell compartment of scurfy mice. Flow cytometric analysis revealed elevated levels of several B cell subpopulations, including germinal center B cells, memory B cells, and plasma cells located in skin-draining lymph nodes or spleen. Focusing on the site of AIBD manifestation, diseased scurfy mice showed significantly higher frequencies and cell numbers of B cells in the inflamed skin. To further specify antigen-specific B cell responses in the context of AIBD, we analysed the development and frequency of antigen-specific B cells in scurfy mice. Using enzyme-linked-immunospot (ELISpot) assays, scurfy mice exhibited antigen-specific B cells against human relevant AIBD-related proteins present in the hemidesmosomal structures. In addition to Laminin 5 and BP180, Collagen type VII (Col7), the immunodominant antigen of the human disease Epidermolysis bullosa acquisita (EBA), was identified.
Taking a closer look at the immune response to this antigen, the spontaneously developed scurfy antibody H510, was previously shown to have a subepidermal blister-inducing capacity in vivo after injection in neonatal C57BL/6 (WT) mice by targeting the murine von-Willebrand-Factor-A-like domain 2 of Col7. Focusing on the pathogenic mechanism, injection into Fc-gamma receptor knock-out mice failed to abolish blister induction by this anti-Col7 antibody. Silencing the Fc part of H510 to completely inhibit immune cell interaction prior to injection into neonatal WT mice confirmed a blistering mechanism independent of inflammatory immune cell help. Injection of this anti-Col7 antibody provides as a new mouse model for classical/mechanobullous EBA that may become an important tool to further study this rare human AIBD, give more insight into autoimmune responses and allow research for potential therapeutic targets.

P119 | The proliferation-associated protein 2G4 (PA2G4) as a multi-functional disease driver in psoriasis, mediating keratinocyte proliferation and inflammation

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Psoriasis is a common type 3-driven chronic inflammatory skin disease (ISD) with a prevalence of 2–3% of the world's population. Although several anti-psoriatic therapies are highly effective, these therapies often suffer from primary and secondary loss of efficacy, so there is still a need for new therapeutic targets. The proliferation-associated protein 2G4 (PA2G4) is an RNA-binding protein regulating cell growth. It's function has been studied in various tumour types and is associated with tumour progression (e.g., neuroblastoma, cervical, brain, breast, prostate, pancreatic, hepatocellular, breast cancer), as PA2G4 regulates cell proliferation, differentiation, and survival—processes that are also dysregulated in psoriasis. However, its role in ISD remains largely unknown. Therefore, this study focuses on the pathophysiological and immunological function of PA2G4 in psoriasis, evaluating its potential as a therapeutic target.
First, expression of PA2G4 in the skin of patients with psoriasis was examined in vivo. Using bulk RNA sequencing of psoriasis skin biopsies, a significant upregulation of PA2G4 compared to non-lesional controls was observed. Spatial transcriptomics and immunohistochemistry revealed a pronounced PA2G4 expression in the basal keratinocytes of psoriatic tissue. Single-cell sequencing further confirmed that keratinocytes are the main producers of PA2G4. Notably, PA2G4 gene expression positively correlated with the severity of psoriasis and its histological characteristics, including acanthosis, hyperkeratosis, and neutrophil migration.
Next, the pathophysiological and inflammatory function of PA2G4 in primary human keratinocytes was analysed in vitro using CRISPR-Cas9 knockouts (KO). Gene set enrichment analysis of bulk RNA sequencing data revealed a suppression of proliferation and inflammation pathways in PA2G4 KO keratinocytes. Under unstimulated and type 3-stimulated (IL-17A/TNF-α) conditions, a significant downregulation of psoriasis-related proliferation and inflammation genes (e.g., Ki67, PCNA, VEGFA, HIF1A) was observed. In addition, functional keratinocyte scratchproliferation assays validated a reduced proliferation capacity in PA2G4 KO cells. In reconstructed human epidermis, PA2G4 KO also showed reduced epidermal thickening upon IL-22 stimulation.
Finally, the commercial small molecule PA2G4 inhibitor WS6 was used to evaluate the potential of PA2G4 blockade as a therapeutic target. Inhibition of PA2G4 in keratinocytes with WS6 resulted in PA2G4-KO-analogous effects on proliferative capacity and regulation of differentiation and proliferation markers.
In summary, PA2G4 is a disease driver in psoriasis mediating keratinocyte proliferation and inflammation under type 3 inflammatory conditions. Targeting PA2G4 may offer a novel therapeutic strategy for treating psoriasis.

P120 | AHR-deficient tumour cells escape CD8+ T cell immune therapy

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The aryl hydrocarbon receptor (AHR) is a ligand-dependent transcription factor that was originally discovered for its ability to bind and detoxify dioxins, a group of undesired highly toxic by-products in the chemical industry. Subsequently, it was found that the AHR also binds to a variety of physiological ligands and is involved in numerous biological functions including the regulation of cellular differentiation and immune defence in the skin. In recent years, evidence has accumulated that AHR signalling promotes melanoma progression and resistance to therapeutic intervention. Here, we investigate the hypothesis that cancer cell-intrinsic AHR signalling limits the efficacy of T cell immunotherapy.

To address this hypothesis, we disrupted the AHR gene in HCmel12 mouse melanoma cells using CRISPR/Cas9 gene editing and employed our established adoptive cell therapy protocol consisting of chemotherapeutic preconditioning, adoptive transfer of melanoma-specific CD8+ T cells together with recombinant adenoviral vaccination, and subsequent adjuvant innate intra-tumoral immune stimulation using the synthetic nucleic acids polyI:C and CpG (CD8 ACT).

Contrary to our hypothesis, we observed that our CD8 ACT therapy was significantly less effective against established HCmel12-AHR-KO melanomas when compared to HCmel12-CRISPRctrl melanomas. In vitro analyses demonstrated that HCmel12- AHR-KO and HCmel12-CRISPRctrl melanoma cells expressed similar levels of MHC molecules after IFNg stimulation and both were able to effectively activate CD8+ T cells. High resolution spectral flow cytometry of single cell suspensions derived from melanomas 7 days after CD8 ACT treatment showed an increased infiltration of macrophages and neutrophils with an immunosuppressive phenotype in HCmel12- AHR-KO melanomas, when compared to HCmel12-CRISPRctrl melanomas. In ongoing work, we are trying to elucidate the underlying mechanisms of how AHRdeficient tumour cells evade death by CD8 ACT therapy.

P121 | Impact of glutamate metabolism and its inhibition on melanoma development and myeloid cells

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Tumour immunity is negatively regulated by metabolites in the tumour tissue. Metabolic reprogramming impacts the activation and maturation of dendritic cells (DC). We work on the transgenic melanoma mouse model tg(Grm1)EPv which spontaneously develops melanoma due to overexpression of the metabotropic glutamate receptor 1 (Grm1) in melanocytes. This aberrant glutamate metabolism drives tumour formation, but might also affect immune cell function. Thus, we study the metabolic changes in progressing tg(Grm1)EPv melanoma and their possible effects on DC and T cell responses.

We performed analyses of myeloid subsets in tumours and draining lymph nodes during tumour progression with multicolor flow cytometry. While cDC2 and macrophages decreased, neutrophils and monocytes increased in the lesions. An investigation of DC precursors in the bone marrow and in vitro DC differentiation assays showed no difference between tumour-bearing tg(Grm1)EPv and C57BL/6 mice.

Metabolic screening of the tg(Grm1)EPv mice at different disease stages with LC–MS technology revealed a shift towards glutamate/glutamine, a shift from Glucose/Pyruvate to Lactate during glycolysis and a decrease in ATP. This data is supported by RNA sequencing results showing an increased expression of enzymes involved in glutamate metabolism and glycolysis. These changes indicate a Warburg effect, disruption of the respiratory chain, and metabolic changes in the tumour microenvironment that are advantageous for the tumour cells and unfavourable for DC.

Current investigations focus on alterations caused by glutamate pathway inhibition in vitro and in vivo. We see a significant increase in cell death in Grm1-positive melanoma cells after treatment with Buthionine sulphoximine (BSO) while not having an effect in Grm1-negative control cells. This knowledge can drive the design of novel therapeutic strategies for cancer patients involving potential modification of tumour glutamate metabolism. Combination therapies with inhibitors of the glutamate pathway might improve response rates in cancer patients.

P122 | Investigating pathogenicity of a N-terminal BP230 autoantibody in human skin models

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Bullous pemphigoid (BP) is the most common autoimmune blistering disease characterised by the presence of autoantibodies against BP180 and intracellular BP230. Autoantibodies directed against these components of the hemidesmosomal complex lead to detachment of the epidermis. In human BP, the pathogenic relevance of anti-N-terminal BP230 antibodies is still under discussion.

20B12, a mouse monoclonal IgG1 antibody, binds to the N-terminus of BP230 and is pathogenic in a mouse model. 20B12 shows cross-reactivity with human BP230. Therefore, we ask whether we can demonstrate the blister formation induced by

20B12 in human skin equivalents (HSE) generated from primary human fibroblasts and keratinocytes. HSE exhibit the distinct skin layers—dermis, epidermis, corneum—which correspond to normal skin morphology. Immunofluorescence analysis showed deposition of 20B12 at the BMZ, but the fluorescence signal was more diffuse instead of a distinct line. Even when using a commercially available antibody, a diffuse expression pattern of BP230 and BP180 as well as proteins important for BMZ stability was observed compared to epidermis of BP patients. Serial sections of HE-stained, untreated HSE showed instability of the dermo-epidermal junction, indicating aberrant assembly of the structural components. In human foreskin from healthy volunteers children, BP230 and BP180 are expressed as expected. Incubation of foreskin biopsies with 20B12 for 1 to 4 days was able to show that the murine antibody can bind its antigen BP230 already on the first day of culture. In the untreated biopsies, no signs of autolysis or instability were observed at the BMZ during the entire culture period. To analyse the potential of 20B12 to induce subepidermal blisters in an ex vivo human skin model, a screening of serial preparations of HE-stained biopsies treated with 20B12 was performed. In the human foreskin biopsy, which was treated with 20B12 for 4 days, we discovered a potential blister. A separation of dermis and epidermis was detected on three consecutive sections.

Therefore, we conclude that the ex vivo model of human skin is an appropriate model to demonstrate binding of 20B12. We hypothesize that the N-terminal BP230 antibody was able to induce blisters, indicating pathogenicity independent of immune cells and inflammation. To confirm this observation, replicates are required. The next steps are to visualise the site of antigen–antibody binding and testing inhibitors for various transport pathways to determine how 20B12 enters the living cells.

P123 | The impact of diet and age on contact hypersensitivity

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Allergic contact dermatitis (ACD) is a T-cell mediated delayed-type hypersensitivity that affects approximately 20% of the general population. During sensitization, the innate immune system is important to induce inflammation in the skin. Pattern recognition receptors, such as Toll-like receptors (TLRs) play a crucial role in the induction of inflammation upon allergen contact. We have shown that mice lacking TLR2 and TLR4 (TLR2/4 mice), TLR4 and IL-12Rβ2, the adaptor protein MyD88, the receptor P2X7 or the IL-1 receptor are resistant to contact hypersensitivity (CHS), the mouse model of ACD. In addition to direct induction of signalling via innate immune receptors, cellular stress such as induction of the unfolded protein response (UPR) is also important for sensitization.

Obesity and aging are both associated with increased systemic inflammation and an increase in inflammatory diseases. We hypothesized that the increase in inflammation caused by obesity or age might overcome the CHS resistance of the TLR2/4 mice.

We were able to show that the resistance of TLR2/4 mice to CHS could be broken by feeding them a high-fat diet (HFD) or a high-sugar diet (HSD) for 4 weeks (Rühl-Muth et al. 2021). These mice show a significant increase in ear thickness. This was associated with an increase in IFNγ producing T cells and increased levels of IL-1β in the ears. We also showed that HFD and HSD increased the initial sensitization response to TNCB in both wt and TLR2/4 mice, together with an increased influx of neutrophils into the ears. In addition, we showed an increase in pro-inflammatory serum cytokines. Feeding HFD and HSD also resulted in a shift in the gut microbiome.

The resistance of TLR2/4 mice can be broken not only by feeding the mice with HFD or HSD, but also by increasing the age of the mice. A significant increase in ear thickness could be shown in 18–30 week old TLR2/4 mice, whereas 6–9 week old TLR2/4 mice are resistant. Interestingly we were not able to show a difference in the initial reaction to TNCB application between young and old mice, even though a difference between HFD and HSD fed mice was observed in comparison to NC fed mice.

This shows that feeding HFD, HSD or increasing age can compensate for the loss of pro-inflammatory signalling via TLR2 and TLR4.

P124 | HOCl-oxidised melanoma cells provide inflammatory stimuli in human dendritic cells

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Preventive vaccination against infectious diseases is considered one of the greatest medical achievements of all time. Therapeutic vaccination of established malignancies has proven much more challenging but ongoing clinical trials are promising. While the efficacy of vaccines based on predefined antigens is limited to the epitope used for immunisation, autologous anti-tumour vaccines provide virtually complete, individual access to the tumour-inherent proteome, including tumour antigens, which are capable of stimulating polyclonal T-cell responses without the need for time-consuming and costly identification of individual immunodominant epitopes. To improve the initiation of adaptive anticancer immunity, oxidative posttranslational modifications (oxPTM) could serve as an intrinsic adjuvant as previously suggested [1]. As such, oxidation of protein antigens using hypochlorous acid (HOCl), which is produced by neutrophils and involved in acute inflammation, can increase antigen uptake, processing, and presentation [2, 3]. Moreover, induction of oxidative stress in cancer cells may involve the induction of immunogenic cell death, further increasing tumour cell adjuvanticity via release of damage-associated molecular patterns (DAMPs). In our present study, we aimed to evaluate in vitro HOCl-oxidised melanoma cells for their immunostimulatory potential in human monocyte-derived dendritic cells (moDCs). In the latter, pulsing moDCs with HOCloxidized melanoma material was found to increase co-stimulatory molecules and inflammatory chemokine and cytokine profiles.

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References
1. Tanyi, J.L., S. Bobisse, E. Ophir, S. Tuyaerts, A. Roberti, R. Genolet, P. Baumgartner, B.J. Stevenson, C. Iseli, D. Dangaj, B. Czerniecki, A. Semilietof, J. Racle, A. Michel, I. Xenarios, C. Chiang, D.S. Monos, D.A. Torigian, H.L. Nisenbaum, O. Michielin, C.H. June, B.L. Levine, D.J. Powell, Jr., D. Gfeller, R. Mick, U. Dafni, V. Zoete, A. Harari, G. Coukos, and L.E. Kandalaft, Personalised cancer vaccine effectively mobilises antitumor T cell immunity in ovarian cancer. Sci Transl Med, 2018. 10(436).
2. Clemen, R., E. Freund, D. Mrochen, L. Miebach, A. Schmidt, B.H. Rauch, J.W. Lackmann, U. Martens, K. Wende, M. Lalk, M. Delcea, B.M. Broker, and S. Bekeschus, Gas Plasma Technology Augments Ovalbumin Immunogenicity and OTII T Cell Activation Conferring Tumour Protection in Mice. Advanced Science, 2021. 8(10): p. 2003395.
3. Clemen, R., L. Miebach, D. Singer, E. Freund, T. von Woedtke, K.D. Weltmann, and S. Bekeschus, Oxidised Melanoma Antigens Promote Activation and Proliferation of Cytotoxic T-Cell Subpopulations. Adv Sci (Weinh), 2024. 11(33): p. e2404131.

P125 | Mechanism of dermal γδ T cells expansion in inflammation

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Psoriasis, with a worldwide prevalence about 2–3%, is a chronic immune cellmediated inflammatory skin disease. A key cytokine driving its pathogenesis is IL-17, produced largely by γδ T cells in the skin within the Imiquimod (IMQ)-induced psoriaslike dermatitis model in mice. We find these cells strongly expanded in the skin and in local draining lymph nodes. However, the expansion and triggering mechanism of the dermal IL-17-producing γδ T cells remain poorly understood.

In order to delineate these mechanisms, we are conducting single cell RNA sequencing of the γδ T cell receptor and other surface proteins. The γδ T cell receptor is generated through V(D)J recombination, a process that generates TCR diversity by recombining variable (V), diversity (D), and joining (J) gene segments. Given that different population of the same γδ T cell subset can utilise distinct Vγ and Vδ gene segments, we aim to reveal clonal expansions, providing insights into the lineage and relationships of these cells. This analysis may reveal specific recombination patterns that drive γδ T cell expansion and IL-17 production, potentially highlighting novel therapeutic targets for psoriasis. We also aim to elucidate the mechanisms driving γδ T cell expansion found in the skin and in the local lymph nodes.

Additionally, we aim to investigate the migratory behaviour of γδ T cells in the skin and lymph nodes using KikGR-expressing mice, a photoconvertible florescent protein model, to track the movement of dermal γδ T cells to local lymph nodes in response to IMQ treatment. This approach will allow us to visualise the migration and dynamics of γδ T cells during psoriatic inflammation, providing further insight into the cellular mechanisms driving their expansion.

P126 | Keratinocyte-derived IL-25 as a regulator of epidermal and dermal function in inflammation

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IL-25 (IL-17E), a member of the IL-17 family, is involved in the pathogenesis of distinct skin inflammatory diseases. We explored the transcriptomic changes in human organotypic skin explants from two healthy donors, which were treated with IL-25 or IL-17A, by performing single-cell RNA sequencing to understand the mechanisms of the specific response to IL-25 in distinct skin cell populations. Our results showed the effect of IL-25 on transcriptomes of each skin cell type was less pronounced than that of IL-17A, which induced multiple proinflammatory pathways. IL-25 had the highest effect on the keratinocyte cluster compared to other skin cell populations. We identified 6 keratinocyte subclusters, which potentially exhibited distinct characteristics and various responses to stimuli. Notably, two proinflammatory keratinocyte subclusters characterised by high expression of KRT6A and KRT16 stood out: one differentiated (KRT10+) and one undifferentiated (KRT5+, KRT14+) subcluster. Differentially Expressed Gene analysis revealed that IL-25 significantly upregulated genes linked to atopic dermatitis (IL-4R and SERPINB2) and itch (SPINK5), particularly within proinflammatory differentiated keratinocytes. In contrast, fibroblasts and other cell types exhibited minimal differential gene expression in response to IL-25. This new information will serve as a basis for future functional experiments on the role of IL-25 in the skin cell crosstalk during homeostasis and pathology.

P127 | Establishment of an ex vivo placental explant model to analyse autoantibodymediated effects on the placenta in pemphigoid gestationis

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Pemphigoid gestationis (PG) is a rare autoimmune blistering disease affecting pregnant and postpartum women, characterised by pruritus and blistering skin lesions. It is caused by a loss of immunotolerance against the hemidesmosomal antigen BP180, with pathogenic autoantibodies targeting its NC16A domain. In a recent large-scale, propensity-matched cohort study, we demonstrated that PG patients have an increased risk of various adverse pregnancy outcomes (APOs). BP180 is expressed not only in the skin but also in amniotic epithelial cells and cytotrophoblasts of the chorioamniotic membranes as well as in syncytial knots of the villous epithelium. Autoantibodies from PG patients have been described to bind to these sites, and IgG and C3 deposition as well as histological abnormalities such as mild villitis and partial basement membrane microseparation have been observed in placentae from PG patients. However, the potential causal role of BP180 NC16A antibodies in these placental changes and their contribution to APOs in PG remains unexplored.

To address this, an ex vivo model of chorioamniotic membrane and placental explant cultures was established. Placentae from healthy, term patients delivered via caesarean section were collected immediately postpartum. Punch biopsies of the chorionic plate with villous tissue and pieces of chorioamniotic membranes were collected and cultured for 6 days. On day five, they were either treated with IgG from apheresis material with a high titre for anti-BP180 antibodies, intravenous immunoglobulins (IVIG), or were left untreated. Cytokine release was assessed after 24 h.

Preliminary results suggest alterations in cytokine production, with a trend towards increased IL-8 release in anti-BP180-IgG-treated explants compared to controls. No significant morphological changes were observed. This is the first ex vivo model designed to investigate autoantibody-mediated effects on chorioamniotic membranes and placental tissue in PG. Further studies are planned to validate the observed cytokine release alterations. The establishment of this model enables not only the analysis and understanding of the pathogenesis of APOs in PG but also in other autoantibody-mediated diseases. This understanding is urgently needed to improve maternal and fetal outcomes.

P128 | Thrombin-dependent and C3-independent mechanisms in experimental pemphigoid disease inflammation

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Pemphigoid diseases (PDs) represent a group of autoimmune blistering skin conditions characterised by the production of autoantibodies that target structural protein of the dermo-epidermal junction (DEJ), leading to the loss of cell adhesion. The presence of linear IgG and C3 deposits is a diagnostic hallmark of these diseases, which include among others, epidermolysis bullosa acquisita (EBA), bullous pemphigoid (BP), and mucous membrane pemphigoid (MMP). While prior research has highlighted the crucial role of the C5/C5aR1 axis in driving skin inflammation in experimental murine models of PD, the role of C3 in these conditions has remained unresolved.

In this study, we utilised antibody transfer-induced PD models in C3-deficient mice to access the functional importance of C3 in the effector phase of development of experimental PD.

Our results reveal that in all three pre-clinical PD models EBA, BP, and MMP, disease pathology can develop independently of C3. These findings suggest that C5a, a potent inflammatory mediator, can be proteolytically generated without the involvement of C3 in these models. To further elucidate the mechanisms underlying C5a generation, we investigated the contribution of alternative C5 convertases, focusing on thrombin and neutrophil elastase (NE), which are known to play roles in complement activation.

Using the antibody transfer-induced EBA mouse model, we demonstrated that blocking thrombin with the thrombin inhibitor argatroban resulted in a significant reduction in clinical disease severity. However, this reduction was less pronounced compared to the effects observed previously in mice deficient in the C5/C5aR1 axis, indicating the likely involvement of additional alternative pathways for C5a production in this model system. In contrast, EBA, BP and MMP developed similarly in NEdeficient mice and wild-type controls, suggesting a lesser role for neutrophil elastase in C5a generation in these models.

In conclusion, our study provides compelling evidence that skin inflammation in experimental PD can develop independently of C3, with thrombin playing an crucial role in the alternative generation of C5a. These findings offer new insights into the complement pathways involved in PD and suggest novel therapeutic targets for mitigating disease progression. Further research is warranted to fully elucidate the contributions of thrombin and other alternative C5 convertases in PD, and to determine the translational relevance of these findings in human patients.

P129 | Generation of albumin nanocapsules and nanocrystals for efficient intracellular drug release

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In order to achieve a therapeutic effect, many drugs have to reach specific cellular compartments, but distributing hydrophobic drugs with a limited solubility across physiological barriers is difficult. Nanoscale drug delivery systems can improve the bioavailability and pharmacological activity of drugs combined with reduced off-target toxicity and alleviated systemic effects through spatiotemporal control over drug release.

By encapsulating the FDA-approved antiprotozoal drug and STAT3 inhibitor Atovaquone (Ato) as a prototype for

hydrophobic small molecules, we have developed two types of albumin-coated nanocarriers to achieve effective intracellular drug release. Tracking of cellular uptake and intracellular enzymatic opening using built-in dyes Cy5 and CMFDA showed delivery and release of incorporated Ato into immune and non-immune cells.

While Ato-loaded ovalbumin-coated nanocapsules (Ato-nCap) preferentially entered human myeloid cells, Ato nanocrystals with a human serum albumin shell (AtonCry) delivered their cargo in all different immune cell types, including T and B lymphocytes with reduced endocytic activities. Analysis of the effect of Ato nanocarriers on induced STAT3 phosphorylation in IL-10-primed human dendritic cells and constitutive STAT3 phosphorylation in human melanoma cells revealed that intracellular Ato release is particularly effective from Ato nanocrystals and at the same time less toxic than equal doses of free Ato. In our study, we found these newly developed nanocarriers and especially the crystallised form of an hydrophobic drug to be an effective tool for intracellular drug delivery which can be engineered for other drugs with similar chemical properties, thus advancing the application of nanomedicine in clinical use.

P130 (OP04/04) | The influence of different immunosuppressive drugs on ex vivo expanded regulatory T cells

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Introduction: The adoptive transfer of ex vivo-expanded regulatory T cells (Tregs) represents a novel therapeutic approach currently being studied in clinical trials for autoimmune and inflammatory diseases, such as inflammatory bowel disease and type 1 diabetes, with potential future applications in autoimmune skin conditions [1,2]. Many patients eligible for the clinical trials in this field already use immunosuppressive medication, raising the critical question of how various immunosuppressants affect the function and migration of therapeutically administered Tregs.

Methods: To evaluate the impact of immunosuppressants on expanded Tregs, the cell product was manufactured using a GMP-compliant protocol applied in clinical trials [3]. Treg function was assessed via a suppression assay, involving coculture of Tregs with autologous CFSE-labelled CD25- responder cells, with drugs added to the medium. Treg-mediated suppression was calculated by comparing responder cell proliferation in the presence and absence of Tregs.

Migratory behaviour was analysed by embedding Tregs in a three-dimensional collagen gel with immunosuppressants present. Cell speed and motile fraction were captured visually using a camera system.

Results: In the presence of prednisolone and the TNFα antibody Infliximab, Tregs exhibited significantly increased suppression of responder cell proliferation. Additionally, the JAK–STAT inhibitor Tofacitinib and the IL12/IL23 antibody Ustekinumab slightly enhanced Treg function, while the antibodies Mirikizumab (IL-23) and Secukinumab (IL-17) showed no significant trends.

In motility assays, mainly motile fraction was affected with a decreasing effect of prednisolone most notably. Also, Tofacitinib, Ustekinumab, and Secukinumab lowered Treg motile fraction, while cell speed was changed significantly only by prednisolone.

Discussion: Given the differing effects on Treg function and migration, determining the most suitable concomitant drug for Treg transfer is essential. While prednisolone enhances in vitro Treg function, its negative impact on migratory behaviour shifts consideration to TNFα antagonists like Infliximab. Further mechanistic studies on how soluble or membrane-bound TNFα affects Tregs via TNF receptors could provide deeper insights. Additionally, confirming these drug effects in antigen-specific settings would help bridge the gap between in vitro and in vivo conditions.

In conclusion, our findings underscore the significant influence of immunosuppressants on Treg behaviour, highlighting the need for further preclinical and clinical studies to optimise Treg-based therapies.

References

1. Qu G, Chen J, Li Y, Yuan Y, Liang R, Li B. Current status and perspectives of regulatory T cell-based therapy. J Genet Genomics. 2022;49(7):599–611. doi:10.1016/j.jgg.2022.05.005

2. Mukhatayev Z, Ostapchuk YO, Fang D, Le Poole IC. Engineered antigenspecific regulatory T cells for autoimmune skin conditions. Autoimmun Rev. 2021;20(3):102761. doi:10.1016/j.autrev.2021.102761

3. Wiesinger M, Stoica D, Roessner S, et al. Good Manufacturing Practice- Compliant Production and Lot-Release of Ex Vivo Expanded Regulatory T Cells As Basis for Treatment of Patients with Autoimmune and Inflammatory Disorders. Front Immunol. 2017;8:1371. Published 2017 Oct 26. doi:10.3389/fimmu.2017.01371

P131 | Standardised diagnosis of pemphigoid diseases with serum autoantibodies against the dermal side of salt-split skin by indirect immunofluorescence microscopy

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Pemphigoid diseases (PD) are characterised by the presence of autoantibodies targeting structural proteins of the dermoepidermal junction. The standard screening test for PD is indirect immunofluorescence (IF) on salt-split human skin, which identifies autoantibodies binding to either the epidermal or dermal side of the artificial split. Dermal binding is typically observed in patients with epidermolysis bullosa acquisita (EBA), mucous membrane pemphigoid (MMP), and anti-p200 pemphigoid, involving autoantibodies against type VII collagen (Col7), laminin 332, and laminin β4, respectively. The objective of this study

was to validate an indirect immunofluorescence (IF)-based Biochip Mosaic(TM) consisting of three biochips with HEK293 cells expressing recombinant laminin β4, the recombinant NC1 domain of Col7, and laminin 332, respectively (Euroimmun, Lübeck, Germany). This novel indirect IF assay was applied to test sera from patients with dermal ($n=42$) or epidermal binding ($n=50$) in indirect IF on salt-split skin, as well as from patients with pemphigus vulgaris (PV, $n=50$) and healthy blood donors (HBD, $n=50$). All 42 sera with dermal binding on salt-split skin reacted with the mosaic. 28 sera reacted with laminin β4, 2 sera with laminin 332, and 15 sera with Col7. No reactivity with any of the substrates was found in the sera of PV patients or healthy blood donors. However, three sera with epidermal binding labelled laminin β4-expressing cells. Anti-laminin β4 reactivity was subsequently confirmed by immunoblotting with recombinant laminin β4. In summary, the novel widely available Dermal Binder Biochip(TM) Mosaic demonstrated a specificity of 100% (excluding the three laminin β4 positive epidermal binder) and a sensitivity of 100% for PDassociated autoantibodies with dermal binding by indirect IF on salt-split skin. This novel diagnostic tool allows for differentiation between anti-p200 pemphigoid, antilaminin 332 MMP, and EBA, thus improving diagnostic accuracy for PD.

P132 | Targeting JAK1/2 with ruxolitinib: A promising therapy for pemphigoid diseases

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Pemphigoid diseases (PD) are severe autoimmune blistering disorders with limited treatment options targeting the disease's molecular structures. As a result, mostly general immunosuppression remains the primary therapy, despite their potentially severe side effects. Epidermolysis bullosa acquisita (EBA), a difficult-to-treat PD, is driven by autoantibodies against type VII collagen, which after the formation of immune complexes (IC), activate neutrophils. Neutrophils are key players in EBA, bind to IC via Fc gamma receptors and trigger signal pathways that ultimately release reactive oxygen species (ROS) and proteases. Therefore, neutrophils and IC-induced signals are promising targets for new therapies.

A kinome analysis of IC-activated neutrophils was therefore performed, screening the activity of 156 kinases over time. This revealed, among other findings, an increased activity of kinases from the Janus kinase signalling family (JAKs), which has already been demonstrated to be implicated in the pathogenesis of several inflammatory and autoimmune diseases. Additionally, RNA analysis and staining for these JAKs demonstrated the explicit expression of JAK1 and JAK2 in immune cells, including neutrophils and T cells, as well as fibroblasts and keratinocytes. Furthermore, a higher expression was observed for phosphorylated and unphosphorylated JAK2 in lesional skin compared to healthy skin. These findings support the role of the JAK signalling pathway also in PDs and suggest that JAK inhibitors (JAKi) may represent a novel therapeutic option.

Following this hypothesis, we examined the impact of distinct JAK inhibitors in several in vitro settings. Of particular interest was the JAK1 and JAK2 inhibitor Ruxolitinib, which exhibited a notable reduction in reactive oxygen species (ROS) production in IC-activated neutrophils at a concentration of 0.01 mM relative to a control group. To further investigate the efficacy of this JAKis, we conducted in vivo testing, initially in a prophylactic topical manner in a local antibody-transfer induced EBA mouse model. Here, Ruxolitinib demonstrated remarkable efficacy, reducing the affected ear surface area, epidermal thickening, split formation, and leucocyte infiltration by over 50% compared to the untreated control group.

In view of these encouraging findings, Ruxolitinib was selected for testing in vivo in an immunisation-induced EBA mouse model using oral administration twice daily for 4 weeks, commencing as soon as the mice exhibited an affected body surface area of at least 2%. Similarly, mice treated with Ruxolitinib exhibited a notable reduction in disease severity compared to the vehicle-treated control group over time. In contrast to the control group, treated mice demonstrated no disease progression from week one and, furthermore, exhibited a decline in the affected body surface area in week four.

In conclusion, the inhibition of JAK1/2 by Ruxolitinib results in the impairment of neutrophil functions and the effective treatment of experimental EBA. Collectively, our results identify JAK1/2 as a potential therapeutic target in the treatment of EBA and other related autoantibody-mediated, neutrophil-driven diseases. Thus, the JAKi Ruxolitinib is a promising therapeutic option for PDs.

P133 | Impact of IgG subclasses on kinase activity in pemphigus: insights into Dsg3 dynamics

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Pemphigus is an autoimmune blistering disorder characterised by the presence of autoantibodies that target desmogleins (Dsg) 1 and 3 – cadherin proteins crucial for maintaining desmosomal adhesion between adjacent keratinocytes. Diagnosis primarily relies on the detection of tissue-bound autoantibodies. The binding of these autoantibodies disrupts cell–cell adhesion through various mechanisms, leading to intraepidermal blister formation in the skin and/or mucous membranes. Although all four immunoglobulin G (IgG) subclasses – IgG1, IgG2, IgG3, and IgG4 – are implicated in the pathogenesis of pemphigus, the autoimmune IgG response is predominantly mediated by IgG4. IgG4 is uniquely characterised by its ability to function as a monovalent, bispecific molecule, a feature that arises from its capacity to undergo Fab-arm exchange facilitated by the transient reduction of disulfide bridges in the heavy chains of the antibody. In contrast, the other IgG subclasses are unable to perform Fab-arm exchange, which enhances their efficacy in

crosslinking Dsg1 and Dsg3. A recent study has revealed distinct binding patterns for IgG1, IgG2, IgG3, and IgG4 in the skin of pemphigus patients, along with a differential distribution of Dsg3 in keratinocytes treated with either a mixture of IgG1, IgG2, and IgG3 or with IgG4 alone.

To this end, we collected serum samples from two pemphigus patients and purified the IgG using protein G Sepharose. Subsequently, IgG4 was separated from the other subclasses through chromatography on a CaptureSelect IgG4 (Hu) Affinity Matrix. NHEK proteins were isolated at different time points (0 min, 5 min, and 30 min) following incubation with serum from a healthy control, as well as with all IgG subclasses, the mixture of IgG1, IgG2, and IgG3, or solely with the isolated IgG4. In parallel, the Dsg3 distribution in NHEK from each treatment group was visualised using confocal microscopy following direct immunofluorescence staining at each time point.

Incubation with the complete IgG subclasses from the pemphigus sera resulted in increased p38 MAPK signalling activity after 5 min, which has been previously associated with Dsg3 internalisation during pemphigus acantholysis. Subsequently, other kinases, including PKC, CaMK4, and IKK-epsilon, exhibited significant increases in activity. Notably, elevated activity of MAP3K8 was also observed in NHEKs treated with either IgG4 alone or with a mixture of IgG1, IgG2, and IgG3. Interestingly, these treatment conditions also revealed an increase in mTOR/FRAP activity after 5 min.

However, the kinome activity profiles differed significantly between the two antibody groups. While IgG4 alone predominantly increased the activities of CDK 4, 6, 9, and 11, as well as FGFR kinases after 5 min, the IgG subclasses (IgG1, IgG2, and IgG3) caused minimal additional changes in the kinome profile. Immunofluorescence staining of Dsg3 showed that both antibody fractions induced internalisation. However, pronounced clustering was absent in IgG4-treated NHEKs, likely due to the reduced crosslinking potential associated with its monovalent, bispecific nature.

This study elucidates the differential activation of kinases in response to various IgG subclasses in pemphigus, particularly highlighting the significant role of p38 MAPK and other kinases, such as CDK4, 6, 9, and 11, in mediating the effects of IgG4 on Dsg3 internalisation. Future research should explore the specific signalling pathways and kinomic profiles associated with each IgG subclass to identify potential therapeutic targets for modulating kinase activity and improving outcomes in pemphigus patients.

P134 (OP05/01) | Overactivation of CdC42 kinase impairs the cytotoxic function of NK cells from old adults enhancing accumulation of senescent fibroblasts in aging skin—A successful rescue by the CdC42 inhibitor CASIN

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Cellular senescence is known as a state of permanent cell cycle arrest, and a prime hallmark of tissue aging. Senescent cells—by the release of bioactive molecules such as inflammatory chemokines, cytokines, extracellular vesicles and matrix degrading metalloproteases, collectively referred to as Senescence Associated Secretory Phenotype (SASP)—even enforce age-related disorders such as non-healing states of wounds, osteoporosis, neurodegenerative disease among other agingrelated conditions. Fibroblasts, which physiologically and anatomically constitute the principal component of the connective tissue, play an important role in organ homeostasis, however—senescent cells—drive organ and skin aging. We previously showed that senescent fibroblasts gradually accumulate in human skin with age. Under transient conditions of senescence during embryogenesis and acute wound healing, senescent fibroblasts are successfully removed by Natural Killer cells (NK cells) which belong to the innate immune system. We here wished to understand whether (1) NK cells fail to remove senescent fibroblasts and—if so, (2) what are the underlying mechanisms and (3) is there any therapeutic approach to rescue the impaired cytotoxic function of old NK cells. Using a NK cell mediated killing assay with magnetic negative selection of NK cells, we were able to show that primary NK cells from old human donors (~70 years) and old mice (~700 days) were less efficient in killing senescent fibroblasts as opposed to those from young human donors (~23 years) and young mice (~100 days), respectively. No change was detected in the number of resident NK cells in the skin between young and old human adults and mice. We also observed, through western blot and flow cytometry analysis, significant reduction in the content of NK cell cytolytic granules—which comprises the pore forming protein perforin and the apoptosis inducing serine protease granzyme B, in NK cells isolated from old individuals. The impaired content of these cytotoxic substances unequivocally leads to a profoundly impaired cytotoxic ability of old NK cells towards senescent fibroblasts. A global approach of transcriptome profiling of NK cells from old and young adults was employed to understand the over- and under representation of pathways the expression of distinct genes and their role in mediating NK cell cytotoxicity. Transcriptome enrichment analysis of old versus young NK cells, identified among others, the upregulation of gene signature sets for Rho GTPases in old NK cells. Pull down experiments found an unrestrained overactivation of Cdc42, a family member of Rho GTPases. Using the small molecule approach, we found CASIN to inhibit the unrestrained Cdc42 activity in old NK cells and, in consequence, profoundly improved the impaired cytotoxic function of NK from old human adults towards senescent fibroblasts. As perforin and granzyme B granules are moved and focused to the synaptic cleft between NK cells and target senescent fibroblasts by the action of microtubules which are regulated by CdC42 activity, unrestrained CdC42 activity—as occurring in old NK cells—disrupts this fine tuning which is remarkably rebalanced to the Cdc42 activity of young NK cells by CASIN.

Collectively, we here unveiled a previously unreported mechanism of the functional impairment of NK cells from old adults and defined a therapeutic strategy to counteract the accumulation of senescent fibroblasts in old skin and likely other organs. In perspective, our data hold also promise to be developed for novel strategies against age-related disorders.

P135 (OP01/04) | Kinase signalling cascades in neutrophils might comprise several potential therapeutic targets for pemphigoid disease

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Neutrophil kinase signalling represents a pivotal mechanism in the pathogenesis of pemphigoid diseases, a group of rare, potentially life-threatening autoimmune bullous diseases. The deposition of pathogenic autoantibodies at the dermal-epidermal junction (DEJ) results in the activation of fragment crystallisable gamma receptors (FcγR) on the surface of neutrophils, the central effector cells in pemphigoid disease. This triggers signalling cascades downstream the FcγR leading to the release of reactive oxygen species (ROS) and proteases, which in turn cause the destruction of the adhesion complex at the DEJ. Patients are usually treated with immunosuppressing agents like rituximab or glucocorticoids causing severe side effects. Thus, new therapeutic options are urgently needed.

Given the incomplete understanding of neutrophil kinase signalling, it is our objective to gain further insight into this process in order to identify and validate novel targets for potential therapeutic applications.

Therefore, we conducted a multiplex kinase activity assay using the PamGene(TM) system to quantify kinase activity in immune complex (IC)-stimulated human polymorphonuclear leukocytes (PMNs), thereby identifying the activity of over 120 kinases in parallel. In addition, a target-selective inhibitor library comprising around 150 different small molecule signal transduction inhibitors (STIs) was screened using IC-stimulated human PMNs in a ROS release assay. The most promising 34 signalling molecules were chosen for further in vitro analyses. Consequently, the impact of selective signalling inhibitors on the following functions was investigated in vitro: surface activation marker expression, chemotaxis, adhesion, ROS release, and cell death.

The present study demonstrates a significant inhibitory effect of multiple signalling molecules implicating an important role in the pathomechanism of pemphigoid diseases. Furthermore, a total of 18 distinct STIs were selected for in vivo validation in a local antibody-transfer mouse model of pemphigoid disease.

In this mouse model, 11 out of these STIs improved the clinical phenotype in a short-term prophylactic setting. Cyclin-dependent kinase (CDK) 9 inhibition by MC180295 showed the most promising effects. Other kinase inhibitors targeting e.g. CDK7, Janus kinases (JAK) 1/2, Akt, p38 kinase, and c-Met significantly improved the health status of the mice, too. The therapeutic use of selected inhibitors in immunisation-induced EBA confirmed the potential therapeutic value in the treatment of pemphigoid diseases.

Taken together, our findings highlight important neutrophil kinase signalling pathways in pemphigoid disease. This research contributes to a more comprehensive understanding of neutrophil signal transduction and facilitates the identification of potential novel therapeutic targets for pemphigoid patients.

P136 | Understanding confined functions of neutrophils in the pathophysiology of diabetic wound healing

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Neutrophils are among the first cells to be recruited to an injury site, where they respond by phagocytosis, degranulation, and the formation of neutrophil extracellular traps (NETs) to prevent infection, and by releasing cytokines and growth factors to stimulate inflammation and tissue repair. Sustained activation of neutrophils has been associated with chronic inflammation and impaired wound healing as found in diabetic ulcers. However, altered or confined functions of neutrophils in the pathophysiological conditions of diabetic wound healing are not well understood yet.

We assessed spatiotemporal organisation of neutrophils in full thickness wounds in wildtype mice. Flow cytometry analysis of Ly6G+ cells showed the presence of viable neutrophil from 12 h up to 5 days after injury with changes in neutrophil subpopulations over time. Neutrophils infiltrate the wound from the wound bed and migrate towards the upper wound area where they accumulate over time in the eschar. Comparatively, neutrophils in diabetic wounds in mice present a different spatiotemporal distribution. Infiltration of neutrophils is delayed and prolonged and most of the neutrophils remain in the wound tissue where they form NETs. Consistent with mice, human diabetic wounds show more neutrophils and NETs in the wound bed, whereas in acute wounds they are found in the upper wound layer in the eschar.

To understand what drives altered neutrophil distribution and function in diabetic wounds we next assess specific subtypes of neutrophils in the peripheral blood. As described in many studies, a specific subset of neutrophils, so-called low density neutrophils (LDN), are found in higher frequencies across numerous pathological disorders. These conditions are often associated with increased inflammation and enhanced NET formation, both typically present in chronic wounds. Our study aims to determine whether low density neutrophils (LDN) are elevated in diabetic conditions and if they contribute to altered neutrophil function in diabetic wound healing. We isolated and comparatively characterised high-density neutrophils (HDN) and LDN from whole blood samples. Our cohorts include healthy and diabetic donors. Latter will be further characterised based on their history of chronic wounds. Using a complex multicolor flow cytometry approach we analyse surface markers to differentiate LDNs from HDNs and to assess changes in neutrophil profiles under healthy and diabetic conditions. In addition, we included markers such as CXCR2 and CXCR4 to evaluate the maturation status of neutrophils.

We defined Neutrophils, both LDN and HDN, as CD11b positive, CD66b positive, and CD14 negative. In contrast to the HDN population, which displays homogeneous CD16 expression, we identified a distinct subset in the LDN population. This subpopulation can be distinguished based on CD16 expression into CD16-high and CD16-low subsets. Notably, the CD16-low subset exhibited higher CD11b and lower CD66b expression compared to the CD16-high subset. Further, CD16-low subsets show less

CXCR2 surface expression while CXCR4 are not upregulated. Interestingly, CXCR2 deficiency on blood neutrophils has been associated with compromised neutrophil tissue infiltration.
In future studies, we want to further differentiate neutrophil subpopulations and characterise their functions in wound healing in healthy and diabetic conditions. In particular, we want to decipher whether specific subpopulations contribute to the spatiotemporal distribution of neutrophils in the wound tissue and what drives their changes in diabetic wounds.

P137 | Characterisation of blood TIGIT+ CD8+ T cells in melanoma patients

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Tumour-reactive CD8+ T lymphocytes are the major effector cells in mediating tumour elimination. However, their features in peripheral blood of cancer patients remain largely undefined. In our preliminary study, we observed an increased expression of TIGIT, an inhibitory receptor, on blood CD8+ T cells from melanoma patients. Here, we perform phenotypic, epigenetic and functional profiling to characterise the tumour-reactive potential of blood TIGIT+ CD8+ T cells in human melanoma. Our multicolor flow cytometry analysis on 32 patient blood samples reveals the antigenexperienced features of TIGIT+ CD8+ T cells, that they are differentiated into the EMRA state (effector memory state re-expressing CD45RA, CD45RA+CCR7-) and can produce high levels of effector molecules, such as IFNγ, TNFα, and granzyme B. Furthermore, we investigated the link between blood TIGIT+ CD8+ T cells and antitumor immune response. Taking advantages of the autologous tumour/T cell patient model, we observed that blood TIGIT+ CD8+ T cells react stronger towards tumour than the other CD8+ subsets, producing more IFNγ/TNFα upon autologous tumour stimulation. Altogether, our data suggest that TIGIT marks circulating tumour-reactive CD8+ T cells and may play an important role in monitoring patient response upon immunotherapy.

P138 | Targeting ERK5 in epidermolysis bullosa acquisita: A novel approach to modulating neutrophil-mediated autoimmunity

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Epidermolysis bullosa acquisita (EBA) is an autoimmune disorder characterised by the formation of autoantibodies targeting type-VII collagen (Col7), resulting in immune-mediated subepidermal blistering at the dermo-epidermal junction. Current treatment strategies rely on immunosuppression, including systemic corticosteroids, mycophenolate mofetil, or CD20 depletion through rituximab, all of which carry the risk of severe adverse effects. Animal models of EBA, involving both immunisation and antibody-transfer, have been widely used to further understand the disease pathogenesis and to identify potential new therapeutic approaches. Our findings indicate that in EBA-affected skin, protein levels of Extracellular Signal-Regulated Kinase 5 (ERK5) are significantly elevated.
ERK5, also known as big MAP-Kinase 1 (BMK1), is a member of the Mitogenactivated protein kinases (MAPK) family, which are serine/threonine kinases involved in a range of cellular processes including proliferation, differentiation, migration, and responses to stress. Encoded by the MAPK7 gene, ERK5 is ubiquitously expressed in mammal tissues under physiological conditions and plays a critical role in embryonic development. It is situated downstream of MEKK2/3 and MEK5, and engaged by a variety of stimuli, e.g. cytokines, hormones, mitogens and stress-factors like shear stress and high osmolarity. ERK5 enhances transcription via phosphorylation of transcription factors and also acts as a transcription factor itself. The pathophysiological role of ERK5 in disease has recently been reviewed. ERK5 has primarily been investigated in cancer, such as haematological malignancies and melanoma. However, its role in autoimmune disorders has been underexplored. To investigate the role of ERK5 in autoimmunity, we utilised a prototypical autoimmune mediated disease. After characterising the impact on effector function of neutrophil granulocytes, we employed the localised autoantibody transfer-induced EBA mouse model for functional assessment. To confirm our results in another neutrophil dependent disease, serum-transfer arthritis (STA) was induced by injection of arthritic serum from K/BxN mice, whereby disease is driven by autoantibodies against glucose-6-phosphate isomerase (G6PI), modelling the effector phase of rheumatoid arthritis (RA). Additionally, we employed a model of passive immune thrombocytopenia (ITP), wherein platelets are tagged by the injected antibodies, leading to their recognition and phagocytosis by reticuloendothelial organs.
While neutrophil dependence is prominent in the EBA and STA models, it plays a lesser role in ITP.
Our study demonstrated increased ERK5 in the lesional skin of EBA mice. Functional analysis of neutrophils revealed a specific impairment in reactive oxygen species release, while other neutrophil functions remained unaffected. Inhibition of ERK5 significantly ameliorated disease severity in the antibody-transfer EBA model, whereas, based on preliminary data, it did not ameliorate STA or ITP. These findings suggest that ERK5 plays a critical role in EBA pathogenesis, particularly in modulating neutrophil effector functions, and highlight ERK5 as a potential therapeutic target for autoimmune blistering diseases like EBA. Further research is warranted to explore the broader implications of ERK5 inhibition in other autoimmune disorders.

P139 | Monoclonal 2G4 antibody fab fragments facilitate cryogenic electron microscopy in pemphigus research

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Pemphigus vulgaris (PV) is an autoimmune blistering skin disease in which IgG autoantibodies, predominantly targeted against Desmoglein (Dsg)-3, disrupt intercellular adhesion, ultimately leading to acantholysis and suprabasal bullae.
Our current research focuses on the characterisation of Fab fragments derived from established monoclonal antibodies (mAbs) such as 2G4 and AK23. This offers advantages in structural analysis, and also reduces non-specific binding, which is particularly important in microscopy. As the hinge region of mAb IgG antibodies is very flexible and therefore difficult to crystallise reproducibly, Fab fragments of the mAb (mAbFab) were established. Verification of Fab-functionality was initially achieved via immunohistochemistry in different tissues such as human and murine skin and monkey oesophagus. Furthermore, ELISA and monolayer dissociation assay were used to verify similar characteristics compared to the respective full antibody, while human control-IgG and its respective Fab fragments remained negative. Currently, a combination of cell culture, PV skin samples, PV sera and mAbFab fragments is applied to analyse the pathogenic antibody-induced morphological changes. Here, we explore the potential use of pathogenic mAbFab fragments for understanding pemphigus vulgaris and demonstrating the versatility of these fragments in different research approaches. Pending research explore, the potential of mAbFab fragments for state-of-the-art cryo-electron microscopy to visualise spatial antibody–antigen binding and subsequent intracellular effects at the molecular level in a near-native state.

P140 | The influence of prednisolone treatment on split formation in the human skin organ culture model for pemphigus vulgaris

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Pemphigus vulgaris (PV) is an organ-specific autoimmune blistering disease of the skin. It is characterised and caused by a pathological formation of circulating autoantibodies against the desmoglein (Dsg) 3 and/or Dsg1. Binding of anti- Dsg1/3-autoantibodies disrupts cell–cell adhesions between keratinocytes. Current therapeutic options target general immunosuppression and the first treatment option is systemic corticosteroids such as prednisolone. As the pathomechanism is not fully understood yet, there are no targeted treatment options available.
In this project, we used the human skin organ culture (HSOC) model for PV to investigate the effect of prednisolone treatment on split formation. In the HSOC model, split formation is induced using the single chain variable fragment (scFv) PX43, which targets Dsg1 and Dsg3. The split formation is quantified using haematoxylin and eosin staining. Additionally, we use immunofluorescence stainings to ensure quality control for the binding of the scFv and expression of Dsgs.
We looked at the effect of prednisolone treatment using 4 h preincubation with prednisolone in different concentrations. In these experiments, no difference in split formation could be observed. In addition, a 24 h preincubation with prednisolone before injecting PX43 was tested. Using this approach, there is a reduction in split formation ($p = 0.015$).
So far, the effect of prednisolone treatment in the HSOC model for PV can only be observed using a preincubation of 24 h. The next step in this project is looking at the transcriptome data to further investigate the differences between samples treated with 24 h preincubation and samples without prednisolone treatment. We hope to further study these differences to gain a better understanding of the mechanism of treatment using prednisolone in PV.

P141 (OP03/02) | Metformin attenuates IFN-gamma-mediated macrophage activation in necrobiotic xanthogranuloma

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Necrobiotic xanthogranuloma (NXG) represents a rare, non-infectious, granulomatous skin condition commonly arising in lymphoproliferative disorders. The pathophysiology of this aberrant macrophage infiltration remains poorly defined. A clinical case of NXG in the setting of paraproteinemia and diabetes mellitus type II (DM) provided us with an opportunity to investigate immunopathology in this entity. Our patient was treated with metformin for the underlying DM, resulting in complete clinical remission of NXG lesions. We obtained lesional and non-lesinal skin before and after 6 months of metformin treatment. Immunhistochemistry showed dense histiocytic, CD68-positive infiltrates in NXG lesions, which were significantly reduced by metformin therapy. Given that IFN-gamma-driven macrophage activation constitutes a hallmark in other granulomatous skin diseases, we hypothesized that an IFN-gamma signature is present in NXG. Thus, we performed bulk RNAseq and qPCR on

the skin samples demonstrating upregulation of IFN-gamma-induced genes, including CXCL9, CXCL10, CYP27B1, SLAMF7, when compared to nonlesional skin and resolved lesions of the patient. A second case of NXG in the context of chronic lymphocytic leukaemia allowed us to confirm upregulation of IFNgamma- induced genes in lesional vs. non-lesional skin. The findings from the first clinical case prompted us to investigate the effects of metformin on macrophages in more detail using in vitro. Metformin downregulated IFN-gamma-induced CXCL 10 protein in human, primary monocyte-derived macrophages (MDM) as shown by flow cytometry. Reduced CXCL10 was seen when using supra-pharmacological metformin concentrations, commonly used experimentally, but also in doses reflecting pharmacological concentrations found in the portal vein and peripheral blood of metformin-treated patients. Proteomics analyses of MDM confirmed suppression by metformin of IFN-gamma-induced targets, including proteins that we found downregulated on mRNA level in our patient under metformin treatment, including CXCL9, CXCL10, CYP27B1, SLAMF7. In sum, our data indicate that metformin inhibits IFN-gamma-activation of macrophages, providing a potential adjuvant therapy in NXG.

P142 | 3D skin equivalents displaying the interplay between Th2-polarised T cells and *Staphylococcus aureus* growth

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Atopic dermatitis (AD) is a frequent inflammatory skin disease marked by skin barrier dysfunction, a skewed Th2 immune response and a microbial dysbiosis with increased abundance of Staphylococcus (S.) aureus. Dupilumab, an anti- IL-4RA monoclonal antibody, improves the clinical phenotype and further reduces *S. aureus* skin abundance and colonisation. Whether and how the microbial dysbiosis contributes to impaired skin barrier and altered immune response or vice versa remains unclear. The causality in AD remains unexplored due to a lack of multi-level functional models.

Here, we introduce a novel in vitro 3D skin model to study the interplay between T cells and cutaneous mibrobiome. We incorporated in vitro-polarised Th2 cells into 3D skin models with simultaneous *S. aureus* colonisation and analysed the effect on skin barrier integrity, inflammatory environment, *S. aureus* load and explored the influence of dupilumab on *S. aureus* growth.

Stimulation of 3D skin equivalents with Th2 polarised T cells promoted increased *S. aureus* growth, which could be reversed by dupilumab treatment. Furthermore, 3D skin equivalents exhibited heightened cytokine secretion to *S. aureus* inoculation when Th2 polarised CD4+ T cells were incorporated. Stimulation of 3D skin equivalents with IL-4 and IL-13 did not promote *S. aureus* growth but, nevertheless, induced AD-like changes in epidermal organisation.

In conclusion, only complex 3D skin models incorporating Th2-polarised cells rather than cytokine stimulated models adequately capture physiological *S. aureus* growth in vitro, providing a robust platform for in vitro AD research.

P143 | SERPINB3 as a novel autoantigen driving a Th2-skewed immune response in psoriasis with eczematous features

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Psoriasis is a chronic inflammatory skin disorder affecting 2–3% of the global population, involving complex interactions between adaptive and innate immunity. While therapies targeting IL-17A highlight the importance of T cells, the specific antigens they recognise remain unclear. Recent studies have identified SERPINB3/B4 as putative autoantigens in a subset of psoriasis patients exhibiting eczematous features (EczPso), suggesting a distinct immunopathological mechanism in this disease subtype. Unlike the typical Th17-dominated immune response in plaquetype psoriasis, EczPso appears to involve a mixed Th17/Th2-driven immune profile. Based on these findings, we hypothesize that SERPINB3 serves as a novel autoantigen driving a Th17/Th2 immune response in EczPso, distinguishing it from the Th17-dominant responses seen in plaque-type psoriasis. Moreover, this antigenspecific response may be additionally modulated by the function of SERPINB3, leading to the unique immunological profile of EczPso.

To explore the role of SERPINB3 in psoriasis pathogenesis, we conducted in vivo studies in wild-type C57BL/6 mice using a well-established mouse model of psoriasis using the TLR7/8-agonist imiquimod creme (IMQ). We found that injection of recombinant murine SERPINB3 into the ears resulted in a significant increase in ear thickness both with and without IMQ, compared to the control group (Mouse Serum Albumin [MSA]). T-cell infiltration was also notably higher in the SERPINB3 group, particularly when combined with IMQ. Immune profiling revealed that application of IMQ induced a shift towards a Th17 response in all groups, confirming its role in driving psoriasis-like inflammation. Meanwhile, SERPINB3 induced a Th2-skewed response, as indicated by increased IL-4 and IL-13 production. These findings highlight the distinct immune pathways activated by SERPINB3, with a clear Th2 bias in the absence of IMQ, and a mixed Th2/Th17 response when combined with IMQ, suggesting a nuanced role of SERPINB3 in psoriasis with eczematous features.

Additionally, proliferation assays performed on ex vivo T cells from the draining lymph nodes revealed that T cells from mice injected with SERPINB3 proliferated significantly more upon co-culturing with SERPINB3-pulsed dendritic cells. This confirms the development of SERPINB3-specific T cells, supporting its role as an autoantigen.

A FACS-based activation-induced marker (AIM) assay further identified lowfrequency SERPINB3-specific autoreactive T cells in the lymph nodes and spleens of untreated wild-type C57BL/6

mice. This independent approach underlies the presence of autoreactive T cells, reinforcing the role of SERPINB3 as a novel autoantigen in psoriasis.
In summary, our findings suggest that SERPINB3 elicits a distinct, mixed Th17/Th2- immune response in psoriasis, which contrasts with the Th17-dominated responses typical of plaque-type psoriasis. SERPINB3 appears to function as an autoantigen in psoriasis with eczematous features, driving a potentially more autoimmune response. Further studies are necessary to elucidate the mechanisms by which SERPINB3 modulates immune activation and to explore its potential as a therapeutic target.

P144 (OP05/03) | Single cell mapping identifies a distinct platelet-phenotype in psoriatic type III inflammation

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Background: The understanding of platelet biology is expanding beyond their wellestablished role in thrombosis and haemostasis to encompass their functions in inflammation.
Objective: To gain insights into the phenotype and possible platelet-subgroups in the context of psoriatic type III inflammation.
Methods: High-parametric single cell spectral cytometry was applied to a prospectively followed cohort of psoriasis patients undergoing systemic therapy ($n=27$), in comparison to atopic dermatitis (AD) patients ($n=4$), and healthy controls ($n=10$). Platelets were further investigated by bulk proteome analysis, and findings validated in a second cohort (psoriasis $n=6$, AD $n=10$, controls $n=6$).
Results: By focusing on the activation profile of platelets and their interaction with immune cells, we identified cell-surface proteins CD32+CD154+ and TLR2+TLR4+, distinguishing unique platelet subsets in psoriasis patients. These subsets demonstrated regression over the course of systemic therapy. Notably, these platelet phenotypes and frequencies differed from those of healthy controls, and patients with atopic dermatitis, a type II inflammation-driven disease.
Conclusion: Our data highlight a specific platelet phenotype responding acutely to the respective inflammatory context. This finding warrants further investigation into platelets as a diagnostic tool in inflammatory conditions, necessitating future insight into the function of these subsets across disease etiologies.

P145 | VSNL1 is a multifaceted driver of psoriatic skin inflammation regulating keratinocyte proliferation, differentiation and cytokine release

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Psoriasis is a chronic inflammatory skin disease characterised by rapid epidermal turnover, resulting in thick, scaly patches that cause significant discomfort and affect patients' quality of life. Current treatments target key cytokines of the adaptive immune system such as IL-17A, IL-17F, IL-23 and TNF-α. However, when patients experience incomplete response or relapses, novel therapeutic targets are needed. We previously demonstrated that VSNL1 (Visinin Like 1), primarily known as a neurological factor, also contributes to skin inflammation. Here, we provide further insight into the role of VSNL1 in psoriatic skin inflammation by focusing on its expression in skin biopsies from patients with psoriasis and lichen planus and examining its function in primary human keratinocytes in vitro. Gene expression data derived from bulk, single cell and spatial RNA sequencing, along with immunohistochemical staining of skin biopsies, showed that VSNL1 is predominantly expressed in keratinocytes and is significantly upregulated in skin lesions, with the highest expression observed in psoriasis. In vitro, gene set enrichment analysis of bulk RNA sequencing data comparing VSNL1 knock-out (KO) to wildtype (WT) keratinocytes revealed a suppression of disease-relevant pathways, including proliferation, differentiation, and inflammation. Subsequent validation demonstrated a reduced proliferative capacity of VSNL1 KO keratinocytes in scratch assays and less pronounced IL-22-mediated acanthosis in reconstructed human epidermis VSNL1 KO models. In addition, bulk RNA sequencing showed a significant downregulation of key neutrophil recruitment markers, i.a. CXCL5 and CXCL8. A chemotaxis assay confirmed that neutrophil migration towards the supernatant produced by VSNL1 KO keratinocytes was significantly reduced compared to their WT counterpart. Consistent with the in vitro data, a positive correlation between VSNL1 expression and clinical attributes of psoriasis (e.g., acanthosis, hyperkeratosis and presence of neutrophils) was observed in patients. Overall, we have shown that VSNL1 is highly expressed in the epidermis of inflamed skin and is a multifunctional regulator of keratinocyte proliferation, differentiation and the release of cytokines. Targeting VSNL1 therefore offers promising potential for addressing key features of psoriatic skin inflammation.

P146 | Immunogenic cell death (ICD) and ICD-dependent dendritic cell activation triggered by extracorporeal photopheresis in CTCL

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Immunogenic cell death (ICD) has emerged as a crucial mechanism in cancer immunotherapy, characterised by the release of damage-associated molecular patterns (DAMPs) that promote dendritic cell (DC) maturation and cytotoxic T lymphocyte (CTL) responses. Extracorporeal photopheresis (ECP) is a chemophototherapy used for cutaneous T-cell lymphoma (CTCL) treatment, involving leukapheresis, 8-methoxypsoralen (8-MOP) administration, and UVA irradiation of leukocytes. This study investigates whether ECP induces ICD in CTCL patients and healthy donors and its ability to induce DC activation.
Using an in vitro ECP model on healthy peripheral blood mononuclear cells (PBMCs) and ECP-treated white blood cells (WBCs) from CTCL patients, we observed significant markers of ICD, including ATP release, HMGB1 secretion, and surface calreticulin (CALR) exposure. Our results demonstrated reduced cell viability and increased cell death in ECP-treated samples. Gene expression analysis showed significant upregulation of ICD-related genes, confirming ICD induction.
In CTCL patients, elevated CALR expression was notably higher in malignant T cells (CD26-), suggesting greater susceptibility to ICD. We further demonstrated that ECPtreated CD4+ T cells were phagocytosed by DCs, and this process was dependent on ICD signals, as blocking CALR and ATP halted phagocytosis.
Our findings reveal that ECP induces ICD in both healthy and malignant T cells, facilitating DC activation and antigen presentation. These results underscore ECP's potential in enhancing targeted immune response to malignant T cells in CTCL, offering new insights into its therapeutic mechanisms and applications in cancer immunotherapy.

P147 | Aryl hydrocarbon receptor-signalling alters metabolic functions in eosinophils, a key player in inflammatory skin diseases

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The aryl hydrocarbon receptor (AhR) plays a role in inflammatory skin diseases and is expressed in keratinocytes, lymphocytes, and myeloid-derived cells. The AhR agonist tapinarof is a novel therapeutic agent used in plaque psoriasis and atopic dermatitis. Tapinarof modulates immune cell responses and proinflammatory cytokines, however, the role of AhR signalling on granulocyte actions remains elusive. We therefore investigated the impact of tapinarof on metabolic and effector functions in bone-marrow derived eosinophils and neutrophils. Tapinarof decreased reactive oxygen species (ROS) secretion in Ca2+ ionophore (Ca-I) stimulated eosinophils and immune-complex stimulated neutrophils. Tapinarof also shifted cell metabolism and reduced adenosine triphosphate (ATP) levels. More precisely, it decreased oxidative phosphorylation and glycolysis of eosinophils resulting in impaired response to Ca-I stimulation. Ca-I-induced secretion of the eosinophil-associated chemoattractants macrophage inflammatory proteins (MIP-1alpha and MIP-1beta) were reduced by AhR-signalling. While eosinophil chemotaxis towards MIP-1alpha/beta was not affected, neutrophils chemotaxis towards MIP-1alpha/beta and complement component C5a was significantly decreased after tapinarof stimulation. Overall, our results demonstrate that tapinarof alters neutrophil and eosinophil metabolism and effector functions. These activities may contribute to its therapeutic effects in psoriasis and atopic dermatitis. In addition, tapinarof might be effective in the treatment of other granulocyte-driven inflammatory diseases.

P148 (OP03/03) | OX40 expression limits suppressive capacity of FOXP3+ T regulatory cells in pemphigus

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Pemphigus is a severe autoimmune disorder characterised by a breakdown in immunological tolerance, driven by autoreactive T and B cells against desmogleins (Dsg) 1 and 3. Patients develop skin and mucosal blistering. T regulatory (Treg) cells, crucial for maintaining immune tolerance, are often impaired

in autoimmune conditions. In this study, we characterised Treg subsets in pemphigus, identifying a FOXP3+ Treg subset, which also expresses characteristic markers known from autoreactive T cells. This subset showed only minimal IL-10 expression and a diminished capacity to suppress the proliferation of effector T cells. Notably, this FOXP3+ Treg subset with impaired suppressive function expressed high levels of OX40, an immune checkpoint protein. Blockade of OX40 restored the suppressive capacity and IL-10 production in this FOXP3+ Treg population. By studying patients with pemphigus receiving B cell depleting therapies, we observed an increase of this non-suppressive FOXP3+ Treg population during clinical relapses. RNA sequencing of these Treg cells revealed a gene expression profile with some specific findings, including elevated levels of certain chemokines involved in lymphocytic recruitment, chemotaxis and inflammatory responses. Furthermore, this Treg subpopulation also exhibited enhanced migratory capacity to the skin, as indicated by increased expression of the cutaneous lymphoid antigen (CLA). We could detect OX40+ Treg cells in lesional skin of pemphigus patients, suggesting that these non-suppressive Treg cells contribute to disease pathology. Our findings highlight the functional diversity of Treg cells in pemphigus, particularly the role of the non-suppressive OX40+ FOXP3+ Treg cells, which present potential new therapeutic targets for restoring immune tolerance in pemphigus.

P149 | Insomnia increases the risk for specific autoimmune diseases: A large-scale retrospective cohort study

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The rise of autoimmune diseases presents a growing global medical challenge. Treatment options for most autoimmune diseases remain inadequate, leading to high morbidity risks and significant healthcare costs, while the underlying mechanisms of disease development are not fully understood. Both genetic factors and lifestyle choices influence the occurrence and progression of these diseases, with sleep being a critical lifestyle factor. Impaired sleep, resulting from insomnia or circadian rhythm sleep disorders, substantially impacts the immune system. To identify the impact of impaired sleep on the risk of developing autoimmune diseases, a global population-based retrospective cohort study was conducted using electronic health records from the TriNetX US Global Collaborative Network to examine the impact of impaired sleep on 20 different autoimmune diseases. The study employed propensity score matching to mitigate bias. The studies' robustness was challenged in three sensitivity analyses. Consistent across all analyses, significantly increased risks were identified for x of the 20 autoimmune diseases. Specifically, cutaneous lupus erythematosus (hazard ratio (HR) = 2119; confidence interval 1674, 2682; $p < 00001$), rheumatoid arthritis (HR = 1404; confidence interval 1313, 1501; $p < 00001$), Sjögren syndrome (HR = 184; confidence interval 164, 2066; $p < 00001$), and autoimmune thyroiditis (HR = 1348; confidence interval 1246, 1458; $p < 00001$). None of the tested diseases showed significantly reduced risks and only 4 out of the 20 tested diseases had never significantly increased HRs at any of the analyses. Our study highlights the crucial importance of recognising and treating sleep disorders as a strategy to prevent the development of autoimmune diseases in the long term.

P150 | In depth characterisation of the lupus pre-disease phase in mice

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Purpose: The complex pre-disease phase of systemic lupus erythematosus (SLE) is largely unknown. However, it is essential to understand how the immune system changes during this time to prevent the outbreak of the disease and slow down its progression.
Methods: Lupus prone NZM2410 mice, B6.Sle1Sle2Sle3 (B6.Sle123), and C57BL/6J mice were weekly sacrificed at the age of 8 to 20 weeks. Organs were harvested and blood, spleen, and lymph nodes were investigated using flow cytometry to identify T cell subpopulations. Additionally, hemograms were analysed. Moreover, body weight and spleen weight were assessed, as well as movement. As glomerulonephritis is a hallmark of SLE in humans, kidneys were stained with Periodic acid-Schiff.
Results: Lupus-prone mice moved less, weighed more, and showed changes in several blood parameters between the ages of 16 and 20 weeks compared to C57BL/6J control mice. Particularly, the number of platelets was higher in lupus-prone mice. Monocytes and granulocytes were rising, starting at the age of 16 weeks, preceding the occurrence of lupus related symptoms. Regulatory T cells briefly increased at the age of 17–19 weeks, before they dropped again. Moreover, kidneys at the age of 18–20 weeks showed structural changes in the glomeruli.
Conclusion: The lupus pre-disease phase is complex and immunologic changes are measurable even before the onset of classical known symptoms such as nephritis. Our findings suggest that identifying the pre-disease phase requires multiple parameters, but employing a combination of them could enable earlier diagnosis and future treatment.

P151 | Potential explanation for severity, atopy and anxiety in generalised pustular psoriasis (GPP) deciphered by transcriptome analysis

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Generalised pustular psoriasis (GPP), palmoplantar pustular psoriasis (PPP) and Acrodermatitis continua suppurativa of Hallopeau (ACH) constitute 3 variants of pustular psoriasis. For the treatment of acute flares in GPP the Interleukin-36 receptor antagonist (IL-36RA) Spesolimab is established, which however is only partially effective or even fails in PPP, indicating that the underlying pathomechanisms differ. Although IL-17, IL-23 and IL-36 have been demonstrated to contribute centrally in both entities detailed molecular analysis is of need.

Our objective was to further decode immunological pathways in GPP as the maximal variant of pustular psoriasis in comparison to localised PPP.

Focusing on transcriptomic differences in GPP versus PPP, bulk RNA sequencing of affected and healthy skin of 3 GPP and 3 PPP patients was performed, and results between entities compared. Principal component analysis revealed fundamental differences in differentially expressed genes, both between GPP and PPP as well as healthy controls. Analysis of heatmaps confirmed the expected up-regulation of IL-17, IL-23 and IL-36 pathways, which was more pronounced in GPP. Interestingly, in GPP patients an unexpected marked atopic milieu with an upregulated Th2 profile was detected: among others the receptor for IL-4, the "itching protein" MRGPRX3 and immunoregulatory IL-19 were profoundly overexpressed. Regarding personalised medicine concepts, these findings may highlight that the inhibition of IL-36 could not be sufficient as a monotherapy in GPP. Addressing PPP patients, the‚typical' immune signatures, although differentially regulated, struck with a notable heterogeneity with atopic markers being less overregulated than in GPP biopsies. Further results closely correlated to clinical features of GPP: Severity of symptoms was related to up-regulated SELL and SERPIN B4. SELL (L-selectin) is a homing factor promoting the invasion of neutrophils into tissues. SERPIN B4 is an innate driver of inflammation. Importantly, overexpression of KYNU (kynurenine) and TDO2 may explain psychological findings like anxiety and depression during an acute flare of GPP. Both are key molecules in tryptophan metabolism and lead to a decrease in serotonin and melatonin production.

Studies on more patients and meticulous transcriptome analysis possibly supported by artificial intelligence will help to increase our knowledge on variants of pustular psoriasis—most fascinating prototype diseases at the border of innate and acquired immunity.

P152 | Chitin sensing promotes the inflammatory activation of human keratinocytes

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Chitin, a large polysaccharide, is an integral component of fungal cell walls, including the human pathogenic species *Cryptococcus neoformans*, *Candida albicans* or Trichophyton rubrum. Previous studies suggest that chitin is a pathogenassociated molecular pattern that promotes a proinflammatory response of the innate immune system. Human keratinocytes express the putative chitin receptor toll-like receptor 2 (TLR2). In addition, infiltrating immune cells or inflamed keratinocytes secrete mammalian chitinases. Chitinase 1 (CHIT1) degrades chitin into small oligosaccharides that may alter TLR2 recognition. However, chitin recognition and degradation in human skin and its potential contribution to skin inflammation are largely unknown.

In the present study, we challenged three-dimensional reconstructed human epidermis with *Candida albicans*. Fungal invasion of the tissue was time-dependent and associated with tissue destruction at later time points (48 h), as determined by two-photon microscopy and histology of tissue sections. At earlier time points (6 h), prior to tissue destruction, we measured an upregulation of the proinflammatory cytokines interleukin-8 (IL-8) and IL-6. At the same time, the expression of TLR2 was also increased. To better understand the molecular basis of chitin recognition and the potential contribution of CHIT1, we used a TLR2 reporter cell line stimulated with purified chitin or *C. albicans* in the presence or absence of CHIT1. We found that TLR2-dependent recognition of purified chitin and *C. albicans* was significantly increased in the presence of CHIT1.

Our data suggest a complex interplay between chitin recognition and chitin degradation in response to fungal skin infections. In addition to TLR2, inflamed keratinocytes also express the putative chitin receptors lysin motif domain containing 3 (LYSMD3) and fibrinogen C domain containing 1 (FIBCD1) and the chitinaselike protein chitinase-3-like 1. Further research will be conducted to understand the molecular interplay of chitin-related proteins and the pathophysiological relevance of chitin sensing during fungal infection and skin inflammation.

P153 | Optimising T helper cell subset analysis from skin biopsies by flow cytometry

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Flow cytometry is a widely used technique to analyse circulating lymphocytes and investigate systemic immune dysregulation observed in autoimmune diseases. Previously, we developed flow cytometry panels to detect circulating CD4+ T helper (Th) cell subsets in skin-targeting diseases. Although the detection of T cells in the periphery is important, the analysis of skin-resident T cells derived from skin biopsies, permits to gain additional insights into tissue-specific immune responses. We initially used already published protocols for cell isolation from skin using DNase I and Collagenase IV for tissue dissociation. For analysis, we implemented a well-established flow cytometry panel to characterise Th cell subsets, focusing on chemokine receptor expression. However, it is known that enzymatic treatment leads to chemokine receptor degradation and limits thorough characterisation of T-cell subtypes. To address this limitation, we transitioned the gating strategy to intracellular transcription factors. Specifically, we stained for T-bet, GATA3, RORγt, and FOXP3 to reliably identify Th1, Th2, Th17 and regulatory T cells (Treg cells), respectively. Although this adjustment improved our ability to distinguish between CD4+ T cell subsets, we faced additional challenges with cell recovery. Unlike blood samples, skin biopsies yielded fewer lymphocytes due to the small sample size (4 mm) and the lower abundance of lymphocytes in peripheral tissues. To overcome this, we introduced a CD4+ T cell pre-enrichment step using magnetic-activated cell sorting (MACS), which significantly improved cell yield for subsequent analysis. By adapting the protocol to prioritise transcription factor identification and incorporating CD4+ T cell pre-enrichment, we successfully optimised both the isolation and characterisation of T cells from skin biopsies. This optimised protocol provides more reliable insights into tissue-resident immune responses and offers a strong foundation for future refinements.

P154 (OP01/05) | TYK2 inhibition improves clinical hallmarks and molecular biomarkers in various subtypes of cutaneous lupus erythematosus

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Background: Cutaneous lupus erythematosus (CLE) is a chronic inflammatory skin disease (ISD) with various clinical subtypes. Most of these share the histological hallmark of an interface dermatitis (ID); characterised by a dense lichenoid T cell infiltrate causing cell death of basal keratinocytes. Type I interferons signalling via tyrosinkinase 2 (TYK2) play a central role in the pathogenesis of CLE. So far, no targeted therapy has been approved for the treatment of CLE.
Objective: The objective of this study was to investigate the potential therapeutic benefit of TYK2 inhibition in CLE patients.
Methods: First, TYK2 gene and protein expression were investigated using bulk RNA sequencing and immunohistochemistry in 4 clinical subtypes of CLE (acute, subacute, chronic discoid CLE, lupus profundus). Second, we investigated the pathophysiological role of TYK2 signalling in T cells isolated from CLE skin biopsies as well as in primary human keratinocytes and reconstructed human epidermis (RHE) using the TYK2 inhibitor deucravactinib. Keratinocytes and RHE were stimulated with either IFN-α or type 1 T cell supernatant (TCS). Analyses were conducted using RT-PCR, bulk RNA sequencing and ELISA. Third, the potential of TYK2 inhibition as a therapeutic option was evaluated ex vivo in skin biopsies of CLE patients and in four therapy-resistant CLE patient cases treated with deucravacitinib.
Results: First, TYK2 and CXCL9/CXCL10 gene expression correlated positively with ID in patients with inflammatory skin disease, and immunohistochemistry revealed a high abundance of TYK2 protein in different CLE subtypes. Second, inhibition of TYK2 significantly reduced pro-inflammatory genes (e.g., type 1 chemo-attractants and interferon response genes) in keratinocytes stimulated with IFN-α. Further, lesional T cells activated by anti-CD3/anti-CD28 showed a markedly decreased capacity to release IFN-γ after treatment with deucravacitinib in vitro. In line, RHE stimulated with TCS exhibited impairment of the epidermal barrier, which was sufficiently normalised upon TYK2 inhibition. Third, ex vivo TYK2 inhibition in CLE biopsies reduced IFN-response genes, type 1 attracting chemokines (CXCL9, CXCL10) and necroptosis-related genes. Finally, four patients with different therapyresistant CLE subtypes (acute, subacute, chilblain- chronic discoid) showed rapid and sustained clinical improvement after initiation of deucravacitinib (6 mg once daily for 12 weeks). Mean PGA decreased from 3.75 at baseline to 1.325 at week 12 and mean DLQI decreased from 17.75 to 6.5 at week 12.
Conclusion: TYK2 inhibition improves molecular hallmarks CLE in keratinocytes and T cells and represents a promising and effective therapeutic target for different clinical subtypes of CLE.

P155 | Immunological characteristics in morphea and lichen sclerosus

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Morphea (localised scleroderma) and lichen sclerosus et atrophicans are two clinically significant yet insufficiently understood dermatological disorders with some morphological similarities. The current paucity of knowledge regarding their immunopathogenesis is reflected in the limited therapeutic interventions available, posing substantial challenges for affected individuals, who may experience severe sequelae such as organ dysfunction and profound psychological distress. Presently, the standard treatment regimen primarily relies on the prolonged administration of potent topical corticosteroids, typically in conjunction with ultraviolet-A (UV-A) phototherapy modalities. Based on the current literature, both type 1 (interferon-γ, IFN-γ) and

type 2 (interleukin 4, IL-4) inflammatory responses are present in these conditions, suggesting the involvement of multiple cytokines in this T-cell-driven inflammation, which results in fibrotic progression. The aim of the current study is to evaluate the immunological characteristics of cytokines and T cells in patients with morphea and lichen sclerosus. We collected peripheral blood mononuclear cells (PBMC) and skin specimen (4 mm punch biopsies) from morphea and lichen sclerosus patients. So far, a total of 19 morphea patients (16 female, 3 male) with the mean age of 60.10 13.4 (range 21–79) and two healthy control individuals (1 female, 1 male, 28 and 37 years) have been enrolled. PBMC were isolated and stained with monoclonal antibodies (anti-CD3, CD45, CD4, CXCR5, CXCR3, CCR6, CD25, CD127, CLA) and analysed by flow cytometry. Further, circulating T cells were analysed for their intracellular cytokine production (IFN-γ, IL-4, IL-10, IL-17). In addition, skin was analysed by immunohistochemistry (CD3, CD4, CD8, CD20, pSTAT1, pSTAT2, pSTAT3, pSTAT4, pSTAT5, pSTAT6) and by real time polymerase chain reaction total RNA (IFNG, TGFB, IL4, IL10, IL12A, IL13, IL17, IL19, IL21, IL22, IL24, IL31, MMP). The data are currently being analysed. Our study will help to improve our understanding of both diseases and help to identify new druggable targets.

P156 | The leukotriene B4 receptor BLT1 on neutrophils is essential for mucocutaneous lesions in a mouse model of anti-laminin-332 mucous membrane pemphigoid

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Mucous Membrane Pemphigoid (MMP) is a mucocutaneous autoimmune blistering disease affecting mucous membranes with inflammatory blisters and scarring, and the skin with inflammatory blisters. MMP is characterised by the presence of the autoantibodies in the dermal epidermal junction, and leads to the infiltration of inflammatory cells, particularly neutrophils, into the dermis of lesional skin. Neutrophils are an integral part of the innate immune system and are first responders at a sight of inflammation. This is due to the expression of different chemoattractant receptors and therby their quick reponse to chemoattractants. LTB4 is a potent chemoattractant for neutrophils and a product of the action of 5-lipoxygenase (5- LO), and is detected by BLT1. Though the LTB4-BLT1 axis plays an essential role on neutrophil effector functions, the significance of LTB4/BLT1 for the pathogenesis of MMP has not been addressed. Therefore, we examined the contribution of the LTB4- BLT1 axis to disease in the anti-laminin 332 IgG antibody transfer mouse model of MMP.

To provide preclinical proof for the role of the LTB4-BLT1 axis and thereby neutrophil infiltration into the dermis during MMP, WT controls were compared to mice deficient in 5-lipoxygenase (Alox5−/−), the key enzyme in the biosynthesis of leukotrienes, or deficient in the leukotriene B4 (LTB4) receptor BLT1 (Ltb4r1−/−). While WT mice displayed clinical signs of the disease, Alox5−/− and Ltb4r1−/− mice were protected and displayed a significant reduction in cutaneous and mucosal lesions. To elucidate the role of BLT1 in more detail, we generated a neutrophil-specific Ltb4r1−/− mice. Absence of Ltb4r1, specifically on neutrophils, significantly ameliorated disease scores indicating that BLT1 is required on neutrophils and the LTB4/BLT1 axis is indispensable for neutrophil recruitment and infiltration into the skin and mucosa. The specific role of BLT1 could be confirmed by the use of BLT1 inhibitor CP-105606. Mice treated daily with CP-105606 displayed significantly ameliorated MMP disease scores. The effect of the BLT1 inhibitor showed a clear dose response, with higher doses (10 mg/kg) of the drug providing significantly more protection than the lower doses (1 mg/kg). Taken together, our results provide evidence that the LTB4/BLT1 axis and neutrophils are key drivers of inflammation in MMP, and serve as promising therapeutic targets.

P157 | Enhanced frequency of EMP1 LMNA/cDC in DC2 and monocytes derived DC3 in lupus patients

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Dendritic cells (DCs) are pivotal players in the immune system, responsible for pathogen sensing, phagocytosis, and the crucial task of presenting antigens to T cells. Recent genetic analyses have unveiled a variety of DC subtypes, revealing a complex and intricate network. Among these subtypes are the conventional DCs (cDC), which are further divided into cDC1 and cDC2. cDC1 specialises in crosspresentation of antigens to CD8+ T cells, while cDC2 primarily activates CD4+ T cells, thus playing a role in the immune response to extracellular pathogens. In contrast, plasmacytoid DCs (pDCs) are capable of producing substantial quantities of interferon (IFN), rendering them a crucial component in antiviral immune responses. A further subgroup is DC type 3 (DC3), which originates from monocyte-derived precursor cells and specialises in polarising CD8+ T cells into tissue-resident memory T cells.

Recently, we identified a novel DC2 subtype characterised by EMP1+ and LMNA+ expression in chronically type I IFN-exposed PBMC of patients with TREX1 deficient familial chilblain lupus erythematosus (FCL). To ascertain whether these cells are also present in multifactorial lupus patients and to study their pathogenic role, we here investigated the DC subtypes and monocytes in the blood and skin of a cohort of lupus erythematosus (LE) patients with systemic and cutaneous manifestations. For the blood analysis, we utilised a complex panel of 21 fluorescently labelled antibodies and conducted advanced flow cytometry on the Cytek Aurora CS device. The patient cohort included individuals with either systemic or cutaneous manifestations of LE. Skin biopsies were examined using a combinatorially staining technique employing six different fluorescently labelled antibodies with analysis using a custom-tailored algorithm for image analysis.

The results of the Flow cytometry analysis revealed that the proportion of EMP1 + LMNA+ cells among DC2 was elevated

in blood of multifactorial LE patients compared to healthy controls. This finding corroborates the data obtained in TREX1 deficient FCL patients. Interestingly, the newly described EMP1+LMNA+subtype was not only present within the DC2 subpopulation but also across various DC3 subclasses in the PBMCs of LE patients. Immunofluorescence analysis demonstrated that EMP1+ LMNA+ DCs were also identified in skin biopsies from LE patients, suggesting a potential involvement of these cells in cutaneous manifestations.

While the overall number of DC2 and monocytes in the PBMCs of LE patients was comparable to that of healthy controls, we observed an increase in the CD163+ CD14- DC3 subclass and a significant reduction in the CD163-CD14- DC3 subclass. The impact of type I IFN on PBMC was demonstrated by an elevated prevalence of CD169 among monocytes and DCs in LE patients. In conclusion, the newly described EMP1+LMNA+ subclass was successfully identified in blood and skin samples from LE patients, indicating a potential role for this IFN-primed population in disease pathogenesis. The markers were not exclusive to DC2, but were also expressed on monocyte-derived DC3, which were more prevalent in LE patients. The data presented herein demonstrate a specific impact of the IFN-rich environment on the evolution of DC and monocyte subsets in LE.

P158 | Inhibition of the JAK/STAT pathway prevents acantholysis in pemphigus

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Background: Pemphigus is a severe autoimmune blistering disorder of skin and mucosa which is elicited by the formation of IgG autoantibodies targeting desmosomal components such as desmoglein 3 (Dsg3). Eventually, keratinocytes lose cytoarchitectural stability and reciprocal adhesion in a process called acantholysis, which manifests as blisters and erosions. It is widely accepted that keratinocytes themselves can produce inflammatory factors such as cytokines in response to mechanical stress. This suggests that stress as induced by the binding of autoantibodies to Dsg3 might initiate similar effects in keratinocytes.

Objectives: Here, we evaluate the cytokine response of human epidermal keratinocytes to anti-Dsg3 antibodies (AK23).

Methods: In this translational research work, human epidermal keratinocytes were treated with AK23. Quantitative gene expression (qPCR) was performed to study cytokine expression induced after AK23 treatment. Activation of the signal transducer and activation of transcription (STAT) factors and the impact of Janus kinase (JAK) inhibitors in AK23-treated keratinocytes were evaluated by western blotting and gene expression assays. Moreover, we studied the functional role of JAK inhibitors during AK23-induced cell dissociation by an in vitro keratinocyte assay. Epidermal activation of pSTATs in pemphigus and control skin was determined by immunohistochemistry. A pemphigus lesion in a steroid-unresponsive patient was treated with a topical JAK inhibitor, the clinical response and epidermal STAT activation were assessed.

Results: Our investigation reveals that loss of epidermal integrity by AK23 is accompanied by increased expression of cytokines (IL6, IL19, IL24, IFNE) and activation of STAT1 and STAT3. Administration of JAK inhibitors in vitro and in vivo prevented AK23-induced STAT activation and cell dissociation in keratinocytes.

Conclusion: Antibodies against Dsg3 increase the expression of IL6, IL19, IL24 and IFNE and activate STAT1 and STAT3 in keratinocytes. JAK inhibitors exert a protective role and hinder cell dissociation and acantholysis in vitro and in vivo.

P159 | Complement factor 3 deposition at the dermal-epidermal junction delays disease onset in a murine model of epidermolysis bullosa acquisita

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Complement C3, the central protein of the complement cascade, is widely recognised for its role in inflammation, particularly in effective opsonization and the clearance of invading pathogens. In autoimmune contexts, local complement deposition is often perceived solely as a marker of inflammation, neglecting its potential role in tissue homeostasis.

In this study we aimed to address the impact of complement C3 in pemphigoid diseases (PD), using a murine disease model system for epidermolysis bullosa acquisita (EBA). EBA, a prototypical autoimmune blistering disease (AIBD) within the pemphigoid spectrum, is characterised by the deposition of anti-collagen type VII IgG antibodies at the dermal-epidermal junction zone (DEJ), initiating local tissue inflammation. Similar to other PDs, the disease-associated IgG deposition is accompanied by co-deposition of complement factor C3. Despite C3 deposition being used as a diagnostic marker for PDs, the biological significance of its local accumulation remains enigmatic.

We employed an active model of experimental murine EBA in complement factor C3-deficient (C3ko) and wild-type (Wt) littermate mice, in which susceptible mice on the B6.SJL-H2s C3c/1CyJ (B6.S) background are immunised with a single dose of recombinant von Willebrand factor type A-like domain 2 of murine collagen VII (mCOL7vWFA2). The mCOL7vWFA2 domain, representing the immunodominant epitope in EBA patients, was administered in emulsion with adjuvant (TiterMax classic) to induce mCOL7vWFA2-specific autoantibodies and fully developed autoimmune disease.

After immunisation the mice were scored weekly for disease progression by assessing the affected body surface area and were sacrificed at pre-defined timepoints: Days 0, 2 and weeks 1, 2, 3, 4, 6, 8 post-immunisation (p.i.). C3ko mice developed active

disease, characterised by clinical blistering as early as week 4 p.i., whereas disease onset in Wt animals was observed starting at week 6 p.i. None of the control mice (C3ko and Wt) immunised with adjuvant alone developed any manifestations of disease.
Local skin deposition of IgG was increased in C3ko mice at weeks 3 and 4 p.i., correlating with the earlier disease onset. In Wt mice, both IgG and C3 were deposited at the DEJ. However, on the level of serum autoantibodies C3ko and Wt mice exhibited no significant differences in anti-mCOL7vWFA2 IgG, IgG1, IgG2b, IgG2c or IgG3 titers at any of the tested timepoints (W1-W8). ELISA-based testing of pooled sera also indicated no difference in the avidity of specific IgG between C3ko and Wt mice at any timepoints. In vitro stimulation of purified neutrophils with immune complexes (IC) generated with sera obtained from mice at week 3 p.i. revealed an increase in reactive oxygen species release from C3ko ICs in high concentrations, indicating a raised potential for inflammation.
Based on literature data, we hypothesized that C3 serves a non-canonical function during local skin inflammation in experimental EBA, providing tissue protection through its crucial role in tissue homeostasis by aiding the effective removal of damaged tissue during early stages of disease. This, in turn, may prevent exacerbated inflammation. Our data suggests that the transition from regulatory complement activation to aggravated inflammation occurs approximately around week 6 p.i. Additionally, despite no apparent changes in autoantibody profile and avidity, we observed an increased inflammatory potential for C3ko sera at week 3 p.i., possibly due to differential glycosylation, which we aim to further characterise.

P160 | Immunophenotyping of peripheral B cell subsets in patients with chronic spontaneous urticaria

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Introduction: Chronic spontaneous urticaria (CSU) is a common skin disorder that is characterised by recurrent hives, wheals, angioedema, which comes with significant impact on patients' quality of life. The presence of IgE autoantibodies directed against endogenous proteins (e.g., tyroid peroxidase) or IgG autoantibodies against the FceRI or IgE, are characteristic features of CSU. B cells play a crucial role in regulating immune responses and contribute to CSU pathogenesis by secretion of autoantibodies, promoting mast cell activation. However, cell subset characterisation, cellular phenotypes and functional features of B cells that might contribute to CSU pathogenesis have not been studied so far. This preliminary study aimed to investigate the characteristics of B cell subpopulations in the peripheral blood of CSU patients compared to healthy controls to provide insight into dysregulation of the B cell compartment in CSU.
Methods: Peripheral blood mononuclear cells (PBMCs) were isolated from fresh blood samples from CSU patients and healthy controls. Circulating B cells were phenotypically characterised by flow cytometry, mainly focusing on total B cells (CD19+), including cells expressing IgG, IgA, IgM, IgD and IgE. Specific B cells subsets were analysed such as switched B cells, unswitched B cells, naive B cells, double negative B cells and additionally other relevant populations memory and antibody secreting cells (ASC). Data obtained was analysed for differences in B cell distribution between patients and healthy controls. Correlation between patients' total B cells and B cell subsets with serum IgE level were also evaluated.
Results: Overall, the total number of B cells was significantly reduced as compared to healthy controls ($p=0.003$). In contrast switched B cells proportion was significantly increased in patients ($p=0.007$), indicating a shift in favour of the activated B cell population. Although naive B cell subset showed a slight decrease, but no significant differences were observed between healthy and patient groups. There was also no difference in the double negative population between the two groups. A slight decline in total IgD expressing B cells was discerned, an increase in IgM, IgG and IgE expression on B cells was observed, though not significant. Conversely, a significant decrease in IgA expressing B cells was observed ($p=0.01$). Furthermore, there was increase in ASC in CSU patients ($p=0.04$).
There was no significant correlation between serum IgE levels and total IgE expression on B cells. However, a weak positive correlation was observed between serum IgE levels and switched IgE expressing B cells ($r=0.4$).
Discussion: These data provide valuable insights into the B cell compartment in CSU. The observed increase in switched B cell populations may indicate enhanced class switching or activation in response to recurrent antigenic stimulation. These data suggest immune dysregulation in patients with CSU, with potential implications for understanding disease mechanisms and identifying novel therapeutic targets. Further investigation is needed to elucidate the functional roles of these B cell subpopulations in the pathogenesis of CSU, as well as their potential utility as biomarkers for disease severity or therapeutic response.

P161 | The role of mitochondrial stress in dermatomyositis

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Dermatomyositis (DM) is a rare autoimmune disease characterised by proximal muscle weakness and atrophy as well as skin inflammation. Several studies have found persistently elevated levels of type I interferon (IFN) in the skin, muscle, and blood of DM patients, indicating that DM is a type I IFN-driven disease. Although the precise molecular pathways involved in the pathogenesis of DM remain to be fully elucidated, the activation of innate immune sensors by self-nucleic acids is a relevant trigger for type I IFN-driven inflammation and cutaneous autoimmunity. Here we asked whether disturbance of mitochondrial function can be involved in the induction of type I IFN patients with DM. We have previously shown that fibroblasts from DM patients harbour mitochondrial stress leading to mtDNA release that can be sensed by the cyclic GMP-AMP synthase (cGAS) / stimulator of interferon genes (STING) pathway leading to type I interferon upregulation.

As performance measurements indicated a reduction in ATP levels in DM fibroblasts, we proceeded to evaluate the downstream effects of AMP-activated protein kinase (AMPK) as a marker of overall energy deficiency. The levels of pAMPK were significantly enhanced in the fibroblasts of 7 DM patients, in comparison to those of seven healthy controls. These levels were further increased by solar simulated irradiation mimicking UV exposure as a strong trigger of disease.

In response to diminished ATP levels, AMPK is activated, stimulating catabolic pathways to enhance ATP production while inhibiting anabolic processes to conserve energy, thereby preserving cellular energy homeostasis. Under these conditions, AMPK activation would typically result in increased mitophagy. However, chronic activation of AMPK can reduce mitophagy and induce cellular senescence. Indeed, RNA sequencing of patient fibroblasts revealed downregulation of the mitophagy marker PTEN-induced kinase 1 in patient versus control fibroblasts. This was associated with an enhanced rate of cellular senescence, which could further intensify mitochondrial stress.

In conclusion, these data show evidence of chronic mitochondrial stress in patient fibroblasts that can explain low-level cytoplasmic mtDNA release and intrinsic IFN induction as a trigger of disease pathogenesis.

P162 | Correlation of transcriptomic and protein changes with split formation and cell detachment in a 3D skin model for pemphigus vulgaris

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Desmoglein 1 (Dsg1) and Desmoglein 3 (Dsg3) are adhesion molecules responsible for maintaining intercellular connections between epidermal, hair follicle, and mucosal keratinocytes. Autoantibodies (AAbs) targeting these molecules ultimately lead to the blister formation characteristic of pemphigus vulgaris (PV).

To investigate the molecular events following autoantibody binding over 24 h, we quantified transcriptome and proteome dynamics during split formation in a human skin organ culture (HSOC) model of PV. The single-chain variable fragment (scFv) PX43 (targeting Dsg1/3) or the mouse antibody AK23 (targeting Dsg3) were injected into the HSOC, with human and mouse IgG as controls. RNA sequencing was performed at 5, 10, and 24 h post-injection, and shotgun proteomics at 24 h.

PX43, but not AK23, induced split formation. In the absence of split formation, no differentially regulated pathways were detected at the transcriptomic level. Split formation, observed as early as 5 h post-injection, was associated with significant and sustained upregulation of IFNγ and TNFα pathways, mediated by upstream NFκB, MAPK, and JAK–STAT signalling. The gene expression changes, corroborated by proteomics data, were strongly correlated with early wounding and keratinocyte detachment, while inversely associated with keratinocyte differentiation and cell stretching.

The co-occurrence of transcriptomic and proteomic responses with split formation, and their absence in the presence of AK23 alone, suggests that PV-related AAbs do not directly induce downstream transcriptomic or proteomic changes. Rather, these changes appear to be secondary effects resulting from loss of adhesion and split formation in the HSOC model of PV.

P163 | Composition of the inflammatory infiltrate and phosphorylated STAT signal in lichen amyloidosis

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Lichen amyloidosis (LA) is a rare and poorly studied skin disorder that presents with disseminated grey-brown/reddish lichenoid or verruciform-like papules. The lesions may coalesce into extensive infiltrated plaques. The disease normally affects the shins, ankles, feet, arms and back. The skin changes seem to occur due to apoptotic degeneration of basal keratinocytes and their liberation of cytokeratin, which is converted into amyloid material constantly deposited in the dermis. Along with macular and nodular amyloidosis, LA belongs to the primary cutaneous localised amyloidosis group. Little is known about the etiopathogenesis of the disease. One peculiar finding was the description that genetic mutations in OMSRβ and IL31RA genes (genes encoding the interleukin (IL-) 31 receptor subunits oncostatin-M and IL-31 receptor A) are associated with the disease. The symptomatic consequence of this disease is a disabling, treatment-resistant, and stable itch, which strongly limits patients' quality of life.

Treatment options are limited and only scarcely effective. Besides application of topical steroids, which are considered the gold standard, topicals (vitamin D3-derivates, retinoids, capsaicin, and tacrolimus), systemic drugs (retinoids, mycophenolate mofetil, ciclosporin A, amitriptyline), phototherapy modalities (narrowband UVB or PUVA) and laser treatments (carbon dioxide-, erbium-, pulseddye) have been reported with mixed results. We previously showed that Upadacitinib, a selective Janus kinase (JAK) 1 inhibitor, rapidly helped to relieve itch and pain in a 41-years old patient affected by a chronic LA. Upadacitinib, which is approved for the treatment of atopic dermatitis, is thought to interrupt the signalling of IL-31, a central pruriginous cytokine in T-cell mediated pruritus. Moreover, JAK1 inhibition will affect IL-4 and IL-13 signalling.

Based on this clinical observation we aimed to study the molecular factors in LA in more detail. We analysed the expression of cytokines in LA by performing qPCR from LA skin samples ($n=8$) and compared these with atopic dermatitis (AD) and lichen planus (LP) (both $n=8$). We analysed Th1, (IFNγ, TNF, IL-12), Th2 (IL-4, IL-13, IL-31) and Th17 (IL-17A, IL-22, IL-23) associated cytokines. By immunohistochemistry, we studied the presence of T cells (CD3, CD4, CD8), B cells (CD20), and expression of phosphorylated Signal Transducers and Activators of Transcription (STAT) proteins (pSTAT1, pSTAT2, pSTAT3, pSTAT4, pSTAT5, pSTAT6) ($n=8$ for each group). While experiments have been completed already, the analysis is still ongoing. This study will contribute to better understand the signature of and find new druggable targets in LA, a scarcely

analysed disease that strongly impairs the quality of life of affected individuals.

P164 | The hair canal serves as an EGFR-regulated antimicrobial gatekeeper

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Hair follicles are crucial to maintain mammalian skin barriers and protect against physical stressors of the environment. They represent a unique niche for commensal skin bacteria but unchecked proliferation of microbiota within hair follicles initiates folliculitis and fosters cutaneous dissemination of infections. This clinical implication is exceptionally displayed in cancer patients receiving anti-EGFR therapy that evokes papulopustular eruptions with concomitant bacterial *Staphylococcus aureus* superinfections.
In this study, we identified the specific cell population facilitating the pivotal microbial gatekeeping function of the hair follicle. We exploited single-cell datasets, deciphered epidermal growth factor receptor (EGFR)-dependent transcriptional signatures, and conducted targeted knock-out experiments in genetically engineered mice to pinpoint the significance of the EGR2/K79-positive hair canal as essential antimicrobial bastion. EGFR orchestrates the expression of antimicrobial peptides (AMPs) such as beta-defensin1/6 and SPRR1a/4 within the hair canal via the ERK signalling pathway. Notably, the presence of EGFR in fully differentiated sebocytes is expendable for the homeostatic defence mechanism of the hair follicle.
Our investigation further revealed that the identified AMP profile translates to the human skin, as AMP homologues are also concentrated in K79-expressing cells, and their overexpression is evident in EGFR-ERK-dominant psoriatic skin conditions. These findings provide crucial mechanistic insights into the microbial defence strategy employed by the hair follicle, with direct therapeutic implications for addressing folliculitis associated with EGFR-inhibitor-based anti-cancer therapy.

P165 | Butyrophilin-like 2 (BTNL2) as a novel candidate player in alopecia areata pathogenesis and management

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Alopecia areata (AA) is the most common inflammatory hair loss disorder. We have previously shown that pathogenic interactions between NKG2D expressed on CD8+, NK, γδ T, and ILC-1 cells and overexpression of the NKG2D-stimulating "danger" signal, MICA, on hair follicle (HF) keratinocytes induces excessive secretion of IFN-γ. The latter induces the collapse of the HF's physiological immune privilege (IP), an absolute prerequisite for developing AA, and thus can promote AA via an autoantigen- independent, non-autoimmune pathway that does not appear to be targeted by JAK inhibitors, the most important advance in recent AA management. Therefore, therapies that antagonise pathogenic NKG2D-MICA interactions must urgently be developed yet remain to be systematically explored in the context of AA.
One promising intervention target is Butyrophilin-like 2 (BTNL2), an immunoregulatory transmembrane protein known to modulate T-cell activation and maintain immune tolerance. It has been shown that epidermal Butyrophilin-γ/δ T cell interactions maintain immunosurveillance and barrier function and contribute to the regulation of cutaneous stress responses, including NKG2D-MICA interactions. Thus, we wanted to understand if BTLN-2 also has such an immunosurveillance function in human scalp HFs.
By IF microscopy, we found BTNL2 to be predominately expressed in the outer root sheath keratinocytes of healthy human scalp HFs. We then organ-cultured "stressed" scalp HFs with a weakened IP or "non-stressed" HFs with autologous Vδ1 T cells and/or CD8 T cells for 3 or 5 days. Ex vivo, BTNL2 expression was lower in scalp HFs with weakened IP than in those with intact IP. This reduction correlated with increased NKG2D expression in Vδ1 T cells and heightened CD8+ T-cell cytotoxicity towards stressed HFs. In contrast, when activated CD8+ T cells were co-cultured with non-stressed HFs expressing higher levels of BTNL2, no AAlike HF cytotoxicity was seen, as indicated by the absence of melanin clumping and premature catagen induction. There were also no signs of HF IP collapse (absence of increased MHC class Ia, MICA and β2-microglobulin, no decrease of IP guardians). Our preliminary data support the working hypothesis that BTNL2 may serve as an immunoregulatory ligand that helps maintain the HF IP by reducing NKG2D expression in AA-pathogenic immune cells, while reduced BTNL2 expression by the epithelium of stressed HFs may promote increased T-cell cytotoxicity and HF IP collapse.

P166 | Cellular and molecular immunoprofiling of lupus panniculitis: Elucidating the roles of cytotoxic T cells, B cells, and complement activation

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Lupus panniculitis (LP) is a subtype of cutaneous lupus erythematosus (CLE) that affects subcutaneous adipose tissue. The

pathomechanisms underlying LP are largely unknown. In this study, we aimed to obtain deeper insights into LP through the characterisation of cellular and molecular immune patterns. Using imaging mass cytometry (IMC), we analysed the cellular infiltrates in deep skin biopsies from LP patients ($n=8$) and healthy controls ($n=6$). Concurrently, we performed nCounter nanostring technology to quantify the mRNA expression of immune-related markers. The IMC analysis revealed that T cells (CD3+) predominated in LP More precisely, they exhibited a predominantly skin-homing (CLA+), cytotoxic phenotype (CD8+, granzyme B+), indicating site-specific immune activation within skin tissue. B cells (CD20+) were also present. They constituted a significant portion of immune cells, alongside with the presence of dendritic cells and macrophages. Differential gene expression analysis revealed an upregulation in pathways associated with adaptive and innate immune responses. This included T cell receptor signalling (upregulation of CD247, LCK), lymphocyte activation (ZAP70, CD3D), cytokine signalling (STAT1, MYD88), and MHC antigen presentation (HLA-DRA, TAP1/2). Additionally, there was an upregulation of genes associated with the Complement system (C1QA/B, C4A/B). Our results suggest that a cytotoxic T- and B-cell-predominated immune response and complement activation is involved in LP. This points towards a pathogenesis that is different from other types of panniculitis.

P167 | Identification and comprehensive analysis of desmoglein-3 specific B cells in patients and humanised transgenic mouse model of pemphigus vulgaris

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Pemphigus vulgaris (PV) is an autoimmune blistering skin disorder in which IgG autoantibodies, predominantly against Desmoglein (Dsg)-3, disrupt intercellular adhesion, ultimately causing acantholysis and suprabasal bullae. In PV immunopathogenesis, the interaction of Dsg3-specific T and B cells, which leads to the formation of autoantibodies, is well established.

The aim of this study was to identify and characterise Dsg3-specific B cells by multicolor flow cytometry using fluorochrome-labelled Dsg-3 proteins in PV patients and a correlating humanised transgenic PV mouse model. In hDsg3-transgenic mice immunised with human Dsg3, we identified Dsg3-specific B cells in spleen, lymph nodes (LN) and bone marrow (BM). Specifically, Dsg3-specific B cells were found within CD19+ CD138+ B220- and CD138+ CD93+ B220- plasma B cells in LN and BM, along with CD19+ CD138+ B220+ plasmablasts in the spleen compared to nonimmunized control mice.

In active PV patients, Dsg3-specific B cells were present at a higher frequency of 0.12%–0.4% of total CD27+ B cell pool compared to patients under Rituximab or healthy controls. In a cross-sectional study, patients under Rituximab displayed a contraction in the total B cell memory pool and a contraction of transitional B cell pool compared to acute PV patients and HC. In the same study, these PV patients also displayed differential B cells profiles under alternative immunosuppressive treatment regimens. A correlation was observed between anti Dsg3-IgG serum concentrations and the frequency of Dsg3-specific B cells in both the humanised transgenic PV mouse model and PV patients. In conclusion, we successfully identified and comprehensively profiled the Dsg3-specific B cells. Future experiments will focus on the affinity, pathogenicity and possible isotype switch of Dsg-3 IgG produced by Dsg3-specific B cells.

P168 | Bullous pemphigoid is associated with increased Th17.1 cells in peripheral blood and Th2 cytokines in skin

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Background: Bullous pemphigoid (BP) is an autoimmune, blistering skin disorder mostly affecting the elderly. Affected patients develop skin-targeting autoantibodies, produced by B cells with the help of T cells. Previous studies have described T cell involvement in other blistering skin diseases, but the exact role of T cell subsets in BP pathogenesis needs further investigation.
Objective: We aim to examine the distribution of Th and Tfh populations from peripheral blood mononuclear cells (PBMCs) and tissue cytokine gene expression in BP patients and non-BP controls according to clinical status.
Methods: Multiparametric flow cytometry was utilised to study the frequencies of circulating Th and Tfh cell subsets. Real-time quantitative PCR was performed for transcriptome analyses of BP lesional versus healthy skin. Patient medical histories were examined to record comorbidities and autoantibody titers against BP antigens BP180 and BP230.
Results: There were 66 BP patients and 65 non-BP controls enrolled in this study. Results showed increased numbers of circulating Th and Th17.1 cells in the peripheral blood of patients with active disease versus those in remission. Controls also exhibited higher levels of circulating memory T cells compared to active and remittent BP patients. In skin, Th2-related cytokine gene expressions (IL-4, IL-5, IL-13, IL-31) were elevated in active BP patients in contrast with controls and were found to have strong positive associations with one another via a Spearman correlation analysis. In addition, IL-10 expression in skin was upregulated in active BP patients as opposed to controls.
Conclusion: Patients with active BP present with higher numbers of Th and Th17.1 cells in peripheral blood and increased Th2-related cytokine gene expressions in lesional skin.

P169 | Testing established MAP kinase inhibitors in a different approach of the human skin organ culture model for pemphigus vulgaris

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Pemphigus vulgaris (PV) is an autoimmune blistering disease of the skin. The pathomechanism involves the production of circulating IgG autoantibodies which are directed against desmosomal adhesion proteins located on epidermal keratinocytes. This leads to disruption of adhesion, resulting in intraepidermal blistering. The clinical manifestation largely depends on the autoantibody specificities. In the presence of anti-desmoglein 1 (Dsg1) and Dsg3 autoantibodies, lesions occur in both the mucous membrane and the skin. To understand the pathomechanism we used the human skin organ culture (HSOC) model for PV which was previously established. Split formation is induced by injection of the pathogenic single chain variable fragment PX43 (scFv) targeting Dsg1 and Dsg3 into biopsies of healthy donor skin. In previous approaches, the MEK1 inhibitor Selumetinib and the p38 MAPK inhibitor BIRB 79 were injected prior to the scFv to ensure their binding to the designated targets. To more closely model a therapeutic approach, we did not preincubate the samples with the inhibitors, but injected the scFv firstly into the donor skin followed by Selumetinib and BIRB 796 2, 4 and 8 h later. After a 24- h incubation period, haematoxylin and eosin (H&E) sections were prepared to quantify split formation. Additionally, samples were collected for RNA sequencing. Immunofluorescent stainings were performed to visualise PX43 binding. We show, that neither Selumetinib nor BIRB796 induce a significant reduction of split formation in this approach of the HSOC model. This suggests that steric hindrance plays a crucial role in the pathogenesis of PV, while signalling pathways seem to play a minor one. To validate this hypothesis, we conducted HSOCs simultaneously by using the inhibitors with and without preincubation. Preliminary results indicate that reduction of split formation are only visible when HSOC models were preincubated with compounds while they don't exert any therapeutic effects on already affected skin. The exact mechanism by which the compounds inhibit the split formation is currently under investigation by the means of RNA-sequencing to identify transcriptomic changes that may explain why preincubation is necessary for these compounds to be effective. Further investigation into the mechanism of split formation inhibition is ongoing.

P170 | Strategies for PET imaging in acidic tumour microenvironments

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Slight changes in the extracellular pH value in the tumour microenvironment (TME) have crucial effects on host defence, metastatic behaviour, immune regulation and cellular metabolism. Due to the high metabolic activity and insufficient perfusion of tumours, acidic metabolites often accumulate in tumours and can influence the pH of the TME. In our study, we describe a new radiopharmaceutical for positron emission tomography (PET) that exploits the increasingly used concept of pH-dependent intratumoral cleavage of probes, to release functional moieties.
Using preclinical models, we were able to visualise small pH differences in the acidic TME of different tumours with our radiopharmaceutical [18F]FDG-4MBA.
Neutralisation of the acidic extracellular tumour pH by sodium bicarbonate prevents pH-dependent cleavage of [18F]FDG-4MBA and thus the visualisation of small pH differences in acidic TME with our system.
The determination of small pH differences in acidic TME may serve as a novel marker for the response of immunotherapies. With our system, even small pH differences in the acidic TME of different tumours can be visualised, and due to its simple tracer synthesis and application, this system could be well suited for implementation in clinical studies.
This finding would then have important implications for the development of new strategies to improve the efficacy of immunotherapy in cancer patients.

P171 | Exploratory study to evaluate changes in inflammatory pattern in patients with active, moderate-to-severe hidradenitis suppurativa after a six-week treatment with adalimumab

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Patients with hidradenitis suppurativa (HS) suffer from a chronic inflammatory skin disease characterised by painful inflammatory nodules, abscesses, fistulas, and scars. Different factors like genetics, environment, lifestyle, hormone balance, or microbiome play a significant role in the pathophysiology of HS. A negative imbalance of these factors leads to an immune activation around the hair follicle and hyperkeratosis as initial

steps. The infiltrate of HS regions includes neutrophilic granulocytes, plasma cells, dendritic cells, and immune cells, which release enhanced several inflammatory modulators such as tumour necrosis factor (TNF) -alpha, Interleukin (IL) -1, IL-6, and IL-23 in the early phase. Adalimumab, a monoclonal antibody against TNF-alpha, has become a key treatment for active moderate to severe HS.
According to the unknown availability of data about the cell infiltrate residing in HS lesions, especially after medical treatment, our study aims to examine the changes of different immune cell types such as dendritic cells, macrophages, and T-cells in skin biopsies of HS patients after adalimumab therapy. Therefore, biopsies were taken from lesional skin before and six weeks after treatment initiation. These skin biopsies were cryoconserved and analysed via multi-epitope-ligand-cartography (MELC), a unique robotic-based cell imaging technology that can show up to 38 immunofluorescence-coated proteins in one single slide. This methodology allows us to investigate inflammatory cell network changes in lesional skin areas before and after Adalimumab treatment.

P172 | The role of neutrophils in the pathogenesis of chronic spontaneous urticaria and delayed pressure urticaria

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Background: Urticaria affects around 20% of the population and significantly impairs quality of life. For patients with chronic spontaneous urticaria (CSU) and delayed pressure urticaria (DPU) who do not respond to antihistamine therapy, treatment options are limited. A deeper understanding of the pathogenesis of these therapy-resistant forms is therefore of great importance. Neutrophil infiltrates are a characteristic feature in skin samples from urticaria patients, but the role of neutrophils, including their interactions with mast cells, is still unclear.
Aim: The aim of the study is to compare the role of neutrophils in the pathogenesis of CSU and DPU and to identify potentially therapy-relevant approaches.
Methods: We analysed skin punches from symptomatic CSU- ($n=18$–19), DPU- ($n=10$–14) patients and healthy volunteers ($n=5$) and investigated the spatial interaction between mast cells and neutrophils. In addition, we quantified neutrophil infiltration, the rate of neutrophil extracellular trap (NET) formation, IL-17A-positive neutrophils and IL-23-positive cells in CSU and DPU samples by immunofluorescence staining.
Results: Neutrophil infiltration in CSU and DPU is similar; however, a significantly higher infiltration of neutrophils in the deep dermis was observed in DPU patients. For the first time, we detected NETosis in CSU and DPU, with an increased rate of NETosis in DPU. IL-23-positive cells were found in close proximity to IL-17Aproducing neutrophils. Preliminary analysis has also shown neuron and neutrophil interactions in both CSU and DPU patients indicating plausible interactions between neutrophils and sensory neurons.
Conclusion and Future Outlook: Neutrophils play a significant role in the inflammatory response of CSU and DPU with the increased NETosis rate in these samples indicating neutrophils as a possible therapeutic target. As IL-23 and IL-17 axis appears to be significantly activated in CSU and DPU, inhibitions of neutrophil associated pro-inflammatory cytokines IL-23 and IL-17 could offer new treatment options. As our preliminary results also show neutrophil neuron interactions, we aim to also look at the molecular mechanisms that facilitate neutrophil neuron cross talk and how this affects inflammatory itch progression in CSU and DPU.

Infectious Diseases

P173 | Determining antimicrobial effects of wound dressings—From planktonic bacteria to 3D-biofilm models

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Introduction: Chronic wounds present a major challenge in medical care, especially if bacterial infections occur in the form of biofilms. Antimicrobial treatment strategies are therefore increasingly utilised in the management of chronic wounds to govern important pathogens such as *Staphylococcus aureus*, *Acinetobacter baumannii* or *Pseudomonas aeruginosa*. The antimicrobial activity of wound dressings have often been judged using challenge or suspension tests with bacteria in the planktonic state. Recently, different biofilm models were proposed to rate the efficacy of wound dressing in a more application-oriented setting. The latest is a 3D biofilm model containing *Staphylococcus aureus*, *Escherichia coli*, and *Acinetobacter baumannii* or *Pseudomonas aeruginosa*.
Methods: Silver- and PHMB-containing dressings were compared for their antimicrobial activity using the challenge test JIS L 1902. Moreover, the dressings were examined for efficacy to eradicate a 2D-biofilm of *S. aureus* grown on a glass surface. Finally, the effect of the dressings was tested in the proposed 3D biofilm models.
Results: A strong antibacterial effect of silver and PHMB-containing dressings was determined according to the challenge test. However, in a 2D-biofilm model of *S. aureus*, only the PHMB dressing achieved a complete eradication while the silver dressing exhibited a lesser reduction of biomass. The established 3D biofilm models closely mimic the chronic wound situation, where bacteria communities occur in clusters of 20–100 µm diameter surrounded by extracellular matrix located not only near the surface, but also deeper in the tissue substitute. The highest antimicrobial effects with significant reduction for all three bacteria compared to the untreated control was again observed for PHMB dressing. However, none achieved complete eradication of the bacteria.
Conclusions: Appropriate biofilm models to improve transferability of benchside data to bedside treatment of chronic wounds are needed. Simplistic models using planktonic bacteria might results in the over interpretation of antibacterial effects. Surface

attachment of the biofilm is a crucial component in most of the established in vitro 2D biofilm models, which certainly plays an important role in other areas of biofilm research like e.g. foreign body infections, but does not occur in chronic wounds. The proposed 3D biofilm models are well suited for antimicrobial testing and can detect differences in the efficacy of antimicrobial substances.

P174 | Growth medium and temperature as critical parameters in microplate-laser-nephelometry (MLN) measurements of itraconazole inhibitory concentrations for dermatomycetes compared to EUCAST E.DEF9.3.2. Or E.DEF11.0 condition

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Introduction: Increased azole tolerance was observed for Trichophyton indotineae isolates1, 2 of the T. mentagrophytes/interdigitale complex, but also for new subtypes of the mouse favus pathogen Trichophyton quinckeanum3. Measurements of inhibitory concentrations were performed according to the EUCAST E.Def9.3.2 protocol4 developed for moulds or E.Def115 adapted for Trichophyton species. The use of the Microplate-Laser-Nephelometry (MLN) method2, 3 allows automated measurements to be carried out throughout the entire growth period. It was used to obtain data sets for growth inhibition as well as for the different growth phases of each isolate.
Methods: MLN methods were adapted to the EUCAST E.Def9.3.2 or E.Def11.0 methods and mainly showed differences in growth temperatures. In addition, different media (RPMI1640, RPMI1640 with additives and Sabouraud Glucose (SG) broth) were compared.
Results: Medium and temperature significantly influenced the growth parameters of the different Trichophyton isolates. The minimal medium RPMI1640 distinctly prolonged the growth time until the growth maximum was reached. This minimised effects between sensitive and tolerant isolates and generally resulted in higher values of itraconazole inhibitory concentrations compared to SG broth. Higher temperatures increased the growth rate and allowed better discrimination between sensitive and tolerant isolates. Addition of a protein source enhanced the growth rate of isolates in RPMI1640, which was accompanied by slightly higher values for itraconazole inhibitory concentrations. The highest discrimination rate for isolates was detected in SG broth in combination with high temperatures.
Conclusions: It was shown that reduction of the growth speed rate by using minimal media or low cultivation temperatures helps dermatomycetes to adapt to higher itraconazole concentrations.

References

1. Ebert et al. Mycoses (2020) 63: 717–28.
2. Burmester et al. Mycoses (2022) 67:97–102.
3. Winter et al. J Fungi (2023).
4. Arendrup et al. (2020) www.eucast.org (accessed 7.7.2023).
5. Arendrup et al. Clin Microbiol Infect (2021) 27:55–60.

P175 | NOD2-induced IκBζ mediates a protective host response against epicutaneous *Staphylococcus aureus* infection

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IκBζ, an atypical and largely unknown member of the IκB family, is a transcriptional cofactor of NF-κB, that can activate or repress a specific subset of cytokines and chemokines within the skin. Here, we investigated the role of keratinocytederived IκBζ upon infection with a multidrug-resistant *S. aureus* strain. Infection of human primary keratinocytes rapidly induced IκBζ expression, leading to an elevated expression of antimicrobial peptides, IL-17/IL-36-responsive genes, and proteins involved in barrier function. Conversely, loss of IκBζ resulted in increased *S. aureus* internalisation, epidermal tissue damage, and severe skin infections in vivo. This impaired host defence upon IκBζ depletion was characterised by reduced antimicrobial peptide expression, and diminished recruitment of neutrophils and CD4+ T-cells. Importantly, *S. aureus*-induced IκBζ expression required the internalisation of the bacteria and its sensing by the intracellular receptor NOD2, which subsequently triggered IκBζ and its target gene expression in a Regnase-1- dependent manner.
Thus, we identified NOD2-IκBζ signalling as a novel pathway acting as a key mediator for a protective host defence against pathogenic *S. aureus* infections.

P176 | The antimicrobial protein RNase 7 directly restricts herpes simplex virus infection of human keratinocytes

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Approximately 22% of moderately to severely affected patients with atopic dermatitis (AD) have a history of eczema herpeticum, a disseminated rash primarily caused by herpes simplex virus type 1 (HSV-1). Reduced activity of antimicrobial peptides may contribute to the increased susceptibility of AD patients to HSV-1. We have previously shown that the antimicrobial protein RNase 7 limits HSV-1 infection of human keratinocytes by promoting self-DNA recognition.

Here, we investigated whether RNase 7 has any effect on HSV-1 infection when infecting keratinocytes without exogenously added costimulatory DNA and which step(s) of the infection cycle RNase 7 interferes with. We quantified viral gene expression by RT-qPCR and flow cytometry, viral genome replication by qPCR, virucidal effects by plaque titration, and plaque formation and subcellular localization of incoming HSV-1 particles by microscopy. Recombinant RNase 7 reduced HSV-1 gene expression, genome replication and plaque formation in human keratinocytes. It was also active in the presence of the Th2 cytokines IL-4 and IL-13 and decreased HSV-1 immediate-early transcripts independently of the induction of interferonstimulated genes. Its main effect was on early intracellular infection processes and not on extracellular virions or virus binding to cells. RNase 7 reduced the amount of cell-associated capsids and HSV-1 envelope glycoprotein D at 3 but not 0.5 h post-infection.

Our data show that RNase 7 directly limits HSV-1 infection of human keratinocytes, possibly by promoting the degradation of incoming HSV-1 particles. This suggests that RNase 7 may limit HSV-1 spread in the skin and that mechanisms that reduce its activity in the lesional skin of AD patients may increase their susceptibility to eczema herpeticum.

P177 | The effect of *Lactobacillus plantarum* 8P-A3 on *Staphylococcus aureus* and a new approach for probiotic use

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The growing global problem of antibiotic resistance in bacterial pathogens calls for the search for alternative therapies. In light of this, the use of probiotic bacteria that produce bacteriocin-like substances for topical applications may be an alternative to the use of antibiotics.

Therefore, we first evaluated the antimicrobial activity of the bacterium *Lactobacillus plantarum* 8P-A3 against common skin pathogens in vitro. Furthermore, monolayers of keratinocytes were infected with *Staphylococcus aureus*, and the efficacy of live *L. plantarum* bacteria or cell-free supernatant in inhibiting *S. aureus* growth was tested. In both cases, a significant reduction in *S. aureus* growth was observed. In addition, by treating different types of skin cells with supernatant of *L. plantarum*, a proliferative effect on fibroblasts was observed.

In preliminary studies, we applied the probiotics by enclosing them between membranes. In this way, only their products, but not the bacteria, can reach the surfaces inoculated with the pathogens. This served as a model for a "probiotic pad" that could be used to treat various infectious skin conditions. Following the successful patenting of this patch, we intend to pursue this approach and test its application in our recently published ex vivo skin model.

P178 (OP03/04) | COL23A1: A potential risk factor for eczema herpeticum

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Eczema herpeticum (EH) is a potentially life-threatening disseminated skin infection caused by herpes simplex virus (HSV) in a subset of patients with atopic dermatitis (AD). The fact that only a subset of AD patients develop EH reflects the crucial role of genetic factors in the development of EH. Therefore, to identify novel genetic risk factors for EH, we performed whole exome sequencing and identified a heterozygous single nucleotide polymorphism (SNP) rs2973744 A/G in the gene encoding collagen type XXIII alpha 1 chain (Col23a1), which was significantly associated with EH in AD patients (χ^2 test: $p=0.034$). Col23a1 is a type II transmembrane protein that exists as a full-length membrane-bound form (75 kDa) or as a secreted ectodomain (60 kDa) resulting from proteolytic cleavage mainly by furin.

Our data show that EH-patient-derived primary keratinocytes carrying the minor allele G of SNP rs2973744 had elevated COL23A1 mRNA and protein levels as well as an increased susceptibility to HSV-1. To study the role of elevated Col23a1 levels in HSV-1 infection, we either treated primary keratinocytes with furin inhibitor or overexpressed COL23A1 in HaCaT cells. Increasing the Col23a1 levels experimentally enhanced HSV-1 susceptibility in both primary keratinocytes and HaCaT cells. Moreover, COL23A1 overexpressing HaCaT cells had enhanced cell surface levels of HSV-1 attachment and entry factors, syndecan-1 and nectin-1, respectively, as well as higher HSV-1 gene expression and cell-to-cell spread compared to control HaCaT cells. To investigate the effect of COL23A1 overexpression on the global transcriptome, we performed bulk RNA sequencing on control and COL23A1 overexpressing HaCaT cells and observed downregulation of genes involved in antiviral responses such as IL1R1, IL32, TLR4, IRF1, S100A9, C3, and CFH and upregulation of AD-associated genes like SPINK5 and TNC upon COL23A1 overexpression.

In conclusion, our data show that the SNP rs2973744 increases COL23A1 expression in the presence of additional risk factors for EH. Enhanced Col23a1 increases cell surface levels of HSV-1 attachment factor, syndecan-1 and entry receptor, nectin-1, as

well as dampens the immune and inflammatory responses of keratinocytes, thereby allowing HSV-1 to infect COL23A1 overexpressing HaCaT cells more efficiently. Therefore, the SNP rs2973744 could be used to identify AD patients at risk of developing EH. In addition, we present a novel role for Col23a1 in HSV-1 infection that could be further exploited as a potential therapeutic target for the treatment of EH.
K.D., L.M.R. and T.W. contributed equally to this study.

P179 | Effect of *Staphylococcus aureus* and *Staphylococcus epidermidis* on herpes simplex virus type 1 skin infection in the context of atopic dermatitis

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In atopic dermatitis (AD) the skin microbiome is altered towards an increased colonisation with the potential pathogen *Staphylococcus aureus* (*S. aureus*) and susceptibility to various skin infections is increased. In a subset of AD patients, herpes simplex virus type 1 (HSV-1) infection can result in a disseminated potentially life-threatening infection, known as eczema herpeticum (EH).
We studied the immunomodulatory effect of two staphylococcal species commonly found in the AD skin microbiome, *S. aureus* and *S. epidermidis*, in combination with a type-2 inflammatory environment on HSV-1 infection in keratinocytes.
A total of 293 AD patients from the TREATgermany registry, 221 without (ADEH-) and 72 with (ADEH+) a history of eczema herpeticum, and 258 healthy controls were included in this study for a 16S skin microbiome analysis. Shannon diversity index was used to assess the microbial diversity. Among other staphylococcal species, the most abundant ones on the skin of ADEH+ patients were *S. aureus* and *S. epidermidis*. Based on these findings, we established an in vitro keratinocyte model. Cells were incubated with heat-killed staphylococci followed by HSV-1 infection, in the presence or absence of the Th2 cytokines IL-4 and IL-13 to mimic AD lesional skin inflammation. To assess the production of infectious HSV-1 particles, supernatants from HSV-1 infected keratinocytes were used to infect Vero cells. Infection rates were analysed by fluorescence microscopy. Transcriptome analysis of keratinocytes incubated with heat-killed staphylococci was performed to investigate the effect of these staphylococci on keratinocyte gene expression.
The diversity of the skin microbiome was reduced in ADEH+ patients compared to ADEH- patients and even more reduced compared to healthy controls. The most prevalent species on the skin of ADEH+ patients, in addition to other staphylococci, were *S. aureus* and *S. epidermidis*. Heat-killed *S. aureus* (HKSA), but not *S. epidermidis* (HKSE), exhibited a slightly dose-dependent protective effect against HSV-1 infection in keratinocytes without affecting cell viability. In addition, HKSA showed an inhibitory effect on the production of infectious progeny virus, as evidenced by reduced HSV-1 infection in Vero cells following infection with supernatants derived from keratinocytes previously incubated with staphylococci. In this model, pre-incubation with IL-4 and IL-13 generally reduced HSV-1 infection compared to unstimulated keratinocytes. RNA-Seq analysis of keratinocytes showed that HKSA activated interferon pathways, which may account for the reduced susceptibility to infection. Additionally, HKSA incubation decreased the expression of the HSV-1 receptor nectin-1 on the transcript level, which may also reduce infection.
In this model, *S. aureus* as well as pre-incubation with the Th2 cytokines IL-4 and IL-13 protected keratinocytes from HSV-1 infection. Transcriptome analysis revealed downregulated receptors involved in viral entry and upregulated antiviral pathways. These findings suggest that *S. aureus* may play a regulatory role in the complex host-HSV interaction and potentially enhance the antiviral defence mechanism of keratinocytes.

P180 | *Borrelia burgdorferi* spirochetes induce vascular dysfunction and neurogenic inflammation in erythema migrans

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The Erythema migrans (EM) is caused by *Borrelia burgdorferi* (*B. burgdorferi*), transmitted by ticks, and represents the first and most common manifestation of Lyme disease. The mechanisms of *B. burgdorferi* migration through the skin, vessels, and along dermal nerve fibres as well as its interactions with skin components remain unclear. This study investigates the distribution of *B. burgdorferi* in the center and border of EM lesions and its effects on local nerve fibres and dermal vessels, comparing findings with healthy skin.
Our findings reveal that *B. burgdorferi* density is highest in the EM center around the bite site and predominantly around vessels, suggesting vascular involvement. We found reduced expression of the glycoprotein von Willebrand factor (vWF) in vessels within the center, indicating potential endothelial cell damage and impaired vascular integrity, possibly due to *B. burgdorferi*.
To assess the impact of *B. burgdorferi* on dermal nerve fibres, we explored the neuropeptide Calcitonin Gene-Related Peptide (CGRP) and its receptor as markers of neurogenic inflammation. Single-cell RNA sequencing analysis reveals upregulation of the CGRP receptor CALCRL in the EM compared to healthy skin, particularly within antigen-presenting cells, T cells, natural killer cells, and melanocytes within the skin. In parallel, the corresponding ligand CGRP was detected around dermal nerve fibres co-localised with *B. burgdorferi* in immunofluorescence staining, indicating neurogenic inflammation caused by *B. burgdorferi*.

Overall, this study demonstrates *B. burgdorferi* migration from the tick bite site in the center towards the rash's periphery and its potential systemic dissemination via blood vessels, leading to endothelial dysfunction and neurogenic inflammation.

P181 | HSV-1 infection modulates human mast cell phenotype and functionality

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Mast cells (MCs) are found throughout the body and are very abundant at hostenvironment interfaces such as the skin where they act as sentinel cells to sense and fight pathogens such as viruses. Herpes simplex virus type 1 (HSV-1) can cause infections in humans ranging from orolabial lesions to life threating conditions such as herpes simplex encephalitis. Primary infection through the mucosa results in life-long infection in sensory neurons and periodic reactivation causing painful blistering and viral shedding. How HSV-1 impacts MCs phenotype and functionality is not known.

In the present study, we found that human skin MCs (hsMCs) are susceptible to HSV-1 infection. Single cell sequencing analysis of infected hsMCs indicated that infection results in a unique pattern of cellular and viral genes that modified receptor expression. Whereas Mas-Related G Protein-Coupled Receptor-X2 expression was maintained, KIT and FcεRI were markedly downregulated leading to changes degranulation patterns and cell death only in HSV-1 infected hsMCs. An increased IgE-independent hsMC activation was found in HSV-1 infected hsMCs compared to uninfected cells. In contrast, no differences in the IgE-dependent hsMC activation were observed.

Our results show that HSV-1 infection modulates hsMC phenotype and functionality. A better understanding of the mechanisms underlying the role of MCs in HSV-1 infections will allow the development of novel therapies to not only control the infection but also to reduce symptom burden.

P182 | Casein kinase 1α (CK1α) inhibition prevents IFNγR1 degradation upon HSV-1 infections in human keratinocytes

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Herpes Simplex Viruses (HSV-1 and 2), as members of the alphaherpesvirus family, are highly prevalent DNA-viruses that often lead to painful vesicular lesions in orolabial- and genital mucosa, causing a ubiquitous disease burden. During HSV-1 infections, keratinocyte cell death is supposed to be induced by apoptosis or autophagy. Our studies demonstrate nevertheless the relevance of keratinocyte inflammatory cell death by necroptosis in the pathogenesis of HSV-1 skin infections. Furthermore, we provide evidence that inhibition of Casein Kinase 1α (CK1α) prevents the downregulation of IFNγR1 upon HSV-1 infections in human keratinocytes. Therefore, by rescuing IFNγ-Receptor 1 (IFNγR1), HSV-1 infected keratinocytes may thereby activate IFNγ-induced necroptosis.

HSV-1 infections cause T cell activation and Interferon gamma (IFNγ) production in keratinocytes. IFNγ induces de novo expression of Z-DNA binding protein 1 (ZBP1) through the JAK/STAT signalling pathway and thereby further activate downstream necroptotic mediators such as RIPK3 and MLKL. Furthermore, through immunohistochemical staining and in vitro experiments, we reveal the activation of STAT3 in IFNγ-mediated necroptosis upon HSV-1 infections and highlight the importance of STAT3 in IFNγ-induced necroptosis in keratinocytes.

Interestingly, we could demonstrate that high viral titers of HSV-1 inhibited IFNγ- mediated necroptosis in keratinocytes through the downregulation of IFNγR1.

Searching for a cause of IFNγR1 downregulation, we discovered that inhibiting Casein Kinase 1 (CK-1) reduced expression of infectious HSV-1 titers as determined by plaque assay and therefore prevented IFNγR1 degradation. Mostly, the combination of CK-1α Inhibitor and IFNγ showed the most effective reduction in HSV-1 titers after keratinocyte infection.

Taken together, our studies: I) depict the role of STAT3 in IFNγ-induced necroptosis in keratinocytes, II) demonstrate the inhibition of IFNγ-mediated necroptosis in HSV-1 infected keratinocytes through IFNγR1 degradation, III) unravel the importance of CK1α as a regulator of viral protein expression and IFNγR1 degradation.

Therefore, inhibition of CK-1α, thereby securing IFNγR1 expression and signalling, may provide a therapeutic approach for treating vesicular lesions after HSV1/2- infections of the skin and in this context identify a druggable target.

P183 | Longitudinal alalysis of persistent anal HPV16 infection in men who have sex with men living with HIV

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Introduction: Persistent infections with high-risk HPV can cause anal intraepithelial neoplasia and anal cancer. The aim of this retrospective study was to analyse whether the repeated, long-term detection of intraanal HPV16 in men who have sex with men living with HIV (MSMLWH) reflects persistent infection with the same isolate or repetitive new infections with different isolates.
Methods: Of 794 MSMLWH participating in anal cancer screening from 2003–2019, 54 patients with repetitive intraanal HPV16-detection were further analysed. Both MSMLWH with continuously HPV16-positive intraanal swabs (group-1, $n=26$) and MSMLWH with HPV16-negative intervals (group-2, $n=28$) were included. Median follow-up time was 82 months (IQR 50.3–116.5). In 155 HPV16-positive swabs of the 54 patients, two variable regions (long-control region, LCR, and non-coding region, NCR) were analysed by Sanger sequencing. The mean number of swabs per patient was 3 (range 2–6). In 12 samples from five patients, additional high-accuracy longread sequencing (PacBioHiFi) of the complete HPV16-genome was performed.
Results: 149 HPV16-LCR- and 150 NCR-sequences of 54 MSMLWH were obtained by Sanger sequencing. The majority of patients, 92.3% (24/26) of group-1 and 71.4% (20/28) of group-2, had identical intra-individual NCR/LCR-sequences during follow-up. 7.7% ($n=2$) of group-1 and 28.6% of group-2 ($n=8$) had probable de novo infections with different HPV16 isolates. PacBioHiFi corroborated the Sanger sequencing results. In phylogenetic analysis, the majority of the sequences grouped with HPV16-sublineages A3 (72.5%) or A1 (24.4%), which differs from the sublineage-distribution reported for European women.
Conclusions: The repeated detection of intraanal HPV16 in MSMLWH probably represents long-term persistence of the identical isolate in the majority of patients. HPV16 redetection of the identical isolate after an HPV16-negative interval suggests low-level persistent infection (latency). This could explain the poor efficacy of prophylactic HPV-vaccination in MSMLWH.

P184 | Tick feeding induces lymphatic emigration and pro-tolerogenic immune response in human epidermal langerhans cells

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Epidermal Langerhans cells (LCs) are the first antigen presenting cells responding to tick vector feeding. We investigated migration and polarisation of LCs in response to tick bites (TB) and *B. burgdorferi* infection and observed strong emigration of epidermal LCs after clinical TB and in an experimental TB model using tick saliva (TS) injection. Consequently, LCs were increased in the dermis and dermal lymph vessels. LCs overexpressed migration markers CXCR4 and CCR7, indicating lymph node homing. In line with this, LCs stimulated with TS showed increased potential to emigrate from epidermal sheets and invade towards collagen gels supplemented with the CCR7-ligand CCL19. Similarly, acute Lyme borreliosis skin harboured activated LCs expressing CXCR4 and CCR7. Interestingly, monocyte-derived LCs exposed to TS or infected with *B. burgdorferi* exhibited a tolerogenic phenotype characterised by the upregulation of the transcription factors IDO1 and IRF4, when compared to uninfected cells or *S. aureus* infection, the latter inducing a strong immunogenic response. When co-cultured with autologous naïve CD4+ T cells, LCs pulsed with TS or *B. burgdorferi* skewed T cell polarisation towards regulatory and type-2 helper, indicating impaired adaptive immune response. In single-cell RNA seq data of Lyme borreliosis skin LCs exhibited increased expression of IRF4, IDO1 and IL4I1 and a T cell composition comparable to autologous healthy skin.
Collectively, our results indicate that TB induces emigration of tolerogenic LCs to lymphatics, resulting in impaired T cell response to tick-borne pathogens. These findings explain low immunogenicity and high transmission/re-infection rates in tickborne infections.

Pharmacology

P185 | In vitro models using human keratinocytes for research topics in dermatopharmacology and drug allergy

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Introduction: Beyond its role as a protective barrier against external factors, the skin also has important immune and metabolic properties. Recent findings suggest that extrahepatic metabolism, especially phase II reactions like sulfation, may contribute to the development of drug-induced cutaneous hypersensitivity reactions. However, the mechanisms driving these complex skin responses remain poorly understood.
Methods: We examined primary keratinocytes isolated from juvenile foreskin or plastic surgery samples, focusing on RNA and protein expression of selected drug-metabolising enzymes. The formation of (reactive) lamotrigine metabolites, potentially involved in cutaneous hypersensitivity reactions, was analysed using high-pressure liquid chromatography coupled with mass spectrometry.
Results: Phase I enzymes, particularly CYPs, were found to have low to undetectable expression at both gene and protein levels, while phase II enzymes were at least detectable at the gene level. N-glucuronidated lamotrigine was identified in cell culture, but other reactive metabolites were only found in enzyme assays.
Conclusion: In vitro keratinocyte cell culture models could be a useful platform for studying the metabolism of drugs known to induce cutaneous hypersensitivity reactions. However, the detection of reactive metabolites in these models remains challenging due to their instability.

P186 | Beneficial effects of angiotensin-(1–7) on wound healing in the human ex vivo wound healing organ culture

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Impaired wound healing is known as a major threat for human health. In an everaging society, the number of patients suffering from chronic wounds increases steadily. As the exact mechanism of impaired cutaneous healing is not fully understood and current treatment strategies are not yet satisfactory, more research is urgently needed.
The wound healing organ culture (WHOC) is a powerful tool to investigate ex vivo wound healing of human skin. Human skin from plastic elective surgeries was manually wounded and cultured over 7 days to study the biological processes involved in wound healing in a controlled laboratory environment. Here, we have investigated different histological and immunofluorescence parameters complemented by different cell-based assays. To discover the effects of angiotensin- (1–7), a heptapeptide exerting its effects through stimulating two receptors, Mas and MrgD, which results among others in anti-inflammatory actions, different concentrations of the peptide were used in the WHOC.
We have seen beneficial effects of increasing concentrations of angiotensin-(1–7) on the macroscopic area and diameter in the top-view analysis of the wounds but without significances. However, we have observed a significantly decreased microscopic area as well as diameter on haematoxylin and eosin (HE) stained slices in angiotensin-(1–7) treated wounds compared to the non-treated controls in the WHOC at day 7, implicating a strongly beneficial effect of the peptide on wound healing. Interestingly, we have observed a significant increase in the area as well as in the length of the outer epithelial tongues but also a significant decreased area and length of the inner epithelial tongues. To better understand the mechanisms behind the controversial data and to further examine the effects of angiotensin-(1–7) on migration, we performed an in vitro wound healing assay with human keratinocytes (HaCaT cells) called scratch assay. We have not observed significant effects on scratch closure between the control and the treatment with the heptapeptide in this assay, concluding that the mode of action is not based on beneficial effects on migration. Furthermore, using immunofluorescence, we have investigated cytokeratin 6 (CK6) as a marker for hyperproliferation, widely expressed throughout wounded epithelia. Congruent to the scratch assay data, we have not seen any significant differences between both treatment groups in the cell culture assay. Importantly, we have observed an increased CK6 expression in the outer epithelial tongues as well as a decreased CK6 expression in the inner epithelial tongues in angiotensin-(1–7) treated wounds compared to the non-treated controls, matching our data of the area and the length of the outer and inner epithelial tongues on the HE stained sections.
In summary, angiotensin-(1–7) has a strongly beneficial effect on wound healing, whereby the mechanism is still not fully explained by our data. Further experiments should determine the mode of action behind the promising effects of angiotensin-(1–7) on human wounds, as our data indicates that the peptide might support the wound healing process in patients.

P187 | Particle-mediated transport of dissolved active agents in the hair follicle

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Introduction: Nanoparticulate systems for the delivery of active agents to the hair follicle have been researched for nearly two decades (1,2), resulting in numerous publications using a variety of particle types, release mechanisms, and active agents. The preparation of a stable dispersion is often time-consuming and expensive. The composition of the formulation investigated here does not require encapsulation or binding to the particle. The active agent resides solely in the outer phase of the formulation. In this way, we aim for a broadly applicable approach to formulation composition, suitable both for follicle disinfection and enhanced drug delivery.
Methods: The enhancement of follicular penetration by the novel formulation concept was investigated based on the particle type and sphericity (nanocrystals (NC) or lipid nanoparticles (LN)) and model drugs (fluorescein sodium, 6-carboxyfluorescein, green fluorescent protein, and FITC-BSA). The penetration depth into the hair follicles was determined using cryosections

and epifluorescence microscopy. All investigations were conducted on porcine ear skin.

Results: In this study, we demonstrate for the first time that the addition of nanoparticles to a drug solution increases follicular penetration depth by >160% to >190%. This effect was observed for all particles and all model drugs studied.

Conclusion: The novel formulation principle can enhance the follicular penetration of active agents in the outer phase of the formulation. The new formulation composition is highly versatile. The system can be applied to various hair follicle-related conditions such as alopecia, preoperative follicle disinfection, and targeted drug delivery.

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References

1. J. Lademann et al., Eur J Pharm Biopharm 66(2) (2007) 159–64.
2. O. Pelikh et al., Journal of Controlled Release 329 (2021) 598–613.

Photobiology

P188 | Influence of age, skin type and melanin distribution on UV induced skin damage

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Sun light is known to induce skin damage especially DNA alterations and free radicals enhancing the risk of developing skin tumours. We know that dark skin is better protected against UV light but little is known about the repair of the DNA damage dependent on skin type and age. Furthermore, is melanin the only relevant parameter or does the distribution of melanin affect the protection as well?

We irradiated excised skin directly [1] and skin healthy volunteers with different age, skin type and took biopsies direct after irradiation, 24 h and 7 days later. For higher age, the repair mechanisms are more slowly compared to younger persons. Interestingly, for darker skin types we found higher DNA damage (CPD, 6,4 PP+) after 24 h when irradiated with one minimal erythema dose. After one week the last damage is the same for both groups.

Using 3D skin models, it could be shown that distribution of melanin within the skin is crucial and it decides on protection or additional DNA damage and radical formation through photosensitization [2]. Here, two photon microscopy and electron paramagnetic resonance spectroscopy was applied.

Thus, we could show that the skin damage is influenced by age, skin type but also by melanin distribution in the skin.

References

1. Far-UVC- and UVB-induced DNA damage depending on skin type. Busch L, Kröger M, Zamudio Díaz DF, Schleusener J, Lohan SB, Ma J, Witzel C, Keck CM, Meinke MC. Exp Dermatol. 2023 Sep;32(9):1582–1587. doi: 10.1111/exd.14902. Epub 2023 Aug 7.
2. Significance of melanin distribution in the epidermis for the protective effect against UV light. Zamudio Díaz DF, Busch L, Kröger M, Klein AL, Lohan SB, Mewes KR, Vierkotten L, Witzel C, Rohn S, Meinke MC. Sci Rep. 2024 Feb 12;14(1):3488. doi: 10.1038/s41598-024-53941-0.

P189 | Effects of titanium dioxide nanoparticles in combination with UV-exposure on human skin with barrier defects

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Titanium dioxide nanoparticles (TiO2-NPs) are frequent components of sunscreens as a physical blocker of UV radiation and can be found in various products like paints, drugs and tooth paste. However, TiO2 was banned as a food additive by the European Union in 2022 due to potential carcinogenic effects. In a process called photocatalysis the electrons of TiO2 receive excitement from highly energetic radiation (such as UV) that triggers redox reactions with water molecules on the particle surface leading to the production of reactive oxygen species (ROS). Increases in intracellular ROS induce oxidative damage to lipids, proteins and in particular nucleic acids that may cause mutations in the genome or acute cytotoxicity.

Safety concerns may also rise for TiO2-NPs in sunscreens due to the exposure to sun light. Several studies investigated the penetration depth of TiO2-NPs on healthy skin with intact skin barrier. These showed that the TiO2-NPs remained in the stratum corneum with no contact to live cells indicating that the use of TiO2-NPs is safe. However, there is a lack of data concerning the penetration of TiO2-NPs in skin with barrier defects in conditions such as psoriasis or atopic dermatitis (AD). In these cases TiO2-NPs may cause damage to live cells when exposed to UV radiation. Therefore, this project aims to close the knowledge gap concerning the penetration depth of TiO2-NPs in barrier-impaired skin and the associated risk of skin cancer development.

Against this background, we first assessed the cytotoxicity of two crystalline forms of TiO2-NPs (anatase and P25) in combination with UV radiation on human keratinocytes (HaCaT) using XTT assay that revealed significant reductions in metabolic activity after incubation of both TiO2-NPs and high doses of UV-A radiation. Another light source (UV-B) was also tested but toxicity on HaCaT was mainly induced by the radiation and was not heavily influenced by TiO2-NPs. We also observed a dependency of toxicity on the incubation time with the TiO2-NPs before UV-A exposure. This may indicate time-dependent uptake of TiO2-NPs into the cells that is crucial for toxicity. In vitro cytotoxicity correlates with increased intracellular ROS levels

confirmed by H2DCFDA assay for both forms of TiO2-NPs after UV-A exposure. Enhanced hyperspectral dark-field microscopy was used to confirm the incorporation of the particles, which is a prerequisite for increased cytotoxicity under UV-A exposure.
To investigating the penetration depth of TiO2-NPs in barrier-impaired skin, we use an in vitro 3D skin equivalent model (epidermis + dermis). To induce a barrier defect in this model treatment with pro-inflammatory cytokines (IL-4, IL-13, IL-31) is conducted. Key features of AD in this model were confirmed by histological and gene expression analyses. To validate this in vitro model for AD results will be compared to results obtained with ex vivo human skin of AD patients. Immunohistochemical/– fluorescence stainings and RT-qPCR are performed to reveal alterations on the protein and gene expression level caused by the combination of TiO2-NPs and UV exposure.

P190 | In vitro characterisation of ectoine in combination with UV-B exposure on human keratinocytes

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Ectoine (1,4,5,6-tetrahydro-2-methyl-4-pyrimidinecarboxylic acid) is a naturally occurring compound that is predominantly found in a number of extremophile species of bacteria, including halophiles like *Ectothiorhodospira halochloris*. Ectoine is classified as an osmolyte that prevents water loss and protects integral structures, including membranes, proteins and nucleic acids, from environmental stressors such as dryness, high salinity or UV radiation. Consequently, it is now used in skin care and medical products for moisturising and protection against sun light and allergens. However, the exact mechanism of action remains largely unclear. The current hypothesis suggests that ectoine creates hydrate envelopes around macromolecules, acting as a diffusion barrier to damaging agents.
In the context of a Jugend forscht project, an investigation was initiated into the potential protective effects of ectoine against UV-B irradiation on human keratinocyte cell line HaCaT. Cells were treated with ectoine either before or after the UV-B exposure (50–150 mJ/cm^2), and in concentrations ranging from 0.1 to 100 mM. Analyses of the metabolic activity after treatment revealed that the addition of ectoine does not protect cells against the harmful effects of UV-B radiation. In contrast with our initial hypothesis, an enhanced reduction in metabolic activity was observed for the highest ectoine concentration (100 mM), particularly following UV-B exposure. A scratch assay showed a reduction in cell migration for 100 mM ectoine after 48 h in comparison to the control cells.
In conclusion, a protective effects of ectoine against UV-B irradiation was not observed. It is possible that this is due to fundamental differences between eukaryotic and prokaryotic cells. While ectoine is mostly well tolerated by eukaryotic cells it exhibits some inhibitory effects at high concentration and especially in combination with UV-B exposure.

P191 | Far-UVC irradiation of the skin for safe eradication of resistant pathogens

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UVC-irradiation, conventionally using discharge lamps at emission wavelengths of 254 nm, has proven to be effective for the eradication of resistant pathogens. However, this wavelength is harmful to the skin and can therefore only be applied in shielded environments e.g. on instruments and surfaces in the absence of humans.
As the absorption and scattering of proteins increases tremendously below 240 nm, the penetration depth of far-UV-C light into the skin is strongly limited. Therefore, it can be safely applied on the skin, as it penetrates only superficially in the stratum corneum and does not reach the basal layer of the skin. This effect has been shown in several studies using far-UV-C irradiation at filtered 222 nm Kr:Cl excimer lamps ex vivo.
We have modelled the penetration depth in the 200–300 nm range, taking account of the absorption and scattering parameters in skin, determined by inverse Monte Carlo simulations performed on transmission and reflection measurements of thin skin sections.
We present results on the skin DNA damage ex vivo and in vivo using far-UVCLEDs emitting at 233 nm at doses effective to eradicate microorganisms, such as Methicillin-sensitive and Methicillin-resistant *Staphylococcus aureus* (MRSA/MSSA) and Candida subspecies *C. albicans* and *C. parapsilosis* in comparison to broadband UV-B. DNA damage were evaluated in comparison to 280–400 nm UVB-irradiation.
At a dose of 40 mJ/cm^2, the far-UVC LED light source could reduce the MSSA/ MRSA load by 5 log10 levels if no organic substances were included in the medium. At 40 mJ/cm^2, the investigated skin models, excised human skin and human skin in vivo showed negligible and only superficial DNA damage which was below 0.1 UVB minimal erythema dose (MED), which is regarded as safe.

References

P. Zwicker, J. Schleusener, S.B. Lohan, et al.; Application of 233 nm far-UVC LEDs for eradication of MRSA and MSSA and risk assessment on skin models; Scientific Reports 12(2022) 2587, DOI: 10.1038/s41598-022-06397-z
J. Schleusener, S.B. Lohan, L. Busch, et al.; Treatment of the Candida subspecies *C. albicans* and *C. parapsilosis* with two far-UVC sources to minimise mycoses in clinical practice; Mycoses, 2023; 66(1):25–2, DOI: 10.1111/myc.13521
D.F. Zamudio Diaz, A.L. Klein, M. Guttmann, et al.; Skin optical properties from 200–300 nm support far-UVC skin-safety in vivo; J Photochem Photobiol B: Biology 247 (2023) 112784, DOI: 10.1016/j.jphotobiol.2023.112784

P192 | Repetitive UVB exposure of keratinocytes to mimic the disease state of actinic keratosis: Evaluation of phenotype switch from KC to AK

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Actinic keratoses (AK) are prevalent skin lesions that develop primarily on skin areas frequently exposed to sunlight, such as the face, scalp, neck, and extremities. These lesions are considered precancerous and exhibit hyperkeratotic features due to the abnormal proliferation and differentiation of epidermal KC. The pathogenesis of AK is complex, involving multiple factors such as alterations in cell growth and differentiation, inflammation, oxidative stress, and impaired apoptosis. The primary risk factor for AK is extensive exposure to solar UV radiation, especially UVB rays, which induces mutations in the DNA of KC through the formation of thymidine dimers. A single AK lesion can follow one of three potential outcomes: stability, spontaneous regression, or transformation into an invasive cutaneous squamous cell carcinoma (cSCC). The probability and rate of progression from AK to cSCC vary greatly between individuals and are difficult to predict. Therefore, it is essential to understand the molecular mechanism behind this transition to develop preventive therapies for AK and cSCC.

To the best of our knowledge, there is only one AK cell line commercially available, which, due to customs regulations, is currently not shipped to Europe. Therefore, the aim of our current study is to generate a novel AK-like cell line for further mechanistic research on the basis of human immortalised HaCaT KC, an established cell line representing UV radiation-damaged epidermal KC. HaCaT cells were repetitively (3×, 9×, 15×) exposed to an apoptosis-initiating dose of UVB radiation (150 J/m^2). Between the irradiation cycles, cells were allowed to recover for up to one week. To monitor the conversion of the KC to an AK-like but non-malignant phenotype, we subsequently analysed cell proliferation, apoptosis, migration, invasion and markers of epithelial-to-mesenchymal transition (EMT). The data derived from the 3× and 9× irradiated cells revealed no differences in proliferation but apparently, the 9× irradiated cells lost their contact inhibition. To assess the potential acquirement of apoptosis resistance, we exposed the cells to 0 and 200 J/m^2 of UVB. In fact, we observed a roughly 50% reduction in UVB-induced apoptosis in the 9× exposed cell population as compared to UVB-exposed parental cells. Results from wound healing assays, measuring cell migration, indicated that the average closure velocity was comparable to control cells. Next, we performed a Matrigel spheroid invasion assay and monitored the spheroids derived from the different HaCaT populations for up to 9 days. In contrast to cancer cell-derived spheroids, none of the tested HaCaT spheroids invaded through the Matrigel, arguing against a malignant transformation of the repetitively UVB-exposed KC populations. This was confirmed by qPCR analysis, showing that neither the 3× nor the 9× exposed cells exhibited an elevated expression of invasion-associated matrix metalloproteases (MMP1, MMP3, MMP10, and MMP13). However, we noticed a slight upregulation of genes associated with EMT (SNAIL, TWIST1) in the 9x irradiated HaCaT KC. These data suggest that the applied protocols for repetitive UVB exposure are effective in terms of inducing phenotypical and functional alterations, but are not sufficient to induce full malignant transformation. To confirm this assumption, we performed a bioinformatic analysis using available RNA sequencing data in the literature and short listed the signature genes that are specifically up- or downregulated in 1). AK lesions but not in healthy skin or cSCCs, or II). cSCCs but not in AKs or healthy skin. We compared the expression profiles of the identified signature genes between the repetitively exposed HaCaT cell populations (3×, 9×, 15×) and parental HaCaT KC, normal human epidermal KC and two cSCC cell lines (SCL-1 and A431). Our findings so far indicate that by applying our irradiation protocol it is possible to transform human KC into AK-like cells. Of course, further investigations, especially comparative analyses with corresponding patient material, are necessary to validate the generated cell line(s) as reliable in vitro test model for AK.

P193 | Sensory re-innervation in human skin ex vivo influences extracellular matrix remodelling in photoaging via the interaction with mast cells

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Photoaging, caused by prolonged exposure to sunlight, negatively impacts the skin's structure and appearance by reducing its elasticity and increasing its fragility. This condition is characterised by the build-up of elastic fibres and alterations in the extracellular matrix (ECM) structure, known as elastotic skin areas. Mediators released by cutaneous mast cells significantly influence ECM remodelling. Notably, mast cells are more abundant in elastotic skin areas, and their functions, in addition to impacting the ECM, are closely associated with nerve fibres. This study aimed to investigate the influence of sensory cutaneous nerve fibres on skin structure and to examine the role of mast cell-nerve fibre interactions in this process, utilising our re-innervated human skin culture model. Therefore, skin biopsies obtained from four healthy donors were cultured for seven days, either alone in co-culture with in vitro differentiated human induced pluripotent stem cell (iPSC)-derived sensory neurons. Chronic sun exposure was mimicked by 100 mJ/cm^2 UV-B irradiation, primarily targeting the nerve fibres in the epidermis. Subsequent analyses included quantitative (immuno)histomorphometry and RNA sequencing (RNAseq). Tryptase immunostaining showed increased mast cell degranulation upon UVB exposure, significantly in the presence of cutaneous nerve fibres. Transcriptomic analysis identified differential regulation of neuronal genes involved in ECM remodelling/ composition, neural migration, and synaptogenesis, specifically in re-innervated skin upon UVB exposure. Additionally, we noted elevated levels of elastin and fibrillin-I in the papillary dermis, along with a more heterogeneous and disorganised fibre structure, following UVB irradiation, particularly in re-innervated samples. Our

preliminary findings suggest that sensory re-innervation may enhance mast cell activation, leading to altered ECM remodelling and the accumulation of dysfunctional elastic fibres in the dermis. Targeting the interaction between mast cells and nerve fibres could thus provide a novel approach to mitigate and manage skin damage associated with photoaging.

P194 | The role of UV-induced cutaneous matrix metalloproteases and mi-RNAs in the pathogenesis of lupus erythematosus

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Cutaneous (CLE) and systemic lupus erythematosus (SLE) are autoimmune diseases with multifactorial pathogenesis. Ultraviolet (UV) radiation is the most important trigger of CLE, and is known to cause DNA damage and cell apoptosis, especially in the upper layers of the epidermis. However, there is variation in the degree of photosensitivity depending on clinical subtypes.

The modulation of matrix metalloproteases (MMPs), important enzymes involved in skin turnover and homeostasis, has often been associated with UV exposure. MMP28, a novel member of the MMP-family, has been shown to influence keratinocyte turnover and wound healing but its reaction to UV radiation and potential involvement in LE have not been thoroughly studied as of now.

UV radiation not only induces altered expression of MMPs but also affects micro- RNAs (mi-RNAs) - a group of short, non-coding RNA molecules that are involved in post-transcriptional control of gene expression. Among those, mir-31 and mir-150 are especially interesting since irregularities in their expression have been observed in LE samples compared to healthy skin. The UV-regulated mir-31 is involved in a variety of cellular processes, including the mediation of inflammatory cytokines (like IL-1β, IL-12, and IL-8) and glucose metabolism involving GLUT1. As for mir-150, its downregulation has been shown to promote keratinocyte proliferation under hypoxic conditions.

In the context of LE, an upregulation of GLUT1 has already been observed in patients with SLE. Inhibition of GLUT1 using T cell-specific knockouts or small molecules that inhibit glycolysis has already improved the phenotypes of various murine autoimmune disease models, such as arthritis, LE, and psoriasis. Deletion or inhibition of GLUT1 blocked T cell proliferation and effector function, antibody production from B cells, and reduced the inflammatory responses in macrophages.

To explore the causality of clinically observed effects of UV radiation in lupus patients in this study, skin samples from patients with different subtypes of CLE and SLE were examined by immunohistochemistry for the expression of MMP1 and MMP28 and compared to biopsies from healthy skin and polymorphous light eruption (PLE). The expression of miR-31 and miR-150, regulators of MMP expression and cellular metabolism, was determined in the samples by in situ hybridization and correlated with the expression of the GLUT1 receptor to explore a potential metabolic regulation.

MMP28 expression was differentially affected by UVA1- and UVB-irradiation in keratinocytes and fibroblasts. MMP28 showed a distinct vertical distribution in Chilblain lupus erythematosus skin samples compared to all other CLE subtypes. This vertical expression pattern coincided with decreased GLUT1 levels and with increased expression of miR-31 and miR-150 in the epidermis of patients with Chilblain LE. This data presents evidence for possible metabolic dysregulation playing a role in the aetiology of LE, especially in the context of tissue reorganisation.

P195 | Exploring UVA1-induced metabolic effects in different in vitro, ex vivo and in vivo settings

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The skin is the largest organ of the human body, that shields and separates us from the outside world. In its function as both a physical and chemical barrier, the skin is exposed daily to a variety of stressors, among others ultra-violet (UV) radiation. UV radiation can cause damage to the skin, ranging from premature photoaging to photocarcinogenesis. On a molecular level, UV radiation exerts its effects via DNA damage, lipide, and protein oxidation, and by dysregulating the cellular metabolism.

In recent years, important knowledge of UV-induced metabolic changes in skin and skin diseases has been accumulated. With the development of new technologies, a variety of model systems have been implemented to achieve a deeper insight into the metabolic effects resulting from the interaction between UV radiation and the human skin.

In the current study, we are exploring the stability and reproducibility of UVA1- induced metabolic changes observed in different in vitro, ex vivo, and in vivo systems with escalating complexity. In vitro, consisting of cell monolayer cultures of primary fibroblasts and immortalised keratinocytes; ex vivo, consisting of donor skin tissue kept under culturing conditions; and in vivo, where we applied a novel, minimally invasive approach to collecting metabolic data in real-time from the skin of healthy volunteers.

Our data show that, despite great metabolic variations between in vitro, ex vivo, and in vivo systems, several metabolites, namely glutamic acid, lactic acid, pyroglutamic acid, succinic acid, threonine, and tyrosine, change in a similar manner across systems after UVA1 irradiation. Some metabolites like glucose and pyruvic acid show similar UVA-mediated metabolic patterns in vitro and ex vivo but not in vivo. Other groups of metabolites, for example, creatinine, phosphoric acid, and threonic acid, could only be detected in in vivo samples.

With the presented data we would like to raise awareness for the importance of careful consideration when choosing the type of experimental system for assaying UV-induced metabolic changes. As we show, the data acquired in vitro, ex vivo, and in vivo cannot be considered unequivocally true or false based on the observed diverging metabolic profiles. Rather, we show that the interpretation of any observed effects should take into

account the type of experimental setup used. Parameters such as donor variability (age, sex, lifestyle, genetic predisposition, medication, accompanying diseases), experimental influences (timepoint of tissue resection and sample collection, duration of treatment, etc.), and system complexity (metabolically open/closed system, cell immortalization, 3D vs. monolayer) should be considered when performing data evaluation.

P196 | Investigation of stress induced metabolic changes in the progeroid Cockayne syndrome (CS) revealed metabolic dysfunctions in CS cells

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Cockayne syndrome (CS) is a rare genetic disease with progeroid symptoms, like, progressive severe neurological defects and UV sensitivity with no efficient treatment up to now. The investigation of metabolic changes, which are associated with aging processes or stressors, can increase the knowledge of the complex processes leading to aging of cells and organisms.
Previous results have shown that exposure of human skin cells to stressors like UVA irradiation or reactive oxygen species (ROS) leads to significant changes in the cell metabolism, especially in the glucose metabolism. The high levels of UVA induced consumption of glucose and pyruvate could be involved in ROS detoxification strategies of these ROS treated cells. Furthermore, it has been shown, that CS cells can exhibit higher levels of cellular ROS damage than WT cells.
In this study, we investigated the impact of stressors (ROS) on the metabolism and oxygen consumption in primary human skin fibroblasts derived from CS patients with the premature aging syndrome or from healthy individuals (WT). These cells were exposed to repetitive low dose UVA irradiation (inducing ROS) with subsequent measurement of cellular oxygen consumption using a Clark type electrode and metabolic changes in the supernatant of the cells, using nuclear magnetic resonance spectroscopy (NMR).
UVA irradiation induced significant changes in many metabolites (glucose, lactate, pyruvate, glutamine, glutamate, choline, alanine, betaine, acetate) in the cellular supernatant. Similar to WT cells, CS cells showed UVA induced higher glucose and pyruvate consumption, as well as higher lactate and alanine secretion. Interestingly, metabolic differences between WT and CS cells are already present without external stressors (UVA irradiation) and many of these differences increase upon UVA treatment. Looking at cellular respiration, differences in the oxygen consumption rate, between CS and WT cells are visible without external stressors. WT cells show a higher oxygen consumption rate than CS cells. The application of stressors (UVA irradiation) enhances these differences between WT cells and CS cells.
These results show differences in metabolism and respiration between CS cells of patients with premature aging symptoms and WT cells. It is known, that, under specific conditions, processes of cellular respiration within the respiratory chain can be a source of ROS. Therefore, it can be speculated, that CS cells try to exploit metabolic ROS detoxification strategies associated to glycolysis (higher glucose and pyruvate consumption than WT cells) and reduce ROS production during respiration (lower respiration rate than WT cells).

Pruritus

P197 | Transcriptomic landscape of chronic nodular prurigo

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Chronic nodular prurigo (CNPG) is a subtype of chronic prurigo characterised by persistent itching and repeated scratching, leading to the formation of multiple itchy nodules. Despite the significant negative impact CNPG has on quality of life, there are currently few effective therapies available. Gaining a deeper understanding of the signalling pathways and genes involved could help identify and validate potential therapeutic targets.
In this study, we systematically analysed the cutaneous expression profiles of pruritic/lesional (PL) and non-pruritic/non-lesional (NPNL) skin from 36 CNPG patients. We also included 24 healthy control subjects (HC) for comparison. Biopsies were taken after physical examinations and the completion of pruritus-specific and related symptom questionnaires (such as assessments of quality of life, anxiety, or depression). RNA was isolated from the samples and analysed using NGSbased mRNA sequencing. Differentially expressed genes (DEGs) were identified using DESeq2, with a threshold of shrunken log2 fold change >1 and s-value <0.01. Pathway analyses were then conducted using the Reactome online database.
Principal component analysis (PCA) successfully differentiated PL from NPNL and HC, indicating distinct expression profiles in lesional skin. Comparison of gene expression between the tissues showed a substantial number of DEGs between PL and NPNL ($n = 2616$) and HC ($n = 2882$), with significant overlap between these comparisons ($n = 1449$). In contrast, only 764 DEGs were identified between NPNL and HC. Of the 1449 overlapping DEGs, 156 were linked to pruritus, 80 to inflammatory responses, and 19 to neurogenic inflammation. Initial pathway enrichment analysis revealed several pathways enriched in PL skin, including cornified envelope formation and Interleukin signalling (IL4/13). Ongoing analyses aim to further explore these expression profiles, potentially enhancing the understanding of the molecular and pathway-level mechanisms underlying CNPG pathology.

P198 | Optimising chronic pruritus assessment: Leveraging machine learning and targeted questionnaires for enhanced precision

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Pruritus is a common dermatological symptom, affecting about one-third of patients who attend to a dermatologists. Chronic pruritus (CP) often persists despite treatment, with 62% of patients reporting it as their primary complaint. Due to its multifactorial causes, diagnosing CP requires an interdisciplinary approach including taking a detailed history and using patients reported outcome measures (PROMs). Machine learning enhances diagnostic accuracy by analysing complex datasets. In this study, spanning March 2012 to October 2023, we examined 9989 retrospective and 1821 prospective patients using hospital data and PROMs. Missing data were imputed, and feature selection employed dimensionality reduction techniques like PCA and SVD. A CatBoost classifier was coupled with SHAP values in order to enable a feature importance analysis and to increase the model's explainability. The models' robustness during training was determined using a 5-fold cross-validation on the retrospective data and achieved AUROC scores between 0.89 and 1.00, with balanced accuracies averaging 90.3%. Age, scratch lesion type, and localization of itch had the strongest effects on classification, while gender and height had minimal impact. The PROMs ItchyQoL and Neuroderm were more predictive than Hospital Anxiety and Depressions Scale, Dermatology Life Quality Index, and Patient Need Questionnaire. Excluding the least informative items led to a slight drop in performance. Validation on prospective data showed a minor decrease in balanced accuracy from 90.3% to 88.5%, with AUC remaining stable. This study demonstrates that machine learning models can effectively classify pruritus subtypes using key features. Excluding less informative items improved diagnostic focus, confirming their limited utility. Prospective validation showed consistent performance, indicating that refined items can enhance pruritus assessment while maintaining predictive power. Although machine learning aids classification, clinical expertise remains critical for accurate diagnosis, improving precision and reducing patient burden.

P199 | Pruritus and neuroanatomy-related gene expression patterns compared across common inflammatory skin conditions

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Pruritus presents a major burden in several inflammatory skin conditions that stem from diverse immunopathological backgrounds. Intensive itching not only has a profound effect on patients' quality of life, but also stimulates behaviours that may exacerbate barrier damage and complicate therapeutical interventions, resulting in vicious feedback cycles. On the other hand, neither the molecular mechanisms nor the neurophysiological alterations behind itch are well understood. Here, we present a multi-layer transcriptomic analysis of a large cohort of atopic dermatitis, lichen planus and psoriasis patients, alongside age- and sex-matched healthy controls. Using RNA sequencing of full thickness biopsies, we define transcriptomic patterns correlated to various aspects of skin inflammation. A combination of unsupervised clustering, linear modelling and spline fitting approaches is used to cross-correlate several distinct data types. These include the detailed clinical characterisation, intraepidermal nerve fibre quantitation and electrically stimulated itch ratings gathered from all conditions. Furthermore, as functional enrichment and pathway analysis is lacking in ground truth information for neuronal and innervationrelated genes, we compile a custom knowledge base for these factors. On the level of cross-disease comparisons, we define disease-specific and shared pathways, as well as gene sets correlated with clinical measures of severity and pruritus. Using immunohistological data on intraepidermal nerve fibre density, a set of highly neuron-specific genes is defined that show strong correlations with innervation of the skin. Moreover, investigating the response of atopic dermatitis patients to electrically stimulated itch on the transcriptomic level hints at subtle changes in the expression levels of neuronal cell surface receptors, but also inflammatory mediators. This suggests a role of local sensitization in patients with highly inflamed lesions. Importantly, genes related to neuronal survival, morphology or sensitization are still scarcely characterised and often only in the central nervous system, but not in the periphery. Thus, our gene sets that correlate to specific facets of pruritic inflammation may serve as valuable candidates for in-depth functional characterisation and may present novel therapeutical targets. Altogether, our findings provide a transcriptomic map for the complex interplay between neuroanatomical, physiological and inflammatory mediators that combine to create a pruritic skin environment.

Skin Biology and Tissue Remodelling

P200 | Medical gas plasma as a modulator of the Nrf2 signalling pathway in diabetic wound healing

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Diabetes mellitus is a chronic disease that can disrupt physiologic wound healing by reducing levels of healing factors or by

impairing the activity of signalling pathways. This may contribute to chronic inflammation, poor neovascularization, and delayed re-epithelialization. Previous studies have shown that increasing the transcriptional activity of the redox regulator erythroid-related factor 2 (Nrf2) in diabetic models can improve insulin sensitivity, reduce blood glucose levels, and ameliorate diabetic complications. To this end, full-thickness dermal ear wounds were created in preclinical models of type I (e.g., streptozotocin (STZ)-induced) and type II diabetes and compared with their native background SKH1- and C57BL/6 strains. Transcriptome profiling was performed in female and male mice at two wound healing stages (days 9 and 20 post-wounding) in type I diabetic mice. In addition, we performed gene and protein expression analyses using immunohistological and—chemical staining of various targets as well as quantitative PCR (qPCR) and protein analysis using WES in all mouse models. Our study showed that repeated in vivo treatment with medical gas plasma supported wound healing, e.g. re-epithelialization, in both sexes. However, diabetic wounds in females healed better than plasmatreated wounds in males suggesting sex-specific differences. Hyperspectral imaging demonstrated that gas plasma therapy altered microcirculatory parameters, particularly oxygen saturation levels during wound healing, presumably due to the gas plasma's tissue delivery of reactive species and other bioactive components. An anticipated stimulation of blood vessel formation was demonstrated via transcriptional and translational increases of angiogenic factors in gas plasma-exposed wound tissue. Our study further evaluated the cellular regulation of key targets of the Hippo and related pathways such as YAP/TAZ, β-catenin, tumour growth factor β, and oxidative stress signalling after plasma treatment. Gas plasma-stimulated changes in Nrf2 signalling in conjunction with downstream targets were supported by the evidence of an impaired wound healing in Nrf2 knockout mice. In addition, gas plasma treatment significantly affected inflammation by modulating local and systemic cytokine levels as well as granulation and extracellular matrix components by stimulating collagen synthesis. Proper regulation of the interplay between focal adhesions and reactive species generated by gas plasma was critical for various cellular processes, including adhesion, and migration as seen in diabetic wounds. In vivo treatment of human diabetic wounds validated the involvement of Nrf2 signalling in protection against the oxidative stress as seen by qPCR analysis. The present finding of accelerated wound healing by the Nrf2 activator gas plasma underscores the importance of gas plasma-assisted diabetic wound therapy in clinical trials.

P201 | Reference mapping of electrical impedance spectroscopy in healthy individuals: An atlas for assessing skin barrier function across clinically relevant body areas

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Background and Aim: Many skin disorders, such as atopic dermatitis and psoriasis, are associated with an impaired barrier function. Several studies have shown that electrical impedance spectroscopy (EIS) was effective in detecting skin barrier defects in patients with atopic dermatitis. EIS measures the resistance of the skin to alternating electrical current at various frequencies, and the values correlate with the severity of skin barrier dysfunction. The EIS MIX value at the volar forearm is used as a reference value. Lower MIX values indicate greater degree of the skin barrier disruption. However, skin lesions can vary across different body areas and the values can potentially be influenced by factors such as skin hydration, epidermal thickness, and the underlying skin structures. There is limited knowledge of normal EIS variations across anatomical sites. Therefore, we aimed to create a comprehensive atlas of EIS measurements in healthy individuals for multiple clinically relevant body areas, providing a reference for assessing MIX values in patients with pathological skin conditions.
Methods: We recruited 39 healthy patients for EIS measurements at 24 relevant anatomical locations. For each location, a mean MIX value with standard deviation was calculated. Differences in the mean MIX values were tested using the one-way ANOVA.
Results: We identified differences in mean MIX values across eleven anatomical locations. These locations were grouped into three categories based on their MIX values relative to the volar forearm. Higher MIX values were observed in the axillary fossa, peri-umbilical region, and posterior cervical region. Intermediate MIX values were found in the deltoid region, fossa poplitea, the area above the malleolus medialis and the plantar foot. Lower MIX values were seen in the frontal region, area zygomatica, dorsal hand, and pre-tibial region.
Conclusion: EIS MIX values varied across different body sites. Compared to the volar forearm, higher MIX values were observed in areas with underlying soft tissue, while lower values were found in regions with underlying bony structures. This suggests that the type of tissue beneath the skin may affect MIX values. These results highlight the usefulness of the reference values at different anatomical locations.

P202 | Gas plasma-enhanced transdermal drug delivery is paralleled by partial skin lipid and protein expression and oxidation modifications

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Gas plasma technology represents a promising option for tackling skin-related diseases, e.g., chronic wound care (established) or actinic keratosis (experimental level). A combination of reactive oxygen species and electrical fields stimulate physiological responses, including cell signalling via JAK/STAT, Nrf2, and HIPPO pathways. However, the immediate consequences of

gas plasma treatment of skin and potential changes in the uptake of pharmacological substances are not clear. To this end, we investigated gas plasma treatment (kINPen, 1 to 5 min cm^{-2}) responses, including drug uptake, in porcine skin ex vivo, as its microanatomy and molecular composition closely reflect human skin. Intriguingly, fluorescence microscopy and mass spectrometry determined enhanced penetration of curcumin (+160%) and ibuprofen (+40%) into the stratum corneum. In contrast, tissue sections collected to assess histology (HE) and cell viability (TUNEL assay) showed no major changes. Next, individual layers of treated skin were then collected by tape stripping and prepared for layer-wise mapping of the lipid profile by UHPLC-HRMS. Ceramides resembled the most dominant lipid class. Alterations remained scarce except for signs of hexosylceramide cleavage and hydrolysis of sebum triacylglycerols. Using the same sampling approach, protein expression profiling was performed by nanoLC-HRMS with an emphasis on biomolecule oxidation. Most of the ≅1900 proteins and some of the oxidative modifications (e.g., methionine oxidation, disulfide oxidation) detected were present in all stratum corneum layers. These data suggest a (transiently) reduced barrier function of the skin, explaining the observed penetration increase, qualifying medical gas plasma technology as a potential amplifier of skin ointments and drug penetration in human skin.

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P203 | Modelling reactive species penetration in hydrogels and human skin

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Cold physical plasma is a partially ionised gas operated at body temperature and generating vast amounts of reactive oxygen and nitrogen species (ROS). Medical gas plasma technology is regularly applied in German University Hospitals across the country to promote wound healing and other dermatology treatments [1]. In addition, there is a keen interest in cancer treatment and ROS damageinduced immunostimulation, with first SCC patients having benefited from palliative medical gas plasma therapy [2]. The latter's main mechanism of action is the deposition of various ROS in a spatio-temporally confined manner [3]. Due to the lack of straightforward research tools for following the trajectories of ROS into tissues, it is, however, challenging to identify the main ROS types responsible for biomedical effects as well as their penetration into target tissues and dependencies of tissue-resident biomolecules. First results have shown an incapacity of the ROS to penetrate through the skin, especially in terms of plasma parameter variations. These include, for instance, gas plasma treatment time, distance to the target (either being in direct contact, i.e., conductive, or not [4]), the composition of the plasmaproduced ROS (modifiable via modulating the plasma feed gas composition), and other physico-chemical parameters such as temperature and leakage currents. To this end, we employed hydrogels as tissue surrogates to address such questions experimentally and quantitatively. Moreover, a range of biomolecules, such as albumin, immunoglobulins, and antioxidants, was added to the agarose loaded with reactive species indicators to study the dependencies of ROS penetration. Also, hydrogel thickness was considered. Human skin was used on top of the hydrogel to simulate and assess ROS diffusion under, e.g., conductive plasma exposure. It was found that gas plasma treatment time and distance-to-target play a major role in introducing ROS into targets, while the target's composition, e.g., type and density as well as content (e.g., biomolecules and antioxidants) critically affects ROS penetration.

References

1. Bekeschus, S., et al., Medical gas plasma-stimulated wound healing: Evidence and mechanisms. Redox Biology, 2021. 46: p. 102116.
2. Bekeschus, S., Medical gas plasma technology: Roadmap on cancer treatment and immunotherapy. Redox Biology, 2023. 65: p. 102798.
3. Bekeschus, S., Gas plasmas technology: from biomolecule redox research to medical therapy. Biochemical Society Transactions, 2023. 51(6): p. 2071–2083.
4. Miebach, L., et al., Conductivity augments ROS and RNS delivery and tumour toxicity of an argon plasma jet. Free Radical Biology and Medicine, 2022. 180: p. 210–219.

P204 | Study of the effect of cigarette smoke on the skin and development of two techniques to analyse the protective properties of cream formulations

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Air pollution represents a significant global health issue, with the potential to cause serious health problems. It has been demonstrated to damage the lining of the lungs, affect skin health, and has been linked to the development of skin diseases. Despite the prevalence of smoking, each cigarette increases free radicals (oxidative stress), which the body must control with antioxidants. Oxidative stress has been linked to premature skin aging, a disturbed skin barrier, pigment disorders, and cell damage. At present, there is no established method for accurately assessing the impact of pollution on the skin or determining the effectiveness of protective substances. In order to address this gap in knowledge, a smoke chamber has been designed for ex vivo and in vivo research. This chamber is used to expose samples of porcine ear skin or the forearm of a volunteer to cigarette smoke under reproducible conditions. Furthermore, the smoke chamber allows the skin to be exposed to varying concentrations of nicotine in a reproducible way.

The use of electron paramagnetic resonance (EPR) spectroscopy with the spin probe PCA represents a promising approach for evaluating the potential risks associated with air pollution on excised skin. Furthermore, the impact of combined smoke and UVA radiation was investigated. To evaluate radical formation, the autofluorescence in the skin, as an indicator of oxidative stress, was quantified using a confocal raman microspectrometer, which can be applied ex vivo and in vivo without

the necessity for additional markers or external stressors. Initial studies demonstrate that cigarette smoke induces an increase in free radicals in the skin, with a positive correlation observed between nicotine concentration and free radical production.
The testing of two antioxidants and a chelating agent in a base formulation and a commercial product, conducted ex vivo, demonstrated that the antioxidant epigallocatechin-3-gallate (EGCG) in the base cream formulation provided superior protection against oxidative stress induced by smoke exposure, irrespective of the technique employed.

P205 | Iron overload and WNT signalling dysregulation drive lipodermatosclerosis progression

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Chronic venous insufficiency often leads to iron overload in the skin, resulting in lipodermatosclerosis, a condition associated with a high risk of leg ulcers. This study investigates the pathological mechanisms underlying lipodermatosclerosis, highlighting iron overload as a key driver. We developed a novel mouse model of local skin iron overload, which mirrors human lipodermatosclerosis, exhibiting iron deposition. In parallel, we conducted in vitro experiments on human cells to understand underlying mechanisms and validate results found in the animal model.
Using a combination of immunofluorescence staining, gene expression and protein analyses, besides in vitro approaches, we demonstrate that iron-induced dermal adipose tissue lipolysis and the release of chemokines contribute to monocyte-driven inflammation. Iron overload also repressed Wnt signalling (confocal microscopy, qPCRs), resulting in decreased expression of pro-adipogenic genes and adipose stem cell loss as shown by multicolour flow cytometry. Additionally, functional studies confirm that iron overload induces oxidative stress while impairing adipogenesis. scRNA Sequencing and flow cytometry analyses reveal changes in the dermal fibroblast populations from iron mice versus control. Dysregulation of Wnt activation in the dermal compartment was associated with disrupted ECM homeostasis and defective hair follicle growth. Collectively, our findings identify iron overload and Wnt signalling dysregulation as critical factors driving dermal fat loss and extracellular matrix dysfunction in lipodermatosclerosis, suggesting potential therapeutic strategies to prevent venous ulcers and reverse tissue fibrosis.

P206 | In vitro evaluation of the cytotoxicity and antimicrobial efficacy of silver- containing nanofiber wound dressings

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Infected chronic wounds pose a significant challenge to both the patient and the healthcare system. In order to prevent and to treat microbial infections and hence to foster the healing process, silver coated antimicrobial wound dressings are commonly used. However, besides the antimicrobial efficacy, silver may also exhibit cytotoxic effects on human cells. Against this background, this study investigates the biocompatibility and antimicrobial efficacy of silver containing nanofiber fleeces. The goal is to compare different structures of electro-spun degradable poly-p-dioxanone (PPDO) fleeces with varying concentrations of silver nanoparticles. Furthermore, three commercially available wound dressings containing silver were included as a reference. Three different cell lines were treated with extracts of the different materials. A significant decrease in metabolic activity (XTT assay), proliferation (BrdU assay), as well as in cell membrane integrity (LDH assay) confirmed the cytotoxicity of silver-containing wound dressings. A PPDO nanofiber fleece without any silver had only a minor effect on the metabolic activity and proliferation of treated cells and the raw material (PPDO granulate) had no effect. This confirms that the observed in vitro cytotoxicity can be largely attributed to the silver components. Ongoing investigations are focusing on the suitability of PPDO as a basic material for silver-containing wound dressings as well as on modifications of the silver-containing nanofiber fleeces in order to reduce cytotoxicity while maintaining antimicrobial properties.

P207 | The adaptive response of S100A8/A9 primed MSC enforces CAP-independent translation with proteins protecting the dermal stem cell niche

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Mesenchymal stromal cells (MSC) play a prime role in tissue regeneration. MSC have entered clinical routine as an approved treatment modality for difficult-to-treat wounds. S100A8/A9 belonging to the family of danger associated molecular patterns (DAMPs), is released from native immune cells during tissue damage. It alarms other immune cells and—currently unexplored—may protect stem cell niches after injury. S100A8/A9 binds to TLR4 and RAGE receptors on MSC and enforces an adaptive response of MSC. Previously, S100A8/A9 primed MSC were reported to accelerate wound healing in mice. However, the underlying mechanisms remain unknown. We here set out

to explore the S100A8/A9 induced adaptive response in MSC employing a multiomics approach with transcriptome and proteome analyses. Transcriptome analysis depicts a significant increase in translation initiation factors such as EIF3I, EIF4A1, EIF4G2 and EIF2A in S100A8/A9 primed MSC. On protein level, we observed that at early time points (6 h and 12 h), the cap-dependent translation related factor EIF4A1 was upregulated, while it was downregulated at a later stage (48 h). By contrast, the cap-independent translation related factor EIF3I was highly upregulated at 48 h after S100A8/A9 priming. These data indicate a possible switch in S100A8/A9 primed MSC from cap-dependent translation to cap-independent translation which is less ATP demanding. S100A8/A9 primed MSC depict enhanced protein synthesis after priming with S100A8/A9 for 48 h, however, without any increase in ATP production as indicated by Seahorse analysis. Liquid chromatography-mass spectroscopy-based proteomics uncovered profoundly enhanced synthesis of collagen processing enzymes and interstitial collagens (type I, III, VI), the most important structural components of the stem cell niche. In addition, proteins involved in autophagy such as ATG9B and PEX5 which protects cells and enhances survival in a hostile environment, proteins regulating proliferation, cell migration and adhesion such as ACP1, LSM2, and proteins involved in inflammation control such as DBN1, NT5C3A were all upregulated in S100A8/A9 primed MSC. Apart from the analysis of S100A8/A9 primed MSC lysates, antibody arrays and ELISA were employed to further explore the difference in the secretome from S100A8/A9 primed and unprimed MSC. We found the angiogenesis promoting angiopoietin-1 and the cell protecting cystatin C to be significantly increased in S100A8/A9 primed MSC, likely protecting MSC under hypoxic stress conditions and enhancing angiogenesis in their endogenous niche.
In conclusion, S100A8/A9 primed MSCs likely switch from cap-dependent translation to cap-independent translation which is favourable for cells exposed to stress conditions. Our data suggest that cap-independent translation of selected mRNAs induces a defence and protective gene program. In fact, S100A8/A9 primed MSC expressed more collagens, angiopoietin-1 and cystatin C, and proteins of inflammation control which contribute to the structurally shaped and sufficiently vascularized endogenous MSC niche, which together with other proteins released in the defence response enhance wound healing as earlier observed. These data may hold promise in the long term to refine already existing MSC-based therapies in clinical routine for the treatment of acute and chronic wounds.

P208 | Endothelial ferroptosis and fibroblast senescence in diabetic angiopathy

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The prevalence of obesity and diabetes mellitus is increasing globally causing huge socioeconomic and clinical burden. Non-healing chronic wounds are serious complications of obesity and diabetes. Impaired angiogenesis is thought to be the leading cause of these non-healing wounds, although the underlying mechanism is poorly understood. To acquire further pathogenic insight and to address the impact of fatty acids such as palmitate and oleate—both increased in obesity and type 2 diabetes (T2DM) - we here set out to study angiogenesis and vessel damage under diabetic condition, employing an array of in vitro and in vivo experiments.
Interestingly, both palmitate and oleate profoundly impaired angiogenesis in a concentration-dependent manner in the in vitro tube formation assay. This assay required the interaction of endothelial cells and stromal fibroblasts, the latter providing the scaffold and secretory factors for differentiation of endothelial cells for vessel formation. When exposed to palmitate and oleate, expressions of p21 and p27, cell cyclin inhibitors indicative of cellular senescence, were increased along with higher senescent associated beta-galactosidase activity in fibroblasts, while Ki67 indicative of cell proliferation was decreased. Interestingly, palmitate and oleate induced cell death of endothelial cells, initially through caspase-3 dependent apoptosis, but at later stage and at higher concentrations through ferroptosis. Of note, palmitate and oleate significantly increased the expression of pro-ferroptotic factors, including SLC11A2 (iron absorption), NCOA4 (ferritinophagy mediation), KEAP1 (inhibit cytoprotective gene expression). In addition, the concentration of intracellular free iron, reactive oxygen species and products of lipid peroxidation increased, likely overwhelming the antioxidant defence. Along this line, reduction of GPX4 and GSH/GSSG ratio was found in ferroptotic endothelial cells. Similar to human diabetic wounds, a severe reduction of vessels in full thickness wounds of diabetic mice (db/db) was observed. Interestingly, immunostaining of sections from uninjured and wounded skin of diabetic mice showed less GPX4 expression in endothelial cells, while increased p27, p21 and decreased Ki67 expression was detected in dermal skin fibroblasts.
In aggregate, our findings suggest that T2DM/fatty acids induce endothelial cells cell death through apoptosis and late onset ferroptosis and concomitantly induce fibroblast senescence which suppress proliferation, migration, sprouting of endothelial cells and, hence, new vessel formation. Our findings hold promise for the development of new strategies for advanced treatment of difficult-to-treat diabetic wounds.

Withdrawn

P210 | Fibroblast-derived TGF-beta1 regulates skin repair and fibrosis

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During wound repair, activation of fibroblasts and their conversion to myofibroblasts are key for granulation tissue formation.

Equally, in fibrotic reactions, activated fibroblasts and their excessive deposition of ECM are central in determining scar formation and functional failure. While a plethora of growth factors and cytokines are involved in the regulation of these repair and fibrotic reactions, TGF-beta1 plays a pivotal and unique role by controlling the immune response and proliferation of many cell types. Its release and activation are governed by several different cell types and, notably, by both the interaction with ECM and mechanical forces.

Our previous work has demonstrated that fibroblasts release TGF-beta1 through an unconventional secretion mode. We also showed that an autocrine loop involving TGF-beta1 is essential for fibroblast activation. In this study, we aimed to investigate whether fibroblast-derived TGF-beta1 is a key contributor during fibrotic processes and wound repair in vivo. The data show that TGF-beta1 is dynamically expressed during tissue repair. Cell-specific ablation of Tgfb1 in fibroblasts resulted in the attenuation of bleomycin-induced skin fibrosis and delayed maturation of granulation tissue in skin wounds. Moreover, in early wound healing absence of fibroblastderived TGF-beta1 gave rise to vascular alterations, associated with altered ECM formation. This can be explained by paracrine regulation of endothelial cells or pericytes by fibroblast-released TGF-beta1 and by impaired expression of proangiogenic factors in fibroblasts lacking TGF-beta1.

Our findings demonstrate the central role of fibroblast-derived TGF-beta1 for early stages of tissue repair and fibrosis in the skin, while providing novel mechanistic insights.

P211 | Analysis of senescent cells in fresh-frozen human skin tissue using singlenucleus RNA sequencing

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The accumulation of senescent cells over time is associated with an increased risk of age-related diseases, including skin cancer and skin aging. In order to perform a risk assessment or to monitor the efficacy of a potential treatment or a dietary intervention targeting senescent cells, a reliable method to quantify senescent cells in skin tissues is needed. As only a small fraction of cells in the tissue are senescent, it is difficult to quantify them using conventional methods such as immunohistochemistry or RT-qPCR. Single-cell RNA sequencing (scRNA-seq) provides an excellent way to quantify different cell types and cell states, including senescent cells, in a cell population. However, cells in tissue need to be dissociated into single cell suspensions before they can be analysed by scRNA-seq. Therefore, the aim of this study was to generate single cell suspensions from fresh-frozen skin tissues and to quantify senescent cells of different cell types in the single cell suspensions. First, we generated single cell suspensions from fresh-frozen skin tissues using a Whole Skin Dissociation Kit (Miltenyi). However, only very few living cells could be recovered from the single cell suspension and the extracted RNA was of poor quality. Therefore, we next tried to generate single nuclei suspensions using Nuclei Extraction Buffer and Nuclei Micro Beads (Miltenyi). In contrast to the single cells, this approach allowed us to obtain a sufficient number of intact nuclei and a good RNA quality for subsequent single-nucleus sequencing (snRNA-seq). Additionally, we prepared three different primary fibroblast cell cultures from different donors and cultured them to a high passage to induces replicative senescence as a positive control. Based on ß-Gal staining, our estimate is that there are about 40% senescent cells in these cultures. The BD Rhapsody system followed by transcriptome sequencing was used for single cell/nucleus analysis. These analyses revealed that senescent cells from fresh-frozen skin tissues can be quantified using a snRNA-seq approach. Taken together, while single-cell sequencing was not feasible with cells from fresh-frozen skin tissue, we successfully sequenced cDNA generated from single nuclei. Hence, snRNA-seq provides an elegant way to quantify senescent cells from fresh-frozen tissues.

P212 | Discovery of new potential therapeutic targets through comparative transcriptomic analysis of acute and chronic skin wound healing ex vivo

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Impaired, or chronic, wound healing significantly reduces the life quality of those affected and it places an immense burden on healthcare systems. Despite persistent efforts to fully discern the complex underlying processes, a significant knowledge gap remains. To contribute novel, mechanistic insights into wound healing processes and to potentially identify novel therapeutic targets, we here utilised established models of physiological (acute) and pathological (chronic) wound healing and delineated their respective longitudinal transcriptomic profiles. Central and partial wounds were generated in healthy full-thickness human skin punches from three donors. Skin punches were cultured under physiological or pathological (hyperglycemic, oxidative and hypoxic) conditions, to mimic acute or chronic wounds, respectively, and RNAseq analysis was performed after 1, 3 and 5 days ex vivo. Longitudinal comparative transcriptomics revealed differential expression of several known wound repair associated genes between acute and chronic wounds (KRT6A-C, PTX3, KRT1, KRT10, COL1A1). Gene set enrichment (GSE) analysis of gene ontology biological processes (GO-BP) terms

demonstrates an enrichment of woundhealing related pathways in both, acute and chronic wounds (e.g., Wnt signalling, ECM remodelling and actin cytoskeleton organisation), suggesting that our acute and chronic wound healing ex vivo models reliably reflect wound healing related processes. Next, we compared the transcriptomic profiles of chronic and acute wounds and found an overall downregulation of gene expression on days 3 and 5 in chronic compared to acute wounds. GSE analysis showed that chronic wounds have a reduced capability to perform key processes of proper wound healing, when compared to acute wounds, including ECM remodelling, Wnt-signalling, angiogenesis, immune responses, transcription & translational processes, epigenetic regulation, or dysregulated protein stabilisation & -folding. Further in-depth analysis of the transcriptomic data demonstrated a significant reduction in keratinocyte growth factor (FGF7) expression in chronic wounds over time, significantly increased expression of MMP10 in chronic wounds on all analysed days, and significantly higher osteopontin (SPP1) expression in acute wounds. As a proof-of-principle, we counteracted the expression of the three selected target genes. Preliminary data on topical administration of a MMP10-neutralising antibody demonstrate a significant increased wound tongue length in acute wounds ex vivo, while treatment with recombinant FGF7 did not exert any effect. Yet, their combined application significantly increased re-epithelization in both acute and chronic wounds. Next, we stimulated the osteopontin pathway by topically treatment with the osteopontin-derived peptide, FOL-005, which contains a similar active site (RGD-domain). Treatment with FOL-005 significantly promoted skin re-epithelization ex vivo in both conditions. Thus, our wound healing ex vivo models show great potential to further investigate underlying mechanisms of non-healing wounds and as novel, pre-clinical drug development tool. Additionally, we show that combinational therapy should be considered for the management of wound healing and demonstrate the therapeutic potential of FOL-005 to improve pathological wound healing.

P213 | Traumatic brain injury induces a novel defence program with faster healing of skin wounds

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Though Traumatic Brain Injury (TBI) and skin trauma often occur together in accidents, there is no information whether TBI changes the quality and velocity of the repair of concomitantly occurring skin wounds. We here addressed the questions whether TBI impacts on the sequence of cellular and molecular events during cutaneous wound healing, and whether it may even raise a coordinated adaptive defence response of the skin wound to protect from superimposed infections. For this purpose, incisional skin wounds from mice subjected to standardised TBI, skin wounds only at 1 and 7 days after injury and non-injured healthy murine skin were assessed employing unbiased transcriptome, and immunostaining for selected proteins. Transcriptome and gene enrichment analysis already at 24 h post injury, detects a profound increase of genes involved in leukocyte adhesion, migration, and corresponding chemokine receptor signalling. In addition, at 7 days post TBI skin injury or skin injury only pathways of Toll like receptor signalling indicative of innate immunity, of antigen processing and antigen presentation indicative of adaptive immunity as well as for the development of a cornified envelope (with enriched hits Cnfn, Lcela, Lcelk, Lor, ...), keratinisation and epithelial proliferation and differentiation (Krt10, Krt6a, Krt2, Wnt16, Krt17, Trp63, Krt84, ...) prerequisites for an effective reconstitution of the epidermal barrier was highly increased in the skin of the combined TBI skin wound group as opposed the skin wounds only. Of note, immunostaining with antibodies against different subpopulations of macrophages, we detected a significantly increased number of F4/80 positive pro-inflammatory M1 macrophages at day 1 post, while at day 7 Arginase positive regenerative M2 macrophages were significantly increased in combined TBI skin wounds as opposed to skin wounds only. These data likely indicate that the removal of wound debris carried out by M1 macrophages and the initiation of matrix deposition of M2 macrophages occurs faster and to a significantly higher extent in the TBI skin wound experimental group as opposed to samples of the skin wound only mice. Of note, numbers of M2 macrophages and other professional antigen presenting cells expressing the major histocompatibility complex (MHC) class II molecules to present processed bacterial antigens to CD4(+) T-lymphocytes are much higher in the combined TBI skin wound group when compared to skin wounds only. MHC class II molecules thereby initiate an adaptive antigen-specific T cell response. In fact, CD3 positive T cells enter skin wounds and at higher numbers at day 1 and are down-regulated already at day 7 post injury of the brain and the skin. We also unveiled the earlier and higher expression of differentiation keratins and proteins of the cornified layer with loricrin, involucrin, filaggrin and keratins which in a complex supramolecular structure form the microbicidal epidermal barrier. Of note, also in the dermis myofibroblasts responsible for wound contraction and tensile strength counteracting wound breaking, are highly increased in the TBI skin wound group as opposed to skin wounds only. In aggregate, we here detect four lines of microbicidal defence with a fast formation of an epidermal barrier, the combined barrier of native and adaptive immunity and a dermal barrier enhancing wound closure and stability. This defence is likely evolutionary optimised to protect from systemic infection to survive and repair TBI injury with the prospect of progenies.

P214 (OP03/05) | Establishment of a novel stem cell-based reconstructed human epidermis

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Background: Current in vitro skin models, such as the Reconstructed Human Epidermis (RHE) and Full Thickness Skin Equivalent (FTSE), provide a viable alternative to animal testing, for studying skin biology, disease mechanisms, and drug efficacy. Despite their utility, conventional models rely on keratinocytes and fibroblasts derived from donor skin tissue that have finite passage numbers, which complicates long-term studies and scalability. To address these limitations, our goal is to establish a novel stem cell-based skin model using cells isolated from human induced pluripotent stem cell (hiPSC)-derived skin organoids. With this, we aim to enhance standardisation and reproducibility of in vitro skin equivalents.
Objective: The primary goal of this study is to establish and characterise an hiPSCbased RHE by comparing the 3D models and their cells, isolated from three different sources: juvenile donors, adult donors, and hiPSC-derived skin organoids. We evaluate RHEs as well as keratinocytes and fibroblasts based on key functional parameters in order to validate the stem cell-based RHE's functional integrity.
Methods: Keratinocytes and fibroblasts were isolated from juvenile and adult donor skin, as well as from hiPSC-derived skin organoids. The isolated keratinocytes were used for building RHEs, and both 2D and 3D cultures were cultivated under similar conditions, respectively. Keratinocytes and fibroblasts were immunohistologically characterised for proliferation markers (Ki67), as well as differentiation potential (loricrin, vimentin, and alphaSMA), to distinguish different subpopulations. Cell viability was assessed by MTT assay. Furthermore, RHEs were analysed for barrier integrity via non-invasive impedance measurement. Histological H&E-staining, and immunohistochemistry (CK10, CK14, ZO1) were used to study stratification, differentiation, and cell morphology.
Conclusion: Through adapted media conditions we were able to isolate keratinocytes from hiPSC-derived skin organoids. Furthermore, our data suggests that hiPSCderived cells hold a higher proliferation potential compared to donor cells. The isolation of fibroblasts from skin organoids also opens the possibility of establishing an FTSE-model that mirrors human skin physiology even more closely than RHEs. In future experiments we are also working towards genetically modified skin organoids, for example with incorporated reporter systems like GFP, allowing for more dynamic and precise experimental designs. Overall, this novel model aims to improve the reproducibility and standardisation of dermatological studies, making it a valuable tool for both basic and applied skin research, while also aligning with the 3R principles (Replace, Reduce, Refine) by reducing the reliance on animal testing in preclinical studies.

P215 | The anti-aging effects of melatonin in human skin ex vivo include the induction of mTORC1-dependent epigenetic changes

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Cell and tissue aging appears to be closely associated with substantial epigenetic modifications. DNA methylation is a crucial epigenetic mechanism that silences gene expression. This process involves the addition of methyl groups to cytosine by DNA methyltransferases (DNMTs), resulting in the formation of 5-methylcytosine (5-mC). Conversely, DNA demethylation is driven by ten-eleven-translocation (TET) proteins, which convert 5-mC to 5-hydroxymethylcytosine (5-hmC), thereby reactivating or inducing the expression of previously silenced genes. This study explored whether these epigenetic modifications also occur during human skin aging ex vivo and how topically applied melatonin impacts these.
Full-thickness skin samples from 4 healthy women (43–75 years) were treated with topical melatonin (200 μM) in a serum-free, "speed-aging" human skin organ culture model and analysed by quantitative immunohistomorphometry. This revealed substantial epigenetic changes in the epidermis after six days of culture ex vivo: increased epidermal hypomethylation, as indicated by a significant decrease in 5- mC and DNMT1 expression. Moreover, the increased expression of the doublestrand DNA damage marker, γH2AX, was associated with the reduced expression of TET3, indicating a reduction of DNA damage repair—another hallmark of aging development. Interestingly, donors over 65 years old show a higher level of DNA damage (indicated by increased γH2AX expression) and a lower expression of DNMT1. Moreover, after six days of treatment, topical melatonin significantly increased the expression of 5-mC, DNMT1, and TET3 proteins, compared to vehicle-treated skin, indicating partial restoration of the DNA methylation and repair machinery. Furthermore, the impact of melatonin was stronger in the donors below 65 years old for 5-mC, while the impact on DNMT1 and TET3 was not agedependent.
Next, we asked if the aging-promoting mTORC1 pathway is involved in this epigenetic modulation and is responsive to regulation by melatonin. mTORC1 activity was stimulated by successfully silencing its key endogenous inhibitor, TSC2, with siRNA, as evidenced by significantly increased S6 phosphorylation. This was associated with enhanced DNA methylation (i.e., elevated 5-mC and decreased 5- hmC level). Notably, co-administration of 200 μM topical melatonin alongside TSC2 silencing led to hypomethylation, evidenced by a significant increase in TET3 and 5- hmC levels, as well as a reduction in DNMT1 and 5-mC expression.
These pilot data strongly suggest that human skin aging is characterised by global DNA hypomethylation, which could facilitate the overexpression of agingpromoting genes. Instead, anti-aging

genes and programs are silenced during this process, as suggested by the increased mTORC1-induced DNA methylation. The ability of topically applied high-dose melatonin to bypass rapid liver metabolism and inactivation, thereby counteracting these epigenetic changes ex vivo, further highlights its well-established potential to slow down human skin aging, with effectiveness varying by age.

P216 | Investigating the mechanism of matrix production in novel in vitro keloid models

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Keloids, pathological scars characterised by excessive fibrosis, pose considerable challenges due to their disfiguring appearance and the limited understanding of their underlying pathomechanisms. Recent research has suggested that the interaction between Schwann cells and M2 macrophages plays a key role in keloid development.
To study keloid formation in in vitro, we have developed a novel 3D model utilising the self-secreted extracellular matrix (ECM) of dermal cells from keloids and compared these to healthy skin. ECM assembly in these models was investigated using multiphoton microscopy with 2nd harmonics generation. Moreover, the changes in ECM components were investigated by ELISA of the supernatants of the models. Additionally, we conducted 3D co-cultures of healthy fibroblasts and Schwann cells with differentially polarised macrophages to explore their interactions and contributions to keloid formation and progression.
Similar to scar tissue in vivo the ECM secreted by the dermal in vitro keloid models displayed a higher degree of alignment of the ECM fibres. Furthermore, the secretion of the ECM components such as collagen I, collagen III and fibronectin were increased in the keloid model compared to models with cells derived from healthy skin. The co-culture of macrophages with Schwann cells showed little if any effects on macrophages polarisation. RNA sequencing revealed that co-cultured with monocytes showed only minor effects on Schwann cells. Contrary, gene expression of Schwann cells changed significantly when co-cultured with M1 or M2 polarised macrophages. Interestingly, co-cultures of Schwann cell with M1 macrophage induced an inflammatory phenotype in Schwann cells, whereas co-culture with M2 macrophages led to the induction of genes involved in the assembly of the ECM, indicating that the macrophage phenotype influence Schwann cell function.
In summary our findings show that our innovative in vitro keloid model can recapitulate the changes in extracellular matrix observed in keloids in vivo, enabling further research on the pathogenesis of keloids as well as potential treatment testing. The interaction of Schwann cells with M2 macrophages might recapitulate the crosstalk leading to an increase in matrix formation by Schwann cells already proposed in vivo, enabling studying this process in more detail in vitro.

P217 | Fixing the percutaneous device dilemma using biologically functionalized titanium implants

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Percutaneous implants, such as osseointegrated prostheses, ventricular assist devices and percutaneous endoscopic gastrostomy (PEG) tubes, penetrate the skin and thereby compromising the skin barrier at the epidermis-implant interface. This disruption increases the risk of pathogen invasion and subsequent infection, causing an unavoidable trade-off between effective treatment and reduction in skin barrier function known as the percutaneous device dilemma.
In this ongoing project, we explore the potential of cadherin-based surface coatings, designed to mimic natural cell–cell adhesion, to enhance the skin-implant sealing. We evaluate the coating's performance by quantifying its ability to immobilise adjacent cells and assess the permeability of the cell-implant junctions, comparing the results to uncoated implants and classical ECM coatings, such as fibronectin and collagen.
As an in vitro skin model, we employ various cell lines including N/TERT-1, MDCKII, HaCaT, as well as primary keratinocytes. Cell immobilisation is assessed by analysing cell migration at the cell-implant interface using live-cell imaging. Barrier integrity is evaluated in a 3D skin model through transepithelial electrical resistance (TEER) measurements and fluorescent dye-based permeability assays.

P218 | The role of skin adipocytes in wound healing

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Background: Wound healing is a complex process involving a wide variety of cell types, such as keratinocytes, fibroblasts and immune cells. Dermal adipocytes are increasingly gaining attention because of their supportive role during wound healing and antimicrobial processes. It is still unclear how dermal adipocytes intervene in the wound healing cascade and whether they influence wound healing directly, through secretion of adipokines, or indirectly through interaction with other skin cell types. Previous in vitro studies have shown that adipokines such as adiponectin and leptin may stimulate skin re-epithelialisation by promoting keratinocyte proliferation and migration. Thus, the aim of our study was to investigate the role of inflammation on dermal adipocyte formation in wounds and to study effects of adipokines on wound healing in vitro and in vivo.

Methods: Pigs were inflicted with dorsal split-skin, full-thickness and burn wounds. To induce an exaggerated inflammatory response, half of the wounds were treated with the TLR7/8 agonist, resiquimod. Biopsies for gene expression analyses and histology were taken at different time points after wounding. The gene expression of (I) adipocyte markers peroxisome proliferator activated receptor gamma (PPARgamma) and fatty acid binding protein (FABP4) (II) adipokines adiponectin, leptin, lipocalin-2, dermatopontin and retinol binding protein 4 (RBP4) were analysed.
Results: Application of the immunomodulator resiquimod to split-thickness, fullthickness and burn wounds induced a strong inflammatory response and resulted in the formation of a distinct adipocyte layer in the dermis and epidermis. Gene expression analysis of adipogenic markers and adipokines showed that FABP4, leptin, and RBP4 were altered in resiquimod-treated wounds.
Conclusion: We found that wound inflammation increased the number of adipocytes in the skin and interfered with adipogenic markers and adipokine expression. Further in vitro and in vivo studies are intended to expand the sparse existing knowledge about the role of dermal adipocytes in wound healing.

Tumour Biology

P219 | Rapid degradation of KDM5B induces an autophagy-dependent vulnerability in melanoma cells

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Melanoma, the most aggressive form of skin cancer, presents a formidable challenge in cancer treatment due to its remarkable adaptability and intrinsic heterogeneity. However, melanoma plasticity has emerged as a major obstacle to long-term treatment success and durability. Within this plasticity lies a subpopulation of drug tolerant persister cells (DTP), which play a pivotal role in the recurrence and resistance to therapies. An important subpopulation of persister cells is characterised by high level of the histone H3K4 demethylase KDM5B. However, the mechanisms of how KDM5B mediates resistance and survival of melanoma cells are not well characterised. Using a PROteolysis-TArgeting Chimeras (PROTAC)-mediated protein degradation against KDM5B, we induced a stress-like-cell state, which is characterised by a decrease proliferation in vitro and in vivo. Furthermore, KDM5B degradation induced an upregulation in autophagy flux, indicating that the increase in autophagy is a needed survival mechanism in order to survive the sudden change in cells' fitness level (due to KDM5B degradation). Combination of KDM5B degradation and autophagy inhibition has led to melanoma cell elimination. We provide first-time evidence of the role of KDM5B in autophagy regulation. Understanding the role and mechanisms of action of KDM5B in melanoma is crucial for developing more effective therapeutic strategies, improving patient outcomes, and providing insights into the epigenetic control of autophagy and its relevance in various biological processes.

P220 | Characterisation of the molecular mechanisms involved in reawakening of dormant melanoma cells in an in vitro model system

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Despite successful initial therapy, melanoma patients often suffer from late recurrence, often due to the reawakening of dormant melanoma cells. Current treatments cannot eradicate all cancer cells, allowing dormant cells to persist in specific niches in a reversible cell-cycle arrested state. Under unknown conditions, dormant tumour cells awaken, acquire proliferative capacity, and disseminate into other organs. The trigger factors involved in the reawakening of dormant tumour cells have still not been identified. Therefore, a more detailed understanding of the interplay of dormant cancer cells and the niche components is needed to understand the mechanism of dormancy induction, maintenance, and escape.
In this study, we established an in vitro melanoma dormancy model by short-term treatment of human melanoma cells with different therapies that induce a reversible G0/G1-cell cycle arrest, followed by an observation period of up to 30 days using live cell imaging. The fluorescence ubiquitination-based cell cycle indicator (FUCCI) reporter system was used to identify the cell cycle state of the melanoma cells over time. We tested several humoral factors as well as extracellular matrix (ECM) and immune cell components for their ability to maintain dormancy or reactivate dormant melanoma cells.
Several humoral factors did not change the dormant state over time. However, conditioned media from proliferating melanoma cells reactivated the cell-cycle arrest melanoma cells. ECM and immune cell components show variable effects, which will be further characterised. Interestingly, cell-cycle-arrested melanoma cells exhibit a higher migratory and invasive capacity than proliferating melanoma cells. Molecular characterisation of cell-cycle arrested melanoma cells indicated that the intracellular molecular pathways are reversibly altered at RNA and protein levels as the cells go through therapy-induced cell cycle arrest over the course of 30 days. Our data indicate that we successfully established an in vitro melanoma dormancy model which can be used to identify niche-related factors involved in the maintenance and escape of melanoma dormancy.

P221 | Role for Girdin and Daple in melanoma progression and therapy resistance

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Melanoma progression and therapy resistance are still a hurdle to overcome. Despite the initial good response after targeted therapy, resistance development eventually appears. Therefore, it is imperative to seek out innovative targets that have the potential to serve as viable treatment alternatives.
Girdin and Daple are guanine nucleotide exchange modulators (GEMs) that transmit signals from diverse receptors including RTKs, can modulate MAPK, PI3K/AKT and Wnt/ β-catenin signalling pathways and couple Gα-protein signalling.
In this study we aim to unravel the expression pattern and mode of action of these GEMs in melanoma tumour progression and to explore their role as potential therapeutic targets.
We found that Girdin and Daple were significantly higher expressed on RNA and protein level in metastatic melanoma cells compared to melanocytes. Database analysis confirmed that Girdin and Daple RNA expression was significantly increased in patients with metastatic melanoma compared to primary melanoma. MAPK signalling pathway may be involved in Girdin and Daple regulation after both GEMs expression was altered when using BRAF and MEK inhibitor treatment. Girdin and Daple expression was also altered after treatment with poly(ADP-ribose)-polymerase (PARP) inhibitor indicating the involvement of Girdin and Daple in DNA damage repair. We performed functional analysis and found that the downregulation of these GEMs did not influence cell viability, however the melanoma cell migratory capacity was reduced when Girdin or both GEMs were downregulated. Phosphorylation of the signalling mediators AKT and β-Catenin was reduced after downregulation of Girdin and Daple respectively, indicating the influence of these GEMs on major pathways.
Our data shows that Girdin and Daple are overexpressed in melanoma metastatic stages. This may be involved in signal modulation of different pathways promoting malignant traits such us higher migration capacity in melanoma.

P222 | Preclinical evaluation of immune checkpoint inhibitor avelumab combined with DC-based vaccination for the treatment of Merkel cell carcinoma

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Background: Merkel cell carcinoma (MCC) is a rare but highly aggressive skin cancer. Almost 50% of the patients show no clinical efficacy to the standard treatment with immune checkpoint inhibitors (ICIs). To increase the response rate of MCC patients to ICIs such as the programmed cell death ligand (PD-L1) antibody avelumab, our research aims to combine checkpoint blockade with therapeutic vaccination using monocytederived dendritic cells (moDCs) resulting in an additional induction of tumour-specific T cells. Previous experiments have shown that in co-cultures of autologous human moDCs and lymphocytes, the number of moDCs decreased in the presence of avelumab.
Hypothesis: We hypothesized that avelumab causes an antibody-dependent cellular cytotoxicity (ADCC) by natural killer (NK) cells against dendritic cells.
Experimental Approach: First, we evaluated the expression of PD-L1 on moDCs that had been matured with different stimuli by flow cytometry. Then we tested the binding ability of avelumab as well as the therapeutic anti-PD-L1 antibodies Atezolizumab and Durvalumab to moDCs. The lytic capacity of NK cells against moDCs in presence of the antibodies was determined by a chromium-51 release assay. To relate the in vitro data to the situation in vivo, the expression of PD-L1 on either immature or activated human primary DCs was investigated. In addition, the frequencies of the DC subsets were compared between healthy individuals and avelumab-treated MCC patients.
Key Findings: Irrespective of the maturation stimuli, all moDCs expressed high levels of PD-L1 and bound avelumab efficiently. Immature moDCs expressed lower, but still substantial levels of PD-L1. Other therapeutic PD-L1 antibodies were also bound by moDCs. The cytotoxicity assay revealed an efficient killing of moDCs by NK cells in the presence of avelumab. Other therapeutic PD-L1 antibodies, which are Fc-engineered to prevent Fc-receptor-binding, mediated no such lytic activities. The lysis of the DCs was independent from the DC maturation protocol and even immature moDCs were efficiently lysed. The ex vivo analyses revealed that PD-L1 expression was low in all analysed DC subsets at steady state, but increased substantially after activation in cDC1, DC2, and DC3. In avelumab-treated patients the frequencies of cDC1, DC3, and pDCs were significantly lower than in healthy donors.
Significance: We show that moDCs are killed by avelumab-mediated ADCC by NK cells in our invitro cell culture system. These in vitro data indicate that the anti-PD-L1 antibody avelumab is likely to interfere with simultaneous DC-based vaccination strategies. Our ex vivo data suggests that the in vitro observed ADCC against DCs could occur in patients treated with avelumab but further investigations are required.

P223 | Actinic keratosis treatment responses by gas plasma jet exposure in vitro and ex vivo

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Actinic Keratosis (AK) is a common skin lesion associated with dysplasia of the keratinocytes that is induced upon chronic exposure of the skin to high levels of UV radiation. This carcinoma in situ is one of the most frequent diseases in elder people in Europe and can potentially develop into an invasive squamous

cell carcinoma (SCC). Many current treatment options are associated with low efficacy, pain, recurrence, and/or high cost. Therefore, we investigate medical gas plasma as a preventive intervention against malignant skin cancer caused by actinic keratosis. To this end, we established an in vitro model of AK by repeated UV treatment of a keratinocyte cell line. We investigated effects mediated by an argon plasma jet treatment using flow cytometry, live cell imaging, and protein phosphorylation analysis. Additionally, biopsies from patients bearing AK were collected and treated with plasma ex vivo to analyse the secretion of signalling molecules and cytotoxic effects using histological methods. The chronic UV radiation of HaCaT cells led to deviating morphology features and enhanced expression of proteins such as p53, EGFR, and Cyclin E2 resembling AK in vitro. We showed that plasma treatment induced the production of reactive oxygen species (ROS) inside the AK model cells, leading to cytotoxicity visible in reduced metabolic activity, cell growth, and motility that could be prevented by the addition of ROS scavengers catalase and N-acetylcysteine. Analysis of cell death revealed apoptosis to be the main mode of action, but also signs of ferroptosis were found. Apoptosis induction could also be confirmed in the ex vivo plasma-treated AK biopsies. Our data suggest that plasma could be an easy-to-handle, efficient, and safe option to prevent SCC by treating AK. Further studies in vitro and in vivo should be performed to investigate probable immune effects in AK mediated by plasma and validate reasonable applications of plasma (e.g., in combination with currently used therapy options) in skin cancer prevention therapy.

P224 (OP04/05) | Multiomics profiling in melanoma before anti-PD-1-treatment: Identifying resistance mechanisms and multimodal biomarkers

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Introduction: The majority of patients with metastatic melanoma develops either primary or secondary therapy resistance, which significantly reduces the effectiveness of systemic treatment. In this context, melanoma cells exhibit a remarkable degree of plasticity. It has been demonstrated that melanoma cells can alter their phenotype and differentiation status in response to changes of the tumour microenvironment, such as inflammation, potentially leading to therapy resistance. Epigenetic regulatory mechanisms play a crucial role in driving the dedifferentiation of melanoma cells under these conditions.
Aims: This project seeks to uncover epigenetically regulated patterns and pathways involved in (de)differentiation, plasticity, and immune cell interactions in melanoma. The overarching goal is to identify clinically relevant targets for overcoming therapy resistance and to find potential biomarker candidates.
Materials & Methods: We collected tumour tissue from a well-annotated cohort of 48 patients with metastatic melanoma prior to the administration of anti-PD-1 immunotherapy, structured as a case–control study (clear responders vs. clear non-responders). Methylome and transcriptome analyses were performed using the Illumina 850k array and 3'-RNA sequencing, respectively. Initial data analysis comprises global and reference-free techniques to identify relevant methylation patterns. In subsequent steps, referencebased techniques such as MethylCIBERSORT, as well as differential methylation/ expression and pathway analyses will be applied to identify biologically significant pathways. Additionally, we plan to integrate data from published melanoma cohorts, such as TCGA-SKCM.
Results: Using RnBeads and the established MeDeCom algorithm for melanoma we have confirmed robust data quality and a variety of differentially methylated CpG sites between the responder ($N=29$) and non-responder ($N=29$) groups. Initial downstream analysis of the differentially methylated genes using more than 200 gene set libraries from the enrichR tool revealed an association with gene sets connected to cell migration, melanoma-related signalling mechanisms and neuronal pathways.
Outlook: The findings from this study may pave the way for future research into clinically relevant pathways, potentially leading to the development of alternative treatment strategies and the identification of novel biomarkers for metastatic melanoma.

P225 | Constitutive expression of IκBζ promotes tumour growth and immunotherapy resistance in melanoma

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IκBζ is an inducibly expressed co-factor of NF-κB, which can activate or repress the expression of a subset of NF-κB target genes in stimulated cells. While its role as a key regulator of several cytokines and chemokines in immune cells has been revealed, its function in solid cancer remains insufficiently investigated. Here we found that up to 30% of primary and malignant melanoma, as well as melanoma cell lines, display constitutive protein expression of IκBζ. This unusual expression pattern of IκBζ did not correlate with its mRNA levels or with any known key

driver mutations but rather seemed to result from changes in its posttranscriptional or post-translational regulation. Deletion of constitutively expressed IκBζ abrogated the activity and chromatin association of STAT3 in melanoma cells, thereby leading to a significant change in the expression of tumour-derived cytokines and chemokines, such as IL6, IL8, or CXCL10. Consequently, loss of tumour-derived IκBζ expression suppressed melanoma cell growth in vitro and in vivo. Furthermore, constitutive IκBζ expression in melanoma promoted the exclusion of NK and CD8+ T-cells from the tumour microenvironment (TME), thus triggering resistance to α-PD-1 treatment in mice. In agreement, the detection of tumour-derived IκBζ correlated with the absence of CD8+ T-cells in human melanoma samples as well as progressive disease during immunotherapy.
Thus, we propose that tumour-derived IκBζ expression represents a new marker in melanoma that characterises tumours with high tumour cell proliferation potential, cytotoxic T-cell exclusion from the tumour stroma, and consequently unfavourable immunotherapy responses.

P226 | PlasmACT: A Marie Sklodowska-Curie Doctoral Network for advancing gas plasma treatment of actinic keratosis

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Actinic keratosis (AK), a common pre-cancerous skin condition, remains a clinical challenge due to limitations in existing treatment methods. PlasmACT, a European doctoral network supported by the Marie Sklodowska-Curie Actions (MSCA), brings together academic institutions from Germany, Belgium, The Netherlands, and France, along with eight industry partners, to explore gas plasma as a novel therapeutic modality for AK. This interdisciplinary collaboration combines expertise in dermatology, plasma physics, and medical technology to develop an innovative, non-invasive treatment approach. Through a comprehensive training and research framework, PlasmACT aims to assess the efficacy, safety, and clinical potential of gas plasma therapy for AK, with implications for broader dermatological applications.
PlasmACT integrates academia and industrial expertise through a collaborative research framework designed to address both fundamental science and clinical translation. The network's research activities span multiple disciplines, including medical physics, theoretical and computational chemistry, the molecular basis of cancer, and medical engineering and technology. Each of the eight doctoral researchers within the network engages in a structured program that includes lab rotations across partner institutions, industry placements, and specialised training in plasma medicine and dermatology.
The PlasmACT projects focus on the effects of plasma treatment on actinic keratosis and related conditions by addressing questions across the fields of biology, chemistry, physics, and engineering. Together, these projects aim to enhance plasma therapy applications in dermatological conditions.
The PlasmACT project seeks to highlight cutting-edge research and innovation in skin care. By advancing gas plasma therapy—a promising, non-invasive treatment for a prevalent dermatological condition—PlasmACT exemplifies how interdisciplinary collaborations and international partnerships can drive scientific and clinical progress in dermatology (https://plasmact.eu/).

P227 | Antibodies targeting a differentiation antigen as effective adjuvant immunotherapy for solid tumours

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Solid tumours are responsible for around nine million deaths globally each year, largely due to their high and early metastatic potential. Post-surgery adjuvant therapy is recommended for patients at high risk of recurrence, but current approaches are variably effective and often associated with severe treatment-limiting side effects. Here, we propose using antibodies against a tumour-associated antigen as an alternative adjuvant therapy with an improved safety profile for solid tumours currently treated with immune checkpoint inhibitors. We identified an association between antitumor B-cell responses and long-term survival in patients with melanoma. We then screened potential tumour-associated antigens in a melanoma mouse model and identified the melanocyte differentiation antigen tyrosinase-related protein 2 (TRP2) as a relevant target. TRP2-deficient Dct−/− mice completely rejected B16F10 tumours after therapeutic immunisation against TRP2, producing abundant TRP2- specific antibodies, which localised to the tumours. We identified TRP2-specific Bcell clones and recombinantly produced monoclonal antibodies against TRP2 that protected wild-type mice from tumour challenge in the absence of marked side effects. The antibodies also reacted with human TRP2 and, when humanised, mediated human melanoma cell killing. Our findings identify TRP2-binding antibodies as candidates for a well-tolerated and potentially highly effective adjuvant

treatment for melanoma. Tumour-specific antibodies represent a promising therapeutic modality for solid cancers with potential for rapid translation to clinical practice, particularly as an adjuvant treatment to enhance existing therapies.

P228 | Dissecting the role of VCAN+ cancer associated fibroblasts in cutaneous squamous cell carcinoma and basal cell carcinoma

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Background: Cancer-associated fibroblasts (CAFs) are critical components of the tumour microenvironment, driving tumour progression and immune modulation in various cancers, including cutaneous squamous cell carcinoma (cSCC) and basal cell carcinoma (BCC). However, the distinct roles of specific CAF subpopulations in these skin cancers remain poorly understood. VCAN is a large extracellular matrix proteoglycan involved in cell adhesion, proliferation, migration, and matrix assembly. It is known for its role in tissue remodelling and is often overexpressed in various cancers, where it contributes to tumours progression, metastasis, and resistance to therapy.
Objective: This study aimed to investigate the therapeutic potential of targeting VCAN + CAF subpopulations in cSCC and BCC tumours.
Methods: Freshly resected cSCC and BCC tumour tissues were dissociated to isolate CAFs. Proteomic analysis of CAF-secreted proteins was performed using mass spectrometry. Additionally, RNA sequencing of isolated CAFs was conducted using the Illumina HiSeq 2500 platform. Tumour samples were analysed for VCAN expression by immunohistochemistry (IHC).
Results: The isolated CAFs were characterised by the expression of typical CAF markers such as α-SMA, FAP, and CD90 to confirm their identity. Proteins secreted into the medium by CAFs cultured under serum-free conditions were analysed by mass spectrometry, revealing a significant upregulation of VCAN across different cSCC and BCC CAFs. IHC staining supports our observations from the proteomics and transcriptomic data. Single epitope staining for VCAN revealed strong expression in the tumour-associated stroma.
Conclusion: These findings suggest that VCAN is a key marker of distinct stromal subpopulation in cSCC and BCC. Targeting these CAF subtypes could represent a promising therapeutic strategy to modulate the tumour microenvironment and improve treatment outcomes for these skin cancers.

P229 | Dissecting metabolic adaptations in melanoma therapy resistance

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Melanoma is an aggressive malignancy characterised by a complex molecular, phenotypic, and metabolic heterogeneity, which presents significant challenges in the treatment of metastatic disease. Although BRAF/MEK-targeted therapies have prolonged patient survival, the issue of resistance remains a significant challenge. One strategy, which involves temporarily withdrawing therapies, is known as a "drug holiday." This approach has been shown to frequently result in relapse. Our study investigates the phenotypic and metabolic changes that occur in melanoma cells during the development of resistance to BRAF/MEK inhibitors and subsequent relapse. We have established a series of targeted therapy-resistant melanoma cell lines, which we have further characterised in detail. The targeted therapy resistant cells demonstrated a reduction in proliferation accompanied by an increase in migration and invasion. This phenotype persisted through the relapse phase when drugs were withdrawn. Furthermore, the resistant cells demonstrated activation of the PI3K/AKT/mTOR pathway, accompanied by metabolic shifts that favoured OXPHOS, enhanced glutaminolysis, and elevated ROS levels, which were followed by antioxidant responses. These findings highlight the metabolic adaptability of melanoma cells in the resistant and relapse stages, indicating potential avenues for novel therapeutic approaches. These include the blockade of the PI3K/AKT axis or the disruption of metabolic dependencies, with the objective of overcoming resistance and relapse in future.

P230 | Assessing metabolic adaptations of melanoma cells in different cell culture models

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The energy and biomolecules produced by the metabolic processes are essential for the maintenance of the organism, its growth and cell division. Cancer cells undergo significant metabolic alterations in order to facilitate rapid proliferation and survival in diverse environmental contexts. In order to gain insight into these changes, a variety of metabolic tools are employed in conjunction with different cell culture models. The metabolic system is highly susceptible to external influences, exhibiting varying responses contingent on the prevailing conditions. The objective of this study is to examine the relative merits and limitations of two-dimensional (2D) and three-dimensional (3D) cell cultures, as well as the chicken embryo chorioallantoic membrane (CAM) assay, with a view to elucidating the metabolic processes associated with melanoma. Two-dimensional and three-dimensional cultures have distinct advantages: while

2D systems facilitate manipulation and analysis, they possess limited predictive capacity due to the non-physiological nature of the environment. Three-dimensional cultures more closely resemble the tumour microenvironment, but are more complex to handle, thereby rendering biochemical analysis more challenging. The CAM assay represents an intermediate model that combines in vivo-like conditions with straightforward visualisation and manipulation. However, this approach is not without its limitations, primarily due to the complexity of its handling. A comparative analysis of the three cell culture models was conducted using a range of metabolic investigations, including isotope tracing, Seahorse and other metabolic assays. This revealed significant differences among others in central carbon metabolism between the models, emphasising the importance of the cell culture model and the metabolic assays employed in the study of cancer cells.
The combination of these models with metabolomic tools offers a comprehensive approach to studying cancer metabolism and developing novel more physiological cancer therapies.

P231 | Novel metabolic vulnerabilities in metastatic melanoma

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Polyamines (PAs) are polycationic alkylamines that are ubiquitous in all living cells, where they regulate a number of crucial cellular processes, including cell viability, growth, proliferation, and antioxidant functions. In melanoma and other cancer types, elevated PA levels are strongly associated with the initiation and progression of the disease. Nevertheless, the precise molecular mechanisms by which the PA pathway is altered in melanoma progression and metastasis remain unclear, forming the basis of our investigation. Ornithine decarboxylase 1 (ODC1) is the rate-limiting enzyme in PA metabolism. We investigate the role of ODC1 using both pharmacological interventions and genetically engineered melanoma cell lines. The proliferation and colony-forming ability of melanoma cells in vitro were significantly impaired by the inhibition or depletion of ODC1. Furthermore, ODC1-deficient melanoma cells exhibited a significant decrease in the formation of metastases when transplanted subcutaneously into NSG mice. In addition, the inhibition of ODC1 with difluoromethylornithine (DFMO) resulted in a notable metabolic alteration in melanoma cells. These findings highlight the pivotal function of PA metabolism in melanoma progression and metastasis and suggest a prospective therapeutic approach for melanoma therapy.

P232 | The sensitivity towards PARP inhibitors in melanoma is increased by downregulation of genes involved in DNA damage sensing and repair

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Melanoma cells frequently exhibit hyperactivation of the MAPK signalling pathway making this pathway an attractive therapy target. However, the effectiveness of targeted therapy with MAPK inhibitors (MAPKi) is limited due to the rapid development of drug resistance in melanoma patients. Interestingly, our previous research indicated that melanoma cells resistant to MAPKi are more sensitive towards treatment with PARP inhibitors (PARPi). We identified the DNA damage sensor ATM as one gene which is downregulated in MAPKi resistant melanoma cells. Indeed, downregulation of ATM in MAPKi sensitive melanoma cells increased sensitivity towards PARPi (Fröhlich et al., 2023) .
In this study we investigated the role of additional DNA repair pathways in PARPi sensitivity of melanoma cells in more detail to analyse potential biomarkers for treatment response. We focused on genes which are downregulated in MAPKi resistant melanoma cell lines and are involved in DNA damage sensing and DNA repair. We performed siRNA-mediated knockdown of candidate genes and analysed the effect on PARPi sensitivity. We identified several genes especially involved in DNA damage sensing, base excision repair and nucleotide excision repair which are involved in increasing sensitivity towards PARPi in melanoma cells. Our study provides further evidence that PARPi treatment of MAPKi resistant melanoma patients might be a new treatment option and that the genes we identified in our study might be potential biomarkers for the treatment response.

P233 | Generating oxidative stress-resistant cells reveals compensatory regulation of IL1R2 and TCA metabolism in SCC

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Many types of reactive oxygen species (ROS) are drivers of skin tumour cell adaptations, leading to therapy resistance. However, replicating complex ROS mixtures is challenging. To address this, we employed gas plasma technology capable of generating high levels of various ROS types simultaneously to mimic complex oxidative stress conditions. Using squamous cell carcinoma (SCC) as a model tumour, the gas plasma exposure in vitro was performed over eight consecutive weeks to generate oxidative stress-desensitised A431 and SCC25 cells. Transcriptomics revealed a strong correlation of cancer cell adaption with increased interleukin 1 receptor type 2 (IL1R2) expression, which has been linked to cancer stem cell self-renewal and poor prognosis before. Engraftment of ROS/RNSresistant cancer cells on the chorioallantoic membrane of chicken embryos revealed

significantly augmented baseline secretion of matrix metalloprotease 2 and several immunomodulatory cytokines. Whole proteome analysis showed a strong correlation between oxidative stress adaptation with enhanced modulation of the tricarboxylic acid cycle (TCA), oxidative phosphorylation, and the ubiquitin-proteasome pathway (UPP). Using small animal magnetic resonance imaging (MRI), tumour growth, as well as gas plasma treatment responses of wildtype (WT) and repeatedly exposed (RE) cells, were further investigated in a xenograft model in vivo. Persistent RE cells showed slow-cycling behaviour and a distinct surface marker expression compared to WT cells. Strikingly, RE cells generated significantly smaller tumours with suppressed inflammatory secretion profiles and increased epidermal growth factor receptor (EGFR) activity. Clinically, combinational approaches together with cetuximab, an EGFR inhibitor, may overcome acquired oxidative stress resistance in head and neck cancers.

P234 | Crucial role of reactive oxygen species in pro-apoptotic therapeutic strategies in skin cancer cells

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Production of reactive oxygen species (ROS) in response to multiple stimuli can result in molecular damage and increased oxidative activity in cells. Therefore, ROS have been described as playing particular roles in various types of neuronal, cardiovascular and nervous system diseases as well as in aging. Besides this, ROS are involved in several signalling pathways and may also contribute to the induction of apoptosis. Production of ROS may therefore represent a crucial step in cancer therapy.

In the last 14 years, our group demonstrated the critical functions of ROS in different kinds of skin cancer cells, as in melanoma, cutaneous squamous cell carcinoma (cSCC) and in cutaneous T-cell lymphoma (CTCL) cell lines. The effects of ROS are thus not specific for a particular tumour type, but rather may apply to cancer cells in general. In melanoma cells, we showed strong induction of ROS in response to treatments with an iron-containing cytosine analogue, the PI3K inhibitor wortmannin, cell cycle arrest, indirubin derivatives as well as the BRAF inhibitor vemurafenib and the ERK inhibitor SCH772984. As for cSCC cells, we described induction of ROS after treatment with the COX-2 inhibitor celecoxib, sinecatechins and indirubin derivatives. Finally, in CTCL cells, ROS was induced after treatment with indirubin derivatives, ingenol mebutate and the Mcl-1 inhibitor S63845. Thus, production of ROS is related to multiple different strategies used for targeting cancer cells.

In most of these studies, we clearly demonstrated that induced ROS was related or even responsible for apoptosis induction through the treatments. The critical contribution of ROS can in principle be proven by certain antioxidants. However, due to many different kinds of ROS molecules and due to different possible locations in the cell compartments, also different kinds of antioxidants may be needed, and not always a suitable antioxidant may be found. For example, the induction of ROS by indirubin derivatives in melanoma, cSCC and CTCL cells was abolished by the wellknown antioxidant N-acetylcysteine (NAC) and, as a result, apoptosis was completely prevented. On the other hand, induction of ROS by either an iron-containing cytosine analogue or by the PI3K inhibitor wortmannin in melanoma cells was resistant to NAC, but here, alpha-tocopherol (vitamin E) was highly effective as antioxidant. It almost completely prevented the induction of ROS and thus also the induction of apoptosis. Similarly, apoptosis and ROS induced by vemurafenib could be at least partly prevented by alpha-tocopherol. In other situations, such as apoptosis induction by celecoxib in cSCC cells, strong ROS induction could not be prevented by any of several antioxidative strategies we tried. We suspect that here ROS was so strong and different types of ROS were produced that no combination of antioxidants was suitable.

When addressing the question, where ROS are linked to other pro-apoptotic pathways such as caspase activation, mitochondrial activation, Bax activation or other Bcl-2 proteins, we always found that ROS were upstream of all other pathways. Thus, ROS usually appeared as early as 1 h after the start of treatment, and when ROS were abolished by antioxidants, all other steps of the apoptosis pathways were blocked. These findings clearly underline the role of ROS as a master regulator in proapoptotic cancer therapy. A better understanding of these effects could enable further improvement of therapeutic approaches in the future.

P235 | Oxidised melanoma antigens promote activation and proliferation of cytotoxic T-Cell subpopulations

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Increasing evidence suggests the role of reactive oxygen and nitrogen species (ROS) in regulating antitumor immune effects and immunosuppression. ROS modify biomolecules and induce oxidative post-translational modifications (oxPTM) on proteins that can alarm phagocytes. However, it is unclear if and how protein oxidation by technical means could be a strategy to foster antitumor immunity and therapy. To this end, cold gas plasma technology producing various ROS simultaneously to oxidise the two melanoma-associated antigens, MART and PMEL, was utilised. Cold plasma-oxidised MART (oxMART) and PMEL (oxPMEL) were heavily decorated with oxPTMs as determined by mass spectrometry. Immunisation with oxidised MART or PMEL vaccines prior to challenge with viable melanoma cells correlated with significant changes in cytokine secretion and altered T-cell differentiation of tumour-infiltrated leukocytes (TILs). oxMART promoted the activity of cytotoxic central memory T-cells, while oxPMEL led to increased proliferation of cytotoxic effector T-cells. Similar T-cell results were observed after incubating splenocytes of tumour-bearing mice with B16F10 melanoma cells. This study provides evidence of the importance of oxidative modifications of two melanoma-associated antigens in eliciting anticancer immunity.

P236 | tRNA pool alterations and their impact on melanoma development

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Tumour development in various cancers has been linked to changes in the molecular tRNA pool that enhance the translation of specific tumour-promoting transcripts. A single human cell contains approximately 60 million tRNA molecules encoded by over 800 genes. In this study, we investigate the coordinated expression of tRNA and mRNA as part of a genetically regulated program that drives malignant growth in melanoma progression. Our research aims to identify variations in tRNA pools between tumour and non-tumour samples.

In initial experiments, we performed tRNA sequencing on samples from a benign melanocytic nevi, a primary melanoma, and a cutaneous melanoma metastasis. We then examined the correlation between tRNA isoacceptor abundance (tRNAs with different anticodons encoding the same amino acid) and codon usage in genes highly expressed in either melanomas or benign nevi. For 9 out of 13 amino acids, we observed a direct correlation between codon usage and the corresponding tRNA isoacceptors in melanoma and nevus samples. A significant correlation was also found when comparing codon usage with tRNA anticodon presence.

To further explore the regulatory relationship between tRNAs and mRNAs in melanoma, we used a novel tRNA sequencing method, LOTTE-seq (long hairpin oligonucleotide-based tRNA high-throughput sequencing). This technique allows us to accurately assess tRNA abundance in tissue samples from benign melanocytic nevi (3x), and primary melanomas (3x). Differential expression of over 40 tRNA isodecoders was found, most of which were upregulated in melanoma. This phenomenon is buffered at the level of the tRNA isoacceptors, so that overall an altered abundance can only be observed for a few amino acids. Additionally, we analyse the mRNA expression profiles of these samples to corroborate the correlations between codon usage in differentially expressed mRNAs and the anticodons of specific tRNA pools. These analyses will help to identify key tRNAs differentially expressed in melanoma development for further functional studies.

At the same time, RT-qPCR was used to analyse the distribution of some tRNA isoacceptors in 9 patient samples (3× nevi, 3× melanoma, 3× metastasis), in which the different distribution has already been demonstrated in other types of cancer. At the moment, a total of 5 amino acids and 15 different tRNA isoacceptors have been analysed in this way. Differences in the distribution of tRNA isoacceptors in the various tumour stages were found. In addition, signals from gene sequences for tRNA isoacceptors were detected in some samples that are not listed in the public genomic tRNA database (GtRNAdb).

In the future, we plan to overexpress and knockdown the most interesting tRNA isoacceptor candidates in melanoma cell lines to characterise their impact on cell mobility and proliferation. Understanding this potential mechanism of malignant transformation could reveal critical insights into protein translation and melanoma biology, paving the way for the development of novel therapeutic strategies.

P237 | Influence of neutrophil granulocytes on hepatic melanoma metastasis

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Background: Liver metastasis mediates therapy resistances to immune checkpoint inhibition in stage IV melanoma. Hereby, the local hepatic immune microenvironment is decisively involved. Neutrophil granulocytes can exhibit different subtypes with both tumour-promoting and inhibitory properties, presenting potential key players in metastasis formation. However, the influence of these subtypes on hepatic melanoma metastasis remains poorly understood and therefore requires further clarification.

Methods: Hepatic melanoma metastasis was modelled by intrasplenic injection of WT31 or B16F10 luc2 melanoma cells. Anti-Ly6G antibody treatment was used to influence neutrophil numbers and function during different time intervals. Peripheral blood neutrophils were analysed weekly by flow cytometry. Melanoma metastases were quantified and further analysed by routine histology and immunofluorescence staining. Besides, hepatic melanoma cell retention 90 min after intrasplenic injection was investigated.

Results: Upon daily treatment with anti-Ly6G, liver metastasis of WT31 melanoma was increased as compared to the isotype control. Anti-Ly6G treatment in the early phase of metastasis had no effect on metastasis formation. However, anti-Ly6G treatment in the late phase of metastasis also resulted in more and larger hepatic metastases. For B16F10 luc2 no difference in the numbers, metastatic area and bioluminescence signal was detected after depletion of neutrophils in all time phases. Hepatic melanoma cell retention was not altered by anti-Ly6G therapy in both WT31 and B16F10 luc2. For WT31 routine histology revealed increased rates of necrotic metastases in the anti-Ly6G group, as well as increased number and microscopic size. For both cell lines the immunofluorescence staining showed no difference in vascularization, ECM-deposits, proliferation, or apoptosis. However, enhanced numbers of neutrophils, monocytes and macrophages per liver area were found in the anti-Ly6G group of all time phases for both WT31 and B16F10 luc2.

Conclusion: Our data demonstrate that treatment with anti-Ly6G antibodies leads to an influx of neutrophils in the liver. As hepatic melanoma metastasis is increased, especially for NRAS-mutant WT31 melanoma, these seem to act immunosuppressive

in the hepatic microenvironment. Currently, the phenotype of these hepatic neutrophils is further investigated.

P238 | Characterisation of neutrophil extracellular traps in ex vivo non-melanoma skin cancers

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Non-melanoma skin cancers (NMSC), including cutaneous squamous cell carcinoma (cSCC), Merkel cell carcinoma (MCC) and basal cell carcinoma (BCC) can take an aggressive course in the metastatic stage. Neutrophil extracellular traps (NETs), chromatin fibres released by neutrophils, can contribute to immunotherapy resistance by impairing CD8+ T cell-mediated cytotoxicity. In this study, we aimed to identify a potential therapeutic target by investigating the release of NETs and their association with CD8+ T cell infiltration in NMSC. Using immunofluorescence staining for neutrophils (CD15) and NETs (H3cit), and immunohistochemistry for cytotoxic T cells (CD8+) in FFPE sections of human cSCCs, BCCs, and MCCs, we found that NETs were present in all of the analysed NMSC primaries, whereas exemplary staining of metastases revealed NETs only in cSCC metastases, but not in MCC metastases. Within the primaries, NETs occurred predominantly in ulcerated areas, but in cSCCs, they also appeared in deeper abscess like structures. The occurrence of NETs in cSCC primary tumours was not linked to established clinical risk factors. To investigate whether NETs may cause immune exclusion in NMSC, the quotient of peri- and intratumoral CD8+ T cells was examined. CD8+ T cell infiltration was not diminished in NET-positive tumours or those with higher neutrophil density.
These results characterise NETs and their influence on T cell infiltration in NMSC for the first time. Whether NETs can actually be a therapeutic target in these tumours must be determined by further functional analyses.

P239 | Cancer associated fibroblast (CAF) subtypes modulate the tumour-immune microenvironment and indicate skin cancer malignancy

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Cancer-associated fibroblasts (CAFs) play a key role in cancer progression and treatment outcome. This study dissects the yet unresolved intra-tumoral variety of CAFs in three skin cancer types—Basal Cell Carcinoma, Squamous Cell Carcinoma, and Melanoma—at molecular and spatial single-cell resolution. By integral analysis of the fibroblasts with the tumour microenvironment, including epithelial, mesenchymal, and immune cells, we characterise three distinct CAF subtypes: myofibroblast-like RGS5+ CAFs, matrix CAFs (mCAFs), and immunomodulatory CAFs (iCAFs). Notably, large cohort tissue analysis reveals marked shifts in CAF subtype patterns with increasing malignancy. Two CAF types exhibit immunomodulatory capabilities via distinct mechanisms. mCAFs synthesise extracellular matrix and have the ability to ensheath tumour nests, potentially limiting T cell invasion in low-grade tumours. In contrast, iCAFs are enriched in latestage tumours, especially infiltrative BCC and high-grade melanoma, and express unexpectedly high mRNA and protein levels of cytokines and chemokines, pointing to their integral role in immune cell recruitment and activation. This finding is further supported by our observation that in vitro exposure of primary healthy fibroblasts to skin cancer cell secretomes induces an iCAF-like phenotype with immunomodulatory functions. Thus, targeting CAF variants, particularly the immunomodulatory iCAF subtype, holds promise for improved efficacy of immunotherapy in skin cancers.

P240 (OP02/03) | Mice expressing KIT D814V in the mast cell lineage show a systemic mastocytosis phenotype with skin lesions that can be reversed by treatment with specific tyrosine kinase inhibitors

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Background: Mastocytosis is characterised by an abnormal expansion of mast cells (MC) in one or various organs such as bone marrow (BM), skin and/or intestine, driven in >90% of patients by the KIT D816V mutation. KIT D816V-specific tyrosine kinase inhibitors reduce symptoms and prolong survival in systemic mastocytosis, but not all patients benefit from this therapy; therefore, there is an urgent need for novel treatment options.
Methods: To advance treatment development, we generated by CRISPR/Cas9 technology a novel pre-clinical mastocytosis mouse model carrying the KIT D814V mutation (homologous to human KIT D816V). KIT D814V expression was restricted to the MC lineage by Mctp5-Cre-mediated recombination. The phenotype resembling mastocytosis, specifically skin MC number, serum levels of MC protease 1 (Mcpt1), peripheral blood count and skin lesions, was evaluated over time. Moreover, mice were monitored for disease progression upon treatment with a specific KIT inhibitor.
Results: Constitutive expression of KIT D814V in the MC lineage resulted in progressive appearance of skin lesions in mice, starting from 7 weeks of age, located on the neck, between the ears and around the eyes. Video recording of single mice showed a scratching behaviour (3–17 episodes in a 2-min time frame) that was absent in control mice expressing WT KIT. MC accumulated in the skin, stomach, spleen and liver, while peripheral blood count was unaltered, consistent with a typical phenotype

of indolent systemic mastocytosis. Expansion of MC also resulted in elevated serum levels of Mcpt1 and markedly reduced body weight already at 6 weeks of age. Correspondingly, MC progenitors in peripheral blood and spleen were elevated in mice expressing the mutant KIT compared to control mice. Olink analysis revealed a significant upregulation of inflammatory cytokines such as TNFalpha and IL-6, chemotactic factors (IL-16, Ccl2 and Ccl12), and immune checkpoint molecules (PD-L1 and PD-L2). In order to investigate the response to clinically relevant tyrosine kinase inhibitors, mice were randomised to receive either avapritinib 30 mg/kg or vehicle by oral gavage for 16 days. Treatment resulted in a significant reduction in MC accumulation in BM and liver, paralleled by a five-fold reduction in Mcpt1 serum levels, and was well tolerated as shown by stable body weight. Moreover, avapritinibtreated mice showed no scratching bouts, indicating resolution of chronic itch, and no skin lesions.
Conclusions: We have generated a novel mouse line expressing the oncogenic KIT D814V mutation specifically in the MC lineage. Mutant mice present an indolent systemic mastocytosis phenotype, with expansion of MC in skin, spleen and liver, and chronic itch as indicated by frequent scratching and appearance of skin lesions. The phenotype is corrected by treatment with the tyrosine kinase inhibitor avapritinib, suggesting the relevance as a preclinical model. Therefore, this mouse line could represent an invaluable tool for the investigation of the pathogenesis of the disease, and the identification of novel targetable pathways.

P241 (OP02/05) | Ultra-sensitive proteomics identifies and quantifies Merkel cell polyomavirus proteins in merkel cell carcinoma tissue

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Merkel Cell Carcinoma (MCC) is a highly aggressive neuroendocrine skin cancer, with approximately 80% of cases associated with the Merkel Cell Polyomavirus (MCPyV) and the remainder linked to excessive UV exposure. MCPyV status has been reported to have a prognostic value, with MCPyV-negative patients experiencing poorer outcomes. PCR and immunohistochemistry are the gold standards for determining MCPyV status in MCC tumours. Here, we present an ultrasensitive mass spectrometry (MS) -based workflow for the direct identification of MCPyV proteins in MCC tissues. This also includes a targeted proteomics pipeline enabling absolute quantification of the viral proteins, using heavy labelled standards. In addition, we aim to elucidate the functional differences between MCPyV-positive and negative tumours by a cell-type resolved approach, contributing to an improved understanding of their distinct pathophysiologies.
Our study includes a sex, age, and anatomical location-matched pilot cohort consisting of 22 MCC patients (12 MCPyV-positive and 10 MCPyV-negative) alongside 11 healthy controls. We dissected bulk MCC tissues from 3 µm thick H&E stained skin sections and analysed them using the latest generation of mass spectrometer (Orbitrap Astral) by which we identified 10 000 unique proteins groups, with a remarkable median depth of coverage of 9502 proteins per sample. We detected 2000 differentially regulated proteins between MCC and healthy skin tissues, with partial separation of MCPyV-positive and negative samples in PCA analysis. Specific proteins were found exclusively in either MCPyV-positive or negative tumours, warranting further functional studies. In addition, we successfully identified the large T-antigen (LTAg) and small T-antigen (STAg) MCPyV proteins. Notably, up to 6 unique LTAg peptides were detected in MCPyV-positive samples, across three orders of magnitude in signal. Remarkably, in certain MCPyV-positive samples, viral proteins were abundantly present, with LTAg ranking among the top 20% of the most abundant proteins in the tissue.
Next, we set out to quantify the viral proteins from the MCC tissue samples in an absolute fashion and established a workflow for targeted proteomics with spiked in heavy labelled viral peptide standards. We quantified up to 300 amol of LTag per MCC sample, from two viral peptides. This experiment confirmed the viral status of the MCC patients in our cohort.
Furthermore, we used deep visual proteomics (DVP) enabling spatial resolution on a single-cell type basis. This identified more than 6000 protein groups from an input equivalent to only 25 CK20 positive tumour cells. Notably, the LTag viral protein was identified already from an input of 5 cells. In an experiment comparing the proteomes of cells with high vs. low viral load from the very same tumour, we were able to identify statistically significant proteomic differences. For example, proteins like CALD1, MCAM and ACOT9 were found to be upregulated in cells with low viral load—of these especially CALD1 and MCAM are reported to be associated with cancer progression.
In summary, the novel mass spectrometry workflows established here not only enable the direct identification and quantification of viral proteins in human samples, but also provide insights into the molecular pathophysiology of viral-induced cancers, in particular MCC.

P242 (OP06/05) | Development of a machine learning-based cytokine score to predict outcomes in melanoma patients treated with immune checkpoint inhibitors

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The approval of immune checkpoint inhibitors (ICI) has revolutionised the therapy of advanced melanoma. However, a relevant proportion of patients do not benefit from this treatment and in addition, immunotherapy is often associated with severe immune related adverse events. Therefore, the aim of this

project was to identify biomarkers that could predict early treatment failure.
In a prospective cohort of 122 melanoma patients, serum samples were collected before and during ICI. 67 patients were in the adjuvant setting and received PD-1 monotherapy, 55 patients were in the palliative setting, 27 received Nivolumab + Ipilimumab, 28 PD-1 monotherapy. The expression of 57 parameters was determined using electrochemiluminescence and predictive models were developed to evaluate disease progression based on baseline and dynamic cytokine changes.
No significant features were identified in baseline parameters for the adjuvant setting. However, dynamic changes in cytokine levels from baseline to 3–4 weeks after ICI initiation (IP-10, IL-12/23p40, IFN-γ, IL-10, IL-6, SAA, sICAM-1, BCA-1) showed high predictive accuracy for disease progression, with Random Forest model achieving the highest area under the curve (AUC = 0.96). In the palliative setting, baseline IL-15 and an increase in IL-1RA and TSLP were predictive for a progress within 24 weeks after start of therapy with an AUC = 0.85. Regarding survival analysis, distinct dynamic changes in Flt-1, IFN-γ, IL-10, IL-6, SAA, and sICAM-1 were predictive for recurrence free survival in the adjuvant cohort, with a concordance index of 0.86 (CI: 0.75.-0.94). In the palliative setting, a higher score of baseline levels of bFGF, PIGF, Tie-2, MCP-1, IL-8, and gro-α was predictive for progression free survival (C-Index 0.73; CI: 0.62–0.83). In conclusion, a score of dynamic cytokine changes was highly predictive for patients experienced an early progress in both adjuvant and palliative settings, while in metastatic patients also baseline parameters played a significant role. These results need to be validated in larger cohorts. Notably, we observed distinct biomarker profiles for predicting outcomes in different therapeutic settings of melanoma patients, highlighting the importance of patient stratification for biomarker analysis.

P243 | The combination of PI3Ki and MEKi as a promising treatment option for melanoma patients with NRAS-mutated tumours

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Introduction: In recent years, new therapeutic approaches such as immune checkpoint inhibitors and targeted therapies have improved the survival rate of melanoma patients. However, response rates are limited and resistance to immunooncology therapies is a major challenge. The PI3K/AKT signalling pathway is deregulated in 70% of melanomas and contributes to the development of resistance, making PI3K a promising target for inhibitor treatment. In particular, the combination of PI3K and MEK inhibitors could be an effective option, as far as MEKi is the only targeted treatment option for patients with NRAS mutated tumours available.
Methods: To investigate the effects of the PI3Ki and the MEKi on NRAS mutated cell lines the viability and apoptosis induction of were analysed via MUH assay, cell cycle analysis and expression of cleaved Caspase 3 and cleaved PARP. In vivo, NSG mice were subcutaneously injected into the right flank with NRAS mutated melanoma cells and the tumour-bearing mice were treated via oral gavage with alpelisib (30 mg/ kg bodyweight daily), trametinib (0.3 mg/kg bodyweight daily), or both treatments combined for 4 weeks. The efficacy and toxicity were analysed by measuring the respective tumour sizes and mouse weights accompanied by histological analysis of the protein levels of phospho-ERK (as a marker for MAPK pathway activation) and cleaved PARP (as a marker for apoptosis).
Results: In monotherapy, the PI3K inhibitor alpelisib (BYL719), an alpha-specific inhibitor, has limited antitumor activity. However, the combination treatment of PI3K and MEK inhibitor resulted in effective growth inhibition and apoptosis induction in the tested cellular melanoma models, surpassing the effect of MEK inhibition as monotherapy. In the in vivo mouse setting, the combination therapy was able to reduce tumour growth and even significantly prolong overall survival in the NRASmutated model.
Conclusion: These data indicate that the combination of PI3K inhibitors with MEK inhibitors may represent a new therapeutic option for melanoma patients with NRASmutated tumours.

P244 | Targeting the deubiquitinase USP7 in melanoma

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Protein ubiquitination and deubiquitination are post-translational modifications that regulate protein turnover in various cellular processes. Deubiquitinases (DUBs) modify ubiquitin-encoded signals and are therefore crucial for the tight regulation of protein homeostasis. Among these DUBs, USP7 has been extensively studied in cancer, because its known substrates are regulators of cell cycle progression.
Accordingly, several USP7-specific inhibitors and recently also USP7-specific degraders like PROTACs (proteolysis targeting chimeras), have been developed to block tumour cell proliferation and disease progression.
Through extensive chemical and biological testing, we discovered tremendous off-target effects of commercially available USP7 inhibitors, affecting melanoma cell proliferation, invasiveness, differentiation and metabolism. In response to this finding, we have developed a novel, highly specific USP7-specific PROTAC, which is currently being tested against USP7 knockouts. So far our data indicate that USP7 affects antigen presentation in melanoma cells, positioning USP7 as a potential therapeutic target in melanoma immunotherapy.

P245 (OP02/01) | Characterisation of IDH-1 mutated melanoma

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Background: Despite the advent of immuno- and targeted therapies for the treatment of advanced melanoma, there is a continued necessity for the development of novel treatment modalities for patients who either do not respond to or progress despite these existing therapeutic options. Isocitrate dehydrogenase 1 (IDH-1) mutations are frequently identified in gliomas, chondrosarcomas, and cholangiocarcinomas, yet have not been documented in melanoma. Somatic mutation of IDH-1 results in the emergence of neomorphic enzyme activity, which in turn leads to the generation of supraphysiological concentrations of D-2-hydroxyglutarate (D-2HG) through the NADPH-mediated reduction of α-ketoglutarate (α-KG). In cells expressing mutated IDH-1, the accumulation of D-2HG inhibits α-KG-dependent enzymes that are involved in the demethylation of histones and DNA. The most common occurrence of heterogeneous amino acid substitutions is in the hotspot locus R132, which suggests that the mutant IDH1 plays an oncogenic functional role in tumour development.
Objective: The objective of this study is to characterise IDH-1 mutations in melanoma both in vitro and in vivo, and to evaluate the potential efficacy of IDH-1 inhibitors as a novel therapeutic option for patients harbouring IDH-1 mutation.
Methods: The frequency of IDH-1 mutations in melanomas sequenced in the Department of Dermatology of the University Hospital Essen was found to be 1.5–2%. We successfully established novel IDH-1 mutant patient-derived melanoma cell lines and conducted in vitro and in vivo characterisation of IDH-1 mutation and the effects of the IDH-1 specific inhibitors in melanoma. Furthermore, the clinical characteristics of an IDH-1 R132 mutated melanoma patient cohort ($n = 51$) were subjected to analysis.
Results: The proof of principle for the functionality of neomorphic enzyme activity in our established cell lines was successfully demonstrated by measuring the concentrations of D-2HG. Our findings revealed a notable accumulation of D-2HG in IDH-1 mutant cell lines in comparison to IDH-1 wild-type, substantiating the enzymatic activity linked to the IDH-1 mutation. Furthermore, the cell state, viability, and death rates were evaluated, and transcriptomic alterations were investigated through RNA sequencing (RNA-seq) to gain deeper insights into the molecular mechanisms linked to the IDH-1 mutation. The present study aimed to elucidate the metabolic alterations associated with the IDH-1 mutation and its oncogenic impact. Our findings revealed significant modifications in pivotal metabolic pathways. The objective is to establish a patient-derived xenograft model for the purpose of evaluating the effects of the mutation on tumour dynamics in vivo. Concurrently, we are assessing the impact of IDH-1-specific inhibitors, with a particular emphasis on their capacity to reduce D-2HG levels and restore metabolic balance in IDH-1 mutant cells, thereby resulting in a reduction of proliferation and an augmentation in apoptosis. Lastly, we conducted a detailed analysis of an IDH-1 mutant patient cohort, comparing and contrasting patient characteristics and survival data with that of IDH-1 wild-type patients.
Conclusion: Our study is the first to describe and characterise an IDH1-mutated melanoma and its metabolic effects, thereby highlighting its potential as a druggable target. These findings provide a foundation for further investigations into targeted therapies in this subset of melanoma patients.

P246 | DIRAS1 overexpression reduces tumour burden in a murine multistage chemical skin carcinogenesis model by decreasing the activity of RAS

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The incidence of non-melanoma skin cancer (NMSC) is rapidly rising with around 1.2 million newly diagnosed cases each year according to the most recent world cancer report. Despite the growing number of patients suffering from advanced stages of NMSC, there are limited targeted treatment options available.
The DIRAS protein family consists of three RAS-related small GTPases (DIRAS1-3) with low intrinsic GTPase activity. DIRAS1 has been reported to act as tumour suppressor in various types of cancers, including glioblastoma, colorectal cancer, and renal cell carcinoma. The biological function of DIRAS1 is not yet fully understood. However, it has been suggested to antagonise RAS signalling by inhibition of the MAPK/ERK and PI3K/AKT pathways.
To study the function of DIRAS1 in vivo we generated a new mouse line which overexpresses Diras1 under the control of the ubiquitous chicken beta actin promoter (Diras1-TG). To investigate if DIRAS1 also acts as tumour suppressor in cutaneous squamous cell carcinoma (cSCC), we utilised an inducible 7,12-dimethylbenz(a)anthracene (DMBA) and 12-O-tetra-decanoylp horbol-13-acetate (TPA) two-stage skin carcinogenesis model. We could show that Diras1-TG mice showed a delayed onset of papilloma development and significantly reduced papilloma burden compared to control littermates. These results suggest that DIRAS1 acts also as tumour suppressor in skin carcinogenesis.
Furthermore, we could show a significant reduction of active GTP-bound RAS protein in papilloma of Diras1-TG mice compared to control littermates. Further analysis of downstream signalling pathways of RAS revealed a reduced phosphorylation of p44/42 MAPK. When analysing DMBA/TPA treated skin in locations without papilloma, we could show increased PTEN expression levels as well as reduced inactivation of PTEN in Diras1-TG mice. These findings suggest that overexpressing DIRAS1 indeed suppresses RAS activation and inhibits the MAPK/ERK as well as PI3K/AKT pathway in vivo, which makes DIRAS1 a potential target for a tumour suppressor therapy in cSCC.

P247 | Comparative analysis of IHC vs. ddPCR for MCPyV detection in MCC

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Merkel cell carcinoma (MCC) is a rare but highly aggressive neuroendocrine skin cancer with a poor prognosis that predominantly affects the elderly and immunosuppressed. Over 80% of cases are associated with the Merkel cell polyomavirus (MCPyV), which plays a central role in tumour development. In MCPyVnegative cases, UV exposure is a significant risk factor and a major cause of tumour development.

Because the viral status of MCC is important for prognosis, the immunohistochemical (IHC) antibody CM2B4, which targets the large T antigen of MCPyV, is currently used to determine viral status. In this study, we analysed 92 samples (51 tumour samples and 24 tumour-free post-excision tissue) from 64 patients using both the conventional CM2B4 method and digital droplet PCR (ddPCR). The advantage of ddPCR for MCPyV detection is that it allows absolute quantification of viral load. This is possible because the PCR reaction of each DNA fragment takes place separately in a droplet and can then be analysed individually. The large T-4 antigen (LT4) was selected for detection of MCPyV in ddPCR.

We found a moderate to high correlation between the percentage of CMB2B4- positive tumour cells and the LT4-positive droplets (Pearson correlation coefficient $r = 0.6612$, $p < 0.0001$), indicating that ddPCR is suitable as a functional marker for MCPyV.

When comparing the sensitivity of the methods, ddPCR showed a sensitivity of 95.8%, while CM2B4 IHC showed a sensitivity of 66.2%. This difference is mainly due to the 50% higher detection rate of MCPyV in tumour-free post-excision specimens when using ddPCR.

We performed a Kaplan–Meier analysis on 38 MCPyV-positive and 13 MCPyVnegative patients and demonstrated a 12-month longer median event-free survival in MCPyV-positive cases (Log-rank chi-squared = 3.886, $p < 0.05$). Interestingly, we found that a higher viral load of MCPyV correlated with later progression in MCC patients (Pearson correlation coefficient $r = 0.3782$, $p < 0.05$).

The superior sensitivity of ddPCR indicates its potential as a complementary diagnostic tool to determine MCPyV positivity or negativity. Furthermore, ddPCR is a valuable tool for accurate quantification of MCPyV viral load. In addition, the results demonstrate that ddPCR has strong potential to be used to confirm the absence of MCPyV-positive tumour cells in areas following surgical resection.

P248 (OP06/01) | Virotherapy as an alternative to chemotherapy-based conditioning for cellular immunotherapies in cancer

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Adoptive Cell Transfer (ACT) of T-cells, such as autologous tumour-infiltrating lymphocytes (TILs) and chimeric antigen receptors (CARs), have shown great promise for the treatment of different cancers. However, chemotherapy-based conditioning regimens and Interleukin-2 (IL-2) administration are frequently associated with substantial morbidity and mortality, limiting the utilisation of these therapies. The present study aimed to minimise conditioning-related complications while enhancing the effectiveness of adoptive T-cell therapies. Here, we propose an alternative to the conventional conditioning method using an arenavirus-based virotherapy. We used the B16F10 murine melanoma model and ACT of T-cells, targeting the melanocyte differentiation antigen TRP2 as a solid tumour model. Conditioning with the replicating lymphocytic choriomeningitis virus expressing TRP2 (artLCMV-TRP2) led to complete rejection of established melanomas, without prior lymphodepletion and IL-2. We identified that replication of the virus is required for persistent tumour control. Our findings further indicated that artLCMV induces activation and expansion of transferred T cells significantly more efficiently than the replicating Pichinde virus (artPICV), translating into complete rejection of established melanomas. artLCMV expressing either a non-tumour antigen or PMEL tumour antigen failed to control tumour growth. Our experimental data shows that effective tumour control requires matching the antigen expressed by the artLCMV with the antigen specificity of the ACT. These findings highlight three key factors for effective conditioning using virotherapy: virus replication, virus type and antigen identity. Our proposed arenavirus-based virotherapy has a favourable safety profile and enhances the efficacy of adoptive cell transfer. These data suggest virotherapy as a promising alternative to conventional conditioning in cellular immunotherapies.

P249 | 1-Methylnicotinamide is an immunosuppressive metabolite in human basal cell carcinoma

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Human basal cell carcinoma (BCC) represents the most common skin cancer. Recent studies suggested that tumour-derived metabolites are key drivers of tumorigenesis and the evasion of T cell-mediated anti-cancer immunity, in part by directly modulating T cell function. Nevertheless, knowledge regarding the metabolic milieu and its impact on T cell responses in human BCCs is limited. In this project, we investigated the direct impact of tumour-enriched metabolites on the function of human T cells. We established a protocol to extract interstitial fluid metabolites from punch biopsies of human BCCs and (adjacent) control skin. Metabolomic analyses using mass spectrometry showed a significant increase of 1-methylnicotinamide (1- MNA) in the BCC samples vs. control skin. 1-MNA is generated from nicotinamide by the nicotinamide N-methyl transferase (NNMT), known to be overexpressed in multiple human cancers. Consistently, we demonstrated overexpression of NNTM in human BCC tumour cells compared to adjacent skin by immunohistochemistry. To investigate the direct impact of 1-MNA on the function of T cells, we studied human blood-derived T cell lines and human skin-derived tumour-infiltrating T cells (T-TIL) from BCCs. We demonstrated by flow cytometry analyses that T-TILs from BCCs express the organic cation transporters SLC22A1, SLC22A2, and SLC22A3, involved in uptake of extracellular 1-MNA. Proteomics analyses of 1-MNA-treated and control T cells revealed significant metabolic and immune functional changes. Among the significant regulated proteins was PD-1, a checkpoint-molecule causing T cell exhaustion. Flow cytometry analyses confirmed enhanced surface PD-1 expression on CD8+ T cells and further showed that Tim-3, and Ox-40 were likewise significantly increased upon 1-MNA-treatment. These changes in the surface expression of immune checkpoint-molecules were paralleled by reduced production of the effector cytokine IFN-γ. Meanwhile, T cell production of IL-10, an immunosuppressive cytokine, as well as T cell proliferation were sustained. In sum, our data demonstrate that 1-MNA contributes to an immunosuppressive environment for T cells in human BCC.

P250 | High expression of neurotrophin receptor CD271 confers an undifferentiated phenotype in cutaneous squamous cell carcinoma and is associated with reduced progression-free survival

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Background: Cutaneous squamous cell carcinoma (cSCC) is the second most frequent skin cancer worldwide, with a rising incidence due to increasing life expectancy. At early stages, cSCC can be cured by surgical excision or radiotherapy. For locally advanced and metastatic cSCC, the anti-programmed cell death protein-1 (PD-1) monoclonal antibody cemiplimab was approved by the European Medicines Agency (EMA) in 2019. However, a significant number of patients are either unsuitable for anti-PD-1 treatment or remain unresponsive. This underscores the urgent need for new therapeutic options.
In this context, the neurotrophin receptor CD271 has emerged as a potential target in various cancers and is associated with a poorly differentiated phenotype in head and neck squamous cell carcinoma (HNSCC), oesophageal squamous cell carcinoma, and lung squamous cell carcinoma. Regarding cSCC, there is only very limited data available, with one study reporting strong expression of CD271 in poorly differentiated pleomorphic epitheloid cells and an association with perineural invasion. Conversely, another study suggested that CD271 activation may prevent progression from low- to high-risk cSCC. To date, CD271 expression has not been thoroughly evaluated in a larger cohort of cSCC patients.
Methods: In this study, CD271 expression was analysed using immunofluorescence on tissue microarrays (TMA). Additionally, we assessed the expression patterns of the stem cell marker CD133. In total, 364 cSCC samples were included. Expression was quantitively analysed by measuring mean grey values. We also conducted in vitro experiments using the human cSCC cell line SCC12. Cells were sorted based on CD271 expression via fluorescence-activated cell sorting (FACS), and the subpopulations were evaluated for their sphere-forming capability. Expression of CD133 was evaluated using flow cytometry. CD271 inhibition was performed by using the small molecule inhibitor LMA11A-31.
Results: TMA: High CD271 expression was significantly associated with inferior progression-free survival (PFS) in a cox proportional hazard model. Poorly or undifferentiated cSCC (G3 or G4) exhibited significantly higher CD271 expression compared to well or moderately differentiated cSCC (G1 or G2). Tumour samples with positive perineural invasion showed increased CD271 expression. The strongest expression was observed at the margins of tumour cell nests, corresponding to the invasive front. Tumour samples with strong expression of CD271 also showed elevated CD133 expression.
In vitro: CD271-positive cells exhibited greater sphere-forming capability compared to CD271-negative cells and also showed higher expression of the stem cell marker CD133. Sphere formation was suppressed by CD271 inhibition.
Conclusions: CD271 is associated with a more undifferentiated phenotype of cSCC, both in vivo and in vitro. It serves as a useful

prognostic marker for predicting PFS and perineural invasion, and is a promising therapeutic target in cSCC.

P251 | Investigation of the anti-tumour humoral immune response in patients with skin cancers by antigens identification and B cell receptor sequencing

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Introduction: Skin cancer is one of the most common cancer types in humans, where the melanoma incidence showed an increasing trend during the past decades. Fortunately, there was a decreasing trend in mortality due to early diagnosis and advanced treatments, like immunotherapy. Nowadays, there are already monoclonal antibodies, working as immune checkpoint inhibitors, being used in melanoma treatment in clinic by modulating T cell mediated immune response. However, antibodies specific for melanoma associated antigens and humoral immune response in melanoma are not well developed and studied so far. Thus, in the project, we aim to search for antibodies targeting tumour associated antigens and study the role of these antibodies in melanoma.
Methods: We cloned premelanosome protein, Glycoprotein 100 (gp100), a well-characterised melanocyte differentiation antigen into CHO cell line and A375 cell line. Supernatant from cell culture was harvested and from which, proteins were purified through Ni- NTA affinity chromatography column in FPLC system. Then recombinant gp100 was used to fish antigen-specific switched memory B cells in patients samples including peripheral blood mononuclear cells (PBMCs), tumour infiltrating lymphocytes (TILs) and lymph nodes by fluorescence-activated single cell sorting. Reverse transcription and nested PCR were used to amplify the variable region of the B cell receptor (BCR) from each B cell. The amplified sequences were used to generate recombinant monoclonal antibodies (mAbs), which were subsequently tested in ELISA, flow cytometry, and various functional assays to assess their binding specificity and biological activity.
Results: From a patient's PBMCs, we successfully identified a monoclonal antibody clone, designated A7, that recognised gp100 on the surface of gp100-positive melanoma cell lines. In addition, when conjugated with a cytotoxic drug (MMAE), the antibody induced apoptosis in gp100-positive melanoma cells in vitro, suggesting its potential as an antibody-drug conjugate (ADC) for targeted melanoma therapy. However, the antibody did not display any NK cell-mediated antibody-dependent cellular cytotoxicity (ADCC) in vitro.
Conclusion: Monoclonal antibodies have revolutionised cancer therapy, especially in the context of targeted treatments such as ICIs. The antibody A7 has demonstrated the ability to mediate tumour cell death through drug conjugation, making it a potential candidate for the development of antibody-drug conjugates in melanoma treatment. Further exploration of the humoral immune response in melanoma, including the identification of additional tumour-associated antigens and their corresponding B cell receptors, may pave the way for more effective antibody-based therapies for melanoma and other cancers.

P252 | Hepatic passaging of melanoma cells acts on cell-cycle regulation and cell proliferation

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NRAS-mutant melanoma shows decreased survival despite novel therapies such as immune checkpoint inhibition or targeted therapies. In addition, liver metastasis is associated with poor response to these therapies. Since it is not known how the metastatic route affects the tumour intrinsic properties of melanoma, our study specifically investigated the effects of liver metastasis on NRAS-mutant melanoma.
Liver metastasis of WT31 melanoma harbouring a NRASQ61K mutation was modelled by intrasplenic or intravenous injection. WT31 melanoma was repeatedly passaged through the liver after intrasplenic injections five times, generating the subline WT31_P5IL. Liver metastases were evaluated macroscopically and by histological analysis. In addition, apoptosis, proliferation and vascularization of melanoma liver metastases were analysed. Bulk RNA-Seq of liver metastases was performed after negative MACS sorting for parenchymal cells. Last, scRNA-Seq of liver metastases was used to investigate intra-tumoral heterogeneity.
Liver metastases of WT31_P5IL were larger compared to WT31, while the number of liver metastases was similar. The percentage of necrotic liver metastases was significantly increased in WT31_P5IL, whereas the cleaved Caspase3 positive area per metastasis was similar between the two cell lines. Cell proliferation was significantly increased in WT31_P5IL as shown by Ki67 staining. A trend towards lower melanoma cell retention in the hepatic vascular bed was observed for WT31_P5IL. Vascular density was similar between WT31_P5IL and WT31 liver metastases.
To investigate whether these effects were liver specific, melanoma cells were also injected intravenously. Interestingly, the resulting lung and liver metastases of WT31_P5IL were also larger than those of WT31 melanoma. BulkRNA-Seq was performed to investigate how hepatic passaging affects tumour intrinsic properties. Pathway analysis revealed regulation of genes involved in cell cycle and oxidative phosphorylation. To further analyse intra-tumoral heterogeneity, scRNA-Seq of liver metastasis of WT31_P5IL and WT31 was performed. 7 clusters were identified.
Overall, our data demonstrate that tumour intrinsic properties such as cell proliferation can be strongly influenced by the liver microenvironment. The resulting melanoma phenotype was robust and observed in lung metastases after intravenous

injection. Currently, intra-tumoral heterogeneity is being further investigated and candidate genes are being evaluated.

P253 | Characterisation of extracellular vesicles to identify blood-based biomarkers of primary and secondary resistance to immune checkpoint inhibition in metastatic melanoma

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Background/Objectives: The isolation of extracellular vesicles (EVs) from liquid biopsy plasma samples in patients with malignant melanoma (MM) treated with immune checkpoint inhibition (ICI) could aid the discovery of new biomarkers to predict treatment response, detect minimal residual disease and track disease progression to potentially improve diagnosis and monitoring. While this tumour entity had a 5-year-survival rate of 5% before the implementation of ICI, 5-year-survival rates are now up to 50%.
Methods: We isolated EVs from the plasma of patients with advanced melanoma (174 samples from 82 patients) undergoing treatment with ICIs at the time of therapy initiation (baseline), six months, and, if applicable, at the timepoint of progressive disease (PD). Moreover, 22 healthy controls were included. Patients were subgrouped according to their treatment response (therapy response, primary non-response [PD before 6 months after treatment initiation] and secondary non-response [PD after 6 months after treatment initiation]).
EVs were isolated by size exclusion chromatography and ultracentrifugation. Nanoparticle Tracking Analysis (NTA) was conducted to measure the particle number and size distribution. Protein concentration was measured by the QuBit assay. The isolation of EVs was validated by electron microscopy. Finally, LC–MS/MS was utilised for proteomic analysis, which allowed the identification and quantification of peptides.
Results: NTA and electron microscopy confirmed the presence of purified EVs. LC–MS/MS identified 1754 proteins. Biostatistical analysis is currently pending, and results will be presented at the meeting.
Conclusion: We aim to discover new biomarkers by isolating EVs in patients with melanoma to detect progression, especially regarding the development of resistance to ICI.

P254 | The mechanistic role of IFN-γ in immunotherapy for melanoma and its clinical relevance in neoadjuvant therapy

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Introduction: Interferon-γ (IFN-γ) is crucial to regulating cellular immunity and antitumoral responses. However, its effect varies depending on the tumour microenvironment (TME), with potential to both suppress tumour growth—e.g., by inhibiting regulatory Tcell apoptosis or angiogenesis - and paradoxically promote tumorigenesis under certain conditions. Understanding the precise role of IFN-γ in the TME is therefore essential. Recently, IFN-γ and associated gene expression scores have been proposed to explain the mechanism of melanoma response to immunotherapy, with clinical IFN-γ scores increasingly used to predict prognosis and optimise patient selection for neoadjuvant immunotherapy.
Aim of the Project: This review aims to (1) characterise the function of IFN-γ and its associated genes in the TME of advanced melanoma patients undergoing immunotherapy and (2) evaluate the clinical utility of IFN-γ scores to determine if preclinical hypotheses on IFN-γ's role align with clinical outcomes.
Materials & Methods: We conducted a systematic search of the MEDLINE database for primary studies on IFN-γ and related proteins in the melanoma TME under immunotherapy. We also reviewed studies assessing clinical scores incorporating IFN-γ gene expression. All abstracts were screened, focusing on studies involving melanoma and immunotherapy.
Results: Several high-quality studies show the dual role of IFN-γ in the TME. IFN-γ can promote tumour progression by facilitating epithelial-to-mesenchymal transition (EMT), angiogenesis via endothelial cells, and immune evasion. Conversely, IFN-γ inhibits tumour growth by inducing cell cycle arrest, activating antigen-presenting cells, and suppressing immunosuppressive cells—actions that are dose-dependent. Multiple clinical studies applying IFN-γ gene expression profiles (GEPs), especially the GEP by Ayers et al., demonstrated significant predictive value for treatment response, with high negative predictive value (NPV) and reasonable positive predictive value (PPV). However, the association with survival outcomes, such as overall and progressionfree survival, remains inconsistent and requires further analysis. Additionally, exploration of GEP utility in aligning with preclinical data is necessary.
Conclusion: IFN-γ plays a nuanced and dose-dependent role within the melanoma TME, offering potential as both a biomarker and a therapeutic target in neoadjuvant immunotherapy. While IFN-γ GEPs show promise for predicting treatment response, further investigation is needed to reconcile these clinical applications with preclinical data. Comprehensive understanding of IFN-γ's dual role could improve prognostic accuracy and patient stratification, supporting more effective personalised therapies for melanoma.

P255 | Inhibition of epigenetic regulators as a potential strategy in melanoma cells resistant to targeted therapies

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Introduction: BRAF mutations are present in approximately 50% to 60% of malignant melanomas. While initially treatable, the emergence of resistance to BRAF-targeted therapies (BRAFi) represents a significant challenge and limits their efficacy. Our previous research has demonstrated that the BRAFV600E signalling pathway is associated with increased expression of EZH2, an epigenetic regulator linked to melanoma progression and worse overall survival. Epigenetic regulators, including EZH2 and HDACs, mediate gene expression by modulating chromatin formation. While these regulators have been shown to contribute to cancer progression, our understanding of their specific contribution to BRAFi resistance remains limited.
Aims: The objective of this study was to investigate whether the inhibition of epigenetic regulators could enhance the response of resistant melanoma cells to BRAFi and to elucidate the underlying molecular mechanisms.
Material and Methods: To validate the results, different Vemurafenib-resistant cell lines were treated with the identified compound candidates at varying concentrations of Vemurafenib, inhibitors of epigenetic regulators, or a combination of both. The viability of the cells was evaluated via cell viability assays, and apoptosis and cell cycle analysis were conducted using flow cytometry. To identify the molecular mechanisms, next generation sequencing was performed.
Results: Our results demonstrated that the orphan drug Tazemetostat, an EZH2 inhibitor, enhances the response to Vemurafenib in BRAFi-resistant cell lines. The combination of Vemurafenib and Tazemetostat resulted in a reduction in cell viability when A375R were treated in comparison to Vemurafenib monotherapy. Our findings demonstrate that the combined inhibition of EZH2 and BRAF signalling results in cell cycle arrest and increased apoptosis.
In a compound screen of 80 different inhibitors against epigenetic regulators, four HDAC inhibitors were identified that demonstrated synergistic effects with Vemurafenib in various BRAFi-resistant cell lines. It was observed that cells with a BRAF mutation exhibited greater susceptibility to HDAC inhibitors than BRAF wildtype cell lines. Next-generation sequencing was conducted on resistant melanoma cells treated with different regimes to identify the downstream pathways regulated by epigenetic regulators. Further functional investigation is being conducted to identify the molecular mechanisms underlying the observed synergistic action of Vemurafenib and inhibitors against epigenetic regulators.
Summary: Epigenetic regulators contribute to the development of resistance to BRAFi in melanoma. The combination of Vemurafenib with small molecules inhibiting these epigenetic regulators or affecting the expression of their downstream targets may represent a novel therapeutic option for melanomas resistant to targeted therapies.

P256 | Exploring melanoma with human skin organoids: Novel in vitro models for drug discovery

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Malignant melanoma is the primary cause of skin cancer-related deaths. Therefore, it is essential to comprehend its pathogenesis and explore new therapeutic approaches. Model systems, such as animal models and human in vitro melanoma cell culture systems, have significantly contributed to understanding the complexities of this disease. However, there are still challenges in the translation of findings from animal models to human pathology and in the reproduction of an appropriate microenvironment in human in vitro models.
To address these challenges, we have successfully generated skin organoids from human pluripotent stem cells that closely mimic the characteristics of native human skin. These hair-bearing skin organoids provide a physiologically relevant basis for studying melanoma progression. Using this system, we aim to establish a thorough in vitro melanoma model. Through the co-culture of melanoma cells, we are currently working to establish a model that captures the nuances of the tumour microenvironment, allowing the study of melanoma progression and the intricate interactions between tumour and stroma.
Eight different melanoma cell lines were tested for their spheroid-forming ability and then co-cultured with 80-day-old organoids to generate tumour-SO. Immunofluorescence staining was used to analyse tumour markers and assess changes in the microenvironment, focusing on the extracellular matrix and cancerassociated fibroblasts. This method offers valuable insights into the interaction between melanoma cells and their environment.
In conclusion, this work presents a new method for melanoma research, using human skin organoids to create a physiologically relevant melanoma model. This model overcomes the limitations of existing systems and allows for the investigation of tumour-stroma crosstalk. The research will contribute to the

development of more effective melanoma therapies through preclinical drug testing.

P257 (OP04/01) | An interferon-induced survival program in dedifferentiated melanoma cells drives resistance to immuno-virotherapy

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Interferons (IFNs) are critical cytokines of both anti-tumoral and anti-viral host defences. The ability of oncolytic viruses to activate the IFN system in tumour tissues is considered to be beneficial, because IFNs promote T cell responses in immune cell-poor tumours and synergize with immune checkpoint inhibition. Since the anti-viral effects of IFNs also limit oncolytic viral infection of cancer cells, oncolytic virotherapy is thought to be particularly effective against cancer cells that have become unresponsive to IFN signalling during the course of tumour evolution. However, the role of IFNs in anti-tumoral and anti-viral immunity is more complex, as the expression of a subset of IFN-stimulated genes has been shown to promote cell survival following viral infection, DNA damaging therapies, and cytotoxic T cell attack. How these complex and opposing effects of IFN-signalling in cancerous and virally infected cells shape responses to combination immuno-virotherapies is only beginning to be understood.
We hypothesized that the variable ability of melanoma cells to respond to IFNs leads to differential induction of anti-viral programs that limit the efficacy of oncolytic viral infection. Consistently, we observed varying levels of IFN-mediated protection against viral oncolysis in a panel of human melanoma cell lines. Since the melanocyte master transcription factor, MITF, can impair the responsiveness of melanoma cells to the inflammatory cytokine TNF, we hypothesized that MITF also participates in the control of the responsiveness to IFNs. To address this, we took advantage of the human MaMel65-tet-on-MITF melanoma cell line that was genetically engineered to express MITF in a doxycycline-inducible manner. We found that doxycycline-induced MITF expression significantly impaired IFN-mediated antiviral protection. To gain insight into the molecular mechanisms of how MITF might regulate IFN-mediated anti-viral responses, we employed RNAseq. We found that induction of MITF dampens the upregulation of IFNa-stimulated genes, including many with known anti-viral functions. Further bioinformatic analyses revealed that a subset of these IFN-stimulated, MITF-dampened anti-viral genes overlaps with genes in the IFN-related DNA damage resistance signature, that have been associated with resistance of cancer cells to radio-, chemo-, and immunotherapy.
We then independently assessed the role of these genes in melanoma cells as a potential predictive marker for the efficacy of immune checkpoint inhibition. For this, we interrogated a recently published scRNAseq sequencing data set obtained in a clinical trial for immune checkpoint inhibition in previously untreated melanoma patients. In our analysis, we focused on the IFN-stimulated genes that we identified in MaMel65 melanoma cells in vitro and compared their expression in melanoma cells from these patients before initiation of anti-PD1 immunotherapy ex vivo. Strikingly, the baseline expression of a core subset of IFN-induced genes was associated with non-response to immunotherapy in these patients. We further demonstrated that the baseline expression levels of IFN-stimulated genes, which were dampened by MITF in vitro, inversely correlated with the differentiation state in larger collections of melanoma cells. Finally, bioinformatics analyses including our own ATACseq data revealed that MITF regulates a transcription factor network that controls the expression of IFN-stimulated genes.
Taken together, our results show that melanoma cell dedifferentiation through MITF downregulation unleashes the expression of a subset of IFN-stimulated genes which contributes to melanoma cell survival, and thereby drives resistance to current therapies.

P258 | ENOX2 (tNOX) is a potential prognostic marker in primary malignant melanoma

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With increasing incidence of malignant melanoma, new prognostic biomarkers for clinical decision making become more important. In this study we evaluated the role of ENOX2 (tNOX), a cancer- and growth-associated protein, in prognosis and therapy of primary malignant melanoma. We conducted protein analysis using a melanoma tissue microarray, RNA expression analysis using The Cancer Genome Atlas (TCGA) as well as viability assays and western blots of melanoma cell lines treated with the ENOX2 inhibitor phenoxodiol (PXD) and the BRAF inhibitor (BRAFi) vemurafenib. We discovered that high ENOX2 expression is associated with decreased overall (OS), disease specific (DSS) and metastasis free survival (MFS) in primary melanoma (PM) and a reduced number of tumour infiltrating lymphocytes. A gradual rise in ENOX2 expression was found with the increase in malignant potential from benign nevi (BN) via PMs to melanoma metastases (MMs), as well as with increasing tumour thickness and stage. These results highlight the important role of ENOX2 in cancer growth, progression and metastasis. The ENOX2 expression was not limited to malignant cell lines, but could also be found in keratinocytes, fibroblasts and melanocytes. Viability of melanoma cell lines could be inhibited by PXD. Reduced induction of phospho-AKT under PXD could prevent the development of acquired BRAFi resistance. In conclusion, ENOX2 may serve as a potential prognostic marker and therapeutic target in malignant melanoma.

P259 (OP05/02) | Engineering stable scTvs: A rational design approach to TCR-based melanoma immunotherapy

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Recent advancements in immunotherapeutic strategies have significantly influenced the treatment landscape for melanoma. Despite the efficacy of immune checkpoint inhibitors (ICIs) in a subset of patients, a large number either do not respond or experience relapse, necessitating the exploration for alternative therapeutic approaches. Among these, chimeric antigen receptor (CAR) T cell therapy has demonstrated considerable success in haematological malignancies. However, its application in solid tumours, including melanoma, remains limited due to challenges such as restricted tumour specificity, accessibility and on-target off-tumour effects. The incorporation of T cell receptors (TCRs), specifically targeting melanoma-associated antigens, has been proposed to overcome these obstacles.

TCR-based CAR therapies offer new possibilities, especially due to their ability to recognise intracellular antigens that often evade the immune system. The TCR is a heterodimeric protein, which limits its handling in research and its application as a therapeutic. In the structurally familiar antigen binding fragments (Fabs) of an antibody, this problem has been resolved by creating stable single chain variable fragments (scFvs). The equivalent single chain variable fragment of a TCR (scTv) would be beneficial combining the binding properties of a TCR and the flexible application of the single chain construct. Here we demonstrate a strategy of rational protein design to increase stability of the scTvs. Mutants were expressed in vitro and evaluated for stability and functionality.

To enhance the stability of scTvs compared to the wild type, the high affinity variant of the 1G4 TCR (specific for the NY-ESO-1157-165 peptide bound to HLA-A*0201) was selected as a template. The scTv was designed rationally using Rosetta, a physics- and statistics-based protein design software. Two approaches were selected to guide the design protocol, an evolutionary approach utilising MSAs (PROSS), and ProteinMPNN, a deep neural network for inverse folding. In addition, we selected mutations only proposed by ProteinMPNN to compare the two protocols with the neural network. The resulting point mutations were introduced into the constructs, mutants were expressed in human embryonic kidney cells, purified and tested in vitro via biolayer interferometry (BLI) to analyse peptide-major-histocompatibility-complex (pMHC) binding kinetics, with wild-type TCR and TCR-mimic antibodies serving as controls.

Computational design led to improved expression of mutated scTvs relative to the wild type. A BLI assay was established to determine binding kinetics, and the affinities of the scTv and control antibodies aligned with literature values, confirming the functionality of the stabilised constructs. This work presents a computational pipeline for stabilising and expressing TCR derived single chained pMHC-binders, enabling efficient functional testing and optimization.

P260 | FICZ induces a sustained activation of AHR signalling in melanoma cells, but not in immortalised melanocytes in vitro

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The aryl hydrocarbon receptor (AHR) is a ligand binding cytosolic transcription factor belonging to the basic helix–loop–helix Per-Arnt-Sim (bHLH-PAS) superfamily. Historically, AHR is best known for mediating the toxicity and tumour-promoting properties of the carcinogen 2,3,7,8-tetrachlorodibenzo-p-diox in (TCDD). During the recent years it has become apparent that AHR activation by dietary compounds and endogenous molecules results in the induction of expression of diverse genes and furthermore plays a role in the major stages of tumorigenesis in a variety of cancer types. In different cancer types, increased levels of AHR as well as constitutive localization of AHR in the nucleus is a frequently observed phenomenon.

In this project, we investigated whether a sustained AHR activation is also present in melanoma cells. In a selected panel of mouse and human melanoma cell lines (e. g. HCmel12, B16-F10, YUMM 5.2, RIM-3, MaMel, MZ7), we tested the duration of AHR activation by the UVB-derived, endogenous AHR agonist FICZ, which is normally degraded by healthy cells via the CYP1A1 enzyme. We observed that exposure of melanoma cells to FICZ leads to persistent AHR signalling activity up to 72 h measured via qRT-PCR by CYP1A1 expression as a surrogate parameter for AHR activity. In contrast, the immortalised melanocyte cell line Melan-A showed only small upregulation of CYP1A1 by FICZ, and CYP1A1 levels returned to baseline after 24 h. This indicates that melanoma cell lines are more sensitive to AHR activation, and that sustained activation of AHR via FICZ is specific to melanoma cells, but not noncancer cells from the melanocytic lineage.

As a potential driver of the increased sensitivity of melanoma cells to FICZ, we hypothesized that melanoma cells might harbour higher expression of AHR. Interestingly, AHR mRNA was highly expressed in all melanoma cell lines. The expression of AHR could be further induced in most melanoma cell lines by exposure to the pro-inflammatory cytokines TNF-alpha or interferon gamma. These results indicate that the increased inflammatory state of melanoma cells directly promotes the expression of AHR, and might mechanistically influence AHR signalling cascades.

In future work, we will further investigate the underlying mechanisms for sustained AHR activation in melanoma cell lines and investigate the potential effect on metastatic progression and therapy resistance.

P261 (OP05/05) | Neutrophil extracellular traps (NETs) as potential biomarkers of immunotherapy in melanoma

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Introduction: Neutrophil granulocytes are the first line of defence upon infections but also promote and interfere with tumour growth. Activated neutrophils eject web-like DNA structures coated with enzymes and proteins to trap and eliminate pathogens and bacteria, neutrophil extracellular traps (NETs). NETs are procoagulant and have become a focus of research, particularly for their potential as biomarkers in melanoma patients undergoing immune checkpoint inhibitor (ICI) therapy. Given that resistance to ICI therapy can develop over time, early detection of such resistance is pivotal for improving treatment outcomes. Here, we aimed to investigate NETs as potential biomarkers in melanoma patients undergoing ICI therapy.
Methods: The study cohort included 40 advanced, non resectable and 40 resectable melanoma patients, with varying responses to ICI therapy, sourced from our melanoma biobank. Patients were divided into three groups according to their clinical characteristics: ICI responders (sustained response), primary resistance (radiologically confirmed disease progress within six months), and secondary resistance (radiologically confirmed disease progress after more than six months). Blood samples were collected at baseline, and each therapy cycle, and, when applicable, at the time of progression. For the resectable melanoma patients, only the baseline blood sample was utilised for comparison with the advanced patients in the analysis.
Initially, plasma samples from melanoma patients at various tumour stadiums were stained with Sytox Green to quantify cell-free DNA. NET quantification in serum was conducted using the Cayman Chemical Citrullinated Histone H3 (citH3) (Clone 11D3) ELISA Kit. In parallel, DNase I activity in patient serum was assessed by Single Radial Enzyme-Diffusion Assay (SRED), and SYBR Safe fluorescence was measured using the Biorad ChemiDoc MP system. Additionally, for tumour tissue staining different tissue sections were stained with DAPI, citH3, and CD16 to assess neutrophil infiltration within different melanoma tissues including primary tumour, metastases, and sentinel lymph nodes. Finally, a fluorogenic thrombin generation assay was performed, to evaluate coagulation system activation. Microparticle (MP) reagent and Tris were used as negative controls, while MP reagent and kaolin assessed the intrinsic pathway and tissue factor (TF) the extrinsic pathway. MP reagent served as a trigger in platelet-poor plasma to initiate thrombin generation and detect microparticles.
Results: Tissue staining revealed substantial neutrophil infiltration and significant NET presence in both primary melanoma and metastatic lesions. Advanced melanoma patients exhibited markedly higher levels of cell-free DNA compared to healthy individuals and those with completely resected melanoma, as quantified by Sytox Green. A pronounced prevalence of NETs was observed within the patient cohort, particularly in responders, who demonstrated low baseline NET levels that further decreased throughout the course of therapy. Responders also exhibited elevated DNase I activity, as indicated by the SRED assay. The thrombin generation assay was inconclusive regarding the intrinsic pathway between advanced melanoma patients and healthy controls, yet it revealed an absence of extrinsic pathway activation in melanoma patients, in contrast to the extrinsic activation observed in healthy individuals.
Conclusion/Outlook: In conclusion, melanoma patients exhibit elevated levels of circulating NETs in their blood, which may serve as a valuable biomarker to distinguish between responders and non-responders to immunotherapy.
To enhance the precision of NET quantification, we plan to combine citH3 and MPO ELISAs in forthcoming experiments. Additionally, a flow cytometry (FACS) analysis of neutrophils isolated from melanoma patients is planned to investigate the mechanisms behind the increased NET release observed in these individuals. Furthermore, analysis of different neutrophil subsets and surface markers is planned as well for a better understanding. There is an unmet need for further investigation of NETs and their role in ICI-treated melanoma in a fundamental and translational manner.

P262 | An investigation of melanoma immunity using single-cell multiomics: Novel cell–cell communication partners and their antitumoral role

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The treatment of malignant melanoma has long shifted to immunotherapy as first line therapy. However, a high rate of non-responders remains, or the treatment success is too often dampened by development of secondary resistance. Therefore, our study focuses on investigating novel cellular interactions between tumour cells in primary melanoma and cytotoxic effector T cells to unravel the workings of immune evasion. With single-cell transcriptomics and the LIANA bioinformatic framework, we were able to predict Ligand-Receptor (LR) pairings that are possible regulators of cell–cell communication. We validated several of these LR-pairings with a versatile spectrum of methods:

Biolayer Interferometry (BLI) confirmed the protein–protein binding of LR-pairings CD58 and CD2, CADM1 and CRTAM, and Galectin-1 and PTPRC. Additionally, these pairings were detected in close proximity in histological melanoma sections by immunofluorescence staining, proving the potential to interact. The immunological functionality was assessed with two different in vitro approaches: one, by autologous co-culture of patient derived melanoma cells and tumour-infiltrating lymphocytes (TILs). And second, by co-culture of melanoma cell lines and TCR-transgenic Jurkat cells. Here, we were able to show that for example the downregulation of Galectin-1 by siRNA results in significant lower Jurkat cell activity.

Furthermore, by utilising single-cell epigenomics (scATACseq), we investigated open chromatin regions upstream of the receptors in lymphocytes and compared it with the epigenetic state of immune cells in nevus. Among others, we found an open genomic region upstream of the PTPRC gene in lymphocytes, whereas this genomic position was not as active in lymphocytes found in a melanocytic nevus lesion. An analysis of binding motifs of this region provided a list of transcription factors that may have a regulatory effect on the expression of PTPRC in TILs.

Taken together, our study provides an overview of possible new cell-cellcommunication mechanisms between melanoma cells and effector T cells in an early stage of tumour immunity. The results point to the importance of the Galectin-1 and PTPRC axis in melanoma and provide information about the epigenetic regulation of PTPRC in TILs.

P263 | MPEG1 mRNA expression predicts prognosis and response to immune checkpoint blockade in melanoma

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Introduction: MPEG1 is a pore-forming protein found in antigen-presenting cells. It is thought to play a significant role in defending against bacterial infections and has been suggested as a key contributor to the process of antigen cross-presentation. However, its significance and specific function in melanoma remains controversial.

Materials and Methods: For cell subset profiling we analysed MPEG1 mRNA expression data from $N=29$ different immune cell types from $N=13$ donors, single-cell RNA-Seq data of $N=4645$ cells from $N=19$ melanoma tissues, and performed immunohistochemistry on melanoma tissue. We used the Human Protein Atlas for cell type clustering and the g:GO St tool on g:Profiler for statistical enrichment analysis of MPEG1-related genes in macrophages. To investigate the expression of MPEG1 in differentially polarised monocytes we used quantitative real-time PCR upon stimulation in vitro. We analysed MPEG1 mRNA expression data from tumour bulk tissue of $N=470$ untreated melanomas from The Cancer Genome Atlas and $N=121$ melanomas prior to administration of anti-PD-1 therapy with regard to overall survival, progression-free survival, and therapy response.

Results: We found that mainly macrophages, dendritic cells, and B cells express MPEG1 in melanoma tissue. Of note, we found a considerable expression of MPEG1 in melanophages. In macrophages, we found MPEG1 to be strongly associated with vesicle trafficking, lysosomal activity, and antigen presentation. Moreover, we found different levels of MPEG1 expression in polarised macrophages upon classical and alternative activation in vitro. Finally, higher MPEG1 expression was associated with favourable OS (hazard ratio (HR) = 0.91, [95% CI 0.86–0.96], $p=0.001$) in untreated melanoma and with PFS (HR = 0.96, [95% CI 0.93–0.98], $p=0.002$) and DC ($p=0.003$) in anti-PD-1-treated melanoma.

Conclusion: MPEG1 is mainly expressed by antigen-presenting cells of the tumour microenvironment and is associated with a favourable survival and response to anti- PD-1 immunotherapy in melanoma. It. Our results suggest that MPEG1 plays a role in anti-melanoma immune response.

P264 | Downstream effectors of TNF signalling as prognostic markers for response to immune checkpoint inhibitors in melanoma patients

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Checkpoint inhibitors (CPIs) have revolutionised the treatment of melanoma. However, predicting patient response remains challenging.

Melanoma cells frequently exhibit varying levels of cytokines and cytokine receptors such as IL-1α, TNF-α and TGF-β throughout different stages of disease progression. TNF signalling has been associated with both inhibition and promotion of melanoma growth.

The aim of this study was to analyse the prognostic value of downstream effectors of TNF signalling in the context of immune checkpoint inhibitor treatment for advanced melanoma.

We hypothesize that downstream effectors of TNF signalling are associate with a better prognosis under treatment with CPIs. This study investigated the prognostic significance of key proteins of cell death mechanisms (apoptosis, necroptosis) and the NF-kB signalling pathway, specifically the expression of CYLD, RIPK1, pRIPK3, and MLKL in patients with melanoma before receiving CPIs.

We analysed a cohort of 230 melanoma patients treated with CPIs and evaluated the association between expression levels of CYLD, RIPK1, pRIPK3 and MLKL with progression-free survival (PFS) and overall survival (OS). Our results demonstrated that high expression of CYLD was correlated with longer PFS in patients. Moreover, patients with brain metastases and high CYLD expression exhibited improved OS.

Furthermore, we observed that elevated RIPK1 expression was significantly associated with longer OS and showed a trend towards improved PFS. In contrast, pRIPK3 expression did not correlate with OS or PFS, but was lower in patients with metastases in more than three organs. MLKL expression did not

significantly impact OS or PFS in our cohort. Additionally, patients with high MLKL expression had a higher likelihood of developing metastases in the lungs, liver, and brain, often presenting with polytope metastases.
Our results suggest that CYLD and RIPK1 could serve as valuable prognostic biomarkers and potential therapeutic targets in melanoma. Currently, a new drug called Daromun is in clinical trials.
Daromun is composed of two antibody-cytokine fusion molecules (L19IL2 and L19TNF) designed to stimulate the immune response directly within the tumour microenvironment. TNF, a component of Daromun, can activate CYLD and RIPK1 via apoptosis and necroptosis pathway, potentially enhancing the anti-tumour response and complementing the effects of CPIs.
The positive correlation between high CYLD and RIPK1 expression and improved survival outcomes, combined with the preclinical data supporting the role of TNF in activating these proteins, underscores the rationale for combining Daromun with CPIs. Further investigations are warranted to elucidate the underlying mechanisms and to evaluate the clinical efficacy of this therapeutic combination.

P265 | Environmentally stressed drug-naïve melanoma cell subpopulations share a molecular signature with drug-induced early persister cells and drug-resistant cells

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Melanoma drug resistance, driven predominantly by dynamic heterogeneity, prevails in slow-cycling rather than proliferative tumour cells. We analysed the transcriptome of a 3D-spheroid melanoma model, where the inner cell-layers are slow-cycling induced by the microenvironment, and the outer cell-layers remain proliferative. The differentially expressed genes (DEGs) and pathways affecting the spheroid cell cycle (inner cells vs. outer cells, IVO) were then correlated with transcriptomics findings in drug-naïve (drug-sensitive) vs. drug-exposed tolerant (TVS) and resistant (RVS) melanoma models. IVO, TVS, and RVS had 1303, 1286, and 1195 upregulated, and 974, 1797 and 890 downregulated genes, respectively. IVO shared 14.7% (192 genes) of their upregulated DEGs and 36.6% (357 genes) of their downregulated DEGs with those of the TVS group, and 9.2% (120 genes) and 15.8% (154 genes) with DEGs from the RVS group. Although some pathways, such as 'lysosomal lumen', showed to be enriched in upregulated DEGs in all three groups, the number of shared enriched pathways in IVO and TVS were higher than IVO and RVS. In addition, the miRNA analysis revealed that 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 this miRNA plays a key role in the 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 cell layers to hypoxia, characterised by upregulation of genes like VEGFA, NFKB2, and FOSL1, mirrors stable resistance (RVS), which focuses on maintaining critical survival pathways and reducing cell migration and invasion. In conclusion, environmentally stressed inner cell-layers revealed gene expression patterns more similar to drug-induced early persister than irreversibly drug-resistant cells.

P266 | Targeting the tumour microenvironment to control melanoma proliferation and invasion

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In solid tumours, such as melanoma, cancer cells crosstalk with their tumour microenvironment (TME) composed of the extracellular matrix (ECM) and a variety of non-neoplastic cells. This process influences survival, proliferation, and dissemination of cancer cells. Undoubtedly, this aspect is crucial for a more accurate and complete understanding of cancer and its management. We demonstrate that melanoma cell plasticity is dictated by the expression and activity of the lineage-survival oncogene MITF by controlling TME composition and organisation and reducing ROCK-driven mechanotransduction. The resulting structural relaxation results in decreased p27Kip1 expression, ultimately reducing cell plasticity. Selective inhibition of ROCK phenocopies the effect of MITF. Our findings place tumour-TME crosstalk as a central driver of melanoma cell plasticity.
We have recently shown that the microtubule plus-end tracking proteins (+TIPs), CLASP1 and CLASP2, are essential for melanoma invasion into the tumour stroma (Ju et al. 2024, Nature Cell Biology). Here, we demonstrate that 'normal' melanoma cells readily invade into the stroma by squeezing through its complex ECM, while CLASP-depleted cells cannot withstand the shear forces of the stroma, consequently they rupture and die due to nuclear damage. We revealed that 'normal' melanoma cells are resistant to this physical stress, as they form a microtubule-dependent mechanoprotective mechanism: a flexible nuclear cage that protects the nucleus from shear forces. This nuclear cage is dependent on CLASP1 and CLASP2.
We propose that reducing melanoma cell plasticity will benefit targeted therapy, while structural relaxation and decreased tumour solid stress will improve immune checkpoint therapy. We further propose targeting CLASP1 and/or CLASP2 as a 'migrastatic' to prevent metastasis.

P267 | 4-Gene signature of circulating tumour-derived cell-free RNA as a relapsestratification biomarker in resectable high-risk melanoma

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Background: Most melanomas are diagnosed at resectable stages, and the risk of relapse varies depending on the stage. From stage IIB onwards, adjuvant therapy can be administered to reduce the risk of relapse and improve overall survival, although not all patients will experience recurrence. To date, there are no valid biomarkers that allow for effective patient stratification according to their risk of relapse, which would enable a more tailored recommendation of adjuvant therapy. Plasma-derived tumour-specific cell-free nucleic acids such as circulating cell-free tumour-specific RNA (cfRNA) are increasingly utilised as a non-invasive and real-time biomarker approach in many solid tumours. In our study, we demonstrated that our 4- gene signature (KPNA2, DTL, BACE2 and DTMYK) exhibited excellent diagnostic accuracy in plasma of advanced melanoma patients ($n=100$) compared to healthy donors ($n=20$), with receiver operating characteristics under the curve (ROC AUC) reaching 100% ($p<0.0001$). Furthermore, we found significantly elevated cfRNA levels of our 4-gene signature in fully resected high-risk melanoma patients ($n=7$).
Objectives: This study aimed to evaluate the role of our 4-gene signature as potential cfRNA-based liquid biopsy biomarker for risk stratification and real-time monitoring of fully resected high-risk melanoma patients.
Methods: We analysed 136 plasma samples from 35 stage III/IV patients with fully resectable high-risk melanoma ($n=19$ without relapse, $n=16$ with relapse). Samples were collected before and after surgery and at standard follow-up time points. Absolute cfRNA copies of the 4-gene signature were quantified on digital droplet PCR (ddPCR). We validated the diagnostic utility of our cfRNA candidates, where plasma samples from healthy donors (HD; $n=20$) served as controls.
Results: We found significantly higher cfRNA copies in resectable melanoma patients ($n=15$ clinical detectable lymph node metastasis; $n=10$ skin metastasis; 10 = sentinel lymph node micrometastasis) both before (ROC AUC 83.3% $p=0.002$) and after resection (ROC AUC 85.4% $p=0.0005$) compared to healthy donors. More importantly, cfRNA copies were significantly higher before (ROC AUC 74.3% $p=0.0136$) and after resection (ROC AUC 77.1% $p=0.0054$) in patients who experienced a relapse than those who did not. Increased baseline cfRNA copies of the 4-gene signature predicted a significantly shorter relapse-free survival (low-risk <2446 copies; intermediate-risk: >2446 and <4074 copies; high-risk: >4074 copies). The hazard ratio (HR) for relapse was significantly reduced in the low-risk (HR = 0.109, $p=0.0041$) and intermediate-risk (HR = 0.191, $p=0.0019$) groups compared to those with high-risk. Furthermore, we found that cfRNA copies significantly increased during follow-up in patients, who did experience a recurrence.
Conclusion: Our study demonstrates the potential role of our 4-gene signature in relapse stratification and monitoring of fully resected high-risk melanoma patients.

P268 | Ultra-sensitive NGS-based cell-free DNA blood test targeting BRAF, NRAS, KRAS, EGFR and PIK3CA in melanoma patients receiving immunotherapy

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Backround: Circulating cell-free tumour-derived DNA (ctDNA) has emerged as a valuable blood biomarker in cancer patients. Here, an ultra-sensitive targeted NGS approach was used to identify known driver mutations and drug gable novel mutations in melanoma patients receiving immunotherapy in a real-world setting.
Methods: Using a NGS approach that covers 1114 different COSMIC mutations (SYSMEX), cell-free DNA in blood plasma was analysed for selected hotspots in five cancer genes (BRAF, EGFR, KRAS, NRAS, PIC3CA) from 33 melanoma patients (unresectable AJCC stages III/IV) receiving immunotherapy. ctDNA results were correlated with clinical data and known risk factors/tumour markers.
Results: The absolute ctDNA detection limit was 6 mutant molecules (MM) and 0.07% mutant allele frequency (MAF). In total, 69.7% of patients were ctDNApositive (detection of at least one mutation). Most frequent mutations detected were BRAFV600E (44.3%), and NRASG12D (36.4%), followed by KRASG15V, A31T (both 9.1%), EGFRA859S and A767D (both 8.0%) and PIK3CAH1047R, E545A (both 20.0%). Interestingly, 12.0% of the ctDNA positive (and progressive) patients were detected by EGFRA859S and A767D (4.4%) and KRASG15V (8.8%) alone. S100 serum levels were significantly higher in ctDNA positive patients compared to ctDNA negative patients ($p=0.0077$). ctDNA-positive patients, whether driver or novel nondriver mutations, showed a significantly shorter progression-free survival compared to ctDNA-negative patients ($p=0.046$). Patients with discordant mutations in tumour tissue and blood showed increased time intervals between tumour and blood analyses ($p=0.0105$).
Conclusion: Detection of ctDNA offers valuable insights into the tumour's evolving genetic landscape and predicts disease progression in melanoma patients, with both driver and novel non-driver mutations associated with a poorer prognosis.

P269 | Downregulation of MHC-I on melanoma cells and decreased CD8+ T cell infiltration are associated with metastatic spread and resistance to immunotherapy

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The success of immune checkpoint inhibitors (ICI) for melanoma therapy has catalysed the introduction of ICI in increasingly early stages of disease. This exposes many patients with lower risk of relapse to the risk of protracted adverse events, highlighting the need for biomarkers guiding the use of ICI. Already many years ago, brisk infiltration of primary melanomas by lymphocytes has been linked to improved patient outcome, but controversial findings due to a high variability in classification systems have been described. CD8+ T-cells have been identified as a primary mediator of antitumor immunity in patients treated with ICI. As CD8+ T-cells require the presentation of antigen via MHC-I on target cells, downregulation and loss of MHC-I has been observed as resistance mechanisms to ICI. Here, we revisit the role of MHC-I expression and CD8+ T-cell infiltration in melanoma evolution using a cohort of advanced primary and matched metastatic melanomas by using an automated immunohistochemistry and digital pathology workflow. Our results show that downregulation of MHC-I expression is a frequent event in advanced primary melanomas that is associated with decreased CD8+ T cell infiltration and early metastatic spread to sentinel lymph nodes. Furthermore, MHC-I downregulation and decreased infiltration with CD8+ T-cells is also associated with resistance to ICI. Our results suggest that analyses of MHC-I expression and CD8+ T-cell infiltration patterns could serve as future biomarkers to guide the decision to treat patients in early stages of melanoma with ICI.

P270 | Influence of melanoma extracellular vesicles on vascular endothelial activation

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Extracellular vesicles (EVs) are crucial mediators of intercellular communication and contain a variety of biologically active molecules, including proteins, lipids, and nucleic acids. Melanoma cells release large amounts of EVs into the tumour microenvironment and the systemic circulation. Previous data suggest that tumour cell EVs educate the vascular endothelium in distant organs to promote hematogenous metastasis.
This work aims to better understand the EV-induced changes in the endothelium. We hypothesize that melanoma EVs lead to proinflammatory, proadhesive, and procoagulatory activation of endothelial cells, facilitating the vascular escape of circulating melanoma cells.
We isolated EVs from the cell culture supernatants of different melanoma cell lines, such as MV3, B16F10, and BLM, by differential ultracentrifugation and ultrafiltration. The size and concentration of EVs were analysed by nanoparticle tracking, and EV morphology was measured by atomic force and electron microscopy. The presence of EV-specific markers (Alix, Flot1, CD81, CD9) was confirmed by Western blot.
EVs were used to stimulate human umbilical vein endothelial cells in doses between 100 and 10 000 EVs per endothelial cell. Quantitative real-time PCR and mRNA sequencing revealed a dose-dependent upregulation of procoagulatory factors, such as tissue factor, and proadhesive molecules, including vascular endothelial cell adhesion molecule 1 (VCAM1). In functional assays, we detected increased vascular permeability using electric cell-substrate impedance sensing and the adhesion of circulating leukocytes using a microfluidic setup mimicking physiological blood flow. In conclusion, our data suggest that melanoma cell-derived EVs promote the activation of the vascular endothelium. In further research, we intend to identify the involved molecular players and confirm the pathophysiological relevance of our findings in a murine melanoma model.

P272 | The diagnostic value of CD4+ CD7- CD26- (double-negative) T-cells from flow cytometry in the diagnosis and follow up of mycosis fungoides and Sézary Syndrome: A prospective study

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Introduction: Blood involvement in mycosis fungoides (MF) and Sézary syndrome (SS) affects clinical decision-making and is generally associated with a poor prognosis. Sézary cells (SC), detected by flow cytometry (FC) express either CD4+ CD7- or CD4+ CD26- by current definition. We hypothesized that double-negative Sézary cells might have a diagnostic value in the diagnosis of MF and SS.
Objective: The aim of the study is to evaluate the sensitivity, specificity and validity of CD4+CD7-CD26- lymphocytes as a tumour marker for mycosis fungoides and Sézary syndrome.
Methods: In this prospective study, patients with the indication of a FC treated at our hospital were included. Parameters for example CD4+ CD7-CD26-, CD4+ CD7-, CD4+ CD26-, were determined using FC. The indication for FC and the (new, modified) diagnoses at the follow-up visit were extracted from the patient records. Data analysis was performed using descriptive statistics, correlation analyses, crosstabs and using the calculation of the area under the receiver operator characteristic curve (ROC-AUC) method.
Results: Between 01/2021 and 05/2022, 264 FC examinations were performed. The mean age of the patients was 68 years. There were 122 female patients. Of the FC, 107 were for SS therapy surveillance. The absolute CD26-CD7-CD4+ present areas of 0.649 when tested for B2 and 0.622 when tested for at least B1

blood-involvement. However, the CD26-CD7- relative to CD4+ T-cells reach an area of 0.593 for B2 and 0.587 for at least B1 blood-involvement.

P273 | Comparison of UV-based combination therapies for cutaneous T-cell lymphoma cell cultures

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Introduction: Skin directed and systemic therapies are frequently combined in cutaneous T-cell lymphomas (CTCL). The most commonly used CTCL cell lines are MyLa, HuT-78, SeAx and HH. The aim of this study is to determine promising therapy combinations and to validate them in these four cell lines.
Methods: In this experimental study cell lines were cultured in vitro and treated with methotrexate (MTX), UVA-light or chlormethine. We then measured the effectiveness using the resazurin viability assay after three days of treatment. After determining the viability and calculating the most promising concentrations, we combined the treatments evaluating possible synergistic effects. Cell viability of the cells after combination treatments was further measured using calcein-AM and apoptosis induction was investigated by a propidium iodide based cell cycle analysis.
Results: We observed a dose dependent cytotoxicity at concentrations ≥2 µg/mL of chlormethin, whereas MTX did not show effects on viability within 72 h at up to 100 µM. In contrast, higher doses of chlormethine are very effective in killing HH cells e.g. using 4 mg/mL reduced the viability to 44%. When doubling the doses to 8 µg/mL only 23% of the cells survive. UVA-irradiation affected the viability of HH and Myla cells but not HuT-78 and SeAx. Further combination therapies are being evaluated.
Conclusion: We conclude that metothrexate and UVA-light might not be potent in inducing apoptosis alone, whereas chlormethine is highly effective in decimating tumour cells even in small concentrations. Using chlormethine as an additional topical agent in treating T-cell lymphomas seems particularly promising. We expect that the cytotoxicity observed with chlormethine could be enhanced, combining it with metothrexate or UVA.

P274 | Liquid biopsy of cfDNA for the detection of genetic alterations through nanopore sequencing in uveal melanoma

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Background: Uveal melanoma (UM) is the most common primary intraocular tumour in adults. Depending on genetic alterations of the primary tumour, at least 50% of patients develop metastases. Especially monosomy 3 is associated with poor prognosis, while tumours with disomy 3 are limited in their potential to develop metastasis. The primary tumour is usually treated by enucleation or radiation and due to advances in local treatment, therapies that preserve the affected eye are increasingly applied. However, this limits the possibility to obtain genetic information from the primary tumour. Here, liquid biopsy represents a promising and minimally invasive alternative. Circulating cell-free tumour DNA (ctDNA) can be obtained from blood samples, allowing continuous and repeatable monitoring of genetic mutations. Furthermore, Oxford Nanopore sequencing offers the advantage of real-time and on-site sequencing. This could enable fast and accurate risk assessment while significantly reducing the burden on the patient by eliminating the need for a biopsy.
Methods: To perform liquid biopsy and detect monosomy 3 as well as relevant mutations using Nanopore technology, 15 mL blood samples from UM patients were collected in EDTA tubes at the Department of Dermatology, Uniklinikum Erlangen. Plasma was obtained and the cfDNA kit (QIAGEN) was used to isolate cell-free DNA (cfDNA). cfDNA was processed according to the cfDNA-specific protocol of Oxford Nanopore and then sequenced using the portable Nanopore sequencing device (MinION). Sample quality control of cfDNA was performed with a TAPE Station system. To determine the content of ctDNA within the cfDNA, Sanger sequencing was conducted to assess the mutation status and frequency of GNAQ and GNA11 in all DNA samples. Since GNAQ and GNA11-mutations occur mutually exclusive in over 90% of UM, they can be used to differentiate tumour DNA from DNA coming from healthy tissue. DNA extracted from matched primary tumour biopsies was used as positive control samples. Generated data was analysed using the EPI2me software.
Results: In total, we sequenced seven samples generating 30.5 GB of data, showing that Nanopore sequencing is a rapid way to gather cfDNA sequencing data (within 2 days, from sampling to result). We successfully validated GNAQ and GNA11 mutations in cfDNA and determined the content of ctDNA, ranging from 20–45%. Initial quality control and analysis show that sequencing provided high quality data (average of median phred score of 13.8).

Conclusion/Outlook: The preliminary data suggest that liquid biopsy combined with Nanopore sequencing is a promising tool for genetic analysis including the detection of monosomy 3 in UM. This might open possibilities for advancements in liquid biopsy techniques for early detection and quick characterisation of genetic changes associated with UM.

Miscellaneous

P275 | Lipid composition of the equine sebum at various body sites

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As in humans and many other mammals, sebum plays an important role in maintaining the skin barrier in horses. Equine sebum has a unique composition, as it includes unusual high amounts of lactones, making it an interesting model for comparative physiology studies. It is well known that in most species, including horses, the size and density of the sebaceous glands depend on their localization. Due to this distinct distribution of sebaceous glands and their hormonal regulation, it can be assumed that the sebum lipid composition is specific to certain regions of the body and might reflect the metabolic situation of the animal in health and disease. However, the exact composition of sebum lipids in horses is only known to a limited extent, in particular regarding specific regions of the body. To close this gap, we analysed the sebum lipid components of hair samples from various parts of the body of healthy horses.
Hair samples were taken by shaving from 6 healthy adult horses (4 mares, 2 geldings) from the pinna, forehead, forechest, medial forearm, fetlock bend, croup, tail rump and lower abdomen. An additional sample was taken from the surface secretions of the sulcus intermammarius from another mare. The lipids were isolated by chloroform/methanol extraction and separated by thin-layer chromatography (TLC). Resulting bands were visualised by primulin staining and analysed according to their intensity (peak height) and size (area under curve). To verify the composition of lipid classes within the different TLC spots, subsequent electrospray ionisation mass spectrometry was performed. In order to compare the composition of lipids between individual horses and different localizations, the lipid fractions were expressed as percentages of the sum of all AUCs of a sample. Results were compared by two-way ANOVA followed by a Tukey's test for multiple comparison.
We detected cholesterol esters, lactones, triacylglycerides (TAG), free fatty acids, cholesterol and phospholipids in sebum lipid samples. The largest proportion of the isolated lipids at all sampling points was made up by cholesterol esters, their proportion varying between 38% and 51%. Differences existed between the lower abdomen, on the one hand, and the pinna, medial forearm and forehead, on the other hand, with lower values of cholesterol esters at the lower abdomen. Lactones were the second largest fraction in most isolates, ranging from 22% to 32%. All other lipid classes accounted for less than 10% of the total isolate. An exception were the TAGs, which showed a significantly higher proportion in samples from the lower abdomen (24%) and the sulcus intermammarius (51%). The stronger occurrence of the TAGs at the lower abdomen was more pronounced in mares compared to geldings.
In this study we characterised different lipid classes from equine hair. In analogy to other species, it is likely that these lipids originate to a great extent from the secretion of the sebaceous glands, and the results can therefore describe the composition of sebum at various sites of the body surface. Future studies will focus on the reason for the high proportion of lactones in equine sebum and on TAGs on the lower abdomen. Furthermore, the sebum composition of horses with various metabolic diseases should be elucidated in order to determine whether an analysis of sebum lipids could represent an ancillary diagnostic tool.

P276 | Non-invasively quantification of meissner corpuscles from human glabrous skin using laser scan microscopy and relations with perception and age

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Motivation: Skin physiology can vary greatly according to anatomical position. However, little attention has been paid so far to the variations in different anatomical positions of glabrous skin. Considering the aging of the population and the importance of glabrous skin in sensory perception, it is essential to understand the effects of aging on receptors. Not all receptors can be quantified with our current techniques, but there is already evidence that Meissner's corpuscles (MCs) can be assessed non-invasively using Laser Scan Microscopy (LSM). This technique is advantageous since it replaces the need for biopsies, which are invasive.
Objectives: Therefore, the aim of this study was to assess how Meissner's corpuscles vary in different anatomical regions of the hand and their impact according to age on the evaluation of perception in pressure tests against the skin.
Study Design: We designed a study ($n = 60$, age range from 18 to 70 years old) to investigate the physiological differences between anatomic positions of human glabrous skin. We evaluate the glabrous skin of the finger from index finger of dominant and non-dominant hand, small finger pads, tenar palm position and volar arm, which represents hairy skin reference. We assessed these anatomical positions using laser scan microscopy (LSM) and evaluated the perception using two-points discrimination. Using LSM we could count the number of MCs per mm^2 using 3 mm x 3 mm images from the dermal-epidermal junction in the glabrous skin.
Results: We observed morphological and physiological differences between fingers, palm and volar arm. During the MCs quantification using LSM we observed that, generally, the MCs

are located next to the ridges. We could not measure the number of MCs from four participants because of the thick SC in all the anatomic positions. From the other 56 participants, we could evaluate MCs at 35 dominant index fingers, 52 non-dominant index fingers, 52 small fingers and 56 from palms. The small finger presented higher number of MCs per mm^2 than the palm ($p < 0.0001$) and similar results with the index fingers. For the two-point discrimination test we could observe the same tendency, with an increase in the threshold perception for the palm and volar arm compared to the phalanges ($p < 0.0001$). In glabrous skin, aging affected the number of Meissner Corpuscles per mm^2 and spatial accuracy. Twopoint discrimination at the volar arm revealed lower tactile resolution compared to glabrous skin. This parameter was correlated with aging only for glabrous skin.

Conclusions and Perspectives: This study brings several information regarding skin physiology for the clinical and cosmetic fields. Dermatological clinics can benefit from this knowledge by enhancing their understanding of skin function and adapting their treatment approaches accordingly. By exploring how differences in skin properties influence the threshold of the 2-points test, the study can shed light on the sensory experience of individuals and contribute to the development of materials with strong tactile appeal. Dermatological clinicians can use these findings to develop targeted interventions for age-related skin concerns, while the cosmetics industry can utilise the knowledge to formulate products that address specific aging-related issues.

P277 | Inducing hair follicle senescence ex vivo as novel tool to investigate hair aging

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Hair follicle (HF) aging not only manifests as hair greying but is also characterised by decreased hair density and thinning resulting from changes in hair shaft structure, extended telogen and/or catagen phases, or HF miniaturisation. Several models to simulate extrinsic hair aging, e.g. induced by oxidative stress, are already available. However, there is an unmet need of experimental models that reflect intrinsic mechanisms causing cell aging, including DNA damage, DNA repair defects, and cellular senescence. Therefore, we aimed to investigate whether the DNAintercalating nucleotide, BrdU, which has been successfully implicated to induce intrinsic skin aging ex vivo, would also promote changes in aging and senescence parameters in human HFs. To this end, human microdissected HFs from 3 healthy donors were treated with three different BrdU concentrations (10 µM, 500 µM, 1 mM) and analysed by quantitative (immuno-)histomorphometry. None of the tested BrdU concentrations induced cytotoxicity, as LDH release and melanin clumping were unaffected, whereas application of BrdU significantly decreased proliferation and increased apoptosis of hair matrix keratinocytes, assessed by Ki-67/TUNEL staining. Additionally, BrdU induced premature catagen development, inhibited hair shaft production and reduced hair shaft quality, demonstrated by a decreased expression of Keratin 85 and keratin associated protein 3.3 (KRTAP3.3) in the pre-cortical hair matrix and inner root sheath, respectively. BrdU treatment also upregulated γH2AX, a marker for DNA damage, and significantly increased the expression of the senescence marker, p21, in the hair matrix, as well as the senescenceassociated secretory phenotype indicator, CXCL10, in the outer root sheath. Most of these effects occurred in a dose-dependent manner throughout the HF epithelium. Thus, our initial findings suggest that BrdU acts as a non-cytotoxic stressor that accelerates intrinsic aging and senescence in human HFs ex vivo. Additionally, our assay provides a tool to study mechanisms of chronological aging and to test novel senolytic compounds ex vivo, which could potentially reduce HF aging also in vivo.

P278 | Anagen prolongation ex vivo, can estetrol serve as new treatment option for female pattern hair loss?

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Female pattern hair loss (FPHL) is the most prevalent type of hair loss in women, marked by early onset of the catagen phase and the miniaturisation of hair follicles (HFs). Effective strategies to combat FPHL remain a significant unmet need in women's health. Although clinical evidence indicates that hormone replacement therapy (HRT) may positively influence hair growth in post-menopausal women, preclinical studies on the effects of estrogens on hair growth regulation have yielded mixed results. Estetrol (E4), a natural oestrogen produced solely by the human fetal liver during pregnancy, is currently being developed as an HRT, but its impact on hair growth has not been evaluated, yet. In this study we treated healthy fronto-temporal, full-length microdissected HFs from 4 healthy female donors (>50 years) with 300 nM, 3 µM or 30 µM E4 and examined the effects on HF function. Quantitative (immuno-)histomorphometric analysis revealed a significant anagen prolongation by 3 µM E4, which was accompanied by significantly increased hair matrix keratinocyte proliferation (Ki-67+ cells). Next, dermal papilla (DP) inductivity was analysed to assess a possible underlying mechanism of anagen prolongation, and treatment with 3 µM E4 significantly increased alkaline phosphatase activity, indicating enhanced DP. The size of the DP and the DP cell density were unaltered by E4, but DP stalk and dermal cup cell numbers were significantly lower, indicating protection from DP fibroblast emigration. Finally, 3 µM E4 expanded the HF stem cell progeny pool, as shown by a significant increase in the percentage of CD34+ cells in the suprabulbar outer root sheath.
These findings show that E4 prolongs anagen, with the potential to decrease HF miniaturisation. Additionally, they warrant further investigation of E4 as a possible treatment option for hair loss conditions like FPHL.

P279 | Innovative perspective in wound management through targeted treatment of wound dressings with tetrapodal zinc oxide and RNase 7

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Difficult-to-heal and chronic wounds are often characterised by bacterial infections, impaired revascularization and the release of self-RNA which can exacerbate inflammation by inducing pro-inflammatory markers. Recently, we developed 3Dprinted alginate-based hydrogel wound dressings loaded with tetrapodal zinc oxide (t-ZnO) microcrystals. We have shown that these wound dressings have no cytotoxic effects on ex vivo skin explants and are antimicrobial active. Moreover, the t-ZnO sticks out of the hydrogel wound dressing and facilitates direct contact between its semiconducting surface and the surrounding tissue. RNase 7 is an antimicrobial active ribonuclease expressed and secreted by human keratinocytes. It exhibits antimicrobial activity against a broad spectrum of bacteria, viruses and fungi. Moreover, RNase 7 degrades RNA, including host RNA. Based on these characteristics, RNase 7 is a promising protein to transfer its beneficial properties to a t-ZnO-containing hydrogel wound dressing.

To facilitate the direct delivery of RNase 7 to wound-located bacteria, we initially evaluated the optimal way to bind RNase 7 to t-ZnO. For this purpose, we used both water alone and a 1:1 ethanol/water mixture as dispersion agent. After incubation and a subsequent drying step, electron microscopy showed an improved binding of RNase 7 to t-ZnO in the ethanol/water mixture, indicated by a ~50 nm thick, homogeneous film on the t-ZnO surface.

Subsequently, the tetrapods were evaluated for their antimicrobial properties against *Staphylococcus* (*S.*) *aureus* in an antimicrobial assay. *S. aureus* growth was inhibited by t-ZnO alone, but when loaded with RNase 7, this activity was significantly enhanced.

In summary, our study revealed that RNase 7 can be bound to t-ZnO resulting in t- ZnO with enhanced antimicrobial activity. A combination of t-ZnO and RNase 7 in a 3D-printed, patient-specific wound dressing may lead to enhanced, more effective and faster wound healing. Further analyses using t-ZnO-RNase 7-laden wound dressings will provide insight into their potential beneficial effects for anti-infective therapy and wound healing.

P280 | Taxane chemotherapy accelerates aging of human hair follicles and their stem cells

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Chemotherapy causes substantial damage to human scalp hair follicles (HFs) and their stem cells on multiple levels. However, it remains unclear whether chemotherapy also promotes premature HF aging. To investigate this, we exposed full-length human scalp HFs from three independent donors for 6 days to the taxane, paclitaxel (100 nM), a known inducer of both acute and permanent chemotherapyinduced alopecia (CIA), which we had previously demonstrated to apoptosis, "mitotic catastrophe", DNA damage, and pathological EMT in human epithelial HF stem cells (eHFSCs) ex vivo.

Quantitative (immuno-)histomorphometry confirmed considerable double-strand DNA damage of hair matrix keratinocytes after paclitaxel treatment, as indicated by elevated γH2Ax expression, reflecting substantial rapidly proliferating hair matrix cell injury. Moreover, a significant reduction in the expression of nuclear Lamin B1, indicating senescence, and two key aging and mitochondrial biomarkers, SIRT1 and PGC1α, within the hair matrix epithelium were observed. Protein expression of collagen XVIIa1, a crucial marker of bulge stem cell niche integrity, was also significantly reduced. Since epigenetic modifications are known to accumulate throughout aging, we also analysed DNA methylation markers, 5-methylcytosine (5- mC), and DNA methyltransferase 1 (DNMT1). Both 5-mC and DNMT1 levels were significantly reduced in hair matrix keratinocytes and HF stem cells, while the DNA demethylation and DNA damage site marker, 5-hydroxymethylcytosine (5-hmC), was markedly increased. Additional analyses are underway to determine if paclitaxel also inhibits the phagocytic clearing of eHFSCs, which is essential for maintaining bulge physiology.

These findings demonstrate that taxanes not only induce epigenetic changes but also promote premature aging of both rapidly proliferating hair matrix keratinocytes and relatively quiescent eHFSCs in the bulge. This suggests that taxanes impair the regenerative and reparative abilities of HFs to a greater extent than previously recognised, encompassing epigenetic modifications, DNA damage, and premature aging processes. These factors could contribute to the development of permanent CIA frequently associated with taxane therapy. Thus, HF-targeting topical therapeutics need to be developed to effectively counteract the above processes and reduce/prevent the development of permanent CIA. This could potentially lower the risk of scalp micrometastases evading chemotherapy by activating senescence programs.

P281 | Low-intensity ultrasound as a novel biophysical strategy to significantly reduce taxane-induced hair follicle damage in vivo: Evidence from a novel humanised mouse model of chemotherapy-induced alopecia

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Chemotherapy-induced alopecia remains one of the most distressing adverse effects of cancer therapy. No pharmacological treatments are available that selectively protect healthy hair follicles (HFs) and their epithelial stem cells (eHFSCs) from acute and permanent chemotherapy-induced damage (note that taxane therapy shows a high incidence of permanent alopecia due to irreversible eHFSC damage). Recognising the fundamental HF biology differences between mice and humans, we have probed the efficacy of low-intensity ultrasound (LIUS) to counter taxaneinduced human HF and eHFSCs damage in vivo in a novel humanised paclitaxel (PTX)-induced alopecia (PIA) mouse model. A single dose of PTX (20 mg/kg) was injected i.p. into SCID/beige mice xenotransplanted with healthy human scalp skin in the presence or absence of LIUS (5 min at 45 KHz applied 2× in a water bath after PTX injection). 30 h after PTX injection, the efficacy of HF damage induction by systemic PTX in the human xenotransplants was demonstrated by the presence of characteristic HF dystrophy signs, incl. pigmentary abnormalities and the appearance of ectopic melanin in the bulge and sub-bulge area, which do not contain any melanin physiologically, indicating substantial cytotoxic in these HF stem/progenitor cell regions. A single injection of PTX in vivo also induced major DNA damage (γ-H2A.x) as well as micronucleation in both hair matrix keratinocytes and K15+ eHFSCs, even though the number of K15+ cells and the expression level of K15 remained high. This indicated that a single PTX injection had not yet significantly reduced the stem cell reservoir at the time of sample, in line with the fact that taxanes primarily affect highly proliferating cells such as hair matrix keratinocytes via mitotic spindle arrest, thus further corroborating the validity and instructiveness of our new PIA in vivo model.

Local LIUS application to the xenotransplants 6 and 24 h after PTX injection reduced melanin clumping in the anagen hair bulb and ectopic melanin localization in the hair bulb and the bulge/sub-bulge area. Moreover, LIUS significantly reduced DNA damage, the % of γ-H2A.x/K15 double-positive cells, and micronucleation in both the bulge and bulb epithelium. Interestingly, LIUS also significantly increased the expression of RARγ and RXRα in the bulge and bulb, i.e. key signalling pathways that have recently been found to promote the proper clearance apoptotic eHFSCs in the bulge of mice, which is vital for the maintenance of bulge health.

These pilot data provide the first evidence that a simple, widely available biophysical intervention, LIUS, can greatly reduce PTX-induced damage on multiple levels in both highly proliferative hair matrix epithelium and quiescent human eHFSCs in vivo. LIUS may also stimulate the RARγ-RXRα pathway to enhance the clearance of chemotherapy-damaged eHFSCs and hair matrix keratinocytes. These preclinical in vivo data strongly support the usefulness of LIUS as a preventive strategy for reducing the risk of both acute and permanent PIA in human scalp HFs.

P282 | Re-examining the forgotten hair growth-promoting properties of an old calcineurin inhibitor: Tacrolimus promotes human scalp hair follicle growth ex vivo

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In dermatology, the fungal-derived immunophilin ligands and calcineurin inhibitors, tacrolimus and pimecrolimus, are mostly used as topical therapeutics for atopic dermatitis. Yet, their good safety profiles invite the exploration of other dermatologically relevant indications. For example, we had previously shown that tacrolimus restores IFNγ-induced human hair follicle (HF) immune privilege collapse ex vivo and profoundly modulates HF cycling in rodents in vivo. However, its impact on human HF growth remains poorly investigated. To explore this, organ-cultured human anagen VI scalp HFs were treated "systemically" with tacrolimus (10, 100 nM; added to the culture medium) for 6 days. Quantitative (immuno-)histomorphometry showed that tacrolimus (100 nM) prolonged anagen/inhibited catagen, just as it did in mice in vivo, yet without significantly affecting hair matrix keratinocyte proliferation/ apoptosis. However, tacrolimus significantly inhibits fibroblast emigration from the dermal papilla, which is expected to counter HF miniaturisation. Furthermore, tacrolimus reduced protein expression of the key catagen-promoting growth factor, TGF-β2, in outer root sheath keratinocytes and increased that of FGF7/KGF in hair matrix keratinocytes. Tacrolimus also significantly increased the keratin 85 protein expression in the precortical hair matrix. We'll also report ongoing gene expression profiling data that point to unconventional, NFATc1-independent mechanisms via which tacrolimus may exert its remarkable, underappreciated hair growth-promoting effects on human scalp HF. Our preliminary data suggest that these may include reduction of known Wnt inhibitors, not unlike but distinct from the NFATc1-indpendent mechanism of action by which cyclosporine promotes human hair growth.

Altogether, our pilot study suggests that topically applied tacrolimus deserves systematic exploration as a candidate hair growth-promoting agent in the future management of a wide range of inflammatory and non-inflammatory hair loss disorders.

P283 | Human hair growth is controlled by chemosensory signalling pathways: Olfactory receptor OR10J1 activation promotes scalp hair follicle growth and epithelial hair follicle stem cell maintenance ex vivo

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Chemosensory receptors like olfactory receptors (ORs), taste receptors (TRs), and transient receptor potential ion channels (TRPs), have increasingly become appreciated as important players in human physiology and pathology well beyond the core sensations of smell and taste. This includes a wide range of functions that are now recognised for selected ORs capable of detecting odorants/fragrances in human skin and suggests that chemoreceptors are not mere evolutionary relics but important modulators of human skin and skin appendage functions and viable targets for dermatological intervention with non-drugs (fragrances/odorants), e.g., hair growth promotion via stimulation of OR2AT4 with the synthetic odorant, Sandalore.

Since OR2AT4 stimulation also changed the intrafollicular transcription of OR10J1, which is stimulated by the synthetic heptanol fragrance, dimetol, a common perfume ingredient and food flavour enhancer, we first assessed whether and where exactly human scalp HFs express OR10J1 protein, using a commercially available antibody against OR10J1, which we validated by demonstrating the expected OR10J1 expression on human spermatozoa. IF microscopy showed prominent OR10J1 protein expression in the bulge and sub-bulge region of the HF epithelium, i.e. the main location of K15+ or CD34+ epithelial stem/progenitor cells in human HFs. OR10J1 activation by dimetol significantly promoted hair growth by retaining a higher percentage of HFs in anagen and suppressing catagen transformation ex vivo, associated with significantly increased proliferation of hair matrix keratinocytes. Dimetol also significantly increased intrafollicular production of the anagenmaintaining key growth factor, IGF-1, and decreased that of catagen-promoting TGF-β2. Interestingly, dimetol treatment also increased the percentage and protein expression level of K15+ and CD34+ epithelial stem/progenitor cells in the HF bulge and sub-bulge regions ex vivo. Gene silencing of OR10J1 by siRNA in the presence of excess dimetol not only largely abrogated all of the above hair growthand HF stem cell-promoting effects of dimetol but also was hair growth-inhibitory (e.g., it induced premature catagen development, reduce IG1 expression). This unequivocally demonstrates that the hair growth- and stem cell-promoting effects of dimetol are specifically mediated by OR10J1 signalling and strongly suggests that human anagen HFs and their stem/progenitors require "tonic" stimulation via this OR. This, in turn, raises the important open question of which (intrafollicularly generated?) endogenous agonists provide this stimulation.

Here, we introduce OR10J1-mediated signalling as yet another functionally important chemosensory pathway for regulation of the growth of human scalp HFs that can be targeted with non-drugs (synthetic fragrances) suitable for both cosmetic and nutraceutical applications, e.g. in the context of managing hair loss disorders associated with telogen effluvium. We also demonstrate that OR10J1-signalling profoundly impacts both the remodelling of a human (mini)organ and the expression/production of potent growth factors (IGF-1, TGF-β2). Finally, we provide the first evidence that selected ORs regulate human HF stem/progenitor cell functions ex vivo. This underscores the fundamental importance and therapeutic targetability of chemosensory signalling pathways in human HF physiology.

P284 | A novel strategy for re-pigmenting grey hair Follicles: The bitter taste receptor TAS2R50 stimulates human scalp hair follicle pigmentation

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Bitter taste receptors (TAS2Rs) are G-protein-coupled receptors historically associated with bitter taste perception. They have increasingly become appreciated as important players in human peripheral organs physiology and pathology, particularly in human skin and its appendages. This suggests that chemoreceptors are not mere evolutionary relics but important modulators of human skin and hair follicles (HFs) functions and viable targets for dermatological intervention with nondrugs (bitter/sweet tastants), e.g., hair growth inhibition via stimulation of TAS2R4 with the sweetener, Rebaudioside A. Since transcriptomic analysis suggested that TAS2R50 which is stimulated by natural herbal bitter amarogentin, is expressed in human HFs, we have followed this up by IF microscopy. We first assessed where exactly human scalp HFs express TAS2R50 protein in human scalp HFs and established the presence of TAS2R50 protein in the hair matrix and outer root sheath of human anagen scalp HFs. Next, we treated human scalp HFs for 6 days for amarogentin (200 μM, a concentration previously showed to activate TAS2R50).

Our quantitative immuno-histomorphometric analyses showed that amarogentin significantly promoted hair growth by maintaining a higher percentage of HFs in anagen ex vivo. This hair growth-promoting effect was also associated with a significantly higher percentage of proliferative hair matrix keratinocytes in amarogentin-treated HFs compared to vehicle-treated HFs. These data are interesting in the hair pigmentation context since they increase the time window where HF pigmentation can be stimulated (pigmentation is only active during the anagen phase). We then next investigate the impact of amarogentin on HF pigmentation. Our data showed that melanin synthesis was significantly increased in amarogentin-treated HFs. This was directly correlated with the significantly increased protein expression of the pre-melanosome marker, gp100, but also of the percentage of gp100+ cells in the HF pigmentary unit. Interestingly, the number of dendrites per gp100+ melanocyte

was also significantly increased. Gene silencing of TAS2R50 by siRNA in the presence of excess amarogentin largely abrogated all of the above effects (e.g., it reduced melanin synthesis, and gp100 expression and cell number).

Given these pro-pigmentary effects of amarogentin treatment in human scalp HFs, we explored, next, whether TAS2R50 activation could stimulate re-pigmentation in grey HFs. Remarkably, some TAS2R50 agonist-treated grey HFs showed signs of re-pigmentation as indicated by the significant increase in melanin production compared to vehicle-treated HFs ex vivo. This demonstrates that the hair pigmentationpromoting effects of amarogentin are specifically mediated by TAS2R50 signalling and strongly suggests that human anagen HFs require "tonic" stimulation via this TAS2R50 for their pigmentation. This, in turn, raises the important open question of which (intrafollicularly generated?) endogenous agonists provide this stimulation.

In summary, our data introduce signalling mediated by the bitter taste receptor, TAS2R50, as an unsuspected, novel regulator of human HF pigmentation, which can even reactivate melanin production in selected grey HFs. Our data underscore the principal reversibility of hair greying and suggest the clinical use of safe tastants, such as non-drug TAS2R50 agonists, in the management of canities and poliosis.

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