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ABSTRACT

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Allergology

P001 | Drug rash to antibiotics and analgesics - causal classification using a modified Naranjo algorithm

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Drug eruptions are among the most common drug-related side effects. A particular challenge is the reliable assignment to a triggering drug. In our retrospective work, we examined the data of 409 patients who were treated in the Clinic and Polyclinic for Dermatology and Venereology, University Medical Center Rostock, due to a cutaneous drug reaction to an antibiotic or analgesic over a period of 20 years. Based on the documentation of the patient files, the suspected diagnosis of "drug eruption" was confirmed using a modified causality assessment based on the decision algorithm by Naranjo et al. By including histologic diagnosis the causal relationship could be classified as probable or certain in 200 patients. Otherwise only 160 would have been classifiable. Among the 128 patients on antibiotics the majority of cases (58.6%) involved beta-lactam antibiotics with ampicillin + sulbactam as the leading preparation. Clinically, 57.8% had a maculopapular rash. Among the patients who responded to analgesics, in 75%, the drug eruption was triggered by NSAIDs, with acetylsalicylic acid as the most common trigger. Clinically, the drug eruptions associated with analgesics most often presented as urticarial rashes (41.7%). Our results showed that the causality assessment scheme used allows a systematic clarification of drug intolerance retrospectively, even in very heterogeneous data situations. Antibiotics, especially beta-lactam antibiotics, typically caused maculopapular rashes, whereas NSAIDs primarily caused urticaria.

P002 (OP05/03) | IgE and anaphylaxis specific to the carbohydrate alpha-gal depend on interleukin-4

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Alpha-gal (Galα1-3Galβ1-4GlcNAc) is a carbohydrate with the potential to elicit fatal allergic reactions in response to mammalian meat and drugs of mammalian origin. This type of allergy is induced by tick bites and therapeutic options for this skin-driven variant of food allergy are so far limited to avoidance of the allergen and treatment of symptoms. Thus, a better understanding of the immune mechanisms resulting in sensitization and elicitation of food allergy through the skin is crucial, especially in the case of a carbohydrate allergen for which underlying immune responses are poorly understood. In this study, we established a mouse model of alpha-gal allergy in which GGTA1-deficient mice devoid of alpha-gal glycosylations are sensitized with the alpha-gal-carrying self-protein mouse serum albumin by repetitive intracutaneous injections. These mice sensitized to alpha-gal displayed increased levels of alphagal- specific IgE and IgG1 antibodies and developed systemic anaphylaxis upon challenge with both alpha-gal-containing glycoproteins and glycolipids. In accordance with alpha-gal-allergic patients, we detected elevated numbers of basophils at the site of sensitization as well as increased numbers of alpha-gal-specific B cells, germinal center B cells and B cells of IgE and IgG1 isotype in skin-draining lymph nodes. With antibody-mediated depletion of IL-4 during sensitization, we could prove for the first time that sensitization and elicitation of allergy to alpha-gal and thus a carbohydrate allergen is dependent on IL-4. These findings establish IL-4 as a potential therapeutic target to interfere with alpha-gal allergy elicited by tick bites.

P003 | In the skin lesions of patients with mycosis fungoides, the number of MRGPRX2-expressing cells is increased and correlates with mast cell numbers

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Abstract

Background: Mycosis fungoides (MF) is an indolent T-cell lymphoma that mainly affects the skin and presents with itch in more than half of the patients. Recently, the expression of Mas-related G protein-coupled receptor X2 (MRGPRX2), a receptor of mast cell (MC) responsible for the IgE-independent non-histaminergic itch, has been shown in lesional skin of patients with pruritic skin diseases, including chronic urticaria, prurigo, and mastocytosis. As of yet, limited knowledge exists regarding the MRGPRX2 expression in the skin of patients with MF.

Objectives: To investigate the number of MRGPRX2-expressing (MRGPRX2+) cells in the skin of patients with MF and its correlation with clinical and laboratory characteristics of the disease.

Methods: MRGPRX2 was analyzed in lesional and non-lesional skin of MF patients and healthy skin tissues by immunohistochemistry. Co-localization of MRGPRX2 with the MC marker tryptase was assessed by immunofluorescence. Public single-cell RNAseq (scRNAseq) data was reanalyzed to identify the MRGPRX2 expression on the distinct cell types.

Results: In the lesional skin of MF patients, MRGPRX2+ cells number was higher than in non-lesional skin and healthy control skin (mean:15.12 vs. 6.84 vs. 5.51 cells/mm2, p=0.04), and correlated with MC numbers (r=0.73, p=0.02). MC was the primary cell type expressing MRGPRX2 in MF patients. The ratio of MRGPRX2+ MCs to MRGPRX2+ cells in lesional and non-lesional skin correlated with the severity of disease (r=0.71, p=0.02 and r=0.67, p=0.03, respectively).

Conclusions: Our findings point to the role of MRGPRX2 and MC in the pathogenesis of MF that should be investigated in further studies.

P004 | Profiling patch tested patients with permanent tattoos - IVDK data 2020 - 2022

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Background: The Information Network of Departments of Dermatology (IVDK) is a clinical epidemiological monitoring system for the prevalence of sensitization to contact allergens in Germany, Austria and Switzerland. Permanent tattooing is an invasive introduction of tattoo ink into the dermis. The ink and aftercare cosmetics applied on pre-damaged skin may contain skin sensitizers. Therefore, sensitization patterns may be special in tattooed patients.

Objectives: To identify patient characteristics and the pattern of sensitization in tattooed patients patch tested within the IVDK.

Methods: Case-control analysis of patient characteristics and proportions of positive reactions to allergens of the baseline series of the German Contact Dermatitis Research Group (DKG) in 1648 consecutive patients with and 8045 consecutive patients without permanent tattoos. Statistical significance of differences in proportions of positive PT reactions and anamnestic items in disjunct groups of patients was concluded from non-overlapping 95% confidence intervals (95%-CIs). In a multivariate logistic regression analysis, gender, age (younger than 40 years or 40 years and older) and being tattooed were used as independent (explanatory) variables, and a positive patch test to nickel sulphate as dependent (target) variable.

Results: Having permanent tattoos was associated with female sex, age <40 years, tobacco smoking, (past or present) atopic dermatitis, (occupational) hand dermatitis and being employed in particular occupational groups (e.g., healthcare workers, mechanics, hairdressers). Sensitization to nickel was increased in tattooed patients

and associated with female sex (OR 4.18 [95%-CI, 3.56-4.95]), age ≥40 years (OR 1.45 [95%-CI, 1.26-1.67]) and the prevalence of permanent tattoos (OR 1.35 [95%-CI, 1.16-1.58]).

Conclusions: No specific (or occupational) sensitization patterns associated with permanent tattoos were identified, except for nickel. The association between nickel sensitization and permanent tattoos is probably confounded by missing data on past reactions to pierced costume jewellery. Socio-economic factors most probably contribute to the connection between tattoos, tobacco smoking, occupational and hand dermatitis and being employed in particular occupational groups.

P005 | Sex-dependent differences of bone marrow-derived dendritic cells in nickel uptake and migration behaviour

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Type IV sensitization to nickel (Ni2+) is the basis of the most common contact allergy worldwide. Although various exogenous and endogenous factors can be considered, it is ultimately unclear why women suffer from allergies to Ni2+ much more frequently than men. In addition to the general mechanisms underlying the development of contact allergies, the activation of Toll like receptor (TLR) 4 is of particular importance in the case of Ni2+. Since wildtype mice do not have the Ni2+ binding sites in their TLR4, they usually do not develop the corresponding sensitization.

In this ongoing pilot study, we investigated whether and to what extent there are sexspecific differences in innate immune functions related to nickel. To this end, bone marrow-derived dendritic cells (BMDC) from mice expressing transgenic human but not murine TLR4 were investigated. These mice are susceptible to develop type IV sensitization to nickel. BMDC from female and male mice were cultured for 8d in the presence of GM-CSF and then stimulated for 1 day with Ni2+ in the absence or presence of LPS.

Our preliminary results showed no sex-dependent differences in cell numbers, purity of populations, and expression of MHC II and the costimulatory molecules CD40, CD80, and CD86, regardless of the presence or absence of Ni2+. The expression levels of human TLR4 were also the same in both cell populations. Surprisingly, however, Newport Green staining revealed evidence of considerably greater nickel uptake in cells from female donors compared with their male counterparts. Additional exposure to LPS leveled out these differences.

Directed migration against a CCL19/CCL21 chemokine gradient in a transwell system was significantly more pronounced at d8 in female cells than in male cells. The sex-dependent difference in directed migration was accompanied by stronger expression of the receptor CCR7 in female BMDC. Again, this difference was no longer detectable after additional stimulation with LPS at d9, whereby this treatment was associated with overall strongly increased migration in both sexes.

Our preliminary results provide to our knowledge the first evidence that true sexdependent immunological differences relevant to the response to Ni2+ may indeed exist.

P006 | Cannabinoid-1 receptors modulate acute and chronic atopic inflammation of the skin

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Atopic dermatitis (AD) is a heterogeneous, multifactorial skin disease accompanied by eczematous skin lesions and intense pruritus. Clinically, eczema may present acutely or chronically. Numerous studies have evaluated the molecular changes in chronic eczema, but only little is known about the transition from acute to chronic lesions or the pathomechanisms that shape chronic inflammatory activity. We recently demonstrated that the endocannabinoid system (ECS), consisting of specific receptors (CB1, CB2) and endogenous ligands is involved in the regulation of cutaneous inflammation, by contributing to skin barrier function and homeostasis. CB1 receptors are abundantly expressed on keratinocytes and peripheral nerve fibres, CB2 receptors are detected mostly on immune cells. Here, we evaluated the impact of CB1- and CB2-receptor signaling on acute and chronic inflammation in an experimental model of oxazolone induced atopic-like inflammation.

CB1-/-, CB2-/- mice and their wild type (WT) littermate controls were sensitized with 5% oxazolone on the shaved abdomen. For acute inflammation, we challenged mice daily with 0.1% oxazolone on both ears and back skin from day 5 to 14 and for chronic inflammation until day 23. Ear swelling and transepidermal water loss were measured daily. For further histological and immunological analyses, we collected skin tissue and blood on day 15 (acute) and day 25 (chronic). CB1-/- animals displayed significantly severe eczema as well as an increased ear swelling and transepidermal water loss in the acute phase of inflammation in comparison to CB2-/- and wild type animals. Flow cytometric analyses revealed an increased infiltration of CD3+/CD4+ t cells and CD11b+/F4-80+/Siglec F+ eosinophils in acute inflamed skin as well as blood eosinophilia in CB1-/- mice. mRNA levels of IL-4 and Tnf-α also were significantly higher when compared to WT mice. In the chronic phase, eczematous skin lesions still were most prominent in CB1-/- mice, although inflammatory ear swelling decreased in all mouse strains. Tissue and blood eosinophilia further persisted and mRNA levels of IL-4, Cxcl10 and IL-10 were elevated in chronic inflamed skin of CB1-/- animals in comparison to WT controls.

Together, these results show that CB1, but not CB2 signaling has an impact on atopic inflammation in the acute and chronic phase of disease. Further investigation into how CB1 in detail controls atopic inflammatory responses could provide important insights into the development of novel therapies for AD.

P007 | CREB is indispensable to KIT function in human mast cells - a positive feedforward loop between CREB and KIT orchestrates skin mast fate

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Skin mast cells (MCs) are critical effector cells in acute allergic reactions, and they contribute to chronic dermatoses like atopic and contact dermatitis, urticaria, psoriasis, prurigo, rosacea and others. The stem cell factor (SCF)/KIT axis represents the cells' most crucial receptor tyrosine kinase, ensuring proliferation, survival, and functional programs throughout their lifespan. cAMP response element binding protein (CREB), an evolutionarily well conserved transcription factor (TF), regulates a plethora of cellular programs throughout the body, but its function in the MC lineage is poorly understood. We recently reported that CREB is an effector of the SCF/KIT axis. Here, we asked whether CREB is not only downstream but could also act upstream of KIT to orchestrate its functional outputs.

Primary human MCs were isolated from skin tissue and cultured in SCF+IL-4. A two-pronged strategy served to interfere with CREB function, i.e., pharmacological inhibition (by 666-15) and RNA interference. KIT expression was studied by flowcytometry and RT-qPCR. KIT-mediated signal transduction was assessed by immunoblotting. Cell survival was determined by scatterplot analysis, and caspase-3 activity. Proliferation, apoptosis, and distribution across cell cycle phases were quantified following BrdU incorporation combined with 7-AAD staining by flowcytometry.

Transient CREB perturbation resulted in strongly reduced KIT expression. Conversely, microphthalmia transcription factor (MITF) was unexpectedly not required for the maintenance of KIT in skin MCs. KIT attenuation secondary to CREB was associated with heavily impaired KIT functional outputs like anti-apoptosis initiated by its ligand SCF. Likewise, KIT-elicited signaling was substantially diminished by preceding inhibition of CREB, as visualized by reduced ERK, AKT and STAT5 phosphorylation. KIT initiated cell cycle progression was likewise reduced despite discontinued CREB inhibition. Surprisingly, long-term interference with CREB led to fully-fledged cell demise in a way even surpassing KIT inhibition (by imatinibmesylate, Gleevec), revealing CREB's non-redundant nature in skin MCs.

Although CREB's significance in MC biology remained barely noticed, our study underlines a key significance of this TF for MC biology. In fact, CREB's absence is incompatible with skin MC existence. Since SCF/KIT regulates CREB activity, while vice versa CREB is required for KIT function, a positive feedforward loop between these elements dictates skin MC destiny. Mechanistic insights into how MCs are functionally maintained in the cutaneous environment may have important repercussions for therapy of skin diseases.

P008 | An effective counterbalance protects human skin mast cells from exaggerated MRGPRX2 activation - ineffectiveness in LAD2 cells explains their hyperresponsiveness to MRGPRX2 ligands

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Background: MRGPRX2 is a recently uncovered multiligand receptor and potential key player in mast cell (MC)-driven disorders. Its expression is confined to MCTCtype mast cells, explaining the reactivity of the skin to MRGPRX2 agonists. Due to the difficulty in obtaining skin mast cells, the LAD2 cell line is the most commonly used model in studies of human MRGPRX2. However, LAD2 cells differ in many respects from skin MCs, displaying chromosomal aberrancies, far less MC proteases, poor stimulability via FcεRI, and altered susceptibility to inhibitors. Evidence is also accumulating that LAD2 cells respond differently to MRGPRX2 agonists. Here, we asked whether LAD2 cells can act as surrogates of skin MCs regarding MRGPRX2- triggered degranulation, internalization, desensitization, and signal transduction. We also explored the molecular basis behind potential differences between the systems.

Methods: MCs were purified from foreskin tissue. LAD2 cells were compared with ex vivo skin MCs side-by-side. Codeine, Substance P (SP) and icatibant were used as MRGPRX2 ligands. Degranulation and desensitization were studied by β-hexosaminidase assay, MRGPRX2 internalization by flow-cytometry, signal transduction and β-arrestin expression/phosphorylation levels were measured by RTqPCR and/or immunoblot.

Results: MRGPRX2 mRNA expression was somewhat lower in LAD2 than skin MCs. Conversely, degranulation was stronger in LAD2 cells with no major ligand preference, while skin MCs degranulated with SP ≅ codeine > icatibant. MRGPRX2 internalization and ensuing receptor desensitization were far more pronounced in skin MCs than LAD2 cells, and the ligand order inducing these effects was likewise distinct. ERK/AKT-phosphorylation triggered by different

MRGPRX2 ligands was substantially prolonged in LAD2 vis–vis skin MCs. Mechanistically, LAD2 cells expressed inferior levels of β-arrestin-1/2 together with a higher pARRB/tARRB ratio, explaining their exaggerated responses towards MRGPRX2 ligands.

Conclusion: The β-arrestin system is highly active in skin MCs and protective against overt and repetitive stimulation. Its perturbation in LAD2 cells explains the various deviations found in the cell line. Therefore, despite their popularity, LAD2 are of modest utility in drug discovery as findings cannot be extrapolated to physiologic settings.

P009 | Oak processionary moth setae contain several potential allergens and induce skin inflammation

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The Oak Processionary Moth (OPM), Thaumetopoea processionea, infests oak trees in almost all European countries. Although the moth is harmless, the setae of the larvae pose a health risk to humans. On contact, the setae can cause a severe skin reaction known as 'caterpillar dermatitis'. Inhalation of setae may provoke airway inflammation and allergic asthma. In recent years, caterpillar dermatitis caused by OPM has increased exponentially in Germany among forest and garden workers, hunters, farmers, and walkers, causing a significant socio-economic problem. Furthermore, children in Waldkindergarten are at high risk of exposure to airborne setae even miles away from caterpillars. However, the immunological mechanisms by which setae toxins or proteins cause severe skin or allergic reactions remain completely unknown. To understand how setae induce skin inflammation, total RNA-seq was employed to obtain the transcriptome of three larval developmental stages. Our proteomic study identified several unknown setae proteins. IgG/IgE from patients sensitized against OPM reacted with the setae proteins. In mice, topical setae application induced immediate vasodilation and ear swelling (ear thickness; setae: 0.068 0.021 mm; PBS: 0.0220.07 mm; n=4), but limited influx of immune cells as detected by histochemistry. The ear inflammation peaked four days after setae application and gradually resolved thereafter. Our study shows that setae contain several uncharacterized allergens and induce skin inflammation.

P010 | The IgEome in Peanut Allergy: Determination of Autoreactive IgE

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Introduction: Specific IgE (sIgE) is a key effector molecule in food allergy. Mast cell and basophil activation is triggered by the interaction of an antigen with its sIgE loaded on the high affinity receptor for IgE. However, till today little is known about the prevalence and relevance of IgE reactivity to autoallergens in food allergy. Autoreactive IgE (autoIgE) has been detected in classical autoimmune disorders, but also in chronic urticaria and atopic dermatitis. In this project we aim to evaluate the presence of autoIgE in food allergic and tolerant patients.

Methods: Samples from well characterized individuals diagnosed either as peanut allergic (n=15) or peanut sensitized but tolerant subjects (n=3) were assessed for their IgEome. Twelve of the oral food challenge proven allergic patients were enrolled for a modified food elimination diet. This cohort was divided into two groups: six individuals were strictly avoiding peanuts (hereafter avoidance), while the other six incorporated modest quantities of peanut into their diet (called consumption) for one year. Serum samples were analyzed for IgE against exoallergens using the ImmunoCap(TM) ISAC Test and for autoIgE using the PEPperCHIP® Autoimmune Human Epitope Microarray.

Results: With respect to exoallergens, all individuals exhibited sIgE reactivity to more than one allergen. Besides peanut, individuals were mainly sensitized to apple, peach, alder, birch, hazel pollen, house dust mite and timothy grass. Concerning autoIgE, half of the peanut allergic patients (7 of 15) but none of the tolerant patients had detectable levels. In individual patients, the number of autoallergens ranged from 0 to 3081 of 5314 possible hits. Carbonic anhydrase 2, collagen alpha, sulfotransferase 1C4, calplastin and alpha-enolase were the most common autoallergens. The number of hits as well as hit intensity changed over the year of intervention in both cohorts (consumption & avoidance). Moreover, it appears that individuals with autoIgE display sensitization to fewer exoallergens compared to those without autoIgE.

Discussion: The role of autoIgE in food allergy is unknown and should be further explored in a larger cohort; for example by applying assays to investigate the functional relevance of autoIgE in peanut allergic patients.

P011 | Relevance of distinct skin-resident cell populations in human contact allergy

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Metal allergies are prime examples of delayed-type hypersensitivity responses, which are divided into two phases: sensitization and elicitation. Sensitization occurs upon initial contact with the allergen and leads to activation of skin-resident cells and formation of metal-reactive T cells. During elicitation, these T cells then mount an immune response causing symptoms such as itchy eczema approximately 72 hours after exposure.

Previous research has identified two main mechanisms involved in sensitization: direct or indirect activation of innate immune receptors such as Toll-like receptor 4 by the respective allergen, and conditional immune activation via the NLRP3 inflammasome. Elicitation is dominated by the activity of cytotoxic T cells, but it is unclear if only circulating T cells or also metal-responsive tissue-resident T cells contribute.

To identify the relevant cell types and signals mediating human nickel hypersensitivity, we performed single-cell RNA sequencing of donor cells from a patient sensitized to nickel (but not chromium), who received 8 or 72 hours of epicutaneous treatment with either diluent, nickel or chromium.

Our results suggest that nickel specifically activates certain populations of endothelial cells, suprabasal keratinocytes, and CCR7+ dendritic cells within 8 hours, a response not observed with chromium exposure. Skin resident T cells were not involved in the early hypersensitivity response, as their gene expression remained unchanged 8 hours after nickel exposure. However, after 72 hours of nickel exposure, substantial changes were observed in the cutaneous T cell compartment, including infiltration of SELL+/CCR7+ central memory T cells, effector T cells, TH cells, and Treg cells.

P012 | GM-CSF-primed eosinophils enhance MC activation

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Introduction: The Allergic Effector Unit (AEU), consisting of mast cells (MCs) and eosinophils (Eos), has been shown to contribute to chronic allergic inflammation. However, the detailed mechanisms underlying the cellular interaction are not completely defined as yet.
Objective: In this study, we investigated whether soluble mediators derived from MCs or Eos can activate them and thus affect their effector status, which might help to understand diseases where both effector cells are involved.
Methods: MCs isolated from human breast skin and Eos from peripheral blood of healthy volunteers and chronic spontaneous urticaria (CSU) patients were either treated with cell culture supernatants or cultured together. MC activation was assessed by β-hexosaminidase and histamine release as well as CD63 expression. Eos activation was assessed by expression of CD69 using flow cytometry. Cytokineblocking antibodies were used to assess the contribution of MC cytokines to Eos activation. To simulate infiltration of Eos into the skin, Eos from peripheral blood were injected into skin explants from healthy donors ex vivo. Histamine content was analyzed in the interstitial fluid extracted with hollow microneedles.
Results: Supernatants of MCs activated by anti-IgE or cortistatin increased CD69 levels on Eos (% CD69+ Eos unstimulated: 3.6±0.9; anti-IgE: 28.6±10.4, P=0.014, and cortistatin: 13.03±17.2). GM-CSF was identified as a key cytokine for Eos activation as its blockade reduced CD69 to the level of unstimulated Eos, whereas blockade of IL-5 or IL-3 had no effect. MCs treated with supernatants of Eos previously activated with GM-CSF showed increased activation compared with no GM-CSF treatment, particularly at lower anti-IgE concentrations. Interestingly, Eos from CSU patients enhanced the stimulatory effects of anti-IgE on MCs more effectively than healthy Eos (% CD63+MCs: CSU vs healthy =8.0 vs 11.5, P=0.026). Similarly, injection of Eos from CSU patients into ex vivo skin resulted in higher histamine release by MCs than Eos from healthy subjects (51.9 vs. 107.9 ng/ml, for healthy and CSU, respectively).
Discussion: Our findings suggest that the bidirectional interaction between MCs and Eos could lead to a reciprocal activation cycle driven by physical contact and the release of GM-CSF by MCs. This promotes chronic activation of these two cell types and contributes to disease pathology. Therefore, disruption of the AEU, e.g., by targeting GM-CSF or its receptor, could be a valuable approach for the treatment of chronic allergic inflammation.

P013 (OP03/02) | Single-cell spatial proteomics identifies the JAK/STAT pathway as a target in Toxic Epidermal Necrolysis

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Toxic epidermal necrolysis (TEN) is the most severe cutaneous drug reaction with substantial mortality. As the molecular mechanism driving keratinocyte cell death and epidermal detachment remains unknown, there is no effective therapy. To address this unmet clinical need, we employed our novel single-cell spatial proteomics technology, termed Deep Visual Proteomics (DVP). To this end, keratinocytes and immune cells were laser-microdissected from FFPE skin-tissue biopsies of three drug-induced skin reactions (TEN, DRESS, MPR) and healthy controls. Following ultra-sensitive mass spectrometry based proteomic analysis, we identified over 5,000 proteins separately in keratinocytes and skin-infiltrating immune cells. Comparative data analysis revealed a strong enrichment for Type-I and -II interferon signaling in the immune cell and keratinocyte compartments of TEN patients. Elevated levels of pSTAT1 further confirmed the presence of interferon signaling in TEN. To investigate the biological relevance of our findings, we developed a novel live-cell imaging assay, using patient-derived keratinocytes and peripheral blood mononuclear cells (PBMCs). In this assay, pan-JAK inhibitor (JAKi) tofacitinib significantly suppressed keratinocyte directed cytotoxicity by activated PBMCs. Additionally, administering oral pan-JAKi tofacinitib or baricitinib mitigated both clinical and histological symptoms in two distinct TEN mouse models. Finally, JAKi proved safe in four patients with TEN and SJS/TEN, and was associated with rapid skin re-epithelialization. Our research uncovers that TEN is driven by interferon signaling and provides a rationale for targeted JAK inhibition in this disorder thereby demonstrating the potential of spatial proteomics for precision medicine.

P014 | Application of hollow microneedles in a model of chronic inducible urticaria

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Dermal interstitial fluid (dISF), rich in disease-specific biomarkers, presents an ideal specimen for biomarker analysis. In contrast to conventional methods relying on serum and punch biopsies, dISF, which surrounds cells at the site of inflammation, offers higher concentrations of mediators. Immunohistochemistry struggles to detect extracellular soluble mediators. Additionally, immune cell released mediators may not reach circulation effectively, becoming diluted or interacting with plasma proteins, limiting their detection in peripheral blood.

To improve diagnostics and advance biomarker discovery, we have developed a sub-pressure-assisted method using sharp hollow microneedle chips (hMNs) for (dISF) sampling. These hMNs, fabricated from monocrystalline silicon wafers, contain 130 hollow microneedles measuring 450 micrometers in length. With a sequential procedure for skin penetration and fluid harvest, we extracted on average 12.6 microliters from ex-vivo human abdominal skin and 15.3 microliters collected from the forearms of healthy volunteers within 15 minutes. In serum transfer models for inducible urticaria, sera from cold urticaria (ColdU), symptomatic dermographism (SD) patients or healthy control (HC) subjects were injected intradermal to ex-vivo skin, followed by application of the corresponding triggers (cooling and rewarming for ColdU, or skin scratching for SD). We were able to detect an increase of histamine in dISF after ex-vivo skin provocation in the areas where patient serum was injected but not HC serum. Importantly, injection of serum alone also did not result in histamine increase in dISF. In addition, we could demonstrate the ability of dISF collected from healthy individuals after cold provocation to induce degranulation in cultured human skin mast cells sensitized with serum from ColdU patients in vitro. In contrast, dISF obtained without cold stimulation did not exhibit this capability.

In summary, we have developed an efficient and minimally invasive method for dISF sampling using hollow microneedles. Our findings also unveiled that the antigen to trigger ColdU is soluble and generated in healthy skin upon provocation. This approach shows significant potential for discovering novel biomarkers, advancing research, and diagnosing diverse skin diseases, thereby enhancing our understanding of cellular and tissue physiology.

P015 | Impact of polycyclic aromatic hydrocarbons and skin sensitizing substances on the oxidative stress response of keratinocytes

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Contact allergies are prevalent in up to 20 % of the general population and are caused by repeated skin exposure to skin sensitizing substances, including various metals, fragrances, preservatives and dyes. These may lead to the development of an allergic contact dermatitis, a common inflammatory skin disease characterized by erythema, swelling and itching. Studies indicate the generation of reactive oxygen species (ROS) by skin sensitizing substances as a key mediator of chemical-induced contact allergies.

On the other hand, ubiquitous environmental pollutants such as polycyclic aromatic hydrocarbons (PAHs) are also known to cause ROS formation by activating the aryl hydrocarbon receptor, inducing cytochrome P450 (CYP) 1 isoforms and undergoing aldo-keto reductase (AKR) 1-dependent redox cycling.

In the current study, we tested whether a co-exposure to the common skin sensitizers isoeugenol (IEG) and p-phenylenediamine (PPD) and the exposure-relevant PAH benzo(a)pyrene (BaP), exhibit additive or synergistic effects on oxidative stress responses of human HaCaT keratinocytes. Whereas a co-treatment with the sensitizers had no effect on the BaP-induced expression of CYP1A1, it resulted in an additive induction of AKR1C isoforms. Further, the co-exposure led to a synergistic induction of heme oxygenase-1 (HMOX1), a marker for activation of the NRF2 antioxidant pathway. Interestingly, this effect was abolished in CRISPR/Cas-mutated keratinocytes lacking functional expression of CYP1A1 and AKR1C3, respectively. In addition, we confirmed a substantial increase of ROS levels in HaCaT cells coexposed to BaP and either IEG or PPD by flow cytometric analyses of superoxide anions. In contrast to the co-exposure with BaP, a combined treatment of the cells with IEG and PPD neither affected HMOX1 mRNA levels nor ROS formation, pointing out the importance of aryl hydrocarbon receptor and downstream metabolic enzymes.

Our in vitro study reveals that co-exposure to skin sensitizing substances and PAHs, present in tobacco smoke and ambient air pollution, results in synergistic effects in ROS formation and HMOX1 expression in an AKR1C3 dependent manner. This suggests that PAHs might enhance the skin-sensitizing potential of contact allergens by additionally elevating cellular ROS levels. Our data might help to improve risk assessment of skin sensitizing compounds.

P016 | Role of the AHR in cutaneous adverse drug reactions: Identification of new clinically relevant AHR activity-modulating drugs

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The occurrence of adverse drug reactions (ADR) is common and often poses a serious risk to the patient's health, as it regularly affects the continuation of the treatment. Furthermore, alternative treatment options and management strategies are often limited. Many adverse drug eruptions seem to be of an allergic nature, hypothesized to be initiated by drug-specific immune responses that activate T cells or induce IgE-mediated mast cell activation. However, the majority of adverse drug eruptions do not necessitate prior immune sensitization. Specifically, although immune cells are involved in the so called off-target non-immune reactions, these reactions are not based on antigen specific sensitization and are not dependent on immunological memory. Previously, our group demonstrated that the drug vemurafenib antagonizes the aryl hydrocarbon receptor (AHR) signaling pathway, leading to the development of an off-target non-immune ADR characterized by inflammatory skin rashes. The AHR is a transcription factor involved in various processes such as skin homeostasis and inflammation and has a wide variety of ligands. Drug-AHR interactions are not unique or limited to vemurafenib, but may represent a more common and relevant mechanism of off-target non-immune ADR.

In the current study, we investigated new clinically relevant AHR activity-modulating drugs in vitro. We focused on drugs that have been reported to induce cutaneous ADR such as Stevens-Johnson syndrome. Potential drugs were screened via reporter gene analyses, which provided initial insights into whether the drug inhibits both basal as well as benzo[a]pyrene (BaP)-induced activity of the AHR-dependent luciferase construct. For further investigations, AHR-antagonistic drugs were preferred to align with the previous findings related to vemurafenib. Furthermore, gene expression of the AHR target gene CYP1A1 was determined after treatment with potential AHR-modulating drugs in HaCaT keratinocytes. We again focused on the drugs that attenuated the BaP-induced activity of the AHR. Moreover, AHRantagonizing effects were further confirmed via AHR translocation assay.

The data demonstrate that not only vemurafenib but also other drugs which were reported to induce cutaneous ADR represent AHR ligands and display antagonistic activity against the AHR in vitro.

Since the AHR plays such a critical role in maintaining skin homeostasis and represents a key regulator in inflammatory processes, its role in cutaneous ADR needs further investigation. Furthermore, the understanding of underlying mechanisms and the identification of drugs inducing offtarget non-immune ADR has tremendous clinical potential.

P017 | Wasp venom-specific immunotherapy-induced changes in T cell receptor repertoire

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Hymenoptera venom is one of the most common causes of anaphylaxis in humans, ranging from systemic reactions to life threatening conditions. The most effective treatment for the patients with hymenoptera venom allergy is venom-specific immunotherapy (SIT). T cells play a critical role in development of tolerance during allergen-specific immunotherapy, however, exact molecular mechanisms behind it are still not completely understood.

Here, we aimed to detect changes in T cell receptor repertoire during wasp venomspecific immunotherapy. CD4+ cells were sorted from the peripheral blood of the patients (n=11) using magnetic cell separation (MACS) before the initiation of SIT and 1 week after the first dose of SIT. Then, alpha and beta chains of T cell receptor (TCR) were amplified and sequenced using next generation sequencing. No significant differences in TCR repertoire diversity as well as various CDR3 properties before and after treatment could be detected. At the same time, principal component analysis of library size-normalized donor effect-corrected TCR cluster counts revealed a clear separation between the samples before and after treatment. We next set off to identify the clone clusters with significantly different prevalence in patients before and after treatment. Here, we detected more than 700 alpha- and 80 beta-chains of TCR. These results suggest that venom-specific immunotherapy affects the prevalence of multiple TCR clonotypes that are potentially involved in the immune response to wasp venom anaphylaxis.

P018 | Understanding the Factors Influencing Birch Pollen Allergenicity and its Health Effects

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Introduction: Environmental stress as with advancing climate change and pollution can affect the plant allergenicity, alter pollen characteristics and influence the occurrence and intensity of allergic respiratory diseases.

Aim of study: This study aims to investigate the correlation between pollen data from birch trees grown under different climatic conditions and the molecular composition of the pollen, as well as the skin prick test (SPT) responses in allergic patients. Comprehending the underlying mechanisms may help in the prediction and management of allergic diseases, especially in the context of climate change.

Materials & Methods: From 2019 to 2022, birch pollen samples were collected from various locations across Europe. Data on abiotic factors (like air temperature, relative humidity, global solar radiation, air pollution) and biotic factors (tree growth parameters, cherry leaf roll virus infection) was gathered. Standardised pollen extracts were prepared, and correlations were drawn between environmental and tree-specific data, parameters related to allergenicity (total protein content, Bet v 1 content, lipid mediators, lipopolysaccharide (LPS), histamine) the results of the skin prick test (SPT) in terms of wheal-size. Three settings were examined: genetically identical birch trees in international phenological gardens (ROI-1), diverse tree clones in a seed plantation (ROI-2), and trees along an altitudinal gradient, exhibiting different genetic backgrounds and climate conditions (ROI-3).

Results: In general, pollen allergenicity was influenced by the genetic background of the trees, with year-to-year variation having an even greater effect. Among the assessed pollen-intrinsic parameters, Bet v 1 and total protein were identified as the strongest predictors of the severity of SPT results. In ROI-1 and ROI-2, wheal size was more strongly correlated with the total protein content than with Bet v 1. In addition, wheal size showed a significant negative correlation with the altitude of the tree location. Finally, we observed a significant positive correlation between Bet v 1 content and the mean temperature of the previous month preceding the onset of flowering.

Conclusion: Our results indicate that the allergenic potential of birch pollen varies annually and is influenced by both, the genetic profile

of the tree and the prevalent climatic factors. While Bet v 1 remains the major birch allergen, the allergenicity of birch pollen may depend on various compounds within the pollen matrix, such as histamine and LPS. Birch trees at higher altitudes or in cooler regions produce pollen with lower allergenicity, indicating that trees in warmer climates tend to increase allergenicity due to environmental stress. In the context of climate change, this may imply that adverse health effects for atopic individuals.

P019 | Efficacy of a Dermocosmetic containing neurosensine, sphingobioma and niacinamide in patients with Atopic Dermatitis - achieving rapid symptom relief after one-day use and sustained improvement of Disease Severity and Quality of Life over time

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Background: Atopic Dermatitis (AD) is a chronic inflammatory skin disease associated with dysfunctional integrity of epidermal barrier, often causing skin dryness, itching, burning sensations and inflammatory lesions. Maintaining and stabilizing the skin barrier is essential for preventing and treating AD. Everyday use of emollient is the basis of AD treatment and helps to restore epidermal barrier functionality.
Objectives: The aim of this clinical study was to evaluate the effects of a face skin care cream (DC) on the functionality of the skin barrier, clinical manifestations, skin condition and quality of life in patients diagnosed with AD.
Methods: 63 adult patients diagnosed with mild to moderate AD without acute eczema on face, neck and décolleté were recruited for the study. They applied the DC cream twice daily on their face, neck, and décolleté for a duration of two weeks. The investigations were performed at three intervals: baseline, after 24h and day 14. These evaluations included clinical parameters such as general SCORAD (Scoring of Atopic Dermatitis) and EASI (Eczema Area and Severity Index) scores and, as well as local SCORAD. Skin physiological measurements such as TEWL (transepidermal water loss), pH, corneometry, sebumetry, chromametry, and electrochemical impedance spectroscopy (EIS) were also performed. Furthermore, subjective and objective assessments of the skin as well as The Dermatology Life Quality Index (DLQI) questionnaire were evaluated at baseline and over time.
Results: The outcomes of the study demonstrate that the application of the DC led to significant improvements in both subjective and objective evaluation of symptoms, evident as early as the first day, with further progress observed after the twoweek period. Patients reported a significantly reduction ($p<0.001$) in symptoms like dryness, itching, redness, desquamation, burning and tightness feeling on the face, neck, and décolleté throughout the study, with the most notable improvements seen in the facial region. Disease severity, as measured by SCORAD and EASI, improved over time, particularly in terms of the reduction in the intensity of skin symptoms and subjective symptoms such as sleep disorders and pruritus intensity. Clinical examinations confirmed the high dermatological tolerance of DC. An improvement in quality of life, as measured by the DLQI, was also observed.
Conclusion: The study demonstrates the high tolerability of a DC containing neuroserine, niacinamide and sphingobioma when applied twice daily. It also underscores its rapid and consistent effectiveness in alleviating the signs and symptoms associated with AD. The integration of a facial skincare cream demonstrated improvements in both the patients' quality of life and the severity of the disease. These findings underscore the significance of emollients to be used on the facial, neck and décolleté areas in the management of AD.

P020 | Peripheral blood IL-23-expressing CD14+ monocytes and CD1c+ myeloid dendritic cells are increased in patients with chronic spontaneous urticaria

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Chronic spontaneous urticaria (CSU) is an inflammatory skin disease characterized by recurrent itchy wheals, which can be accompanied by angioedema. The therapeutic management encompasses antihistamines and the anti-IgE antibody Omalizumab in non-responsive cases. However, a substantial part of patients are still not well controlled by these measures. So far, the pathophysiology of CSU is not well understood. In general, an autoallergic subtype, driven by IgE antibodies against endogenous antigens, and an autoimmune form with IgG against the high-affinity IgE receptor, are distinguished. As the IL-23/IL-17 axis plays an important role in different autoimmune diseases we wondered if IL-23 might be overexpressed in peripheral blood cells of CSU patients. Peripheral blood mononuclear cells of both 20 CSU patients (CSU; median age: 35 years (19-69); 11 females, 9 males) and 19 agematched healthy controls (HC) were analyzed by flow cytometry for the expression of IL-23p19 in CD14+ monocytes and CD1c+ myeloid dendritic cells, both considered to be main producers of IL-23. IL-23p19 expression was also determined in CD3+ T as well as CD19+ B lymphocytes. In addition, clinical parameters of CSU patients including urticaria control tests (UCT) were recorded. While all cell types exhibited basal levels of IL-23p19 expression in both CSU patients and HC, the expression was significantly elevated in monocytes and myeloid dendritic cells of CSU patients. Of note, elevated levels of IL-23p19 expression correlated with disease activity, measured by UCT (mean: 6, range 0-10). However, ex vivo stimulation with the TLR4 agonist LPS resulted in equally increased

IL-23p19 expression in the investigated cells. Expression of IL-23p19 in T and B lymphocytes showed no difference between CSU patients and HC, regardless if cells were unstimulated or stimulated by PMA/ionomycin or CpG. In summary, peripheral blood CD14+ monocytes and CD1c+ myeloid dendritic cells of CSU patients exhibit increased IL-23 expression, which might reflect a chronic inflammatory activation status of these immune cells in CSU, regardless of disease severity. Subsequent studies should further explore the role of IL-23 in the development of CSU including the contribution of IL-23-expressing immune cells in the affected skin of patients with CSU. The new insights into the role of the IL-23/IL-17 immune axis may also lead to a better understanding of the pathogenesis of CSU.

Cellular Biology

P021 | Inhibition of Btk blocks neutrophil effector functions in pemphigoid diseases

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Background: Neutrophil effector functions are crucial for blister formation in pemphigoid diseases, i.e., via production of reactive oxygen species (ROS), neutrophil extracellular traps (NET)s and secretion of soluble factors including leukotriene B4 (LTB_4), after activation by immune complexes (IC)s and complement. Bruton's kinase (Btk) is known to be involved in Fc receptor signaling and could thus be a feasible target for inhibition of neutrophils.

Methods and Results: Human and murine neutrophils showed increased phosphorylation of Btk after stimulation with IgG ICs but not LTB_4 or complement component C5a. After incubation with the irreversible Btk inhibitor ibrutinib, we hence demonstrated complete inhibition of ROS release, release of NETs and secretion of LTB_4 from human and murine neutrophils activated with ICs (IC_{50} for ROS: 2.04 nM). Mechanistically, this was accompanied by a lack of Ca^{2+} influx resulting from downstream signaling of Fcγ receptors in neutrophils. Further, we could uncover changes in metabolic states of neutrophils with ibrutinib mediating a lower basal glycolysis rate measured by extracellular acidification rates with glucose. In a passive antibody-transfer model of epidermolysis bullosa acquisita (EBA) using C57BL/6J mice subcutaneously injected with anti-collagen VII-IgG, we demonstrated a complete prevention of skin blistering and erosion by treatment with ibrutinib. Neutrophils were observed in lower numbers in the skin infiltrate of treated mice.

Conclusion: Btk plays an important role in mediating neutrophil activation upon engagement with ICs deposited at the dermoepidermal junction in pemphigoid diseases. Inhibition of Btk by ibrutinib blocked neutrophil functions in vitro, and further, prevented a disease phenotype in a passive mouse model of EBA. Additional dose-finding studies and testing of new generation Btk-inhibitors are needed for clinical translation.

P022 | Differing sensitivity of two human skin cell lines to reactive oxygen species

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As the largest organ and an important barrier unit, human skin is exposed to a variety of stressors such as oxidative stress on a daily basis. This oxidative stress can be triggered by so-called reactive oxygen species (ROS), which in large amounts can lead to cellular demise, but in lower amounts can also mediate signaling processes. The effects of ROS are also of interest in the treatment of skin diseases such as skin cancer, for example by chemotherapy, radiotherapy or photodynamic therapy. Still, the processes triggered by the presence of ROS as well as the conditions that make different cells more or less susceptible to ROS-mediated effects are not fully understood. Our study aimed to compare two types of human skin cells typically used in dermatology research regarding their sensitivity towards different ROS. To this end, HaCaT keratinocytes as well as dermal fibroblasts were treated with increasing concentrations of either H2O2, HOCl or a gas plasma-mediated multi-ROS mixture, and inhibitory concentrations (IC25) were calculated based on metabolic activity. Further, basal expression profiles of 33 surface markers in total were investigated using flow cytometry. Additionally, basal levels of gene expression were quantified with an in-house transcriptomic microarray. HaCaT cells showed 2-3 times higher robustness towards all three tested ROS-conditions compared to dermal fibroblasts, and the largest difference was observed towards HOCl treatment. Basal surface marker expression varied a lot, and especially higher levels of aquaporins, oxidases and catalase were found in HaCaT cells. Basal transcriptome analysis revealed further differences in the gene expression of redox-associated enzymes such as dual oxidases, glutathione peroxidase or superoxide dismutases. In summary, we found differing sensitivities to ROS in two healthy skin cell lines, what should be considered e.g. when ROS effects in skin cancer cells are compared. Further investigations on markers of ROS susceptibility should be performed in the future to improve approaches to use ROS for therapeutic applications in dermatology.

P023 | Oxford Nanopore direct RNA sequencing reveals changes in ribosomal RNA modifications in senescent human epidermal keratinocytes

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Introduction: Environmental factors, like air pollution and radiation exposure, contribute to several age-related pathologies in humans.

The skin is one of the organs most profoundly exposed to those extrinsic factors, which lead to functional loss of the skin barrier and accelerated aging. Extrinsic skin aging is associated with the accumulation of senescent cells, which elicit a senescence-associated secretory phenotype (SASP), negatively impacting the surrounding tissue's integrity. Cellular senescence is additionally accompanied by protein expression changes that sustain, for example, the SASP. In recent years, the ribosome has gained interest as a potential source of translational regulation, leading to an altered proteome. Around 200 2'-O-methylations and pseudouridines decorate human ribosomal RNA (rRNA). Studies show varying rRNA modification levels between different cell types and tissues, raising the possibility of altered translation depending on the rRNA modification level.

Methods: We induced cellular senescence by stress exposure in primary human keratinocytes to study potential rRNA modification changes in extrinsic skin aging and senescence. We used Oxford Nanopore direct RNA sequencing to determine changes at known rRNA modification sites between senescent and proliferating human keratinocytes.

Results: Direct RNA sequencing revealed subtle differences in rRNA modification levels at sub-stoichiometrically modified sites. Several of these variable positions, for example, Um354 and Gm867 on 18S rRNA, have been previously implicated in cancer and development. The validation of differentially modified sites with orthogonal methods and mechanistic studies on the snoRNAs required for guiding these modifications are currently in progress.

Conclusions: Our data show the variability of rRNA modification sites in treatment-induced cellular senescence in human keratinocytes. This will be important for a better understanding the impact of environmental factors like radiation and pollutants on extrinsic skin aging. Moreover, our study will also aid the better characterization of senescent cells, which accumulate in our body throughout aging and contribute to age-associated diseases.

P024 | FasL enhances AK23-induced Acantholysis in a Caspase-dependent Manner in Pemphigus Vulgaris

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Pemphigus vulgaris (PV) is a chronic, life-altering autoimmune bullous disease, characterized by the presence of circulating antibodies specific for desmosomal structural proteins (desmoglein (Dsg) 1 and Dsg3). The binding of these pathogenic antibodies occurs ubiquitously on the skin and mucous membranes, while suprabasal epidermal acantholysis only manifests at certain predilection sites. This suggests the presence of local augmentation factors. Previous studies have shown a possible influence of FasL (CD95L) on acantholysis: firstly, increased levels of FasL were detected in sera from untreated pemphigus patients, and secondly acantholysis has been shown to be reduced in mutant mice which lack soluble FasL. FasL is a ligand of TNF-superfamily receptor Fas, which is an activator of the extrinsic apoptotic pathway, activating caspases 8/ 10 and caspase 3, consecutively. Caspases are serin-proteases involved in the regulation of both, apoptotic and inflammatory signaling pathways. The role of cell death in the development of PV and specifically the question whether keratinocytes cell death is a cause or a consequence of acantholysis has been widely discussed in the past.

Here, we show that prolonged incubation of immortalized human keratinocytes (HaCaT cells) with the specific anti-Dsg3 antibody AK23 does not result in increased cell death. Furthermore, AK23 does not affect the extent of FasL-induced cell death. In a dispase-based keratinocyte dissociation assay (method for testing the stability of a monolayer), AK23 induces acantholysis, whereas FasL (sublethal concentration) does not affect the stability of the monolayer. In contrast, simultaneous stimulation with AK23 and FasL leads to significantly increased acantholysis. The addition of pan-caspase inhibitors (Z-VAD-fmk/ Q-VD-Oph) suppresses this enhancing effect. This was confirmed in HaCaT cells overexpressing the short isoform of cFLIP which is known as a specific caspase 8 inhibitor.

As shown previously, FasL leads to caspase-dependent cleavage of membranebound Dsg 3, whereas AK23 induces a caspase-independent reduction of full-length Dsg 3. Dsg3 distinction and cleavage appear to be two independent processes that operate synergistically. Interestingly, upon combined treatment with FasL and AK23, the reduction of the full-length Dsg3 protein was significantly enhanced, probably as a result of the combination of these two independent processes. Furthermore, in an ex vivo skin model, we show that simultaneous stimulation of AK23 and FasL leads to acantholysis and an increase in TUNEL-positive epidermal cells, while stimulation with AK23 alone has no visible effect.

In summary, our findings provide evidence that early acantholytic processes occur independently of cell death, while a sublethal concentration of FasL enhances the acantholysis in a caspase-dependent manner. Our data warrant future studies to dissect the impact of other potentially caspase regulated factors which might be involved in the manifestation of PV.

P025 | Modification of transglutaminase-1 during cornification of epidermal keratinocytes

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Transglutaminase 1 (TGM1) cross-links proteins in the epidermis. Mutations in the TGM1 gene cause autosomal recessive congenital ichthyosis (ARCI1) of the lamellar form. To determine the dynamics of TGM1 expression and activity in the epidermal cell layers and in cultured keratinocytes, we established a protocol for the

antibody-dependent detection of TGM1 protein and the parallel detection of TGM activity. TGM1 immunoreactivity initially increased and co-localized with membrane-associated transglutaminase activity during keratinocyte differentiation. Unexpectedly, further differentiation of keratinocytes was associated with the loss of TGM1 immunoreactivity while transglutaminase activity persisted. Similarly, when HEK293T cells were transfected with TGM1, the recombinant protein was detected by the anti-TGM1 antibody only transiently whereas transglutaminase activity remained present after the loss of TGM1 immunoreactivity, suggesting that binding of the antibody was prevented by a modification of active TGM1. To screen for candidate proteins controlling this TGM1 modification, we performed a virotrap assay in which proteins binding to TGM1 are trapped in viral particles. Mass spectrometry identified the CAPNS1 subunit of calpain as interaction partner of TGM1. Consequently, we treated keratinocytes and TGM1-transfected HEK293T cells with chemical inhibitors of calpain. Both N-acetyl-Leu-Leu-norleucinal (ALLN) and calpeptin suppressed transglutaminase activity and allowed the maintenance of immunodetection of TGM1. Thus, TGM1 undergoes a modification which is controlled by calpain and may affect epidermal cornification.

P026 | S100A8/9 Potentiates Wound Healing Properties in ABCB5+ MSCs by RAGEmediated STAT3 activation

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S100A8/9 is a potent danger molecule that is secreted by many cells including immune cells among others. MSCs priming with a variety of molecules including alarmins as S100A8/9 has been suggested to ameliorate different phases of wound healing. S100A8/9 can be recognized by pattern recognition receptors such as TLR4 and RAGE. Although the involvement of TLR4 is established in response to S100A8/9 in MSCs, it is unclear whether RAGE-mediated signaling triggers alternative MSCs activation. In this study, we employed an in vitro model with S100A8/A9 priming of ABCB5+ MSCs, a skin-derived MACS isolated MSC subpopulation of high purity. Results of immunoblotting and immunostaining indicate that priming MSCs with S100A8/9 for 24 h significantly increased activation of the transcription factor p-STAT3, while inhibition of RAGE receptor mitigated this effect. Furthermore, we observed that pharmacological inhibition with TTP88 and siRNA knockdown of RAGE reduced STAT3 protein and gene expression. This highlights the causal role of RAGE in relaying S100A8/A9 signaling to the downstream effector STAT3. Data from secretome analysis revealed that priming MSCs with S100A8/9 increased the release of the STAT3-induced cytokine IL-22 which regulates extracellular matrix (ECM) formation. Moreover, transcriptome profiling of S100A8/9-primed MSCs showed a significant upregulation of STAT3 target genes such as, BMP1 and MST1 as factors involved in ECM formation and CSF3 as well as SOD3, known as regulators of angiogenesis. Overall, S100A8/9 - by binding to the RAGE receptor - activates STAT3 signaling in MSCs. This may contribute to accelerated wound healing by alleviating the expression of regenerative signaling and by controlling unrestrained inflammation, leading to balanced ECM remodeling, epithelial barrier protection, improved angiogenesis and stem cell proliferation.

P027 | Loss of translational accuracy in aging and aging-associated disease

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In previous studies, our lab identified disturbed ribosomal biogenesis in premature aging diseases like Cockayne syndrome and trichothiodystrophy as a pathomechanism that could contribute to 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 characterizes most neurodegenerative diseases of the aging body. Now we hypothesize that an error-prone protein translation might contribute to aging-associated diseases like Parkinson´s disease (PD). To investigate this, we use fibroblast cells from PD patients with a familial G2019S mutation in the LRRK2 kinase, leading to hyperphosphorylation of the ribosomal protein S15. RPS15 is located at the decoding site of the ribosome and its phosphorylation might influence the accuracy of protein synthesis. Inhibiting the mutant and hyperactive LRRK2 kinase reduces RPS15 phosphorylation and results in a decreased error rate of the ribosomes. Comparing skin fibroblasts from healthy young and old donors, we find, in contrast to our initial hypothesis, a decreased error rate of the ribosomes with aging. In line with this result, we find a reduced protein aggregation in fibroblasts from old donors. We now hypothesize that healthy aging might depend on the sustained accuracy of protein synthesis by the ribosome. As cellular compensation mechanisms that balance the proteome decrease with aging, an elevated error rate of translation might overwhelm the homeostasis mechanisms of cells and organs.

P028 | The role of mechanotransduction in the integrin-dependent TGF-beta activation in psoriatic inflammation

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Psoriasis is a chronic inflammatory skin disease, affecting 2 to 3% of the population. TGFβ1 is elevated in the plasma and skin lesions of psoriasis patients, and can drive the maturation of Th17 cells into IL-17A producing cells, a process crucial for the psoriasis development. Initiation of the TGFβ signaling requires prior TGFβ activation from the latent form by the mechanosensitive αVβ6 and αVβ8 integrin receptors, which promotes the release of the TGFβ Latency-Associated Peptide (LAP).

Increasing evidence shows that epidermal keratinocytes take an active role in the regulated production of TGFβ alongside immune cells and dermal fibroblasts, but their role in TGFβ activation and the implications for pathological processes in the skin are largely unknown. Our previous work indicates that inflammation upregulates mechanotransduction pathways in the epidermis. Here, we hypothesize that the increased mechanotransduction might have a functional significance for the activation of αVβ6 integrin within the epidermis resulting in increased release of active TGFβ from the epidermis.

We used N/TERT-1 normal human keratinocytes cultured as non-differentiated 2D monolayers and 3D reconstructed human epidermis (RHE) and stimulated with a specific cocktail of inflammatory cytokines M5 (IL-17A, IL-22, Oncostatin M, TNFα, IL-1α) to induce psoriasiform inflammation.

We observed increased total and surface expression of αVβ6 integrin and also increased ability of cells to bind synthetic LAP (αVβ6 integrin ligand) under M5 treatment. Inhibiting mechanotransduction with small molecule inhibitors KD025 (for ROCK2), Y-27632 (for pan-ROCK), and blebbistatin (for myosin II) abrogated these effects. On the contrary, mechanostimulated cultures that were grown under cyclic stretch showed elevated LAP binding, even in the absence of the M5 stimulation. Interestingly, IL-26, which has been recently shown to induce TGFβ expression by keratinocytes, also promoted TGFβ activation pathway in our experimental system. At the same time, external stimulation of keratinocytes with TGFβ1 led to the activation of Rho-dependent cell contractility and mechanotransduction pathways.

In conclusion, our data indicate that psoriatic inflammation drives synergistic changes in the affected epidermis contributing to mechanodependent positive feedback loops for TGFβ signaling upregulation in the skin.

P029 | Differential activation of mast cells by human effector T cells and regulatory T cells

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Introduction: Skin-infiltrating or tissue-resident T cells and their secreted mediators are known to be involved in mast cell (MC) recruitment (e.g. IL-9), activation (e.g. IL-4, IL-5, IL-13), and survival (e.g. IL-3). In contrast, regulatory T cells (Treg) have been described to dampen mast cell activation. Thus, T cells modulate MC responses and might contribute to chronic MC-driven diseases such as chronic spontaneous urticaria. However, previous studies on MC-T cell interactions mainly used murine cells or knock-out mouse models while comprehensive studies investigating the influence of different human T cell subsets (i.e. Tm and Treg) on primary human MCs are lacking.

Objective: We aimed to dissect the response of primary human skin MCs after direct contact with peripheral T memory (Tm) and Treg and their respective secreted mediators.

Method: Human peripheral blood CD4+ Tm and Treg were activated overnight using antibodies against CD3 and CD28, and cell culture supernatant was collected. Primary human skin MC were activated using anti-IgE or cortistatin to activate FceRI or MRGPRX2 respectively in the presence or absence of Tm or Treg (ratio 1:1) or their respective culture supernatants. MC activation was assessed by measuring CD63 surface level via flow cytometry.

Results: In the presence of activated Tm and Tm-supernatant and FceRI activation, CD63 expression on MC was increased by Tm and Tm-supernatant (fold change of CD63 expression: MC+aIgE = 1, MC+aIgE+Tm = 1.27, MC+aIgE+Tm-supernatant = 1.88). Tm and Tm-supernatant also strongly enhanced cortistatin-induced MRGPRX2 activation (fold change of CD63 expression: MC+cortistatin = 1, MC+cortistatin+Tm = 2.13, MC+cortistatin+Tm-supernatant = 3.42). However, Tm or Tm-supernatant did not activate MCs in the absence of FceRI or MRGPRX2 activation. Activated Tregs did not affect FceRI-mediated MC activation, while MC activation was reduced from 45.3% to 34% in the presence of Treg-supernatants.

Discussion: We observed a differential influence of primary human T cell subsets on skin MC activation, which should encourage attempts to identify and characterize the underlying molecular mechanisms of these effects. Targeting these mechanisms and MC-T cell interaction could benefit the development of novel treatments of diseases where MC and T cell, together, contribute to the pathogenesis.

P030 | Uncovering Microtubule-driven Mechanisms of Melanoma Invasion

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Cells migrating through complex 3D environments experience considerable physical challenges including tensile stress and compression. To move, cells need to resist these forces whilst also squeezing the large nucleus through confined spaces. This requires highly coordinated cortical contractility. Microtubules can both resist compressive forces and sequester key actomyosin regulators to ensure appropriate activation of contractile forces. Yet, how these two roles are integrated to achieve nuclear transmigration in 3D is largely unknown. Here, we demonstrate that compression triggers reinforcement of a dedicated microtubule structure at the rear of the nucleus by the mechanoresponsive recruitment of CLASPs (cytoplasmic linkerassociated proteins) which dynamically strengthens and repairs the lattice. These reinforced microtubules form the mechanostat: an adaptive feedback mechanism that allows the cell to both withstand compressive force and spatiotemporally organise contractility signalling pathways. The microtubule mechanostat facilitates nuclear positioning and coordinates force production to enable the cell to pass through constrictions. Disruption of the mechanostat imbalances cortical contractility, stalling migration and ultimately resulting in catastrophic cell rupture. Our findings reveal a new role for microtubules as cellular sensors which detect and respond to compressive forces, enabling movement and ensuring survival in mechanically demanding environments.

P031 | Deletion of keratin 77 causes increased expression of keratin 1

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Keratins are intermediate filament-forming proteins of epithelial cells and are mostly expressed in keratinocytes maintaining their structural stability and integrity. The differential expression of keratins is associated with development and homeostasis of the skin and its appendages. Keratin (KRT) 77 is a type II keratin and it is structurally closely related to KRT1, the prototypical type II keratin of the suprabasal epidermis. Apart from the epidermis KRT77 is also expressed in luminal cells of eccrine sweat gland ducts in both human and mouse skin. Furthermore, KRT77 is supposed to be functionally similar to KRT9 and it may contribute to an increased mechanical stabilization in differentiating epidermal cells. It is known that despite the structural and evolutionary relationship between KRT77 and KRT1, KRT77 cannot compensate for the loss of KRT1 in the epidermis of Krt1-null mice. To study the function of KRT77 in skin we have successfully generated a constitutive Krt77-knockout (KO) mouse model using clustered regularly interspaced short palindromic repeats (CRISPR)- CRISPR-associated (Cas) protein 9 technology. The absence of Krt77 expression in Krt77-KO mice was confirmed by RT-PCR. No noticeable clinical changes were observed in the skin of Krt77-KO mice. Likewise, no significant histological differences could be observed in the skin of Krt77-KO mice and control littermates. Our data indicate that KRT77 is dispensable for embryonic development and adult tissue homeostasis. In addition, we analysed expression of various keratins by RT-qPCR in skin that have a direct relationship with KRT77 or are associated with KRT77. RT-qPCR analysis revealed significant up-regulation of Krt1, Krt10, and Krt9 mRNA in Krt77-KO mice compared with control littermates. Overall, we showed that the loss of Krt77 expression in the murine skin is balanced by an increased expression of Krt1 and Krt9 - two keratins that are closely related to KRT77. Moreover, deletion of KRT77 is concomitant with a higher expression of Krt10 - the putative type I partner of KRT77 and KRT1. In conclusion, the observed upregulation of Krt1, Krt9, and Krt10 in the skin of KRT77-deficient mice may functionally compensate for the loss of KRT77 and provide an explanation for the absence of specific defects.

P032 | The ion channel TRPV4 contributes to epidermal functionality by regulating tight junctions and keratinocyte maturation

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Calcium is a major regulator of keratinocyte function. In healthy epidermis, a Ca2+ gradient promotes differentiation as keratinocytes migrate through the different epidermal layers to form a functional, protective barrier. The Transient Receptor Potential Vanilloid 4 (TRPV4) is a mechanosensitive Ca2+ channel expressed in murine and human keratinocytes and knockout mice display severe defects in tight junction formation. However, the exact role and function of the channel in human skin is not well understood. Therefore, we explored the role of TRPV4 in human keratinocytes.

Using different immortalized keratinocyte cell lines (HaCaT and N/TERT1) as well as primary keratinocytes, we found that TRPV4 is stringently upregulated during keratinocyte differentiation in vitro. Thus, we assume that the channel plays a crucial role during keratinocyte maturation. Indeed, reducing the amount of TRPV4, either by siRNA-mediated knockdown or CRISPR/Cas9-mediated genetic knockout, resulted in decreased expression of tight junction

proteins claudin and occludin. However, on top of that, we detected a significant reduction in differentiation markers unrelated to tight junctions, including involucrin and cytokeratin 1/10.

Furthermore, we generated epidermis equivalents and modulated TRPV4 either by siRNA or CRISPR/Cas9 or pharmacological agonists such as 4aPDD or GSK1016790. Barrier integrity was assessed by Lucifer Yellow penetration and transepithelial electrical resistance (TEER) and overall maturation was evaluated by immunohistochemical staining of various proliferation and differentiation markers. Initial results show that TRPV4 seems to contribute to proper barrier function as well as keratinocyte maturation in reconstituted epidermal models.

Overall, this data suggests that TRPV4 might be an interesting target to study in inflammatory skin diseases, where patients suffer from an imbalance between keratinocyte proliferation and differentiation. Thus, we will investigate whether manipulation of TRPV4 function could represent a beneficial strategy for the treatment of inflammatory skin diseases.

P033 | Activation of STAT3 through the mTOR and MAPK pathways contributes to inflammatory skin diseases

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The transcription factor STAT3 (signal transducers and activators of transcription 3) has recently emerged as a key player in the pathogenesis of inflammatory skin diseases and hyperactivation of STAT3 on Y705 has been found in virtually all cell types that are involved in the initiation and maintenance of the psoriatic inflammation.

Pro-inflammatory cytokines can activate kinases of the Janus kinase (JAK) family that in turn phosphorylate STAT3 on Tyr705, which allows STAT3 dimerization, nuclear translocation and DNA binding. Thus, phosphorylation on Tyr705 has been regarded as the essential mechanism of STAT3 regulation. However, for maximal transcriptional activity, additional phosphorylation of STAT3 on Ser727 is necessary, which is more complex and regulated by various kinases integrating different stimuli.

Therefore, we investigated the degree of full STAT3 activation in inflammatory skin diseases by measuring the phosphorylation of STAT3 at Tyr705 as well as Ser727 in the epidermis of psoriasis, atopic dermatitis and Hidradenitis suppurativa patients. We found that, in addition to phosphorylation on STAT3 Tyr705, inflamed skin displayed strong phosphorylation on Ser727 in keratinocytes in comparison to healthy skin. To investigate which signaling pathways mediate this additional phosphorylation in the epidermis, cultured keratinocytes were treated with proinflammatory cytokines and specific inhibitors. While IL-6 and IL-22 induced strong phosphorylation on Tyr705 via JAK2, no activation of Ser727 could be detected. In contrast, IL-1beta and TNF-alpha were able to mediate phosphorylation of this site, which could be blocked by pre-incubation with the MEK inhibitor U0126 and to some degree by mTOR inhibitors.

Hyperactivation of STAT3 has previously been shown to impair keratinocyte differentiation. Therefore, we investigated the effects of ERK1/2, mTORC1, and STAT3 activation on epidermal function in reconstituted 3D models. We could show that inflammatory stimuli impede ordered keratinocyte maturation and epidermal barrier function, which is dependent on activation of ERK1/2 and mTORC1 to fully phosphorylate STAT3. This demonstrates a novel mechanism of how inflammatory cytokines mediate their pathological effects beyond the known activation of Janus kinases. While JAK/STAT pathway has recently attracted some attention as a novel therapeutic target for oral small molecule inhibitors, our data suggest to not only inhibit STAT3 activation through JAKs but to also explore therapeutic intervention through the mTOR and MAPK pathways to fully block STAT3 activity.

P034 (OP06/04) | The volume-regulated anion channel LRRC8 participates in initiating keratinocyte differentiation and represents a novel target for topical psoriasis treatment

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Psoriasis is a common chronic inflammatory skin disease which is characterized by hyperproliferation and aberrant differentiation of keratinocytes. Since changes in cell volume are an integral part of both processes, the aim of our study was to analyze one of the key players of cell volume regulation - the volume-regulated anion channel LRRC8 - with regard to its involvement in the pathogenesis of psoriasis.

Immunohistochemical staining of the essential subunit LRRC8A in healthy and psoriatic skin showed, that LRRC8A is mainly located in the basal layer of healthy and non-lesional epidermis. Interestingly, in lesional psoriatic skin its expression is severely decreased. In accordance with its localization in vivo, we found that LRRC8A is upregulated in the transiently amplifying keratinocyte population and during the onset of epidermal differentiation in vitro. In addition, LRRC8A protein levels declined with further keratinocyte maturation, giving a bell-shaped expression pattern of LRRC8A. Strikingly, simulating a Th1 inflammation by treating keratinocytes with a cytokine mix abrogated LRRC8A upregulation in differentiating keratinocytes.

As LRRC8A is mainly expressed in transiently amplifying cells, we were interested whether LRRC8A is functionally involved in initiating the switch from proliferation to differentiation. Indeed, siRNA-mediated knockdown of LRRC8A in primary keratinocytes resulted in a reduction of late differentiation markers but had no effect on proliferation. Strongest effects were achieved, when LRRC8A was

specifically targeted in keratinocyte stem cells - thereby inhibiting its upregulation - which did not only reduce involucrin or filaggrin expression but also reduced the expression of FOXM1 and NGFR, two important initiator factors of differentiation. These data suggest, that LRRC8 contributes to the ability of transiently amplifying keratinocytes to initiate differentiation.

As in psoriatic lesions numbers of transiently amplifying cells are highly increased while differentiation is decreased, we wondered whether activating LRRC8 could mitigate the psoriatic phenotype. To identify small molecule activators of LRRC8, we performed a compound-screening, which revealed tioxolone, a known active ingredient in topical formulations for the treatment of psoriasis, as an enhancer of LRRC8 ion channel activity. Furthermore, another study identified zinc pyrithione, which has been described to improve PASI score in psoriasis patients. To analyze whether these LRRC8 activators can indeed improve inflammatory effects, we generate epidermal equivalents with a psoriasis-like phenotype by adding Th1 cytokines, which results in acanthosis and reduced expression of keratins. These epidermal equivalents will be treated with tioxolone and zinc pyrithione and effects on thickness, barrier function and various proliferation and differentiation markers will be monitored.

In summary, our work identifies LRRC8 dysregulation in lesional psoriatic skin, which we were also able to mimic in vitro using Th1 cytokine mixes. In turn, dysregulation of LRRC8A impairs epidermal differentiation. Most significantly, two compounds that are known to improve psoriatic phenotype are activators of LRRC8. Overall, our data suggest that LRRC8 might serve as a novel therapeutic target for the topical treatment strategies of psoriatic lesions by restoring the capacity of keratinocytes to initiate differentiation.

P035 | Development of 3D epidermal spheroids with a dermal core as a highthroughput suitable alternative to classical full-thickness skin models

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Dermatological research relies on in vitro skin models as a crucial tool to study skin physiology as well as its dysfunction in dermatological diseases. In classical reconstructed three-dimensional (3D) skin models, keratinocytes (NHK) are cultivated in a transwell system, where lifting the cells to the air-liquid-interface (ALI) and elevation of Ca2+ concentration induces three-dimensional growth as well as epidermal stratification. These models can further be refined by adding a fibroblastembedded dermal layer to generate full-thickness skin models (FTMs), which are able to recapitulate many features of native skin. However, these are labor-intensive and time-consuming models that require a large number of cells and are not suited for high-throughput applications such as drug screenings. To generate 3D models for other tissues, newer developments are focusing on organoids or spheroids as alternatives. Spheroids can be easily generated through the liquid overlay technique (LOT) by seeding cells in low-attachment microwell plates, which promote a rapid self-assembly into discrete, multilayered spherical structures. Spheroids are not only suitable for high-throughput analysis in drug screenings but also allow a more complete analysis of the 3D microenvironment by whole-mount immunostaining as well as live-cell imaging.

Thus, we aimed at generating skin-like spheroids with a dermal core and outer epidermal layers and compare these to classical FTMs regarding keratinocyte differentiation and stratification as well as barrier formation.

We used HaCaT cells and two immortalized keratinocyte cell lines (NHK-SV/TERT and NHK-E6/E7) with and without fibroblasts to create spheroids and compared them with classical FTMs.

While HaCaT cells were able to form spheroids by themselves, they did not reconstitute a stratified epidermis even if involucrin expression increased over time. In contrast, both NHK cell lines required a fibroblast core to assemble, which also induced proper distribution of differentiation markers in all cell lines, with stronger expression of keratin 14 in contact with the fibroblasts, involucrin mainly located on the outer rim, and keratin 10 in between. In addition, the NHK-E6/E7 spheroids displayed cell flattening and loss of nuclei on the exterior surface, which increased over time. Thus, these cells were able to reconstitute a proper epidermal architecture similar to the superior differentiation and stratification obtained with this cell line in FTMs. Furthermore, both NHK cells lines reconstituted a functional barrier in spheroids as well as FTMs as measured by lucifer yellow penetration, which was absent in HaCaT models.

In summary our novel 3D skin spheroid model displays important characteristics of keratinocyte differentiation and maturation. It permits fast, non-destructive imaging with high spatio-temporal resolution. Therefore, these types of 3D epidermis models will be particularly suitable for preclinical testing of novel drugs and may complement existing classical epidermis models for streamlining preclinical investigations.

P036 | Beneficial effects of a plant extract mix in a psoriasis-like in vitro model: role of acting as antioxidant AhR ligand

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The arylhydrocarbon receptor (AhR) is a transcription factor which can be activated by a variety of different exogenous or endogenous ligands. In skin, activation of the AhR leads to epidermal differentiation by upregulation of barrier molecules. Some AhR ligands also activate the antioxidative nuclear factor erythroid-2-related factor (Nrf) 2 pathway, which regulates the expression of antioxidative enzymes and plays a crucial role in eliminating reactive oxygen species

(ROS). Dual AhR/Nrf2 ligands are therefore presumed to be beneficial in cutaneous inflammatory diseases with barrier defects. It has already been shown, that in psoriasis vulgaris, a common chronic inflammatory skin disease, activation of AhR/Nrf2 by the antioxidative AhR ligand tapinarof is of advantage.

Recently, we experienced that a standardized plant extract mix had beneficial effects in a psoriasis-like 2D in vitro model by restoring skin barrier molecules and lowering inflammation. Plant occurring flavonoids are known to have AhR modulating effects. Thus, the aim of our study was to investigate whether the plant extract mix acts as antioxidative AhR ligand and if its activation is responsible for the beneficial effects in the 2D psoriasis model.

Differentiated normal human keratinocytes were stimulated with a psoriasis-like cytokine mix (interleukin (IL)-17A, tumor necrosis factor (TNF)-alpha, IL-1 beta and IL-22) with or without the extract mix for 24h. Gene expression measurements revealed that the extract mix induced a strong cytochrome P450 family 1 subfamily A member (CYP1A) 1 expression. CYP1A1 is a direct downstream AhR target gene. Gene expression level of antioxidant enzyme NAD(P)H quinone dehydrogenase (NQO) 1, a downstream marker of Nrf2, was slightly downregulated by the cytokine mix, whereas simultaneous stimulation with the extract mix upregulated NQO1 expression again. Furthermore, the plant extract mix induced a ten-fold higher gene expression of heme oxygenase (HO)-1, another Nrf2 downstream marker, in comparison to the control.

Both treatments with AhR inhibitor CH-223191 and AhR knockdown via siRNA totally abolished the induction of CYP1A1 by the extract mix, confirming that the CYP1A1 expression was dependent on AhR activation. In line with that luciferase activity assays showed a direct activation of AhR by the extract mix.

Supplementary, the impact of AhR inhibition on the beneficial effect of the extract in the psoriasis-like in vitro model was evaluated. Interestingly, AhR inhibition had a slight negative impact on the ability of the extract to improve the expression of barrier molecule filaggrin, but it had no impact on the ability of the extract to reduce the expression of inflammation markers like IL-17C. Additionally, AhR inhibition had only little effects on NQO1 and HO-1 expression hypothesizing that the extract may directly target Nrf2.

Nrf2 activation may also be supported by the fact that stimulation with the extract reduced intracellular ROS production in cytokine-treated keratinocytes, which was shown by 2',7'-dichlorofluorescein (DCF) assays.

In summary, the plant extract mix activates the AhR and increases the antioxidative capacity in keratinocytes. AhR activation is involved in the upregulation of filaggrin by the extract, but its anti-inflammatory effect seems to be AhR independent. Further experiments will show if the Nrf2 pathway with its antioxidative effect is also responsible for the anti-inflammatory effects of the plant extract mix. This could be an interesting topical treatment option in the future.

P037 | Combined Skin and Chest Trauma Accelerates Cutaneous Wound Healing - Role of Released MDC in Shaping Macrophage Polarization

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Trauma is one of the leading causes of mortality worldwide. Epidemiological studies on trauma have shown that 81% of injured patients suffer from bone fractures of the extremities, 58% from thoracic trauma, and 50-65% from traumatic brain injuries. Although defined injuries, like traumatic brain injury or hemorrhage, are associated with higher mortality, the combination of multiple injuries aggravates the outcome even more. Blunted chest injury and soft tissue injury of the skin - though frequently occurring together - have less well been studied, and it is unclear whether the traumatic chest injury may impact on wound healing of the skin or vice versa. Skin wounds enhance the propensity for severe infections with the development of life threatening sepsis. Therefore, we are particularly interested whether combined chest and skin trauma - via the release of MDC and altered macrophage polarization - aggravate or possibly limit each other.

Notably, we observed that a preceding chest trauma significantly accelerates healing of full thickness wounds in mice. We also found that the contraction phase with activation of α-smooth muscle actin positive myofibroblasts is stronger and highly accelerated in skin wounds after chest trauma. This is of major clinical relevance as enhanced cutaneous wound healing is the best prevention against the threat of systemic infections. Enhanced cutaneous wound healing may be due to the extensive release of cytokines, growth factors and chemokines after chest trauma which may prime the cells in cutaneous wounds. In fact, we found that Macrophage Derived Chemokine (MDC) was highly up-regulated in the peripheral blood after blunted chest trauma. To dissect the question whether MDC systemically released after chest trauma may impact on cutaneous wound healing, we explored its role on macrophage function and polarization.

Our in vitro results suggest that MDC not only represses pro-inflammatory signaling in macrophages, but also polarizes them towards the M2-like subtype. At the molecular level, MDC abrogates STAT1-IRF1 axis, thereby reducing pro-inflammatory activity (suppression of pro-inflammatory cytokines IL-1, TNFα; IL-6; M1 cell surface markers) and in parallel switching the polarization towards a M2-like macrophage subset (CD206, CD163) with enhanced TGFβ1 expression. TGFβ1 plays a key role in converting wound fibroblasts into contractile myofibroblasts. Collectively, our data highlight that MDC is critical in shifting the wound microenvironment from the inflammatory phase to the resolution phase, which subsequently accelerates tissue repair. Thus, MDC qualifies as an excellent candidate which - after a preceding chest trauma - mediates the beneficial

effects on accelerated healing of skin wounds. To further explore whether our in vitro findings are relevant in vivo, we now intend to investigate the potential benefits and the causal role of MDC in a murine skin wound healing model. In independent experiments, we will employ neutralizing antibodies against MDC in the combined chest skin trauma model to test whether the beneficial effect on accelerated wound healing of the skin can be abrogated. The knowledge gained from these studies will advance our understanding of interorgan relationships after trauma.

P038 | Natural Killer Cells from Old Donors Fail to Efficiently Remove Senescent Fibroblasts from Aged Skin

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Cellular senescence, a state of permanent cell cycle arrest, is a prime hallmark of tissue aging. Senescent cells - by the release of 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 aging-related conditions. Fibroblasts, which physiologically constitute the principal component of the connective tissue, play a pivotal role in organ homeostasis, however - if senescent - 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 either senescent fibroblasts in aging skin are resistant to the cytotoxic removal by NK cells or, alternatively, NK cells themselves fail to remove senescent fibroblasts. Using a newly developed NK cell mediated killing assay with negative selection of NK cells, we were able to show that the NK cell line 92 as well as primary human and mouse NK cells preferentially killed senescent fibroblasts and not young fibroblasts. 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 donor (~23 years) and young mice (~100 days), respectively. No change was detected in the number of NK cells in the skin between young and old human adults. However, synapse formation between old NK cells and senescent fibroblasts was significantly impaired, and at the synapse old NK cells release significantly less perforin responsible for the induction of holes in senescent fibroblasts. In addition, old NK cells release significantly less granzyme B which physiologically enters target cells through the perforin induced holes and initiate their apoptosis. The impaired release of these cytotoxic substances unequivocally leads to a profoundly impaired killing ability of old NK cells. RNAseq and subsequent enrichment analysis of old vs young NK cells, identified that oxidative phosphorylation- and Rho GTPase signaling is decreased, respectively, increased in old NK cells. Synapse formation and the focused release of perforin and granzyme B into the synaptic cleft depends on the reorganization and of the cytoskeleton, a high energy demanding process which is fine tuned by Rho GTPases. Interestingly, ATP concentrations were significantly reduced in NK cells from old donors as opposed to young donors (n=6). In conclusion, these findings suggest that reduced cytotoxicity of old NK cells likely contributes to the accumulation of senescent fibroblasts in skin and other organs. In the long term, our data hold promise to be exploited to develop novel strategies against age-related disorders.

P039 | Understanding the Adaptive Response of MSCs upon Priming with S100A8/ A9

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Mesenchymal stromal cells (MSC) are stromal cells of mesenchymal origin. MSC so far are isolated from a variety of tissues such as bone marrow, adipose, umbilical cord, dental pulps among others. Because of their multipotent differentiation potential and immunomodulatory function, MSC play a major role in tissue regeneration and inflammation control. MSC exerted beneficial effects on tissue repair following experimental injury and have entered clinical routine as approved treatment modality for difficult-to-treat chronic wounds. S100A8/A9, an important danger associated molecular patterns (DAMPs), is released from native immune cells such as neutrophils and monocytes during tissue damage and functions as an alarming signal for the immune system. S100A8/A9 could bind to TLRs and RAGE receptors which are expressed on the surface of MSC and many immune cells to regulate inflammation. Former studies in our lab showed that S100A8/A9 primed MSC significantly accelerate acute wound healing in mice. To understand the molecular mechanism of the S100A8/A9 mediated adaptive MSC response, we have performed transcriptome analysis of S100A8/A9 primed MSC. To verify the differentially expressed gene sets in S100A8/A9 primed MSC, protein profiling with flow cytometry and western blot analyses were performed. Transcriptomic, flow cytometry and western immunoblot analyses revealed a significant increase in cap-independent related translation initiation factors (EIF3I), and a decrease in cap-dependent translation initiation factors (EIF4A1). Furthermore, MSC primed with S100A8/A9 showed a higher level of translation initiation. Of note, metabolic analyses with seahorse real-time FLUX analysis showed no changes in the rate of glycolysis and oxidative phosphorylation compared to unprimed MSCs. Cross-linking and immunoprecipitation (CLIP) and co-immunoprecipitation (Co-IP) studies revealed a transition from cap-dependent to

cap-independent translation in S100A8/A9 primed MSCs. In conclusion, these findings suggested that cap-dependent translation which is the most common way for cells to synthesize protein may be less favored and switched to cap-independent translation in S100A8/A9 primed MSCs. It is likely that a cap-independent translation of selected mRNAs induces a defense and protective gene program. This study helps us to understand the adaptive response of MSCs upon S100A8/A9 treatment and is helpful for developing more strategies in MSCs related application.

P040 | Alzheimer´s disease: CRISPR-Cas9-Mediated Knockdown of Presenilin1 Leads to Disturbed rDNA Transcription and Impaired Cell Proliferation

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In former work with skin cells, we could show that premature aging children suffer from a loss of proteostasis mediated by ribosomes. Asking if this pathological mechanism is also active in age-related diseases, we are currently conducting a study on Alzheimer's disease (AD) using skin fibroblasts. Alzheimer's disease (AD) is a complex neurodegenerative disorder characterized by the accumulation of amyloidbeta plaques and neurofibrillary tangles in the brain. Mutations in the Presenilin1 (PSEN1) gene have been linked to familial forms of AD, implicating its role in disease pathogenesis. In this study, we employed CRISPR-Cas9 technology to knockdown PSEN1 expression in human fibroblasts, creating a cellular model of AD. Our findings reveal a significant change in ribosomal DNA (rDNA) transcription levels in these cells, indicative of a disturbed transcription. Furthermore, we observed a concomitant rise in ribosomal error rate. Importantly, these molecular alterations were associated with a profound reduction in cell proliferation rates. These results provide valuable insights into the potential role of PSEN1 in regulating rDNA transcription, translational accuracy, and cell proliferation, shedding light on the complex molecular mechanisms underpinning AD pathogenesis. Understanding these processes may pave the way for the development of novel therapeutic strategies to combat this devastating disease.

P041 | rDNA transcription disturbances in xpg mutated Cockayne syndrome

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Cockayne syndrome (CS) is a rare autosomal recessive disease with pre-mature aging symptoms. There is some evidence that CS is not only a nuclear excision repair (NER)-related disease but also a ribosomal biogenesis and ribosomal function disorder. Previous studies in our group showed that CSA and CSB-deficient CSpatient cells display severe disturbances in ribosomal biogenesis and function leading to the accumulation of misfolded proteins CS can be provoked by mutations in the xpg gene that can also cause the skin-cancer prone disease Xeroderma pigmentosum. Now we asked the question if xpg-mutation affects ribosomal biogenesis and function. We investigated rRNA expression in a XPG/CS cell line (94RD27) via qPCR. The CS cell line showed a decreased expression of 47S, 5.8S/ITS2 and 28S/ETS when compared with healthy control. The results suggest that XPG deficiency could lead to a decrease of rDNA transcriptional initiation, elongation and termination. In addition, we also detected that the XPG/CS cell line showed a significant increase in eIF2α phosphorylation, which indicates that CS cells suffer from cellular stress. The results suggest that the XPG/CS patient cell line has a dysfunction of rDNA transcription, probably because of cellular stress. Our work revealed an underlying pathomechanism of XPG/CS and helps us to understand the mechanisms of premature aging.

P042 (OP02/01) | Importance of the xCT system and lipid peroxidation in systemic sclerosis.

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Systemic sclerosis (SSc) is a chronic autoimmune disease, resulting in skin and organ fibrosis. It is known that skin cells from SSc patients exhibit higher levels of reactive oxidative species (ROS) compared to healthy donors (HD). In turn, the xCT system transports cystine into the cell in exchange for glutamate to synthesize the antioxidant glutathione (GSH). GSH then activates glutathione peroxidase 4 (GPx4), preventing lipid peroxidation and iron-dependent cell death.
We observed an overexpression of the soluble carrier 7 a11 (Slc7a11)/xCT system in RNA bulk sequencing of CD45- skin cells in SSc patients compared to HD. Further, Cystine Biotracker revealed high xCT activity in ROS stressed fibroblasts. For functional analysis, we induced chronic oxidative stress in xCT KO mice by daily intradermal injections of hypochlorous acid (HOCl), resulting in dermal fibrosis. Interestingly, HOCl-treated xCT KO mice showed significant reduced collagen accumulation and myofibroblast activation compared to WT animals. In line with these results, in vivo PET/CT based detection of [68Ga] fibroblast activating protein inhibitor (FAPI) demonstrated diminished tracer expression in xCT KO skin compared to WT. Furthermore, fibroblasts isolated from xCT KO

mice could only survive in vitro by adding a ROS scavenger and a Nanostring array confirmed a reduced fibrotic gene signature in xCT KO mice.

Based on these findings, fibroblasts might undergo lipid peroxidation induced cell death due to the absence of xCT and the associated lack of Gpx4 activity. Moreover, treatment of SSc mice with the xCT inhibitor imidazole keton erastin (IKE), known to drive lipid peroxidation, resulted in a reduced fibrosis in vivo. We also detected an increased lipid peroxidation in HOCl-stressed murine fibroblast by BODIPY C11 and Liperfluo staining.

To conclude, loss or inhibition of xCT prevent skin fibrosis and might be a new therapeutic target in SSc.

P043 | Filamin B modulates fibrosis-associated proteins and functional properties in cancer-associated fibroblasts

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Cutaneous squamous cell carcinomas (cSCC) interact with stromal fibroblasts in a way that facilitates their transdifferentiation into cancer-associated fibroblasts (CAFs). In turn, CAFs affect tumor cell proliferation, migration and invasion centrally by generating a protective tumor scaffold.

The dynamics of the actin cytoskeleton of CAFs determines the physical properties of the tumor scaffold including the contractile forces therein. Detailed knowledge of cytoskeletal regulations will improve our understanding of tumor pathophysiology and may even contribute to new therapies. Initially, we isolated CAFs from cSCC and analyzed gene expression of central pro-tumorigenic and profibrotic markers, focusing on actin cytoskeleton dynamics. In addition to the induction of various profibrotic and pro-tumorigenic markers, the actin-binding protein Filamin B (FLNB) was significantly upregulated compared to normal dermal fibroblasts. FLNB crosslinks F-actin and binds to the cytoplasmic domain of integrin β1 (ITGB1). This interaction is thought to modulate the stiffness of the matrix. Given the frequent mutations of FLNB in cSCC, we are currently studying its functional impact on tumorigenesis. To examine its CAF-related function, we perform siRNA-mediated knockdown in dermal fibroblasts and fibroblasts stimulated with CAF activators activin A and TGF-ß, respectively. Intriguingly, activin A and TGF-ß induce not only typical CAF activation markers such as α-smooth muscle actin, plasminogen activator inhibitor-1 (PAI-1) and collagen IAI, but also FLNB gene expression itself.

siRNA-mediated knockdown of FLNB showed efficient reduction both at the transcriptional and translational level along with high specificity. There were no compensatory changes of the filamin homologs A and C. FLNB suppression led to the modification of gene expression of pro-fibrotic markers PAI-1 and ITGB1, whereas cell viability remained unchanged in normal and CAF-like states. Functional experiments revealed modifications in migration following FLNB knockdown. Currently, G/F actin dynamics, cellular contraction and motility are examined more closely and will be analyzed in primary CAFs.

Immunohistochemical analyses of human cSCC show FLNB expression in tumor keratinocytes and surrounding stroma cells suggesting a role for FLNB in tumor-CAF interaction and cSCC pathogenesis.

P044 (OP02/05) | Inactivation of RNase 7 by RNase inhibitor influences the inflammatory response in atopic dermatitis

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Atopic dermatitis (AD) is an inflammatory skin disease commonly characterized by barrier disruption and increased colonization and risk of infection by Staphylococcus aureus (S. aureus) due to microbiota dysregulation. In the case of skin barrier disorders such as AD, cell disruption leads to the release of self-RNA which promotes the inflammatory response by inducing pro-inflammatory mediators. RNase 7 is an antimicrobial active ribonuclease expressed and secreted by keratinocytes and abundantly present on lesional AD skin. It exhibits antimicrobial activity against a broad spectrum of bacteria, viruses and fungi. Moreover, RNase 7 degrades RNA, including host RNA. It is has been reported that the endogenous RNase inhibitor (RI), which is also expressed in keratinocytes, binds to RNase 7 and blocks its antimicrobial and ribonuclease activity. Therefore we aimed to investigate whether the inactivation of RNase 7 by RI may have a regulatory influence on the RNAmediated inflammatory and antimicrobial response associated with AD.

To this end, we stimulated human primary keratinocytes with the RNA analogue poly I:C in the presence or absence of RNase 7 and examined the induction of proinflammatory mediators. These data revealed a decrease in poly I:C-induced proinflammatory factors after addition of RNase 7. In line with these data, endogenous siRNA-mediated downregulation of RNase 7 in keratinocytes led to enhanced expression of pro-inflammatory mediators after addition of poly I:C. In contrast, siRNA-mediated downregulation of RI had the opposite effect. Our results suggest that inactivation of RNase 7 by RI leads to RNA-mediated inflammation.

RI was released by damaged keratinocytes and damaged epidermis and increased RI gene expression was detected in biopsies of lesional AD skin. Western blot analyses of skin rinsing fluids derived from lesional AD skin revealed an enhanced release of RI. Ribonuclease activity of RNase 7 was strongly inhibited in these fluids suggesting that the RI binds RNase 7 on the surface of the damaged epidermis

in AD patients and consequently, that released RNA triggers the pro-inflammatory cytokine response. The antimicrobial activity of RNase 7 was blocked by RI and this prevented S. aureus outgrowth. As AD patients often struggle with S. aureus colonization and infections, the inhibition of the antimicrobial activity of RNase 7 by the released RI on lesional AD skin could be an important influencing factor of these pathophysiological dependencies.

In summary, we could demonstrate that inactivation of RNase 7 by RI has an impact on both, the RNA-mediated pro-inflammatory response and S. aureus growth. These new findings suggest a previously unknown modulatory influence of the RNase 7 - RI interaction in AD pathophysiology.

P045 | 1064 nm Nd:YAG laser induces pro-fibrotic traits in collagen-embedded dermal fibroblasts

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Neodym:YAG (Nd:YAG) laser is routinely used to treat pigment and vascular lesions. By combining classical incision and expression therapy with 1064 nm Nd:YAG laser treatment, we significantly improved the outcome of digital mucous pseudocyst therapy. Since pseudocysts are associated with fibrogenesis, we hypothesized that laser treatment affects pro-fibrotic traits in skin fibroblasts. During fibrosis, they transdifferentiate into contractile, alpha smooth-muscle-actin (α-SMA)- and extracellular matrix (ECM)-synthesizing myofibroblasts.

To test our hypothesis, we examined 1064 nm Nd:YAG laser effects on dermal fibroblasts. To mimic real-life conditions, fibroblasts embedded organotypically in a collagen matrix were laser irradiated using clinically relevant settings (50 ms pulse, 90 Joule/ cm^2, 6 mm spot size). Cells were analyzed either within the matrix or after collagenase A digestion. We assessed myofibroblast marker expression on both transcriptional and protein expression levels as well as hyaluronan (HA) secretion shortly (1 h) as well as later (24 h) after laser treatment. When the cells were stained with calcein-AM and visualized by confocal spinning disc microscopy to study cytotoxic effects, we saw no changes of viability or morphology. While short-term analysis revealed induction of pro-fibrotic markers such as plasminogen activator inhibitor-1 (PAI-1) and hyaluronan synthase 2 (HAS2) as well as constant HA secretion, laser-treated fibroblasts secreted significantly less HA but revealed constant pro-fibrotic marker expression after 24 h.

Our data indicates initial activation of dermal fibroblasts to a transitional pro-fibrotic state in response to Nd:YAG laser treatment. At later stages, this appears to be counterregulated, thus reducing pro-fibrotic traits of dermal fibroblasts. Nd:YAG laser treatment is currently being investigated to elucidate the long-term effects on healing and fibrogenesis in mucous pseudocysts.

P046 | Mechanotransduction via myosin II isoforms contributes to TGF-β-driven fibroblast activation within Scleroderma skin fibrosis.

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Introduction: Scleroderma (SSc) is an autoimmune disease characterized by fibrosis in the skin and internal resulting from immune dysregulation, endothelial damage, and activation of fibroblasts prompted, at least in part, by an autocrine TGF-β loop. Nonmuscle myosin II isoforms (NMIIA, NMIIB, NMIIC) are motor proteins executing virtually all cellular processes that require force, such as cell migration, adhesion, and mechanotransduction. NMII motor activity is regulated by phosphorylation of the myosin regulatory light chain (MLC), mainly through the Rho-ROCK pathway. Recent studies portray a possible role of NMII in lung fibrogenesis. In scleroderma (SSc), there is evidence of the Rho-ROCK pathway activation; nevertheless, the specific role of NMII in SSc has never been investigated.

Material and Methods: The expression and the cytoskeletal distribution of NMIIA and NMIIB and their activation through MLC phosphorylation were evaluated by immunofluorescence in primary fibroblasts from eight SSc and eight healthy donors (HD), which were stimulated with rhTGFβ. The NMII activation pathways were inhibited (i) with Y-27632, a small molecule inhibitor of pan-ROCK that phosphorylates MLC, (ii) with KD025, a small molecule inhibitor of ROCK2 isoform, or (iii) with blebbistatin, a small molecule that inhibits NMII motor. The production of IL-6, type-I collagen, and fibronectin by fibroblasts was assessed by ELISA.

Results: SSc fibroblasts exhibited altered re-distribution of NMII isoforms within the actomyosin cytoskeleton (n = 8, NMIIB filament/ cytoplasm intensity median (range) HD 1.2 (0.8-1.7) vs SSc 1.8 (1.5-2.2), p = 0.02). HD fibroblasts exhibited a similar pattern of redistribution of NMII isoforms (n = 8, NMIIB filament/ cytoplasm intensity median, (range) ctrl 1.2 (0.8-1.7) vs TGFβ 1.9 (1.8-2.3), p = 0.02) and increased phosphorylation of MLC at Thr18/Ser19 upon TGFβ stimulation (n = 8, ppMLC fluorescence intensity/cell median, (range) ctrl 10000 (8000-1200), TGFβ 15000 (12000-17000), p 0.002). Treatment of HD fibroblasts with blebbistatin or Y-27632 substantially reduced the stimulatory effect of TGFβ on IL-6 (n = 10, pg/mL median, (range) TGFβ 192.6 (72-572) vs TGFβ+Y 15 (4,6-81) vs TGFβ+blebbistatin 25 (12-200), p = 0.003, p = 0.02 respectively) and collagen I (n = 10, ng/mL median, (range) TGFβ 248 (109-572) vs TGFβ+Y 109 (73-206); TGFβ vs TGFβ+blebbistatin 174 (77-244), p = 0.005, p = 0.04, respectively) production. Treatment of HD fibroblasts primed or not with TGFβ with KD025 did not affect IL-6 production while impaired the type-I collagen production already in resting fibroblasts (n = 10, ng/mL median, (range) ctrl 136 (51-165) vs KD025 84(23-95), p = 0.03).

Conclusion: Our data point to an altered actomyosin cytoskeleton dynamics and force distribution in SSc fibroblasts and indicate that NMII isoforms are a downstream mediator of the stimulatory effects of TGFβ in fibroblasts. These findings suggest that NMII assembly and activation pathways may be a possible target for novel antifibrotic therapies.

P047 | Unveiling Fascia Dual Deformation: Catalyst for Healing and Scarring

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Skin fascia plays a critical role in wound healing and scar formation of deep skin wounds. Fascia mobilization driven by swarming-like collective migration of fascia fibroblasts brings the pre-made cellular and matrix material to initiate wound repair. The detailed process of fascia-mediated wound healing and scarring has not been comprehensively elucidated.

Here, we use ex vivo fascia explant model and in vivo lineage tracing coupled with multiphoton time-lapse imaging to demonstrate that fascia driven skin wound healing requires sequential fascia swelling and contraction phases. Swelling and contraction take place primarily in fascia but not in dermis or panniculus carnosus muscle. Fascia swelling and contraction are cell-driven processes, and are adaptive injury responses of fascia fibroblasts to the changes of environmental mechanical stress.

Mechanistically, we identify Procr+ fascia fibroblasts are responsible for fascia swelling and are dependent on the changing extracellular matrix architecture at the early phase of wounding. Whereas, PDPN+ pro-inflammatory fascia fibroblasts initiate fascia contraction via TRPV4-evoked calcium oscillations and subsequent activation of mechanosensory YAP signaling. Ablation of Procr+ or PDPN+ fascia fibroblasts in mouse prevented fascia swelling or contraction, respectively.

In summary, our study reveals a biphasic fascia deformation process during fascia steered skin wound healing. Fascia swelling initiates wound repair by transferring fascia material into wounds, which imprints the amount of scar tissue after wound healing. The subsequent fascia contraction closes wounds and forms scars. Targeting signaling pathways in fascia fibroblasts controlling fascia swelling and contraction provides novel strategies to improve wound management with faster healing and less scars.

P048 | Distinct protein kinase activity across age and sex in murine skin

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Protein kinases are key molecules in intracellular signal transduction, and are involved in the regulation of cellular processes including proliferation, differentiation, migration and apoptosis. However, since the activity of kinases is heavily regulated on a posttranslational level, their activity cannot be correctly assessed using expression data alone. Additionally, due to the fact that all kinases catalyze the same type of reaction, that being the transfer of a phosphate group from adenosine triphosphate (ATP) to a hydroxyl group of an amino acid side chain, it is difficult to accurately assess the activity of multiple kinases within the same sample.

This issue has been addressed by the development of peptide array chips, which employ spatially separated oligopeptides to distinguish the activities of multiple kinases within the same mixture based on their substrate affinities. Differences in kinase activity can be inferred from differences in the phosphorylation patterns of the oligopeptides on the chips. The availability of this method allows for broad, screening based approaches in kinome profiling, since preselecting kinases of interest is no longer necessary. Our aim is to utilize this methodology to uncover differences in the baseline activity of kinases in the skin of mice of different ages and sexes.

A total of 12 mice separated into four groups according to age and sex (adolescent males, adolescent females, old males and old females, 3 mice per group) were killed and transcardially perfused with PBS. Various organs and tissues, including skin, were harvested and used to prepare total protein lysates. These lysates were analyzed using the above described peptide chip based multiplex kinase activity assay. Variations in the activity of a total of 154 kinases were evaluated by comparing peptide data between the groups. From among the assessed kinases, 122 displayed elevated activity in young mice relative to old mice, while 14 kinases showed higher activity in females when compared to males. Many of the kinases which showed higher activity in younger mice were associated with cell proliferation and differentiation, such as members of the MAP Kinase family as well as cyclin dependent kinases (CDKs) and Aurora kinases, whereas a large subset of the kinases showing higher activity in females were receptor tyrosine kinases associated with vascular tissues, such as vascular endothelial growth factor receptors (VEGFR) 1 and 2, platelet derived growth factor receptor beta (PDGFRβ) and Eph family receptor EphB1. The link between these differences in kinase activity and differences in tissue structures will now be investigated further using tissue staining experiments and supplemented with transcriptomics data.

These differences, upon further investigation, have the potential to provide valueable insights into both changes the skin undergoes

with age as well as differences between the sexes. This, in turn, can aid in our understanding of sex- and agespecific variations in the progression of disease and tissue structure.
Chemokines/Cytokines

P049 | IL-9 induces a type-1 interferon signature in pathogenic skin-tropic Th2 cells

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Background: IL-9 is a pleiotropic cytokine, for which an overarching role in humans remains elusive. Both interleukin 9 (IL-9) and its receptor, IL-9R, are specifically expressed by skin-tropic T helper 2 (Th2) cells. This suggests that IL-9 signals play an important role in cutaneous immunity and allergy. Yet, the mechanism of action of IL-9 remains incompletely understood.
Objective: Here, we aimed at deciphering the effect of IL-9 signals on Th cells in allergic skin inflammation. We isolated human IL-9R+ Th2 cells from blood and skin of acute allergic contact dermatitis and performed transcriptional profiling after stimulation with IL-9.
Results: IL-9 induced differential expression of approx. 800 genes in Th2 cells. Upregulated genes were associated with conventional Th2 immune response. Surprisingly, it also showed a strong induction of i) interferon-stimulated genes (ISGs) such as IFIT3, RSAD2, and OAS1, and ii) subunits of ISGF3 and DTX3LPARP9. The latter are powerful transcription factor complexes that amplify type-1 interferon responses. Since ISG expression is mediated by STAT1 phosphorylation, we next investigated IL-9R signaling in detail. Interestingly, we found that signal transduction by IL-9R indeed leads to a unique STAT1 phosphorylation profile that is not comparable to other members of the common gamma-chain-cytokine receptor family.
Conclusion: We show that IL-9 signals are able to induce a type-1 interferon signature in Th2 cells through a unique STAT1 signaling profile. This suggests that IL-9 enhances interferon-dependent immunity in type-2 inflammation and plays an important role in protecting against viral infections.

P050 | Microdialysis in common inflammatory skin diseases highlights abnormalities in nonlesional skin of patients with atopic dermatitis

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Background: Pathophysiological insights into chronic inflammatory skin diseases are still incomplete, especially on a proteomic level, which mandates better, minimally invasive profiling techniques.
Methods: Skin microdialysis was performed on atopic dermatitis (AD, n=6), psoriasis (PSO, n=7), prurigo nodularis (PN, n=6), and healthy controls (n=7) for proteomics and multiplex cytokine analysis. Single-cell RNA sequencing data from skin biopsies were used for identification of cytokine sources.
Results: Three of the top 20 enriched GO annotations (NAD metabolic process, regulation of secretion by cell, pyruvate metabolic process) were elevated in microdialyses of lesional AD vs. nonlesional skin, and equally of lesional AD vs. controls. Enriched top 20 KEGG pathways between lesional AD, nonlesional AD, and controls overlapped almost completely. In contrast, nonlesional PSO and PN skin vs. controls revealed no overlap of altered top 20 KEGG pathways compared to lesional skin. Cytokine analysis revealed significantly increased IL-22 and MCP-1 levels in lesional vs. nonlesional PSO, but not in AD or PN. In AD and PSO, IL-8 was elevated in lesional vs nonlesional skin, whereas IL-12p40 was increased in lesional PSO only. Integrated single-cell RNAseq data from skin biopsies revealed that cellular sources of these cytokines remained identical in AD, PSO, and PN.
Conclusions: Microdialysis fluids of lesional PSO and PN skin, but not lesional AD skin, differ significantly from the corresponding proteome of nonlesional skin and controls. IL-8, IL-22, MCP-1, and IL-12p40 may be suitable disease markers for minimally invasive profiling techniques.

P051 (OP01/02) | Blocking the IL-22RA1 improves the molecular and histological signature of Atopic Dermatitis- insights from in vivo and in vitro AD models

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Background: Atopic dermatitis (AD) represents a heterogeneous, type 2-immune response-driven, chronic inflammatory skin disease. Overexpression of several cytokines and molecular mediators, notably IL-4 and IL-13, as well as IL-22, have been observed in AD patients. IL-22 is known for its diverse functions, including pro-inflammatory effects and effects on keratinocyte differentiation. Despite several therapeutic options for AD such as IL-4/IL-13 blockade, or JAK inhibition, a subset of individuals are non-responders, underscoring the disease's complexity and the need for alternative treatment modalities.

Objective: This study aimed to elucidate the pathophysiological role of the IL-22/ IL-22RA1 signaling axis in the context of AD-like skin inflammation and to assess the potential of inhibiting this pathway as a treatment strategy.

Methods: In situ hybridization for IL-22RA1 were conducted on biopsies obtained from AD patients. T-cells were isolated from lesional AD skin and three-dimensional (3D) skin equivalent models were stimulated with AD-derived T-cell supernatant and IL-22. The impact of inhibiting IL-22RA1 through a humanized immunoglobulin G (IgG) antibody was examined in skin equivalents, and in a murine model of chronic cutaneous inflammation. Histological analysis, FACS Analysis, RNA Sequencing and rtPCR was performed.

Results: Elevated expression of IL22RA1 was detected in the epidermal layer of AD skin biopsies in comparison to healthy controls. This expression of IL22RA1 was positively correlated with increased epidermal thickness and inversely associated with barrier function integrity. In 3D skin equivalents, IL-22 stimulation induced a unique molecular signature characterized by alterations in terminal differentiation, immune response and lipid metabolism, corresponding with the compromised barrier integrity observed in AD. Pharmacological blockade of IL-22RA1 prevented epidermal hyperplasia and hypogranulosis, two histological characteristics of IL-22. Molecular changes of IL-22RA1 antagonism predominantly modulated cellular cycle activities and metabolic processes. Therapeutic targeting of IL-22RA1 in a TPA-induced murine model led to a downregulation of key inflammatory markers, implicating the involvement of the IL-22/ IL22RA1 axis in skin inflammation.

Conclusion: The data indicate that the IL-22/IL-22RA1 signaling axis plays an important role in AD and hence, inhibition of IL-22RA1 axis might represent a potential therapeutic strategy for AD.

P052 (OP06/02) | Topical application of CCL22-specific aptamers as a treatment option to reduce allergic symptoms in contact hypersensitivity

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Around 20% of the general population suffer from allergic contact dermatitis (ACD) - a prevalent occupational disease with limited therapeutic options. The chemokines CCL17 and CCL22, which both bind to the chemokine receptor CCR4, have been associated with allergic diseases such as ACD. Despite their differential functions, we observed that not only CCL17- but also CCL22-deficiency reduced allergic reactions in contact hypersensitivity (CHS), the mouse model for ACD, effectively by reducing ear swelling and migration of activated T cells. To investigate the therapeutic potential of CCL22 blockade in CHS, we developed a 29-nt DNA aptamer, named AJ102.29m, to impair CCL22-mediated chemotaxis.

The aptamer AJ102.29m effectively inhibits the migration of CCL22-dependent Tcell migration in in vitro transwell assays and ameliorated allergic reactions in vivo when injected intraperitoneally in the CHS model. Moreover, CHS associated allergic symptoms could successfully be reduced by topical application of AJ102.29m in DAC cream. Additionally, microscopic analysis of an ex vivo skin assay demonstrated that the aptamer can penetrate the epidermis and dermis of murine skin.

Suppression of allergic symptoms by CCL22 blockade via the newly generated aptamer AJ102.29m proves the therapeutic potential of CCL22 targeting in treatment of ACD. The effective topical application of aptamers thereby offers a non-invasive local treatment alternative to systemic application with lower risk of adverse effects and the advantageous properties of aptamers.

Thus, with the prospect of finding an aptamer-based ACD therapeutic we will next test aptamers specifically targeting human CCL17/ CCL22.

P053 (OP03/04) | The antimicrobial peptide RNase 7 induces the expression and secretion of the pro-inflammatory cytokine IL-6 by keratinocytes

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Keratinocytes are the most abundant cell type in the skin and serve as a protective barrier against many pathogens. They secrete effector molecules including cytokines, chemokines and antimicrobial peptides. RNase 7 is one of the antimicrobial peptides secreted by keratinocytes that restricts the growth of bacteria, fungi and viruses and, when combined with human self-DNA, activates a potent immune response. Interestingly, the lesional skin of patients with atopic dermatitis and psoriasis expresses and secretes RNase 7 at higher levels than healthy skin (Harder et al., 2010).

To study the effect of RNase 7 on keratinocytes, we performed microarray analysis. RNase 7 upregulated the expression of several transcripts encoding proteins potentially involved in skin integrity (KRTAP2-3, keratin-associated protein 2-3; MMP3 and 10, matrix metallopeptidase 3 and 10) and inflammation (CXCL3, CX- C motif chemokine ligand; IL13RA2, IL-13 receptor α2; IL-24; SLCO2A1, solute carrier organic anion transporter family member 2A1). Since IL-24 is involved in the pathogenesis of atopic dermatitis and psoriasis and was reported to be induced by IL-6 (Jin et al., 2014; Mitamura et al., 2020), we further characterized the effect of R7 on IL-6 and IL-24 expression and secretion.

RNase 7 enhanced the expression and secretion of the pro-inflammatory cytokines IL-6 and IL-24 by keratinocytes as confirmed by quantitative RT-PCR and ELISA. The secretion of IL-6 correlated with the secretion of IL-24 in RNase7-stimulated keratinocytes. Interestingly, a function-blocking antibody against IL-6 blocked IL-24 secretion, suggesting that RNase 7 induces IL-6 release, which then triggers IL-24 secretion. Pre-stimulation of keratinocytes with the T cell cytokines IL-4 or IL-13 and IL-17 or IFN-γ, involved in the pathogenesis of atopic dermatitis and psoriasis, respectively, further enhanced RNase 7-induced IL-6 secretion.

In contrast to the expression and secretion of certain cytokines and products of interferon-stimulated genes (Kopfnagel et al., 2018, 2020), DNA did not further increase the RNase 7-induced IL-6 secretion. However, inhibiting intracellular DNA sensing reduced RNase 7-induced IL-6 secretion. Moreover, RNase 7 stimulates IL-6 release from undifferentiated but not from differentiated keratinocytes, suggesting that in the epidermis, RNase 7 released from the stratum corneum may induce expression and secretion of pro-inflammatory cytokines by basal undifferentiated keratinocytes.

In conclusion, our data suggest that the increased release of RNase 7 may contribute to the pathogenesis of atopic dermatitis and psoriasis by enhancing the release of pro-inflammatory cytokines.

P054 | Expression of IL-17 receptors by skin cell populations and their significance in cutaneous inflammation

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Background: Results of clinical studies with IL-17A/F inhibitors show unequivocally that IL-17A is an essential mediator of the pathogenesis of psoriasis and hidradenitis suppurativa (1, 2). Together with IL-17E and IL-17C, IL-17A and IL-17F, belong to the IL-17 cytokine family. IL-17A forms homodimers as well as heterodimers with IL-17F. Both, as well as IL-17F homodimers, act primarily through a receptor complex made up of receptor chains IL-17 receptor A (IL-17RA) and IL-17 receptor C (IL-17RC). Interestingly, IL-17RA is also part of the receptor complexes for IL-17E (IL-17RA/IL-17RB) and IL-17C (IL-17RA/IL-17RE). Apart from the well-established importance of IL-17A/F in skin inflammation, little is known about the expression and regulation of the receptor chains mediating the IL-17A/F effects and about the overlap of the IL-17A/F effects with the effects of the other IL-17RA-using cytokines.

Objective: Detailed characterization of the target cells in the skin and associated effects of IL-17A/F and IL-17C.

Methods: Keratinocytes, fibroblasts, and endothelial cells isolated from the skin as well as neutrophilic granulocytes, monocytes, and lymphocytes isolated from the blood of healthy donors were used in expression and stimulation studies. Moreover, Th1, Th2, Th17 and Th22 cells were generated from blood-derived naïve CD4- positive T cells. The expression of IL-17 cytokine receptor chains was assessed using RT-qPCR. Cells were analysed for effects of IL-17 cytokines using RT-qPCR, ELISA, and RNA sequencing.

Results: Robust IL-17RA expression was found in keratinocytes and dermal fibroblasts. The lack of expression of this receptor chain in dermal endothelial cells implies no functional regulation of these cells by IL-17A/F or IL-17C. Immune cells showed high IL-17RA expression that even exceeded the expression in keratinocytes and fibroblasts. IL-17RC and IL-17RE were also found to be expressed by keratinocytes, dermal fibroblasts, and immune cells, qualifying these cells as relevant target cells of IL-17A/F and IL-17C.

Among immune cells, monocytes turned out to have an outstanding expression of IL-17RA and IL-17RC, and the expression of both receptor chains increased under pro-inflammatory conditions. With respect to Th cell subsets, the expression IL-17RA/IL-17RC implies an influence of IL-17A/F mainly on Th2 and Th22 cells, while IL-17C doesn't seem to favor any of the T cell subsets. There was no impact of pro-inflammatory cytokines on the IL-17RA/RC expression in keratinocytes and fibroblasts. In contrast, IL-1beta and a mixture

of TNF-α, IL-17A, and IL-36α downregulated the IL-17RE expression in fibroblasts.

Regarding the effects of IL-17A and IL-17C, the number of regulated gene expressions induced by these cytokines was much lower in monocytes than in keratinocytes. There was a relevant overlap between regulations induced by IL-17A and IL-17C in both cell populations. IL-17A-regulated genes in keratinocytes were involved e.g. in chemotaxis, keratinocyte differentiation, and signaling pathways that are more typical of neuronal processes. In monocytes, the IL-17A-regulated genes were involved in IL-1 processing. The strength of validated effects of IL-17C was significantly smaller than that of IL-17A.

Conclusion: This study revealed that IL-17A/F and IL-17C do not influence dermal endothelial cells, which implies that the positive effects on vascular inflammation observed as a result of anti-IL-17A therapy are more of an indirect nature. Furthermore, there is a relevant overlap in the effects of IL-17A/F and IL-17C, yet the majority of regulated genes expressions appears to be specific for the cytokines. Finally, of all cells examined, monocytes showed the strongest IL-17RA expression; our current work focuses on clarifying the effects of IL-17A/F and IL-17C on these cells and their significance.

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P055 | Preferential endogenous regulation of skin inflammation by IL-37 seems to be specific for psoriasis.

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Sharp demarcation between lesional and non-lesional skin is characteristic for psoriasis. Endogenous control mechanisms seem to regulate growth of the psoriatic plaque into healthy skin areas. In this study, we collected epidermal samples from lesional skin, as well as paralesional and nonlesional skin from patients not under systemic treatment for their skin condition. Samples were then analysed by mass spectrometry and ELISA to assess their protein profile.

Our findings confirmed a significant upregulation of inflammatory proteins in the lesional skin, including IL-36γ, CXCL1, S100 proteins and CCL20. The antiinflammatory cytokine IL-37, however, showed a unique pattern of expression that stood out from all others measured. IL-37 expression was low in lesional samples but increased significantly in paralesional skin compared to both lesional and nonlesional skin. Sequential tape-stripping of the perilesional skin adjacent to the distinct psoriasis lesion border with 2 subsequent samples taken at an increasing distance from the lesion shows IL-37 is highly expressed right at the lesion border and trends downwards as the distance from the lesion increases. Measurement of these cytokines in eczema lesional, paralesional and non-lesional skin as well as bullous pemphigoid and pemphigus vulgaris perilesional and non-lesional skin failed to show a similar pattern of expression.

Building upon these results, we propose that the sharp demarcation between psoriatic skin lesions may be a result of the highest anti-inflammatory activity of IL-37 present in paralesional skin, which "clashes" with the inflammatory pattern within the lesions. This insight highlights the potential for harnessing the anti-inflammatory properties of IL-37 to enhance control psoriatic inflammation. Further investigations are required to fully comprehend how this anti-inflammatory activity can be leveraged to manage the disease more effectively.

P056 | Atopic dermatitis and psoriasis associated cytokines influence HSV-1 infection of primary keratinocytes depending on their differential status.

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Herpes simplex virus (HSV-1) establishes primary infections in the epithelium after invasion of the skin or mucosal surfaces before becoming latent in neuronal ganglia. Apart from epithelial integrity and loss of barrier function the influence of inflammatory cytokines is discussed as important factor influencing infectability. Atopic dermatitis (AD) and psoriasis (Pso) two of the most common chronic inflammatory skin diseases show a complex pathophysiology characterized by impaired epidermal barrier and disregulated immune response. Especially in children with AD primary HSV-1 infection is frequently followed by disseminated HSV skin infections (termed eczema herpeticum). In this study, we asked whether Th-1 and Th-2 cytokines (typical for AD or Pso) as well as interferon-γ (IFN-γ) have impact on HSV-1 infection in normal human epidermal keratinocytes (NHEK). Furthermore, we were interested if differentiation of NHEK is relevant for HSV-1 infection.

NHEK were stimulated for 48hr with AD or Pso relevant cytokines (single cytokines or in combination) in a concentration range of 6.25ng/ml-100ng/ml. Differentiation was induced in parallel by increasing the calcium concentration in the culture medium. Consecutively, NHEK cultures were infected with HSV-1 (MOI 0.01) for 48hr. HSV-1 infection was monitored by immunohistochemical staining and quantified by correlating the number of infected cells to the total cell number of each individual culture.

In general we observed that differentiated NHEK showed a lower amount of infected cells in the respective virus controls compared to

the non-differentiated NHEK. Whereas IL-22 and IL-31 did not influence HSV-1 infection, IL-4 and IL-13 increased it significantly. A combination of IL-4 and IL-13 further increased the number of infected cells in comparison to the respective virus controls. Comparison of NHEK treated with an established AD cytokine mix (IL-4, IL-13, IL-31, TNF-α) or Pso cytokine mix (TNF-α, IL-22) lead in both to an increased infection. Of note, the AD-cytokine mix showed significantly higher infection than the Pso-cytokine mix independent of the differential status. Pre-treatment with IFN-γ reduced HSV-1 infection in a concentration dependent manner.

Our results suggest that especially IL-4 and IL-13 might be key players in HSV-1 infection in AD skin. Furthermore a regular differentiation of the epidermis might protect against HSV-1 infection.

P057 | Altered keratinocyte differentiation and increased chemokine and antimicrobial peptide expression in palmoplantar keratoderma due to keratin 9 variants

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The clinical phenotype of palmoplantar keratoderma (PPK), due to keratin (KRT) 9 variants, consists of debilitating diffuse hyperkeratosis on palms and soles. However, the hyperkeratoses also exhibit an erythematous periphery, indicating an accompanying hyperinflammatory response. We used single cell RNA (scRNA) sequencing of palmar skin biopsies combined with confirmatory PCR and immunofluorescence to characterize the repair response in PPK due to KRT9 mutations.

After scRNA sequencing, we first analyzed keratinocyte (KC) differentiation in KRT9mut PPK compared to normal control skin. Basal KRT5 and KRT14 were upregulated in KRT9mut differentiated KCs. Suprabasal KRT1 and KRT10 were downregulated in KRT9mut in clusters of differentiated KC. KRT2 was strongly downregulated in KRT9mut cells, and KRT6 and KRT16 were strongly upregulated in KRT9mut skin. Interestingly, CALML5, encoding a calcium-binding protein, was downregulated in KC of KRT9mut palmar skin biopsies. Comparing chemokine expression, CCL2 and CXCL12 were increased in KRT9mut PPK in multiple KC clusters, as compared to normal controls. In contrast, CXCL1 and CCL19 were selectively decreased in fibroblasts, whereas CCR7 was decreased in KRT9mut dendritic cells as compared to control. Finally, dermcidin was upregulated in KRT9mut sweat glands and DEFB1, S100A8 and S100A9 were additionally increased in keratinocytes in KRT9mut palmar skin biopsies.

Based on these data, we hypothesize that altered keratinocyte differentiation in KRT9mut skin initiates a previously unrecognized hyperinflammatory response in KRT9mut PPK. As KRT9 mutations put skin barrier function at risk, epidermal keratinocytes (KC) respond with an exaggerated, futile repair response, resulting in chronic inflammation.

Clinical Research

P058 | Oil-in-water emulsions containing a combination of Zingiber officinale root extract and cannabidiol with potent in vitro anti-inflammatory effects ameliorate symptoms of dry and eczema-prone face and hand skin in a clinical trial

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In total, 24 plant extracts of different polarities and phytochemicals were screened for potential anti-inflammatory and -oxidative activities in primary cells and cell lines. Effects were assessed and applied to a principal component analysis. Cannabidiol (CBD) and a Zingiber officinale root extract were found to be extraordinarily active and subsequently tested alone and in combination in relevant assays for skin inflammation.

In three different in vitro assays (TNF-α-stimulated NF-κB activation, poly(I:C)- stimulated cytokine and chemokine secretion and transcription of cytokines and -receptors as well as chemokines stimulated by a mixture of TNF-α, IL-4, IL-13, IL-22), the anti-inflammatory activities of Zingiber officinale root extract and CBD were confirmed. Notably, all three assays revealed that a combination of both enhanced the anti-inflammatory effects.

The analysis of endocannabinoids suggested a positive modulation of the endocannabinoid system exhibiting relevant effects regarding inflammation and pruritus.

Therefore, two oil-in-water (O/W) emulsions containing CBD and the Zingiber officinale root extract were developed and investigated in a clinical study including 65 subjects, of whom 32 used the face cream (BNO 3733) and 33 the hand cream (BNO 3734) at least two times daily. Skin condition was assessed objectively by a dermatologist as well as by the subjects themselves at Day 1 and Day 15 using a symptom severity score from 0 to 3 (0: none; 0.5: very slight; 1: slight; 2: moderate; 3: strong). For BNO 3733 a statistically significant reduction of dryness (from 0.9 ±0.1 to 0.4 ±0.1; $p<0.001$), itching (from 0.5 ±0.1 to 0.1 ±0.1; $p=0.002$), tension (from 1.2 ±0.2 to 0.7 ±0.2; $p=0.008$) and feeling of dryness (from 1.5 ±0.2 to 1.0 ±0.2; $p=0.010$) was observed. Similarly, BNO 3734 application led to a statistically significant reduction of dryness (from 1.2 ±0.1 to 0.6 ±0.1; $p<0.001$), itching (from 0.7 ±0.2 to 0.2 ±0.1; $p=0.007$), tension (1.0 ±0.1 to 0.4 ±0.1; $p=0.001$) and feeling of dryness (from 1.8 ±0.2 to 0.5 ±0.2; $p<0.001$). Both products were very well tolerated.

In summary, the identification of a combination of CBD and Zingiber officinale root extract with proven antioxidative and anti-inflammatory activities enabled the development of two new O/W

emulsions, which improved key symptoms of dry and eczema-prone skin clinically after application on hands and face.

P059 | Therapeutic effects and impact of individual skin care regimes as basic therapy in the management of atopic dermatitis: a remote prospective observational study in adults and children suffering from AD

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Background: Atopic dermatitis (AD) is one of the most prevalent chronic skin diseases and characterized by flares of dry, inflamed and itchy skin. In order to reduce the need for pharmacological intervention with immunosuppressants such as corticosteroids, the regular basic therapy with topical skin care products including emollients represents a cornerstone in AD management. While prescribed pharmacological therapies typically involve defined dosage regimes, basic skin care is subject to individual factors such as patients' education, usage habits and product preferences making its therapeutic success challenging to quantify.
Objectives: The aim of this remote 3-months prospective observational study was to assess the real world effectiveness of individual common skin care regimes as basic therapy in AD. Therapeutic effects on skin condition, itch sensation and eczema control were assessed in subjects suffering from AD symptoms. Moreover, the occurrence of flare-ups, as well as the frequency of flare-up related corticosteroid use and doctor's visits were monitored.
Methods: Subjects were asked to apply their respective individual skin care regimes and provide data regarding their skin condition in an online survey monthly over 3 months. Symptom severity was evaluated by subjective dermatological assessments including itch intensity, rated by NRS-11 (numerical rating scale with score from 0-10, 0 = no itch, 10 = worst imaginable itch). Eczema control was captured by the recap of atopic dermatitis (RECAP) questionnaire. The occurrence of flare-ups, as well as the frequency of flare-up related corticosteroid use and doctor's visits, respectively, were documented in twice-weekly online diary reports.
Results: The study 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). During the 3-months study period itch sensation decreased by 0.9 points (17%) on the NRS-11 rating scale with 40% of subjects experiencing a minimal clinically important improvement of at least 2 points. Experience of eczema control measured by the RECAP mean sum score improved by 2.4 points (20%). Flare-ups occurred in nearly the entire study population with a mean of 5 flare-ups per subject documented in the online diary during the study period. More than 70% of subjects required the use of corticosteroids and over 25% visited a doctor at least once due to a flare-up.
Conclusion: Individual common skin care regimes may provide significant and appreciable positive effects on skin condition, itch sensation and eczema control in AD management. Nonetheless, the frequently observed occurrence of flareups and flare-up related corticosteroid use and doctor's visits, respectively, clearly demonstrated an insufficient real world effectiveness of the individual basic therapy applied by AD patients. The results of this prospective observational study revealed substantial room for improvement, which can be addressed by intensified patient training regarding flare-up prevention, appropriate frequency of emollient use and careful selection of suitable high-quality products.

P060 | Reduced killing-capacity of NK cells in atopic dermatitis

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Natural killer (NK) cells play an important role in early responses to infections and tumors. In many autoimmune disorders the function of NK cells is reduced or even turned down. Atopic dermatitis (AD) is a chronic inflammatory skin disease associated with allergic inflammation. Current treatment strategies in AD have focused on either broad or selective immunomodulation to combat pathologic type 2 inflammation. Studies over the past two decades have identified various abnormalities in NK cell populations in patients with AD. However, the nature of these defects and the reason for reduced activity of NK cells in AD are is elusive. Here, we analyzed blood NK cells (CD56dim) from AD patients and healthy controls for their ability to induce apoptosis of effector cells. NK from AD patients revealed a reduced expression of the proapoptotic molecule Fas-ligand. Furthermore, we found in AD skin high expression of HLA-G which ligates the inhibitory NK receptors ILT2 and ILT4 which were significantly upregulated on peripheral and cutaneous NK cells of AD patients. HLA-G can be induced by IL-10 which we found significantly upregulated in circulating NK cells of AD patients. Recently it was published that NaCl concentration in AD skin is extremely high. We found that stimulation with NaCl reduced significantly the apoptotic potency of blood NK cells and additionally induced HLA-G expression. It is conceivable that in such an environment (high NaCl, high HLA-G) NK cells are inhibited and cannot pursue their role in cutaneous defense. Future studies will address by which means this deleterious environment can be changed.

P061 | Effects of tirbanibulin on keratinocytes and squamous cell carcinoma cell lines in vitro

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For the treatment of actinic keratoses (AK) topical treatment with tirbanibulin 1% is approved since 2021. Within the approval study a single treatment cycle with application on 5 consecutive days lead to complete clearance in approximately 50% of patients. Two modes of action of tirbanibulin are already described: (i) inhibition of the oncogenic SRC-kinase and (ii) interference with tubulin-polymerization.

The aim of this study was to further characterize the mechanism of action of tirbanibulin in keratinocytic cells.

We analyzed effects of tirbanibulin in a keratinocyte (NHEK) and two differently dedifferentiated cutaneous squamous cell carcinoma (cSCC) cell lines (A431, SCC-12) in vitro. Concerning proliferation (MTT-assay), in all cell lines a dosedependent reduction was detected following tirbanibulin treatment. A tirbanibulindependent inhibition of migration (scratch-assay) and G2-cell cycle arrest (7AAD) was only present in cSCC cell lines. In addition, apoptosis (7AAD/Annexin V) was induced by tirbanibulin in the A431 cell line. In contrast to the published data in breast cancer cell lines, we could not detect a reduced phosphorylation of SRC or other proteins within the same signaling pathway (FAK, ERK, AKT, STAT3) via western blot. Therefore, we performed a phosphokinase-array (NHEK, A431). Here, tirbanibulin reduced activity in SRC and STAT3 in NHEK, as well as a slightly increased SRC phosphorylation in the A431 cell line. The most prominent effect of tirbanibulin, however, was seen in our analyses concerning beta-tubulinpolymerization (microtubules / tubulin in vivo assay kit). Polymerization of betatubulin to microtubules was disrupted the most pronounced in A431 cells and could also be visualized using immunocytochemistry.

In conclusion, we could show that tirbanibulin inhibits proliferation and migration in keratinocytic cell lines, as well as promoting apoptosis and G2-cell cycle arrest. The effects were predominantly detectable in the A431 cell line; healthy keratinocytes (NHEK) as well as more dedifferentiated cSCC cells (SCC-12) were less affected. Whilst we did not detect involvement of SRC-kinases in the examined cells, the antitumorous effect of tirbanibulin in cSCC cells is most likely mediated via dysregulation of beta-tubulin polymerization.

P062 | Across the Skin Depths: Increased Absolute Levels of Bacteria within Atopic Dermatitis and Psoriasis, but not Chronic Spontaneous Urticaria

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Introduction: Atopic Dermatitis (AD), Psoriasis, and Chronic Spontaneous Urticaria (CSU) are inflammatory skin diseases with different suspected pathogenesis. AE and Psoriasis have been previously associated with microbiome changes, while little is known about CSU. Furthermore, the microbiome changes across the different skin layers (epidermis and dermis) for these three skin diseases are under-examined.

Method: To investigate the skin's microbial load across its layers, tape strips (TS) 2 and 20 were used to sample 64 adults (28 healthy participants, 14 AD, 5 Psoriasis, and 17 CSU patients). For a subgroup of 6 AD and 7 HE participants, TS40, TS60, and TS80 were used to observe changes past the stratum corneum. Lesional and non-lesional samples were collected from AE and psoriasis participants. Absolute quantification of bacteria was measured by qPCR of the 16S rRNA gene.

Results: The bacterial load decreased with increasing depth of the tape strip, regardless of the skin's health status. Compared to healthy samples, at the skin's surface (TS2), the bacterial load was higher among AD and psoriasis lesions but not CSU samples. Only within the stratum corneum (TS20) did AD and psoriasis nonlesional skin have a significantly higher bacterial load than healthy samples. Absolute bacterial abundance correlates to skin pH only in healthy individuals.

Conclusion: By absolute quantification, we could show that high skin pH and diseased skin (AD, psoriasis) are associated with higher bacterial burden. Thus, absolute quantification provides crucial supplementary information for nextgeneration sequencing.

P063 | Skin Microbiome dysbiosis associated with inflammation and body malodour are hallmarks of Darier's disease

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Darier disease (DD), is a rare autosomal dominant genodermatosis with a prevalence range between 1/30.000 and 1/100.000. It is characterised by a mutation of the ATPA2 gene leading to disrupted intracellular Ca2+ signalling, a loss of keratinocyte adhesion (acantholysis) and apoptosis (anoikosis). Recurrent episodes of skin infection and inflammation with a characteristic foul odour in DD indicate a role for cutaneous dysbiosis. Nevertheless, DD microbiome has not been investigated so far and possible connections between dysbiosis and inflammation are not yet established. In this study we investigated for the first time the cutaneous dysbiosis and its consequences in DD. We collected 115 skin swabs from 14 patients and healthy matched volunteers and analysed them using a 16S metabarcoding approach. We also assessed microbiome changes in the context of DD malodour, and explored inflammation and dysbiosis signatures in patients' skin transcriptomes. We identified a disease-specific cutaneous microbiome characterised by a loss of microbial diversity and decreased abundances of beneficial commensals. Inflammation associated microbes, notably S. aureus and S. warneri dominated the DD landscape and showed strong correlations with disease severity scores. DD dysbiosis was further characterized by an expansion of species belonging to Corynebacteria, Staphylococci and Streptococci groups displaying strong associations with malodour intensity. Transcriptome analyses revealed an upregulation of epidermal-repair, inflammatory and immune defence pathways reflecting epithelial and immune mechanisms to DD dysbiosis. In contrast, the downregulation of key barrier genes including claudin-4 and cadherin-4 indicate a skin barrier impairment. These findings highlight the role of cutaneous dysbiosis in DD inflammation and associated malodour and also suggest potential biomarkers and intervention targets for DD.

P064 | Transcriptome analysis defines combinatory pathway activity in keratinocytes necessary for split formation in pemphigus vulgaris

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Pemphigus vulgaris (PV) belongs to the group of autoimmune blistering diseases of the skin and/or mucous membranes. After the loss of tolerance, autoantibodies are produced, which predominantly target either desmoglein 3 (Dsg3) or Dsg1/3. The epidermal, hair follicle, and mucosal keratinocytes express these molecules. Autoantibodies targeting these proteins disrupt cell-cell-adhesion, leading to blister formation in the skin and mucous membranes. PV pathogenesis is not known in detail yet, so we here unravel want to unravel the early onset of split formation, focusing on the first steps occurring right after autoantibody binding.

Here, we study the transcriptome and proteome changes occurring after autoantibody binding and split formation under single-chain variable fragment (scFv) PX43 (directed against Dsg1/3) stimulation in a human skin organ culture (HSOC) model. Keratinocytes respond within 24 hours with activation of NFκB, JAK-STAT, EGFR/MAPK, and TGFβ, as well as Interferon α/β signaling on the transcriptome and proteome level. Repression of split formation through targeted inhibition of either p38 MAPK, MEK, PIK3, VEGFR, or the NGF receptor TRKA resulted in inhibitor-specific pathway (de-)activation. While NFκB and JAK-STAT gene expression remained unaffected with all inhibitors as a general stress response, secondary to split formation, inhibition of p38 MAPK. TRKA or VEGFR inhibition activated upstream WNT signaling, and MEK inhibition downregulated the upstream EGFR/MAPK pathways. PIK3 signaling was negatively affected through PI3K, p38 MAPK, and VEGFR inhibition. The results hint at different pathways and pathway combinations necessary for PV cell detachment. The results will help to define the clinically relevant transcellular tissue communication code driving tissue remodeling during injury and shedding light on the pre-disease state of PV.

P065 | C5aR expression is increased in the skin of patients with chronic spontaneous urticaria

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Background: Chronic spontaneous urticaria (CSU) is defined as the recurrent appearance of itchy hives (wheals) and/or angioedema for more than 6 weeks. Histamine release upon skin mast cell (MC) activation and degranulation is key in the pathogenesis of CSU. The exact mechanism by which MCs are activated in the skin of CSU patients remains unclear. IgG autoantibodies to IgE and to FcεRI have been shown to activate complement with subsequent C5a production. C5a activates MC via C5a receptor (C5aR) leading to the amplification of MC activation and the development of CSU signs and symptoms.
Methods: We characterized C5aR expression by immunohistochemistry in the skin of CSU patients and assessed blood levels of C5, C5a and sC5b-9 by ELISA in patients compared to healthy controls. For this, fifteen CSU patients (median age 41 [range 21-72]; 61 % female) with poor disease control, i.e. average urticaria control test scores of 5.5 of 16 points, were investigated. Ten healthy individuals (median age 41 [range 21-64]; 50 % female) served as controls.
Results: C5aR expression was significantly increased in skin lesions of CSU patients compared to healthy individuals. Very few C5aR+ cells were observed in healthy individuals. Furthermore, C5aR expression in the lesional skin of CSU patients was found in both tryptase+ and tryptase- cells. However, C5a, C5, and C5b-9 levels in peripheral blood were not significantly different in CSU and healthy controls.
Conclusions: Increased C5aR expression in the skin of CSU patients may indicate the involvement of complement activation in the pathogenesis of CSU. C5a levels in peripheral blood may not represent their concentration in the skin. Our results highlight the potential role of anti-C5 and anti-C5aR therapies as novel treatment for CSU patients.

P066 | Clinical Profiling and Prognostic Factors of Mucosal Melanoma: Insights from a Comprehensive Cohort Study

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Mucosal melanomas, a distinctive and infrequent subset of melanoma, present unique challenges in clinical management due to their generally poor and highly variable prognosis, which is influenced by the primary tumor's specific anatomical location. In contrast to the wide landscape of well established systemic therapies for advanced cutaneous melanoma, there is little data available for the mucosal variant of this disease. Furthermore, risk factors and pathogenesis of the disease are poorly understood.
Here, we collected a cohort of 48 mucosal melanoma patients treated at at the University Skin Cancer Center Hamburg from 2008 up to today. We collected a wide array of clinical data including all mutational data, therapies, imaging and stagings, radiation treatment, blood analysis, surgeries, thromboembolic events among others. We aimed to characterize the subtypes of mucosal melanoma and identify potential risk factors and protective factors associated with this rare malignancy.
Our findings reveal that anal mucosal melanoma exhibits a significantly worse prognosis when compared to other locations such as aerodigestive or genital melanoma. Furthermore, patients receiving combined immunotherapy (Anti-PD-1 + Anti-CTLA-4) showed improved outcomes compared to other treatments. Analysis on the effect of radiation, local treatments, thromboembolic events and other clinical risk factors is still in progress, with anticipated completion within the next two months.

P067 (OP04/04) | High serum levels and dynamics of Protein Y are associated with impaired overall survival and recurrence in melanoma patients receiving immunotherapy

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Background: Immunotherapy has revolutionized melanoma therapy and significantly improved prognosis in both fully resectable as well as advanced melanoma. However, not all patients benefit from the therapeutic advances and experience primary resistance or develop secondary resistance. Additionally, some patients experience severe side effects (grade 3-4 CTCAE) or irreversible side effects. Biomarkers are therefore urgently required to predict response to therapy prior to initiation and to monitor disease progression.
Methods: In a proteomic analysis of plasma samples from an advanced melanoma patient discovery cohort (n=40) we identified Protein Y (protein is called at ADF) as a suitable biomarker for primary resistance under immunotherapy. For validation, Protein Y concentrations in serum of 261 melanoma patients were monitored by ELISA during therapy (baseline, 3 months, 6 months, 12 months after therapy initiation, respectively) and correlated with clinicopathological parameters and disease related outcomes.

Results: An increased Protein Y concentration (>0.23 pg/mL) at baseline was associated with a significantly impaired overall survival (38.23 months (95% CI: 33.42-43.04) vs. 51.36 months (95% CI: 47.00-55.71) (p=0.009) and could be identified as an independent negative prognostic factor for overall survival (Hazard Ratio: 2.15, p=0.006) in multivariate cox-regression. Moreover, a decrease in protein Y concentrations after 3 months during treatment was associated with improved progression-free / relapse-free survival (30.61 months (95% CI: 23.99-37.22 months) vs. 24.90 (95% CI: 20.16-29.63 months) with no change and 21.54 (95% CI: 16.77-26.31 months) with increase) (p=0.045).

Conclusion: Protein Y may represents a novel biomarker for the prediction of prognosis in melanoma under immunotherapy. Subsequent validation of the promising results using an independent multi-center cohort is pending and further prospective randomized trials are needed to establish protein Y as a biomarker in melanoma.

Dermato-Endocrinology

P068 | Melatonin exerts synergistic responses in targeted therapy for 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, unwanted side effects are a problem. Melatonin, a well-known endogenous synchronizer of the circadian biorhythm has a variety of promising effects for melanoma biology. It regulates proliferation, apoptosis and oxidative phosphorylation via melatonin-receptors, and receptor-independent pathways due to its lipophilicity. Herein, by using human melanoma cell lines in vitro, we show that melatonin enhances anti-tumor effects of commonly used BRAF/MEK inhibitors, i.e. vemurafenib (VF) and cobimetinib (CB), respectively. Our results demonstrate that compared to VF/CB alone, melatonin significantly reduced proliferation read-outs (colony, drop, scratch or migration assay) and induced of apoptosis (cl. Casp-9, -3, PARP) in melanoma cells. Concurrently, VF/CB+melatonin decreased melanoma invasiveness-related protein (E-cadherin), inducible nitric oxide synthase (iNOS), epithelial cell adhesion molecule (EpCAM), and proliferating cell nuclear antigen (PCNA) which are important players in melanoma tumorgenesis, tumor growth, invasion and metastasis. In addition, we also show that combined treatment with the above inhibitors and melatonin results in significant mechanistic changes in cellular bioenergetic by (i) uncoupling of oxidative phosphorylation (OXPHOS), (ii) attenuation of glycolysis (Seahorse assessment), (iii) dissipation of mitochondrial transmembrane potential (mtΔΨ) (FACS), (iv) changes in mitochondrial morphology (TEM) and (v) supported by a xenograft model in vivo using zebrafish embryos. These findings extend previously published data and provide new perspectives for the introduction of melatonin as an add-on therapy in future treatment of melanomaaffected patients.

P069 | Endothelial cells - new targets for the anifibrotic action of the alpha7 nicotinic acetylcholine receptor?

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Systemic sclerosis is an autoimmune chronic disease characterized by early occurring vascular dysfunction followed by fibrosis in skin, joints and internal organs. Endothelial to mesenchymal transition (EMT) describes the transformation of functional endothelial cells to collagen producing myofibroblasts and is considered to play a crucial role in the pathogenesis of SSc.

Previously, we reported that activation of the alpha7 nicotinic acetylcholine receptor (alpha7nAChR) has a beneficial effect on fibrosis in human dermal fibroblasts in vitro and more importantly in various models of erxperimentally induced skin fibrosis.

Here, we investigated the effect of the α7nAChR on EMT in human dermal microvascular endothelial cells. First, we examined the expression of the alpha7nAChR in primary human dermal microvascular endothelial cells (HDMEC) at RNA level using semi-quantitative RT-PCR. We also confirmed the presence of this receptor in the endothelial cell line HMEC-1, a tool cell line for EMT research due to a high expression of the endothelial cell marker CD31 as shown by FACS analysis. At protein level we detected expression of this receptor via Western immunoblotting in HDMEC as well as HMEC-1. Immunofluorescence analysis with an alpha7nAChR antibody disclosed cell membrane-associated immunoreactivity of this receptor in both cell types. We further demonstrated functionality of the alpha7nAChR in HDMEC by measurements of calcium influx using alpha7nAChR agonists and the antagonist alpha-bungarotoxin. Finally, first functional experiments revealed an induction of EMT in HDMEC after stimulation with transforming growth factor (TGF)-beta1. Accordingly, downregulation of the endothelial cell markers CD31, van Willebrand factor and VE-cadherin and upregulation of the mesenchymal marker collagen type I after TGF-beta1 treatment was detected in HDMEC at RNA level as shown by quantitative PCR. Moreover, in HMEC-1 TGF-beta1 also led to a slight suppression of CD31 expression at protein level as shown by FACS analysis. Whether activation of the alpha7nAChR can modulate these effects will be answered by our ongoing investigations. Intensified future

research on the dermal endothelial cell system is currently being conducted to precisely dissect the role of the alpha7nAChR in EMT.

P070 | Meta-Analysis of European Clinical Trials Characterizing the Healthy-Adult Serum 25-hydroxyvitamin D Response to Vitamin D Supplementation

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Because 80-90% of the human bodies requirements in vitamin D have to be fulfilled by the UV-B-induced cutaneous synthesis of this prohormone, the vitamin D status of individual populations is of mayor concern for skin cancer prevention campaigns. To obtain reliable data that allow health authorities to re-evaluate recommendations for oral vitamin D uptake, we conducted a meta-analysis to investigate the impact of supplementation on serum 25-hydroxyvitamin D (25(OH)D) levels in healthy adults in Europe. Of publications identified (n = 4005) in our literature search (PUBMED, through 2 January 2022), 49 primary studies (7320 subjects, 73 study arms) were eligible for inclusion in our meta-analysis. The risk of bias was assessed using the Cochrane RoB tool based on seven categories, according to which each study is rated using three grades, and overall was rated as rather low. The median duration of intervention was 136.78 days (range, 1088 days); the mean weighted baseline 25(OH)D concentration and mean age were 33.01 vs. 33.84 nmol/L and 46.8 vs. 44.8 years in the vitamin D and placebo groups, respectively. Using random-effects models, 25(OH)D levels were increased by 36.28 nmol/L (95% CI 31.97-40.59) in the vitamin D group compared to the placebo, with a relative serum increment of 1.77 nmol/L per 2.5 μg of vitamin D daily. Notably, the relative serum 25(OH)D increment was affected by various factors, including the dosage and baseline serum 25(OH)D concentration, decreasing with increasing vitamin D doses and with increasing baseline serum levels. We estimate that supplementation in all healthy adults in Europe with appr. 25 μg of vitamin D (1000 IU) daily would raise serum 25(OH)D levels in 95% of the population to ≥50 nmol/L. Our work provides health authorities with reliable data that can help to re-evaluate recommendations for oral vitamin D supplementation.

P071 | Differential regulation of circadian clock genes by UVB radiation and 1,25- dihydroxyvitamin D in human epidermal keratinocytes: a pilot study during different stages of skin photocarcinogenesis

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Increasing evidence points at an important physiological role of a time-keeping system, known as the circadian clock (CC), not only for our sleep- awake rhythm but also for many cellular processes in peripheral tissues, including carcinogenesis. UV-radiation regulates expression of CCGs in many cell types, and it has recently been shown that CCGs modulate susceptibility for UV-B-induced cellular damage. It was the aim of this study to investigate the circadian clock in a cell culture model representing different stages of skin photocarcinogenesis. We compared cultured normal (Normal Human Epidermal Keratinocytes - NHEK; p53 wild type), precancerous (HaCaT keratinocytes; mutated p53 status) and malignant (Squamous Cell Carcinoma SCL-1; p53 null status) keratinocytes. First, following treatment with 1,25(OH)2D3 and UV-B (alone or in combination), expression of two core clock genes (brain and muscle ARNT-like 1 (BMAL1) and Period-2 (Per2)) was measured over several time points (0-60 h) in HaCaT keratinocytes. Moreover, expression of these two CCGs differed significantly comparing NHEK, HaCaT and SCL-1. In summary, we demonstrate a circadian rhythm in expression of these two major CCGs in human skin cells (HaCaT), that was modulated following treatment with UV-B. Expression of BMAL1 and Per2 was increased in UV-B-treated as compared with untreated HaCaT cells ($p<0,001$). In contrast, treatment with 1,25(OH)2D3 alone resulted only in marginal modulation of expression of CCGs in HaCaT cells (no visual effect in BMAL1, trend for a reduction of overall Per2 expression in nonradiated HaCaT), and co-treatment with 1,25(OH)2D3 had only marginal effects on UV-B-induced modulation of CCG expression,. UV-B treatment was associated with reduced cellular viability (24h after treatment, $p=0,024$). Our findings are only in part in line with the hypothesisthat the UV-B-induced upregulation of these CCGs may be mediated via UV-B-induced synthesis of vitamin D. In Conclusion, we provide further evidence for an independently operating timekeeping system in human skin cells, that is physiologically regulated by UV-B. The disruption of this mechanism in malignant keratinocytes points to a contribution of CCGs for skin photocarcinogenesis.

P072 | Dermal white adipose tissue induces interleukin 4/13 expression in myeloid cells during inflammation in an IL-33 dependent manner

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White adipose tissue (WAT) has long been known as a vital reservoir of energy in the body. However, recent research has revealed a diverse role for WAT that goes beyond energy storage. In particular, dermal white adipose tissue (dWAT), a fat depot in its own right, has been shown to play a critical role in several physiological processes. Dermal WAT has unique characteristics, including a specialised gene expression profile, strong local expansion in response to injury and infection, and a role in tissue repair. The strategic location of this tissue allows it to interact with different skin cells and influence skin homeostasis, immune responses and inflammation.

This study examines the intricate relationship between dWAT and myeloid cells, in particular neutrophils, monocytes and macrophages, which are central components of the innate immune system. Neutrophils actively scan the body for signs of microbial infection and migrate to sites of inflammation, while monocytes and macrophages play a critical role in both local and systemic inflammation. This work investigates how soluble mediators from dWAT influence the activation of myeloid cells under steady-state conditions and during inflammation. In addition, the possible effect of obesity on the interaction between dWAT and myeloid cells will be investigated, as inflammation is dysregulated in obese individuals.

The results presented here shed light on the critical role of dWAT in immune response, inflammation control and tissue repair, and highlight its importance in maintaining skin homeostasis and overall health. Understanding the dynamics of interactions between adipocytes and myeloid cells in dWAT may have implications for the development of new therapeutic approaches to modulate immune responses and combat inflammation-related diseases.

P073 | The association between Vitamin D Status and Melanoma Risk and Prognosis: Meta-Analyses and Systematic Review

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Introduction: Solar UV radiation represents the most important environmental risk factor for skin cancer. On the other hand, UV-B-induced cutaneous vitamin D synthesis has been reported to exert anti-carcinogenic (anti-proliferative, antiangiogenic and pro-apoptotic) effects on melanocytes and keratinocytes in vitro. This anti-tumor effect has been reported to be mediated not only by the vitamin D receptor (VDR), that has been described as a tumor suppressor in skin, but also by other nuclear receptors, including Peroxisome-Proliferator-activated Receptor (PPAR). As Melanoma is the deadliest skin cancer and its incidence is on the rise, identifying potential risk and prognostic factors is of utmost importance.

Objective: It was the aim of this study to assesss the relevance of the Vitamin D status for melanoma risk and prognosis.

Materials & Methods: A systematic review and meta-analyses were conducted according to PRISMA guidelines, using Databases Medline (via PubMed) and ISI (Web of Science), until December 31st, 2022. Study quality and risk of bias were evaluated by applying the "Newcastle Ottawa scale" and level of evidence was assessed based on the recommendations of the "Oxford Center for Evidence-based Medicine". The standardized mean differences (SMD) and odds ratios (OR) with 95% confidence intervals (95% CI) were derived from random-effects meta-analyses to account for possible heterogeneity across studies. Furthermore, moderator analyses were used to investigate systematic differences in the effect sizes and subgroup analyses were performed. 9 different meta-analyses were carried out, assessing association (OR and SMD) of vitamin D status (25(OH) D serum concentration) with melanoma risk and various prognostic factors (Breslow thickness, mitotic rate, tumor stage and ulceration status).

Results: 26 studies were eligible for inclusion in this investigation. Significantly lower mean serum 25(OH)D levels in ng/ml were found comparing patients with melanoma and healthy controls (SMD: -0.40 [-0.74; -0.06]). There was a not significant trend for an increased melanoma risk in patients with Vitamin D deficiency (OR: 1.79 [0.95; 3.37]), comparing study participants with ≤20 vs. >20 ng/ml 25(OH) D serum levels. Due to significant heterogeneity across studies and no indicative funnel plot and Egger´s test, subgroup analyses were carried out. Interestingly, restricting the geographic region to Southern European studies resulted in significant results (SMD: -1.02 [-1.48; -0.56]; OR: 5.08 [1.80; 14.33]) comparing melanoma risk in study participants with ≤20 vs. >20 ng/ml 25(OH)D serum levels. Funnel Plots and Egger´s tests did not indicate any publication bias. In terms of prognosis, low serum 25(OH)D serum levels in ng/ml were associated in melanoma patients with higher Breslow thickness (SMD: -0.14 [-0.22; -0.07, comparing >1mm vs. ≤1mm]). Low mean 25(OH)D serum levels were significantly associated with presence of mitoses (SMD: -0.30 [-0.57; -0.02, comparing mitoses present vs. absent] and with ulcerated primary tumors (SMD: -0.20 [-0.30; -0.11, comparing ulceration present vs. absent]), and were not significantly associated with higher tumor stage (SMD: -0.33 [-0.69; 0.03, comparing highest vs. lowest tumor stage]). In agreement with these findings, we observed significantly increased risks for thicker tumors (OR: 1.85 [1,23; 2,8]), for mitotic tumors (OR: 2.02 [1.21; 3.36]), and for higher tumor stage (OR: 1.54 [1.01; 2.38]), in patients with Vitamin D deficiency (comparing patients with ≤20 vs. >20 ng/ ml 25(OH)D serum levels). Among the studies analyzing melanoma prognosis, the heterogeneity tests and tests for Funnel plot asymmetry were not significant, except for heterogeneity tests for the analyses on tumor stage and mitotic status and mean serum levels.

Conclusion: In these meta-analyses, we show an association of vitamin D status with melanoma risk and prognosis. This study adds to the constantly growing body of evidence revealing a tumor-protective role of vitamin D. These findings need to be considered when developing recommendations for skin cancer prevention.

P074 | Is the alpha-MSH-MC1R axis relevant for cutaneous photoaging?

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Alpha-melanocyte-stimulating hormone (alpha-MSH) plays a key role in the tanning response of the skin. Moreover, alpha-MSH directly antagonizes genotoxic stress in both melanocytes and keratinocytes after ultraviolet (UV)A irradiation by modulating the DNA damage response and apoptosis. This biological effect of alpha-MSH is mediated by a functional melancortin-1 receptor (MC1R) that induces canonical cAMP signalling. In individuals with the red hair pale skin phenotype loss of function (LOF) mutations of MC1R result in impaired alpha-MSH-MC1R-cAMP signalling in melanocytes which explain their increased risk for melanoma. Interestingly, epidemiologic studies suggest a link between alpha-MSH-MC1R signaling and dermal photoaging, the latter mainly mediated by UVA. To address the hypothesis as to whether alpha-MSH may modulate UVA-mediated effects and thus potentially may reduce dermal photoaging we used human adult dermal fibroblasts (aHDFs) as an initial in vitro tool. All primary cell lines were screened for MC1R single nucleotide polymorphisms (SNPs) including p.V60L, p.D84E, p.V92M, p.R142H, p.R151C, p.I155T, p.R160W, p.R163Q, p.T314T and p.F196L using a TaqMan SNP genotyping assay. Only aHDFs with a wild-type (wt) MC1R were used for subsequent studies. Compared with the antioxidant vitamin C pretreatment (or even cotreatment) of aHDF (n>8 donors) alpha-MSH did not reduce UVA1-induced accumulation of intracellular oxidative stress as measured by FACS anaylsis. Moroever, the alpha- MSH-related tripeptide derivative KdPT - not acting via MC1R - and beta-endorphin - a neuropeptide suppressing cAMP signaling - likewise did not reduce UVA1- induced oxidative stress. Interestingly, forskolin, an artificial cAMP inducer, failed to reduce UVA1-induced oxidative stress in these cells. Of note, MSH had some time dependent effects on the mRNA expression of p21, MMP1/3 and sirtuin 1 but no significant downregulation of these parameters. Our findings provide a first base for further studies elucidating the impact of MC1R but also the role of the cAMP signalling in fibroblast biology and dermal photoaging.

P075 | Does a functional, peripheral equivalent of hypothalamic-pituitary-thyroid axis exist in human hair follicles?

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The hypothalamic-pituitary-thyroid (HPT) axis is originally responsible for preserving the circulation and the production of thyrotropin-releasing hormone (TRH), thyrotropin (TSH), and thyroid hormones (TH) in the human body. Since key HPT axis players have been identified and are functional in human skin and/or hair follicles (HFs), we have asked if organ-cultured human scalp HFs that are isolated from the central, endocrine and neural inputs, exhibit a functional HPT axis. Immunofluorescence microscopy and in situ hybridization confirmed the presence of all HPT axis components (TRH, TSH, their respective receptors, and TH receptor beta (THRβ)) in the outer root sheath keratinocytes of freshly frozen anagen scalp HFs. Next, scalp HFs were treated with TRH (3, 30nM) or TSH (1, 10mIU/mL) for 7 days. Quantitative immunohistomorphometry showed that 3nM TRH upregulated TSH expression. Unexpectedly, TSH upregulated also both TRH and TRHR protein expression, suggesting a positive feedback loop (in striking contrast to its recognized negative feedback on hypothalamic TRH expression). While TRH also stimulated intrafollicular TRHR and TSHR protein expression ex vivo, TSHR expression was unaffected by TSH treatment. Just as in the central HPT, THRβ protein expression was upregulated by both TRH and TSH. Altogether, these preliminary data confirm the presence of a functional peripheral HPT axis in HFs and the role of human scalp HFs as peripheral neuroendocrine organs is currently characterized by TRHR/TSHR silencing analysis ex vivo.

P076 | Topical T3 and T4 promote hair follicle growth in intact human scalp skin ex vivo

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We have previously shown that the thyroid hormones triiodothyronine (T3) and thyroxine (T4) prolong anagen, mitigate stem cell apoptosis, and stimulate mitochondrial functions in microdissected human scalp HFs ex vivo. To circumvent the systemic adverse

effects of T3/T4, we have asked in the current pilot study whether topically applied T3/T4 retains hair growth-promoting properties. To probe this, we have topically treated healthy full-thickness human scalp skin with T3 (1, 10nM) and T4 (1, 10μM) for 6 days in serum-free organ culture, using an HFtargeting vehicle that contains only FDA-approved ingredients. This showed that, at distinct doses, topical T3 and/or T4 significantly increased the percentage of HFs in anagen and/or the percentage of proliferative (Ki-67+) cells in the hair matrix, enhanced gp100 expression in the pigmentary unit of anagen HFs, yet without promoting melanogenesis (as measured by quantitative Warthin-Starry histochemistry), and significantly increased keratin 15 expression in the bulge. Finally, T3 and T4, at high concentrations, increase the expression of the hair growth promoter FGF-7. Of note, contrary to what showed previously, T3 and T4 at both concentrations, decrease the expression of the subunits of respiratory complex IV, MTCO1, indicating a downregulation of the mitochondria activity. These preliminary results strongly suggest that topically applied thyroid hormones promote hair growth in intact human scalp on multiple levels ex vivo. This invites the intermittent pulse application of topical T3 and T4 as a novel therapeutic intervention for managing hair loss disorders associated with telogen effluvium, such as androgenetic alopecia.

P077 | Melatonin unfolds prominent, differential epidermal and dermal anti-aging properties in aged human facial and scalp skin ex vivo

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The well-tolerated neurohormone, melatonin is known to unfold anti-aging properties in cell culture and in some animal species. However, it remains unclear whether these translate to aged human skin. Here, we have probed whether high-dose melatonin (100 and 200 μM, administered for 6 days) alters a battery of core aging biomarkers in aged, full-thickness human eyelid or scalp skin (both genders; age range: 49-67 years). This was examined under serum-free organ culture conditions, since we had previously shown these to accelerate human skin aging ex vivo, thus rendering the skin particularly sensitive to candidate anti-aging actives.

Quantitative immunohistochemistry revealed that, compared to vehicle-treated control skin, 200μM of "systemic" melatonin (= added to the medium) significantly down-regulated intraepidermal activity of the aging-promoting mTORC1 pathway ex vivo, as demonstrated by reduced S6 phosphorylation. Melatonin also reduced matrix-metalloprotease (MMP-1) protein expression in the epidermis. Conversely, the transmembrane collagen 17A1, a crucial stem cell niche matrix molecule that declines with aging, and mitochondrial key markers (e.g., TFAM and VDAC/porin) were significantly upregulated by melatonin. Interestingly, 100 μM melatonin also significantly increased epidermal expression of VEGF-A protein, which we shown to be both required and sufficient for human skin rejuvenation in vivo and ex vivo. In aged human dermis, "systemic" melatonin significantly increased collagen I and fibrillin-1 protein expression and improved fibrillin-1 structural organization, indicating improvement of the collagen and elastic fiber network. Instead, other key aging biomarkers (SIRT-1, lamin-B1, p16INK4) remained unchanged. Given that aging is associated with lower general DNA methylation, we also explored if melatonin impacted on intracutaneous DNA methylation. This showed that 200 μM melatonin significantly reduced the level of DNA methyltransferase 1 and its product 5-methylcytosine in epidermis indicating DNA hypomethylation and suggesting that only some genes may be epigenetically affected (as showed by impact of melatonin of selected markers). Furthermore, expression of DNA demethylation 5-hydroxymethylcytosine, which also marks DNA damage sites, was decreased by melatonin, indicating reduction of DNA damage-associated with aging. When we applied 100 μM melatonin topically to human scalp skin ex vivo for 6 days, this also reduced mTORC1 activity and increased TFAM and VDAC expression in the epidermis, compared to vehicle-treated control skin (effects of topical melatonin on additional aging biomarkers are currently being analyzed). Taken together, our available data strongly suggest that "systemically" or topically applied melatonin indeed exerts substantial, yet unexpectedly differential epidermal and dermal antiaging effects in aged human facial and scalp skin ex vivo, namely at the level of mTORC1 activity, mitochondrial function, and MMP-1, collagen 17A1 and VEGF-A protein expression.

P078 | Melatonin significantly regulates arylhydrocarbon receptor, p27, pH2AX and COX-2 with consecutive impact on UVR-induced inflammation, skin aging and carcinogenesis

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UV-radiation (UVR) is a known stressor for the skin and leads to inflammation, photoaging and skin cancer. Melatonin is one of the most potent protective agents to reduce UVR-mediated oxidative, mitochondrial and DNA-damage and to ensure non-damaged keratinocyte survival. The damaging role of the arylhydrocarbon receptor (AhR), a UVR-activated transcription factor, and its downstream signaling molecules leading to inflammation, cellular aging, and carcinogenesis has previously been described. However, the

influence melatonin might have on this UVR-activated transcription factor and its consecutive and/or parallel events is not known yet.

We studied in human ex vivo full skin irradiated with different UVA/B doses (0, 100, 300 mJ/cm2) differential UV-effects at different time points after UV exposure (0, 24, 48h post UVR) and compared skin pre-incubated with melatonin with non-pre-incubated skin. Cryosections of the skin were analysed by immunofluorescence staining with AhR and downstream signaling molecules such as the tumor suppressor p27 and phosphorylated H2AX, a DNA double strand break marker. Furthermore, we studied the gene expression levels of AhR, p27 and cyclooxygenase-2 (COX-2) by real-time PCR in equally treated skin samples of 5 independent individuals. COX-2 is known to play a critical role in UV-induced inflammation as well as in tumor initiation and promotion.

As expected, UVR-exposure increased AhR positivity in contrast to non-UV-exposed skin with a peak at 24h post UVR. On the other side, melatonin-incubated skin showed a prominent decrease of AhR positivity directly after irradiation (0h post- UVR), as well as 24h and 48h post UVR compared to non-melatonin-treated samples. Also in non-irradiated skin samples, melatonin lead to a slight reduction of AhR positivity.

Second, UVR induced a slight decrease of tumor suppressor p27, while melatoninincubated skin samples showed a distinct increase of p27 positivity in UVR-exposed skin (300mJ/cm2) 24h post exposure time point.

Third, pH2AX positivity was enhanced in UVR-exposed skin compared to nonirradiated ones, whereas melatonin lead to a prominent decrease of pH2AX positivity directly after irradiation (0h post-UVR), as well as 24h and 48h post UVR. The pH2AX-reducing melatonin-effect was seen in UVR-exposed (100 and 300mJ/cm2) as well as in non-exposed skin (0mJ/cm2).

In parallel, gene expression showed a similar pattern of regulation compared to the protein level. Pre-incubation with melatonin prior to 300mJ UVR lead to a downregulation of AhR by 20.7% ($p<0.01$) at 24h post UV. At the same time point and UV dose melatonin downregulated p27 by 24.8% ($p<0.01$). Also, COX-2 gene expression was downregulated by 42.9% ($p<0.001$).

Aside from that, a reduction of AhR expression by 9.4% ($p<0.05$) and p27 expression by 8.6% ($p<0.05$) was observed in pre-incubated skin without any UVR exposure, implying an additional pure melatonin effect.

Melatonin prevents the UV-dependent activation of AhR and consequently reduces COX-2 expression leading to less cell damage, such as the UVR-induced formation of cyclobutane pyrimidine dimers (CPDs) as shown earlier. Consequently, the gene of tumor suppressor p27, a key player in the removal of harmful UV photoproducts is downregulated in melatonin pre-incubated skin due to lack of CPDs. Also, inhibition of AhR results in lower levels of phosphorylated H2AX, thus reducing DNA double strand breaks. To conclude, melatonin significantly reduces AhRmediated inflammation, cellular aging and carcinogenesis in UV-irradiated human skin.

Dermatopathology

P079 | Metabolic intervention with 2-DG ameliorates symptoms in a model of atopic dermatitis

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Atopic Dermatitis (AD) is a chronic, Th2-type-mediated disease often manifesting in infancy. Current treatment options are limited to topical glucocorticoids and systemic immunosuppressants solely alleviating the detrimental symptoms. These therapies fail to target disease-driving factors and innovative treatment approaches are needed. It has been shown that immune cell activation is coherent with cell metabolism alterations, such as aerobic glycolysis and mitochondrial activity. 2-Deoxyglucose (2-DG) and oligomycin are known to efficiently inhibit aerobic glycolysis and mitochondrial oxidation in diverse autoimmune diseases. Here, we investigated the effect of 2-DG and oligomycin on symptom severity in a model of AD. C57BL/6J wild-type mice were randomly allocated into four treatment groups (Control (CTRL), 2-DG, oligomycin, and 2-DG+oligomycin) and AD-like symptoms were induced with epicutaneous application of the vitamin D3-analogue Calcipotriol (MC903). Our results show that 2-DG- and 2-DG+oligomycin-, but not oligomycintreated mice, display significantly alleviated AD-like symptoms compared to the CTRL group. The ear thickness in the 2-DG- and 2-DG+oligomycin-treated mice was significantly reduced compared to the CTRL group. Histological evaluation revealed a significant decrease in the epidermal and dermal thickness in 2-DG- and 2-DG +oligomycin-treated mice compared to the CTRL mice. Whereas transepidermal water loss was only significantly reduced in the 2-DG+oligomycin-treated compared to the CTRL animals. Lastly, no differences in immune cell population were detected between the groups in different organs. Taken together, our results provide evidence that inhibition of aerobic glycolysis results in significant symptom amelioration in a murine model of AD. We suggest that aerobic glycolysis plays a crucial role in the pathogenesis of AD and constitutes a promising novel therapeutic target.

P080 | Staphylococcus hominis is beneficial for skin architecture, barrier and inflammation in an atopic dermatitis model with reconstructed human epidermis

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Commensal skin microbes are essential components of the epidermal barrier. However, an imbalance of skin microbes is associated with inflammatory skin diseases such as atopic dermatitis (AD). In AD, Staphylococcus (S.) aureus is highly abundant and knowingly contributes to disease pathology. In contrast, S. hominis is more abundant in non-lesional and healthy skin than in AD, suggesting a beneficial role. To investigate the effects of S. hominis on skin barrier, reconstructed human epidermis (RHE) was inoculated with S. hominis under type 2 inflammatory conditions, using T cell supernatant (TCS) isolated from T cells derived from AD skin biopsies. AD-TCS led to thickening of the epidermal layer, induction of spongiosis, and upregulation of inflammatory genes (e.g. CCL2, S100A7, SERPINB3). These effects were reversed by S. hominis inoculation in a dose-dependent manner. Furthermore, expression of genes related to physiological epidermal differentiation (e.g. KRT10, COL1A1, DSC1, MMP2) increased after S. hominis inoculation, indicating positive effects on barrier formation. These findings were validated in AD patients, where the trans-epidermal water loss - a marker for epidermal barrier damage - correlated negatively with S. hominis abundance. In conclusion, S. hominis normalized type 2 related changes of the epidermal architecture, barrier and inflammation in AD. Therefore, skin treatment with S. hominis may represent a new potential therapeutic strategy. Moreover, established AD-RHE models allow the analysis of interactions between microbes, keratinocytes, and T cells and can contribute to study the effect of microbes on disease pathology.

P081 (OP01/01) | Deletion of both ERBB2 and ERBB3 in mouse skin affects epidermal differentiation and causes inflammatory lesions

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The tyrosine kinase receptors ERBB2 (HER2, neu) and ERBB3 (HER3) are members of the epidermal growth factor receptor (EGFR, ERBB1, HER1) family, which further includes ERBB4 (HER4). These receptors are usually activated by ligand binding to the extracellular domain followed by homo- or heterodimerization, which activates the intracellular kinase activity and initiates a downstream signaling cascade. While the EGFR has an established role in the physiology and pathology of the skin and its appendages, the functions of ERBB2 and ERBB3 are less well known. ERBB2 is an orphan receptor and ERBB3 has no kinase activity. In contrast to the autonomous ERBB receptors (EGFR and ERBB4), ERBB2 and ERBB3 need to dimerize with other receptors of the family to be activated. The two receptors often dimerize with each other and are frequently upregulated and/or hyperactive in tumors. Monoclonal antibody (MAB) therapy with antibodies against ERBB receptors are frequently associated with severe dermatological toxic side-effects, causing inflammation, papulopustular rush, nail inflammation, dry skin, alopecia, strong pruritus and increased growth of eyelashes and facial hair. Mice lacking both ERBB2 and ERBB3 would thus represent an attractive model to study the mechanisms and side effects of a monoclonal antibody approach to cancer therapy.
To ablate ERBB2 and ERBB3 expression the epithelial compartment of mouse skin, we crossed mice carrying conditional Erbb2 and Erbb3 alleles with transgenic animals expressing cre recombinase under the control of the keratin 5 (KRT5) promoter. K5Cre;Erbb2del;Erbb3del mice (ERBB2/3 knockouts) were born at the expected mendelian ratios and showed no obvious abnormalities for the first weeks of life. After 6-8 weeks the double knockouts showed scratching behavior and develop small skin sores around the neck and throat and on the tail. At six months of age, wounds increased in size and skin inflammation developed, and animals were killed for ethical reasons. ERBB2/3 knockout animals showed increased epidermal thickness and enlarged sebaceous glands with increased proliferation rate compared to control mice. The basal and suprabasal layers were thickened, while the cornified layer of the epidermis was unchanged, as assessed by KRT5, KRT10, and loricrinstaining, respectively. Furthermore, KRT6 positive cells were found in the epidermis of ERBB2/3 knockout mice, indicating that the loss of ERBB2 and ERBB3 disturbs the differentiation of the epidermis. These observations indicate that ERBB2 and ERBB3 are important for the homeostasis of the epidermis and its appendages. ERBB2/3 knockout mice show a skin phenotype resembling that of ERBB inhibitortreated cancer patients and enables us to address the impact of ERBB2 and ERBB3 in skin homeostasis and skin cancer, and the skin toxicity during ERBB inhibitor therapy in cancer patients.

P082 | Comparative in vivo imaging of blood flow and inflammation in skin tumors including follow-up under immunological therapies using dynamic optical coherence tomography and line-field confocal optical coherence tomography

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Introduction & Objectives: In the present project the blood flow of skin tumors in vivo was investigated by line-field confocal optical coherence tomography (LC-OCT) and dynamic optical coherence tomography (D-OCT), in particular of basal cell carcinoma, squamous cell carcinoma, Bowen's disease and malignant melanoma. Our hypothesis is that noninvasive high-resolution in vivo imaging of blood vessels, blood flow, and the immunological microenvironment can be used to characterize skin tumors, predict their therapeutic response to immunotherapy, and monitor them during therapy.

Materials & Methods: The blood flow, blood vessels as well as the blood cells are examined quantitatively and qualitatively in the individual tumors with LC-OCT and D-OCT from May 2023. The migration of leucocytes on the vessel wall as well as in the center of the lumen and their speed were observed and evaluated. Furthermore, the flow of erythrocytes was examined. In addition, a possible differentiation of leucocytes, erythrocytes and lymphocytes were investigated. For this purpose, a cooperation with basic research, especially physiology for the visualization of blood cells in channels and with data science for the AI-based evaluation of the videos of blood flow and blood cells will be pursued. Automated quantification of blood cells, their localization and velocity using artificial intelligence will be performed as well as artificial intelligence guided visualization of their changes in terms of inflammation, plasticity and angiogenesis under therapy. This information will also be compared with clinical and dermoscopic images. Furthermore, this complex of topics offers the possibility, to assess melanomas under neoadjuvant therapy with regard to their blood flow in the course of time. In addition, changes in inflammation, plasticity and angiogenesis in the primary tumor under immunotherapy can be visualized preoperatively. In basal cell carcinoma (BCC), for example, these same processes could be evaluated under topical therapy with Imiquimod, a Toll-like receptor agonist, and/or in BCC/squamous cell carcinoma (SCC) under an intralesional combination preparation of the immunocytokines L19IL2 and L19TNF.

Results: The recruitment of patients should be completed by the end of 2023. Descriptive results are already available and prove the reduction of the blood flow of subcutaneous melanoma metastases in comparison to before and after 4 weeks after local immunotherapy with proleukin.

Before https://1drv.ms/v/s!AoCJAdXSY-tHibl2WBUdJDvWuSpz1A?e=oMSgSv

After https://1drv.ms/v/s!AoCJAdXSY-tHibl4YbJqq8z_hDHKeA?e=0IzKqH

Differences between the various tumor entities and between benign skin lesions were found. Statistical and quantitative results are currently carried out.

Conclusion: The overall aim of the study is to demonstrate that non-invasive imaging allows the differentiation of tumor entities by visualizing blood flow, blood vessels and the immunological microenvironment thus providing individual monitoring of treatment response and valuable information for treatment decisions (neoadjuvant or adjuvant therapy, surgery).

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

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Autoimmune bullous skin diseases (AIBDs) represent a group of chronic skin diseases associated with autoantibodies directed against proteins of the epidermal/ epithelial desmosome (pemphigus group) and the cutaneous basal membrane zone (pemphigoid diseases). Despite the widespread distribution of target antigens in the skin and mucosa, blister formation is not equally distributed to different body sites. It remains largely unexplored whether these predilection sites differ in terms of expression of the corresponding target antigens. To address this, we examined the antigen expression levels of BP180, BP230, COLVII, Dsg1, Dsg3, laminin α3, laminin β3, laminin β4, integrin α6, integrin β4, cytokeratin 14, and plectin across various anatomical sites affected by AIBDs. Skin and mucosal tissue biopsies were obtained from 13 different anatomical regions of 10 body donors without AIBD. The strength of antigen expression was evaluated through serial dilution of antibodies using indirect immunofluorescence (IF) microscopy. Our data indicated a significant difference (pvalue <0.05) in the expression of integrin β4, laminin β4, and cytokeratin 14 at least at one body site. Subsequently, expression levels of these proteins in the skin of the arm (as a reference site) were compared with those in the back, chest, cheek, foot sole, hand palm, leg, lower conjunctiva, mucosa, and right flank. The results revealed a significant variation in the laminin β4 expression in the hand palm, foot sole, and mucosal tissues, while the expression levels of integrin β4 and cytokeratin 14 remained consistent across all body sites when compared to the arm. This study suggests that AIBD predilection sites are mostly unaffected by regional variations in antigen expression. Consequently, additional external factors, such as microbiota, mechanical stress, or sunlight exposure may trigger and modulate the localization of lesions in AIBD.

P084 | Immunoglobulin D - Does it play a role in chronic spontaneous urticaria?

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Introduction: Immunoglobulins (Ig) are one of the hallmarks of the adaptive immune system. While the biology of IgM, IgG, IgA and IgE has been extensively studied, the function and regulation of IgD is less well known. In addition to acting as a B cell receptor, IgD can also appear secreted in circulation. Secreted IgD can be found on the surface of myeloid innate effector cells, including basophils, mast cells, and monocytes. In recent years, abnormalities or dysregulation of IgD have been identified to play a critical role in the pathogenesis of autoimmune, allergic, and other inflammatory diseases. To date, the role of IgD in chronic spontaneous urticaria (CSU), a mast cell-driven disease, has not been systematically investigated.
Methods: Immunohistochemistry staining from lesional and non-lesional CSU skin biopsies (n=10) were performed to identify IgD+ skin mast cells. Furthermore, serum samples from well-characterized adults with CSU (n=260) and age and sex-matched healthy controls (n=136) were analyzed and compared for IgD serum levels as well as for IgG, IgM, IgA and, IgE. To determine whether there is a signature of cytokines, chemokines and growth factors associated with a possible IgD phenotype in CSU patients, a 45 multiplex assay was performed from the serum of CSU patients (n=85) and healthy controls (n=35).
Results: No IgD+ skin mast cells could be detected. IgD serum levels in CSU patients and healthy controls varied considerably, from 0.18 to 287 μg/ml and 1.14 to 270.9 μg/ml, respectively and did not correlate with serum levels of IgG, IgM, IgA, or IgE. Overall, patients with CSU had significantly lower median (IQR) serum levels of IgD as compared to healthy controls, i.e. 22.02 (38.51) vs. 39.06 (63.29) μg/ml ($p < 0.0001$). Moreover, low serum IgD levels, i.e. <10 μg/ml, were more common in patients with CSU (26%) as compared to healthy controls (10%, $p = 0.0001$). In addition, the multiplex assay performed revealed significant upregulation of granulocyte macrophage-colony stimulating factor (GM-CSF; $p = 0.0094$) and hepatocyte growth factor (HGF; $p = 0.0023$) in CSU patients.
Discussion: Our findings indicate that low or very low levels of IgD may be more common in CSU and thus suggest that dysregulation of IgD may be involved in the pathogenesis of CSU in a subset of patients. Further research is required to characterize the functional significance of IgD in CSU and to investigate a possible impact of IgD on the pathogenesis of CSU.

P085 | Epigenetic alterations of proliferation-associated molecules in psoriatic keratinocyte subtypes

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Introduction & Objectives: Psoriasis is a chronic inflammatory disease characterized by sharply demarcated erythematous and scaly skin lesions resulting from keratinocyte hyperproliferation and impaired differentiation. The molecular processes underlying the altered cutaneous proliferation in psoriasis are still incompletely understood (1). Therefore, this study aimed to characterize the methylome and transcriptome pattern of proliferating keratinocyte subtypes in psoriatic and healthy skin.
Materials & Methods: Keratinocyte subtypes were isolated from whole skin biopsies of patients with psoriasis and healthy donors. Characterization of methylome and transcriptome pattern was carried out by methylation as well as RNA sequencing. Skin biopsy sections were analyzed by immunohistochemistry and quantitative RTPCR.
Results: A shift in keratinocyte subtype composition was observed in psoriasis lesions compared to skin from non-lesional sites or from healthy participants. In fact, the number of transit amplifying (TA) cells, the primary proliferating keratinocyte subpopulation in the epidermis, was increased in psoriatic lesions. A whole genome analysis of these cells revealed substantial methylome and transcriptome alterations in psoriatic lesional TA cells compared to those from healthy donors. Interestingly, only 17% of differentially expressed genes in TA cells from psoriatic lesions were associated with respective methylation changes. In non-lesional TA cells, considerably less methylation changes were found. Interestingly, only <1% of those methylation changes were associated with respective transcriptional changes, suggesting external triggers not present in non-lesional skin to be essential for the development of psoriatic lesions. Further bioinformatics analyses identified potential proliferation-associated molecules with differential methylation and respective transcriptional changes in TA cells from psoriatic lesions. Detailed analysis of those molecules by qPCR and immunohistochemistry on whole skin biopsies confirmed their differential regulation in non-lesional and lesional psoriatic areas compared to healthy skin. Functional studies using gene knockdown of these molecules in keratinocytes are currently being conducted.
Conclusion: In summary, our data suggest that TA cells, known to mediate epidermal renewal and psoriatic hyperproliferation, are altered at the epigenetic level already in the non-lesional skin of

psoriasis patients. The occurrence of psoriatic lesions is associated not only with an increase of these cells but also with a strongly altered transcriptome that already comprises features characteristic for the suprabasal psoriatic epidermis. Our results furthermore imply that longterm persistence of psoriatic lesions may affect the epigenetic pattern of keratinocyte subtypes.

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P086 | Supporting B cells in hidradenitis suppurativa lesions, BAFF secreted by GCSF activated neutrophilic granulocytes is a pathogenetic factor

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Introduction & Objectives: Hidradenitis suppurativa (HS) is a chronic inflammatory disease characterized by inflamed nodules, abscesses, and pus-draining tunnels that develop in the skin in axillary, inguinal, and perianal sites (1). HS leads to a profound impairment of the quality of life as well as the ability to work and the work productivity of those affected (2, 3). Unfortunately, HS pathogenesis is poorly understood and treatment options are limited. The identification of mediators responsible for the survival and activity of immune cells in the inflamed skin is the prerequisite for the development of new innovative therapies. Therefore, this translational study aimed to identify mediators that support the lesional B/plasma cell persistence in HS lesions.

Materials & Methods: Keratinocytes, dermal fibroblasts, neutrophilic granulocytes (neutrophils), monocytes, B cells, cultured skin biopsy samples and blood plasma specimen were analyzed in mechanistic studies. Skin samples from several cohorts of HS patients and control participants were assessed by mRNA sequencing, quantitative RT-qPCR, flow cytometry, and immunohistofluorescence. Complex systems biology approaches were used to evaluate cutaneous bulk and single-cell RNA sequencing data.

Results: First, by means of flow cytometric and 'cybersort' analyses of whole skin biopsies obtained from HS patients and healthy participants, we discovered that HS lesions show elevated proportions of B/plasma cells, neutrophils, CD8+ T cells, as well as M0 and M1 macrophages compared to perilesional skin and intertriginous skin from healthy participants. In contract, the percentages of CD4+ T cells, dendritic cells, and mast cells were decreased. Multiplex immunofluorescence analysis demonstrated B/plasma cells exclusively in the lesional dermis. Here, these cells mostly formed clusters next to high endothelial venule-like blood vessels, characteristic of tertiary lymphoid structures. Second, histological and RT-qPCR analysis revealed an association between B/plasma cells, neutrophils, and Bcell activating factor (BAFF, aka TNFSF13B) in HS lesions. The expression of BAFF was elevated in HS lesions, particularly in nodules and abscesses, and exceeded the expression in cutaneous lesions of patients with psoriasis and atopic dermatitis. Mechanistically, granulocyte colony-stimulating factor (G-CSF), an immune mediator produced by fibroblasts and keratinocytes as a result of IL-1β, IL-17, and/or TNF-α stimulation, was the major stimulus for the neutrophils' BAFF secretion. The G-CSF effect on neutrophil-derived BAFF was further amplified by bacterial products. The upregulation of BAFF receptors in HS lesions was attributed to B cells (TNFRSF13C/BAFFR and TNFRSF13B/TACI) and plasma cells (TNFRSF17/BCMA) as demonstrated by single-cell skin RNA sequencing data. Third, Characterization of the lesional BAFF pathway revealed molecules involved in migration/adhesion, proliferation/survival, activation, and reactive oxygen species production of B/plasma cells. Accordingly, the application of a soluble form of BAFF receptor to ex vivo cultured samples from HS lesions led to reduced expression of CXCR4, CD53, SELL, PRKCB, and NCF1. Furthermore, stimulation with recombinant BAFF elevated the expression of L-selectin on B cells. B cells from HS lesions were also able to produce TNF-α, a key mediator in HS pathogenesis.

Conclusion: In HS lesions, BAFF secreted by neutrophils supports the persistence and function of B/plasma cells that, in addition to producing auto-antibodies, contribute to the TNF-α production. This translational study demonstrated that targeting the BAFF pathway might be an innovative therapeutic strategy for HS management.

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P087 | A primary melanoma orchestrates premetastatic niche formation via neutrophils

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Introduction: Malignant melanoma is an extremely aggressive tumor characterized by a rapid tumor spreading to the liver, the brain and the lung. One initial step in the metastatic process is the conversion of a distant organ into a premetastatic niche based on a complex interplay between inflammation and coagulation.

Hypothesis: We postulate that a primary tumor mediates the secretion of the procoagulatory protein von Willebrand factor (VWF) in the premetastatic lung to promote coagulation, inflammation and metastases.

Methodology: For evaluation of the clinical relevance of VWF, we analyzed plasma and tissue of malignant melanoma patients. To characterize the premetastatic lung and the impact on the metastatic process, different mouse models were developed. Proteomics, immunofluorescent analyses and kinomics were conducted.

Results: Elevated plasma concentrations of VWF in stage III melanoma patients (lymph node metastases, no organ metastases) were associated with elevated neutrophil infiltration into the primary tumor. For evaluation of the pathophysiological mechanisms, a mouse model was applied. We could show that a primary tumor promotes VWF secretion associated with elevated neutrophil counts and neutrophil extracellular traps (NETs) in the premetastatic lung. A subsequent increase in experimental lung metastases, neutrophil infiltration and neutrophil activation was abolished by therapeutic anticoagulation and pharmaceutical inhibition of NETosis in preclinical animal models.

Conclusion: Our findings provide a novel mechanism by which VWF promotes premetastatic niche formation and suggest neutrophils as a promising therapeutic target for the treatment of malignant melanoma.

P088 (OP06/05) | Interferon and tumor necrosis factor alpha are upregulated in the human skin organ culture model for pemphigus vulgaris

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Pemphigus vulgaris (PV) is an autoimmune blistering disease of mucous membranes and/or skin. Once the immune system loses tolerance, autoantibodies targeting mainly desmoglein 3 (Dsg3) or Dsg1/3 are produced. The antigens support strong cell-cell adhesion between epidermal, hair follicle as well as mucosal keratinocytes. Accordingly, autoantibodies targeting these proteins uncouple trans- or cisadhesion which ultimately leads to blister formation. So far, the molecular mechanism of PV pathogenesis is not fully understood and needs further investigation.

In this project, we focus on the events occurring directly after binding of autoantibodies to their antigens. The human skin organ culture model (HSOC) for PV is used to establish a comprehensive and integrative biomolecular and biochemical Dsg3 network. Injection of the single-chain variable fragment (scFv) PX43 (directed against Dsg1/3) or the antibody AK23 (directed against Dsg3) with (as a control) or without exfoliative toxin A (ETA) replicate the clinico-morphological features of PV. HSOCs were harvested 5, 10 and 24 hours after injection and prepared for quantification of split formation as well as bulk RNA-sequencing (RNA-seq). It was evident from hematoxylin and eosin-stained sections of HSOCs that PX43 and AK23 with ETA led to quantitatively different split formation: after titration of AK23 with 0.8 microgram of ETA, pre-experiments showed that 34 microgram AK23 in combination with ETA lead to 20 % less split formation than 85 microgram of PX43. Furthermore, twenty-four hours after injection of PX43, RNA-seq from microdissected basal keratinocytes revealed 775 differentially expressed genes (DEGs; ≥2 fold change; padj ≤ 0.05). Of these, 488 were up- and 287 downregulated, with prominent upregulation of IFNgamma and TNFalpha pathways, the latter coinciding with upregulation of NFkB and JAK-STAT-mediated circuits. In contrast, when applying the same threshold, no DEGs were identified in RNA-seq of AK23-injected HSOC. Proteome analysis of PX43-injected HSOC is currently ongoing while changes in the epigenome will be analysed by chromatin immunoprecipitation (ChIP) and in the kinome by PamGene technique.

Hereby we hope to shed light on the initial signals in PV pathophysiology and identify a clinically relevant tissue communication code to develop new therapeutic strategies.

P089 | Degree of Actinic Elastosis Is a Surrogate of Exposure to Chronic Ultraviolet Radiation in melanoma and NMSC

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Background: Keratinocyte cancer (KC) is associated with exposure to ultraviolet (UV) radiation. Also UV radiation and sunburns are associated with a higher number of acquired nevi and melanoma. However, data are controversial as to whether chronic UV exposure or high intermittent UV exposure are key drivers of carcinogenesis in melanoma, cutaneous squamous cell carcinoma (cSCC) and basal cell carcinoma (BCC). Prolonged sun exposure of the skin causes photoaging, which is associated with actinic elastosis, a condition characterized by the degeneration of elastin in the upper dermis, which is assessable via conventional histology. In this study, we aimed to compare the degree of actinic elastosis in different types of KC and in different subtypes of malignant melanoma with regard to various patient characteristics.
Methods: We defined a semiquantitative score for the degree of actinic elastosis ranging from 0 = none to 3 = total loss of elastic fibers (basophilic degeneration). The extent was measured histometrically by two independent dermatohistopathologists in the immediate vicinity of 595 melanocytic lesions and 353 KC. The scores were merged and matched with tumor types (Nevi, Melanoma with subtypes, cSCC and BCC with subtypes), and clinical variables such as body site, sex and age.
Results: As expected, the degree of actinic elastosis correlated with age. We saw signifacantly differences in subtypes of malignant melanoma. degree of actinic elastosis was also significantly higher in cSCC compared to BCC irrespective of age, sex, body site and tumor subtypes.
Conclusions: Lifetime sun exposure may be estimated via routine histology using this scoring technique for actinic elastosis as a surrogate marker. There are differences in subtypes of malignant melanoma. cSCCs are more strongly associated with chronic UV exposure than BCCs, even in sun-exposed localizations such as the face.

P090 | The role of lactate and its receptor Gpr81 in the resolution of inflammatory blistering skin diseases

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Granulocytes, especially neutrophils, dominate the inflammatory infiltrate in many antibody-induced autoimmune diseases (AADs) and are often the primary effector cell population inflicting tissue injury in these diseases. This also includes pemphigoid diseases (PDs) like Epidermolysis bullosa acquisita (EBA), a rare autoimmune skin disease that develops when autoantibodies against collagen type VII are produced. The tissue micromilieu dictates the progression of disease and is not only shaped by inflammatory mediators but also products of metabolic activity. Neutrophils integrate external stimuli to a large extent via cell surface receptors, dominated by GPCRs (Gprotein coupled receptors). We hypothesized that neutrophil adaption to local tissue conditions may be regulated by metabolite-sensing GPCRs, like Gpr81, a receptor for lactate. Therefore, we investigated a role of Gpr81 in neutrophils and the effector phase of inflammatory skin blistering diseases.
Our findings suggest that lactate diminishes neutrophil migration towards LTB4 via GPR81 interaction in vitro, however lactate induced modulation of immune complex induced neutrophil effector functions such as ROS release appears to occur independent of Gpr81. Moreover, there were no changes observed in mRNA expression of Gpr81 in neutrophils upon stimulation with immune complexes. Additionally, comparison of Gpr81 knock-out and litter mate wild type mice, in the antibody transfer-induced EBA mouse model, demonstrated that disease manifestation and resolution are independent of Gpr81.

P091 | Depletion of mast cells with barzolvolimab, an anti-KIT antibody, does not affect mast cell progenitor numbers or function

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Background: Mature mast cells (MCs) express the KIT receptor (CD117), which is essential for survival, maturation and function. As MCs are the main drivers of the development of signs and symptoms in chronic inducible urticaria (CIndU) a recent study investigated the effect of barzolvolimab, an anti-KIT antibody, to treat this disease. CIndU patients showed a significant depletion of skin MCs, and 95% achieved complete response to provocation testing after a single injection of barzolvolimab. Whether barzolvolimab affects the numbers or function of circulating MC progenitors is currently unknown.
Methods: Blood samples from CIndU patients (n=10) before and four weeks after barzolvolimab treatment were analysed for blood MC progenitor numbers by flow cytometry. Isolated MC progenitors before and after treatment were assessed for their capacity to differentiate into mature MCs, and then tested for degranulation capacity, viability, and proliferation.
Results: Barzolvolimab had no significant effect on the numbers of CD34+ cells, i.e. pluripotent including MC progenitor cells, at four weeks after treatment as compared to before treatment (before

treatment: 2.44 0.45*104/mL vs after treatment: 1.78 0.26*104/mL; p = 0.24). In vitro generated MCs from patients were not affected by barzolvolimab treatment in terms of their expression of KIT, FcεRI or MRGPRX2 receptors (40% vs 49% for FcεRI+/MRGPRX2+ MCs; p = 0.52), viability in presence and absence of SCF (-28.3% vs -14% viability in starved MCs before and after treatment, respectively; p = 0.1), proliferation rate (8.4% vs 12.1% at day 21 of proliferation assay; p = 0.13) and degranulation capacity upon IgE-mediated (36% vs 30%, p = 0.78) and non-IgE-mediated stimulation (26% vs 29% for substance P; p = 0.98).

Conclusion: KIT inhibition leading to skin MC depletion with barzolvolimab does not affect MC progenitor numbers or their capacity to give rise to mature MCs. This suggests that MC populations can fully recover after depletion via KIT-targeted treatment and as evidenced by the observed recovery in serum tryptase.

P092 | Interleukin-19: a key pathogenic factor in palmoplantar pustulosis?

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Introduction & Objective: Palmoplantar pustulosis (PPP) is a chronic inflammatory skin disease with painful erythematous scaling or crusting lesions and neutrophilic granulocyte (neutrophil)-filled pustules on the palms and soles. The pathogenesis of PPP is poorly understood. The aim of this translational work was to identify and characterize pathogenic players in PPP.

Materials & Methods: Immune mediators elevated in the blood of patients suffering from PPP were screened. Keratinocytes, dermal fibroblasts, endothelial cells, melanocytes, monocytes, neutrophils, and three-dimensional reconstituted epidermis cultures were analyzed in mechanistic studies. Blood and skin samples from murine DNFB model and from the phase 2, multicenter study of apremilast (PDE4 inhibitor) in 21 patients with moderate-to-severe PPP (APLANTUS; EudraCT 2016-005122-1) (1) was used for validation.

Results: Interleukin (IL)-19 was found to be the most upregulated immune mediator in the blood of PPP patients. IL-19 blood levels were independent of patients' age, gender, and BMI but were associated with high IL-19 expression in the skin lesions and positively correlated with the number of palmoplantar pustules. Mechanistic studies in vitro demonstrated that IL-19 was produced by neutrophils activated with even small amounts of microbial products, but not with inflammatory cytokines like IL-36, TNF-α, or IFN-γ. Furthermore, despite strong IL-17 receptor A (IL-17RA) expression, IL-17A, IL-17C, and IL-17E did induce the neutrophil IL-19 expression either and had no effect on the IL-19 expression induced by microbial products. In contrast, IL-19 was induced by IL-17A as well as IL-1β in keratinocytes. TNF-α and IL-22 amplified this IL-17A effect, however, they had no effect on the IL-17RA expression in these cells.

IL-19 acts via a receptor complex composed of IL-20R1 and IL-20R2. The strongest expression of these receptor subunits was found in keratinocytes. In contract, immune cells, endothelial cells, or melanocytes did not express these proteins. In epidermis models, IL-19 induced the neutrophil-attracting chemokine CXCL6 and this effect was amplified by IL-17A. The expression of CXCL6 was elevated in cutaneous PPP lesions compared to skin taken from the palms of healthy participants. Furthermore, CXCL6 blood levels in PPP patients positively correlated with IL-19 and IL-17 blood levels.

Using an in vivo murine DNFB model of skin inflammation, we found elevated IL-19 levels in the skin but not in the lymph nodes of these animals. Application of anti-IL-20R1 antibodies in this model reduced the skin thickening. In patients with moderate-to-severe PPP, oral treatment with apremilast significantly decreased those patients' PPPASI, with a median reduction of 57.1% after 20 weeks of treatment (end of treatment) (1). Interestingly, apremilast reduced pustules more effectively than erythema and scaling. In these patients, the apremilast treatment also significantly reduced the IL-19 blood and skin levels, and the reduction in IL-19 blood levels at week 4 correlated with the reduction in pustule counts at week 20.

Conclusion: The results of this translational study documented increased levels of IL-19 in the blood and skin of PPP patients. We postulate a relevant role of this cytokine in the communication between neutrophils and keratinocytes that is partly amplified by IL-17 and leads to pustule formation in this disorder.

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P093 | Loss of T antigen expression results in cell cycle arrest and neuroblastic transdifferentiation in Merkel cell carcinoma

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Merkel cell carcinoma (MCC) is an aggressive skin cancer frequently caused by genomic integration of the Merkel cell Polyomavirus (MCPyV). Among the MCPyVnegative cases, many present as combined MCCs, which represent a distinctive subset of tumors characterized by association of an MCC with a second tumor component, mostly squamous cell carcinoma. Up to now, only a few cases of combined MCC with neuroblastic differentiation have been reported.
Herein we describe two combined MCC with neuroblastic differentiation and provide comprehensive morphologic, immunohistochemical, transcriptomic and genetic characterizations of these tumors, which both arose in elderly males and appeared as an isolated inguinal adenopathy. Microscopic examination revealed biphasic tumors combining a poorly differentiated high-grade carcinoma with a differentiating neuroblastic component lacking signs of proliferation. Immunohistochemical investigation revealed expression of Keratin 20 and MCPyV T antigen (TA) in the MCC parts, while neuroblastic differentiation was confirmed in the other component in both cases. A clonal relation of the two components in both combined tumors can be deduced from shared copy number variations as well as from 20 and 14 shared acquired point mutations detected by whole exome analysis, respectively. Spatial trancriptomics demonstrated a lower expression of stem cell marker genes such as SOX2 and MCM2 in the neuroblastic component. Interestingly, although the neuroblastic parts lacked TA expression, the same genomic MCPyV integration and the same TA-truncating mutations were observed in both tumor parts. Given that neuronal transdifferentiation upon TA repression has been reported for MCC cell lines the most likely scenario for the two combined MCC/neuroblastic tumors described here is that neuroblastic transdifferentiation resulted from the loss of expression of the two TAs in a subset of MCC cells.

P094 | Detrimental effects of the disinfectant Octenisept® 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 needed.
The wound healing organ culture (WHOC) is a powerful tool to investigate ex vivo wound healing of human skin. However, it is prone to infections, because the plates must be opened for top-view evaluation in a nonsterile environment. Therefore, the culture medium was supplemented with penicillin and streptomycin to avoid bacterial contamination. In addition, the antimycotic Amphotericin B was added from day 0 to day 1. As still infections, especially with yeast, were observed, Octenisept® was investigated as a possible additional disinfectant for the WHOC. It seemed to be the optimal choice for our ex vivo wounds, as it is non-alcoholic and frequently used in the clinics as a disinfectant.
Octenisept® was sprayed at the wounds at day 0 before the culture started as well as every time after the top-view microscopy at day 2, 4 and 6. The control WHOC were not disinfected with Octenisept® at all. We have investigated different parameters to determine the wound healing in the WHOC. Moreover, the effects of Octenisept® were studied in an in vitro scratch-wound assay to measure its influence on keratinocyte migration.
Unexpectedly, the ex vivo wounds disinfected with Octenisept®, showed noticeably less top-view wound closure in the WHOC. While we have observed a dense dermis with an intact epidermis and growing epithelial tongues in the control group, in wounds disinfected with Octenisept® almost no epithelial tongues could be observed. Moreover, a partly disrupted and often detached epidermis was found. In addition, relative cytokeratin 6 expression was strongly decreased in Octenisept® disinfected wounds in the ex vivo model. Disinfection with Octenisept® also seemed to decrease the cortactin expression.
Besides, we have observed a significantly reduced in vitro scratch-wound closure in with Octenisept® disinfected scratch-wound assays.
In summary, all so far investigated parameters strongly indicate that Octenisept® has a detrimental effect on wound healing in the WHOC and negatively influences the keratinocyte migration. Further experiments should determine the consequences for the treatment of human wounds, as our data indicates that the use of Octenisept® prevents the wound healing process in patients.

P095 | Testing the effect of kinase inhibitors in the human skin organ culture model

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Pemphigus vulgaris (PV) is an IgG-mediated autoimmune disease of stratified squamous epithelia, such as the skin and oral mucosa. Autoantibodies against cell-cell adhesion molecules, namely desmoglein 3 (Dsg) and/or Dsg1, cause acantholysis. Subsequently, the disease predominantly manifests in blistering of the skin and/or mucous membranes.

It is hypothesized to involve several intracellular signalling pathways downstream of the binding of PV autoantibodies. These signalling molecules, including several kinases, might be targetable within more specific treatment approaches.

This project aims to further identify the role of kinases in intracellular signalling cascades in PV by investigating the dose-dependent effects of kinase inhibitors. These inhibitors were previously selected using a technology called Pamgene. Proteins were extracted from skin samples, whereupon the activity of the kinases containing in those proteins was detected. A significant increase in activity was measured for overall eight kinases, including several representatives of the Src and FAK family. In the following, seven specific inhibitors for those kinases were selected and keratinocyte dissociation assays using HaCaTs and primary human epidermal keratinocytes were performed.

Based on these preliminary investigations of the effect of kinase inhibitors in cell lines, the human skin organ culture model was performed. Within the in vivo model, PX43, a single chain variable fragment, targeting both Dsg1/3 was injected - mimicking the key molecular and morphological characteristics of PV.

The kinase inhibitors were introduced to the skin samples, as well as intravenous immunoglobulin and (IVIG) and anti-BP230 as negative controls. The kinase inhibitors were injected in three concentrations: 0.01 mM, 0.1 mM, 1 mM.

The skin samples were semi-quantitatively evaluated on the histological development of intraepidermal split formation.

Eight skin specimens have been tested for each inhibitor, including controls.

The qualitative analysis of Dsg 1/3 and PX43 verify the hypothesized binding behaviour of the antibody fragment and the resulting split formation corresponding to PV.

The evaluation of split formation in the H&E stained sections demonstrate that six out of seven kinase inhibitors significantly reduced the acantholysis in the skin samples of the human skin organ culture model in a dose-dependent manner. The inhibition of FAK-family kinases is significant and dose-depended throughout. The reduction of split formation was significant and dose-depended for four out of five kinases from the Src-family.

The results of this work may serve as a foundation for further research concerning the molecular role of kinases in the pathogenesis of PV, possibly leading to more targeted treatment options of the autoimmune disease.

P097 | The single chain variable fragment PF1-8-15 induces acantholysis in the human skin organ culture model

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Pemphigus foliaceus (PF) is an autoimmune blistering disease of the skin. Within the framework of the disease autoantibodies directed against desmoglein 1 (Dsg1) are produced. Dsg1 is a glycoprotein and part of desmosomes, cell-cell contacts which are providing strong intercellular adhesion. The binding of the specific antibodies causes a disruption of the cell-cell adhesion and leads to acantholysis. Parts of the pathogenesis (e.g. signaling pathways) of PF are still the subject of current research.

The research goal within this project is the establishment of a human skin organ culture model (HSOC) for pemphigus foliaceus. Therefore, we tested a previously established single chain variable fragment (scFv), cloned from an active PF patient, in the HSOC, to evaluate its pathogenicity. We started with the establishment of the best dosage of PF1-8-15 by injecting the scFv in different concentrations into the HSOC model. For positive control we used the scFv PX43, directed against desmoglein 3 and 1 and as a negative control human immunoglobulin G (hIVIg). The HSOCs were harvested after 24 hours post injection. We performed hematoxylineosin staining of HSOCs to confirm split formation caused by PF1-8-15-treated samples in the granular layer of the epidermis. We also took samples for the analysis of kinase expression via PamGene system. The largest split formation could be shown at a concentration of 1.6 microgram per microliter of PF1-8-15. The analysis of kinase expression will be completed soon.

The established HSOC will serve as a testing tool for active substances interfering with the pathogenesis of PF.

P098 | Screening of an ion channel inhibitor library to unravel new therapeutic targets for pemphigus vulgaris

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Pemphigus vulgaris (PV) is an autoimmune blistering disease of mucous membranes and the skin, targeting desmoglein 1 (Dsg1) and Dsg3 in the epidermal keratinocytes. After the loss of tolerance, autoantibodies targeting these desmosomal adhesion molecules are produced. So far, there is no treatment that targets the loss of Dsg specifically in PV. Common ways to treat the disease are a high dose corticosteroid and rituximab administration downregulating the immune system. Since the development of new substances as therapeutics is a long process, this research project focuses on repurposing already approved ion channel inhibitors which are currently used for the treatment of other diseases involving ion channels. Previous experiments in the keratinocyte dissociation assay (KDA) in the context of PV could show that ion channels play an important role in the disease. In this project, we want to identify new potential therapeutics already used in other diseases and repurpose them for the treatment of PV.

The KDA is a well-established method and widely used to investigate cell-cell adhesion. Besides normal human epidermal keratinocytes (NHEKs), HaCaT cells are most commonly used in cell culture research. To mimic the effect of PV, the single chain variable fragment (scFv) PX43 targeting both Dsg1 and Dsg3 was used. This project was performed in two steps. The first step was to perform a rough screening of the complete ion channel inhibitor library provided by Selleckchem. Hence, all substances were tested in a concentration of 1 micromolar on HaCaT cells in the KDA. Promising inhibitors were identified and selected to be tested in a second step. Therefore, the promising inhibitors were tested again, this time in a concentration of 0.1, 1 and 10 micromolar to check for an optimal working concentration. So far, the investigations have shown a dose dependent reduced rate of fragmentationand other inhibitors have been eliminated.

The next steps include the further testing of these promising inhibitors in the KDA using NHEKs and further investigations of promising inhibitors in the human skin organ culture model for PV to reduce split formation in human skin.

Genetics

P099 | Serum analysis of patients with Netherton syndrome shows significant differences compared to other subtypes of ichthyoses: implication for novel therapeutic approaches

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Netherton syndrome (NS) is a syndromic subtype of ichthyosis which is characterized by scaling, erythroderma, hair shaft anomalies, growth retardation and multiple allergies. Moderate to severe pruritus is very common. Some patients show a proneness towards infections due to an associated immune defect. NS is caused by mutations in the SPINK5 gene, encoding a serine protease inhibitor called lymphoepithelial Kazal-type 5 related inhibitor (LEKTI). Treatment options are symptomatic and often insufficient.

We aimed to characterize this rare disorder in more detail to find novel targeted therapeutic approaches.

In a greater heterogeneous cohort of 57 patients with hereditary cornification disorders we included 4 patients with NS. We also recruited 63 patients with inflammatory dermatoses (psoriasis and atopic dermatitis (AD)). We analyzed 19 serum parameters (cytokines and chemokines) by Luminex 200 (TM) System (Millipore) based on manufacturer's instructions.

Two serum parameters showed significant elevated levels compared to other subtypes of ichthyosis: Interleukin(IL)-17A and thymic stromal lymphopoietin (TSLP). Of note, it is known that in AD TSLP is involved in pathogenesis of itch via activation of neurons. In our cohort TSLP levels tended to be higher than in AD. IL-15 was elevated in all ichthyoses compared to AD and psoriasis. IL-33, IL-1ßeta, IFN-γ were significantly decreased in NS. These findings were not in line with previous reports about increased IL-33 in the epidermis of patients with NS. IL-21 and CCL27 were lower than in ichthyosis vulgaris. IL-18 was reduced compared to AD and psoriasis, however, did not differ from other ichthyoses.

In a first immunological analysis we identified serum markers that were significantly different in NS compared to other ichthyoses or inflammatory dermatoses. However, as serum levels do not always correlate with findings in the skin and we could only include a small number of patients with NS, the results must be interpreted with caution. We plan to complement these findings with a healthy control group and gene expression patterns in the skin. We also aim to investigate a greater cohort with NS and would like to finalize

immunohistological stainings. Our results are in line with cases of successful anti-IL17A biologic therapy of Netherton syndrome and perhaps TSLP turns out to be a novel therapeutic target.

P100 | Nanopore sequencing for T-cell receptor repertoire analysis in patients with cutaneous T-cell lymphoma

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Mycosis fungoides (MF), a subtype of cutaneous T-cell lymphoma (CTCL), has a good prognosis if diagnosed in an early stage (IA-IIA), with an average survival time of about 10 to 20 years. A diagnostic method for early detection is therefore crucial to ensure the best treatment decisions in a timely manner. CTCL is usually characterised by a clonal expansion of T-cells and in addition, the TCF (tumor clone frequency) factor provides information about the prognosis of the CTCL patient.

The available analytical methods differ greatly in terms of accuracy, time and cost. The most accurate method is the detection of clonality in T-cell receptor gamma chain (TCRγ) and beta chain (TCRβ) by sequencing.

Illumina sequencing, the current sequencing gold standard method, is a costintensive method for TCR repertoire analysis and is therefore often not used for reasons of economy.

In this study, the sequencing accuracy of T-cell receptor amplicons using the MiSeq sequencing method (Illumina) was compared with Oxford nanopore sequencing (ONT) using short-read sequencing.

The analysis were performed with FFPE samples (n=27), fresh tissue samples (n=10), CD3 isolated T-cell samples (n=9) and different dilutions of Jurkat T-cells in peripheral blood mononuclear cells (n=6). The results from the FFPE sampels showed a significant correlation of the data generated by ONT compared with the MiSeq data with an R2=0.98 (p=0.001) for the TRG and TRB primer set. The clonality detection limit is equally between 3-10% for both sequencing methods.

In this study we demonstrated a strong correlation between the data generated by Oxford Nanopore sequencing with the gold standard MiSeq sequencing (Illumina). Therefore, ONT could represent an alternative sequencing technology for TCR repertoire analyses in the future. This may allow a more cost-efficient application for also small laboratories and clinics, as the cost of sequencing can be individually adjusted to the number of samples. This may result in higher chance of early detection of CTCL.

P101 | 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 segmental progeria, progressive severe neurological defects, UV sensitivity and short life expectancy with no efficient treatment up to now.

It is known, that in humans aging processes as well as the development of cancer can be associated with severe metabolic changes. Furthermore, it has been 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. In this study, we investigated the impact of stressors (ROS) on the metabolism of cells of patients with the premature aging syndrome CS. The cells (primary human skin fibroblasts) of CS patients as well as cells of healthy individuals (WT) were exposed to repetitive low dose UVA irradiation (inducing ROS) with subsequent measurement of metabolic changes in the supernatant of the cells, using nuclear magnetic resonance spectroscopy (NMR).

UVA irradiation induced many significant changes in metabolites (glucose, lactate, pyruvate, glutamine, glutamate, choline, alanine, betaine, acetate) in the cellular supernatant. Similar to metabolic changes in WT cells, CS cells showed UVA induced higher glucose and pyruvate consumption, as well as higher lactate and alanine secretion.

Interestingly, many of these metabolic differences between WT and CS cells occur even without applying external stressors (UVA irradiation) and aggravate upon UVA treatment.

These results point to important metabolic differences between CS and WT cells. Previous results have shown, that some UVA induced metabolic changes (glucose and pyruvate consumption) could be involved in ROS detoxification strategies of WT cells. Taken this into account, CS cells could be in need of a higher level of ROS detoxifying systems even without external stressors. These findings could be used for the development of ROS quenching therapeutic strategies to ameliorate symptoms of CS.

P102 | CRISP-Cas double nicking targets disease-causing keratin 9 variant in keratoderma to prevent intermediate filament collapse

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Keratin 9 variants are known to cause excessive thickening of palmoplantar skin (keratoderma). We report previously unknown keratin 9 variant p.E283X (c.847G>T), which generates a STOP codon. Patients carrying the latter variant do not have keratoderma, but show a lower threshold for developing skin blisters after physical stress. Accordingly, heat exposure of primary cultured patient-derived keratinocytes showed that p.E283X carrying keratinocytes were more resistant to heat stressinduced keratin intermediate filament clumping than those expressing N1615S KRT9. Thus, comparing newly-described p.E283X and the known dominant-negative keratin 9 variant p.N161S (c.482A>G), causing severe keratoderma, indicates haplosufficiency of keratin 9.

Consequently, we reasoned that introducing a stop codon into p.N161S (c.482A>G) should be a feasible gene-editing approach for mitigating keratoderma in severely affected patients. We therefore tested whether such STOP codon can be introduced into p.N161S by using a ribonucleoprotein based double-nickase approach in primary patient-derived keratinocytes. After harvesting clones with an intact wild type allele and a frameshift-induced STOP codon on the mutated strand we demonstrated improved keratin 9 intermediate filament integrity comparable to p.E283X or wild type cells as assessed by immunofluorescence staining. Upon heat stress 90% of p.N161S keratinocytes exhibited abnormal keratin aggregates, whereas in p.E283X keratinocytes and in gene edited p.N161S keratinocytes the frequency of keratin aggregates was low. To evaluate risks of unintended small insertions/deletions or single nucleotide alterations, we employed comprehensive CAST-Seq and NGS, which did not reveal any off-target translocations or mismatches.

These results (1) demonstrate a previously uncharacterized mild phenotype in patients with keratin 9 variants generating a STOP codon, and (2) they show that carrier-free electroporation of double-nickase allele-specific ribonucleoptroteins generating such STOP codon mitigates keratin filament instability with an excellent safety profile and with major therapeutic potential.

Immunology

P103 | Crosstalk between Keratinocytes and Neutrophils shapes skin immunity against S. aureus infection

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Introduction: Staphylococcus aureus infection of the skin leads to a rapid initial innate immune response with keratinocytes in the epidermis as the initial sensors. Neutrophils are the first innate immune cells to infiltrate infection sites where they provide an effective first-line of defense. Previous work of our group showed that in inflamed skin, a crosstalk between neutrophils and keratinocytes results in enhanced S. aureus skin colonization [1].

Objectives: In this work, we studied the crosstalk between keratinocytes and neutrophils in a sterile environment and upon S. aureus infection. We investigated the influence of keratinocytes on the activation of neutrophils by analyzing neutrophil lifespan, expression of degranulation markers and induction of proinflammatory cytokines. Moreover, we analyzed the influence of neutrophils on the inflammatory response of keratinocytes. Finally, we investigated the influence of the skin microbiome on neutrophil-mediated skin inflammation.

Material and Methods: Using an in vitro co-culture model we investigated the interaction of neutrophils and primary human keratinocytes by studying the neutrophil lifespan by Sytox Green staining and Annexin-V staining. Furthermore, we studied the induction of proinflammatory mediators in keratinocytes and neutrophils via Legendplex analysis and RT2 Profiler PCR arrays.

Results: We show that a crosstalk between keratinocytes and neutrophils in the skin shapes the immune response against S. aureus infections. On the one hand, the crosstalk delays the induction of apoptosis in neutrophils by an IL-8-dependent mechanism and primes neutrophils for enhanced activation and responsiveness against S. aureus. On the other hand, the crosstalk with neutrophils induces inflammatory responses in keratinocytes, which are further exacerbated in the presence of S. aureus. Finally, we show that the skin commensal S. epidermidis reduced the inflammatory effect of neutrophils in the skin and exhibits an antiinflammatory effect.

Conclusion: Our data indicate that skin infiltrating neutrophils and keratinocytes influence each other in such a way to enhance skin inflammation and that commensal bacteria are able to reduce the inflammatory effect.

1. Bitschar K. et al. Staphylococcus aureus Skin Colonization Is Enhanced by the Interaction of Neutrophil Extracellular Traps with Keratinocytes. J Invest Dermatol. 2020 May;140(5):1054-1065.e4

P104 (OP04/05) | AhR agonism by tapinarof regulates TH2 and TH17 cell function in human skin

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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 healthy skin, AD, PSO, and allergic contact dermatitis (ACD).

Using a short-term human skin explant model, we cultured skin biopsies from PSO, AD and ACD with tapinarof for 24 hours. 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. In ACD, tapinarof decreased IL-13 levels in TRM and CD4+ T cells without affecting IFN-γ expression.

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. These findings were corroborated by transcriptomic analysis of tapinarof-treated PBMCs and CD4+ memory T cells, which interestingly showed a strong concerted downregulation of fatty-acid beta-oxidation and cholesterol metabolism.

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. Since memory T cells rely heavily on oxidative phosphorylation for energy production, treating memory T cells with tapinarof could lead to a metabolic impairment.

In summary, our ex vivo model demonstrates that treatment with tapinarof significantly reduces disease-relevant cytokines in skin T cells from AD,PSO and ACD biopsies. Furthermore, we provide mechanistic evidence that this is mediated by impairment of glycolysis and oxidative phosphorylation, revealing a previously unknown mechanism of action.

P105 | IL-9 sensitizes human pathogenic Th2 cells to pro-inflammatory IL-18 signals in atopic dermatitis

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Background: Pathogenic CRTh2+ T helper 2 (pTh2) cells are crucial contributors to the pathogenesis of atopic dermatitis (AD) by secreting interleukin (IL-)13 and IL-22. Yet, IL-18 as one putative upstream regulator of pTh2 cells, is linked to AD pathogenesis by multiple lines of evidence, however, its role in activating pTh2 cells in AD skin remains unknown.

Objective: We investigated the role of IL-18 in human Th2 responses in AD by analyzing pTh2 cells from AD patients, and ex vivo skin explants of human lesional AD skin.

Results: Of all the cytokines which pTh2 cells express the receptor for, only IL-9 was able to induce high levels of IL-18R via a previously unknown TYK2/STAT1 signaling pathway. Consistently, IL-9R+ pTh2 cells were increased in blood of AD patients. Functionally, stimulation of circulating pTh2 cells with IL-18 induced secretion of IL-13, which was enhanced by co-stimulation with IL-9. Mechanistically, IL-18 induces rapid and strong activation of both NF-κB and AP-1 signaling in pTh2 cells. In human skin explants from lesional AD skin, neutralization of IL-18 downregulated IL-13 and IL-22 secretion from pTh2 cells. Finally, IL-18 protein levels correlated positively with IL13 expression in lesional AD skin.

Conclusion: Collectively, our data reveal a previously underappreciated role of IL-9 and IL-18 as positive regulators of Th2 cell responses in human AD. Further, we uncover a novel signaling cascade downstream of the IL-9R on pTh2 cells involving TYK2 and STAT1. These findings may guide future therapeutic approaches aiming at inhibiting aberrant activation of pTh2 cells in human skin.

P106 | JAK1/2 pathway-specific treatment of generalized granuloma annulare with baricitinib

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Granuloma annulare (GA) is an idiopathic non-infectious granulomatous skin disease. Unlike localized GA, which is usually self-limited and responsive to topical corticosteroids, generalized GA is often refractory to treatment and recurs frequently.

We present the case of a 66-year-old Caucasian woman with a ten-year history of generalized GA. The patient underwent various treatments including topical corticosteroids, cryotherapy, UVB therapy, methotrexate, ciclosporin, and adalimumab, but experienced only short-lived and insufficient improvement. To provide targeted therapy, a literature search on the pathogenesis was performed. A recent study using single-cell RNA sequencing revealed increased production of interferon-gamma and oncostatin M, along with upregulation of IL-15 and IL-21. These cytokines signal through the Janus kinase (JAK)-signal transducer and activator of transcription (STAT) pathways. (Wang A., J Allergy Clin Immunol. 2021) Consequently, off-label treatment with a JAK 1/2 inhibitor (baricitinib) was initiated, resulting in rapid clearance of skin lesions within six weeks. However, in the seventh week of treatment, the patient developed herpes zoster. Baricitinib administration was paused and herpes zoster was treated with valaciclovir and analgesia. After complete recovery without lasting neurological effects, the patient received the first dose of a recombinant zoster vaccine (Shingrix®). During treatment pause of baricitinib, GA skin lesions recurred. Baricitinib was resumed one month after the initial zoster vaccination without side effects, and within two weeks, its skin-clearing effects were fully restored.

This case highlights the importance of zoster vaccination prior to initiating JAK inhibitors, especially in patients over 50 years of age and those lacking varicella immunity. The first dose should be administered four weeks prior to treatment. As a non-live recombinant vaccine, the second dose can be safely administered during treatment, two months after the initial dose. In conclusion, pathway-targeted therapy presents great therapeutic potential in addressing rare inflammatory skin diseases.

P107 | Specific reduction of the viability of motile human monocytes upon tattoo ink exposure but not of skin resident cells

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Macrophages assume a pivotal role in maintaining the longevity of tattoo ink within the human skin. Nevertheless, there exists an unmet medical need for comparing these processes with those involving other skin-resident and circulating blood immune cells, as well as for conducting an in-depth analysis of REACH-compliant tattoo ink. In our study, we aimed to address the critical role played by macrophages in the long-lasting presence of tattoo ink in human skin. To provide a comprehensive perspective, we compared macrophages with other resident skin and circulating blood immune cells. We also conducted an in-depth analysis of tattoo ink compliant with the Registration, Evaluation, Authorization, and Restriction of Chemicals (REACH) framework. Our investigation began by characterizing the size distribution of ink particles using physicochemical methods. We then delved into the uptake of tattoo ink by key human skin cells and blood-derived immune cells through optical and electron microscopy as well as flow cytometry. Scanning electron microscopy unveiled the crystalline structure of the ink, with indications of aggregation tendencies upon dilution. Flow cytometric analyses of skin and immune cells after exposure to tattoo ink revealed increased cellular granularity upon uptake, particularly with red ink, which also triggered fluorescent signals. Human macrophages were most potent in internalizing ink in full thickness 3D skin models. Notably, macrophage cultures did not exhibit heightened levels of inflammatory mediators and showed no signs of toxicity, even after nine days of exposure. Interestingly, monocytes displayed the highest efficiency in ink uptake but exhibited reduced viability. In contrast, granulocytes and lymphocytes exhibited only temporary ink uptake, with flow cytometric signals diminishing after one day. Our mechanistic studies involving corticosteroids or dexpanthenol in macrophage cultures revealed that these compounds did not lead to ink excretion but, in fact, slightly increased ink retention within macrophages. These findings underscore the potentially underestimated role of highly mobile monocytes, which serve as macrophage precursors, in the translocation of tattoo ink from dermal blood vessels into internal organs.

P108 | NDP-MSH improved the integrity of inflamed endothelial cell barriers

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Dysregulation of endothelial cell (EC) barriers occurs in a variety of inflammatory diseases, destroying the normal cellular functions and leading to disease initiation. Increased levels of pro-inflammatory cytokines, such as Interlukin-1ß (IL-1ß), are a common characteristic of diseases showing increased EC permeability, suggesting that these pro-inflammatory cytokines play a role in disrupting the integrity of the EC. Of particular note, TGF-ß was found to exert its anti-inflammatory effect by decreasing the production of IL-1ß. We have previously shown that the neuropeptide derivative Nle4-D-Phe7-alpha-melanocyte-stimulating hormone (NDPMSH) reduced

the inflammation by increasing the secretion of TGF-ß. However, it still remains unclear, whether NDP-MSH has an anti-inflammatory effect on EC, therefore modulate the EC barrier function. To investigate whether NDP-MSH might modulate EC barrier integrity and potentially decrease the permeability of EC, we performed Trans-Endothelial Electrical Resistance (TEER) assays on TNF- and IL-1ß-stimulated EC. Interestingly, treatment with NDP-MSH markedly increased the electrical resistance of EC monolayers, suggesting a decreased permeability of the EC monolayers. To further analyze whether this effect was based on the NDPMSH- mediated induction of TJ proteins, we quantified the expression of Zonula occludens-1 (ZO-1) and claudin-5 in tissue sections. Immunofluorescence staining showed an increased expression of ZO-1 and claudin-5 after NDP-MSH stimulation, which resulted in decreased transmigration of CD45+ leukocytes. In support of this, real-time PCR revealed an elevated mRNA expression of claudin-5 in the tissues after NDP-MSH treatment compared to controls. Altogether, these data indicate that NDP-MSH enhanced the EC monolayer integrity by upregulating TJ proteins, such as ZO-1 and claudin-5.

P109 | The role of cyclin-dependent kinase signalling in experimental epidermolysis bullosa acquisita

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Epidermolysis bullosa acquisita (EBA) is a rare life-threatening autoimmune blistering disease characterised by the binding of pathogenic autoantibodies against human collagen VII (hCOL7) at the dermal-epidermal junction. The binding of immune complexes (IC) consisting of hCOL7 and anti-hCOL7 immunoglobulin gamma (IgG) to fragment crystallisable (Fc) receptors expressed on the surface of neutrophils is one of the key pathogenic events in the effector phase of the disease. Consequently, this Fc receptor binding triggers kinase cascades leading to the release of proteases and reactive oxygen species (ROS). This, in turn, gives rise to tissue damage manifested as skin lesions, erosions, and blisters. Currently, EBA patients are normally still treated with high-dose corticosteroids, which is often associated with partly severe side effects and immunosuppression. This raises the need for novel, targeted therapeutic strategies that require a deeper understanding of the disease mechanisms underlying EBA.

Previous studies from our group have revealed cyclin-dependent kinases (CDKs) to be differentially activated upon stimulation of neutrophils with immobilised ICs. Despite the fact that CDKs are involved in regulating essential cellular processes such as the cell cycle, transcription, and mRNA processing, their signal inhibition is a promising therapeutic option in many diseases including cancer. Although they have long been thought to play a negligible role in the terminally differentiated neutrophils, newer studies suggest the opposite, supporting our data. Moreover, it was demonstrated that CDKs can function in immune regulation. Therefore, we hypothesised that CDKs may comprise several potential therapeutic targets for EBA. Especially, topical inhibitors are promising candidates.

Hence, we analysed the expression of CDKs on RNA level in an in vitro model of EBA using IC-stimulated neutrophils. Using the same model, we decided to further investigate the effect of pharmacological CDK inhibition on human neutrophils in functional assays. Furthermore, we tested the most potent inhibitors in an in vivo mouse model of local antibody-transfer EBA.

Our results revealed significant effects in vitro regarding mRNA expression as well as ROS release, expression of surface activation markers, and adhesion for several inhibitors. Only very slight cytotoxicity was detected for some of the inhibitors at high concentrations. Three inhibitors, which were effective in suppressing ROS release, were tested in vivo showing a significant reduction of the disease score for a selective CDK9 inhibitor, MC180295, and an inhibitor selective for CDK7, THZ1 2HCl.

Taken together, our work contributes to the characterisation of kinase signalling cascades in EBA. It underlines the importance of CDKs for many cellular processes and thus, the therapeutic potential of their inhibition.

P110 (OP03/03) | Role of neutrophil extracellular traps in Epidermolysis bullosa acquisita

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Epidermolysis bullosa acquisita (EBA) is an acquired, autoimmune blistering disease (AIBD) clinically characterized by erosions and blisters on the skin, and the presence of IgG class autoantibodies to type VII collagen. EBA, when compared to other AIBD, has a decreased responsiveness to therapy, making it essential to investigate the role of various disease contributing factors.

The generation of the autoimmune responses in EBA is facilitated by the effector functions of neutrophils. Release of reactive oxygen species (ROS) and neutrophil extracellular traps (NET) are among the effector functions of PMN which contribute to tissue damage and the resulting inflammation in AIBD. Various factors are involved in the generation of NETs by PMN. Among them, myeloperoxidase (MPO), peptidylarginine deiminase 4 (PAD4), and Rab27a play an important role in degranulation, citrullination, and histone modification during the process of NET formation.

To provide preclinical proof for the role of neutrophil effector functions during the disease, a mouse model for bullous pemphigoid (BP)-like EBA was utilized. WT controls were compared to Mpo-/-,

Padi4-/-, and Rab27a-/- mice during the course of the disease. While WT mice displayed clinical signs of the disease, Mpo-/- mice were completely protected and did not develop skin lesions. While no signs of neutrophil infiltration or NETs was seen in the skin from Mpo-/- mice, skin samples from WT mice stained positive for Ly6G and CitH3 during various time points, indicating the involvement of NETs during disease development. Padi4-/- and Rab27a-/- mice showed no difference in disease scores when compared to their respective WT controls. To study the in vitro role of these factors towards neutrophil effector function, bone marrow derived PMNs from Mpo-/-, Padi4-/-, and Rab27a-/- mice and their respective WT controls were used. Mpo-/- PMN stimulated with immune complexes (IC) indicated a complete reduction in radical oxygen species (ROS) release, NOX dependent neutrophil extracellular traps (NET) formation, and leukotriene B4 (LTB4) release when compared to their WT counterparts. These differences were not observed in PMN from Padi4-/-, and Rab27a-/-mice. PMN from WT and Mpo-/- mice stimulated with IC did not indicate differences in expression of cell surface receptors CD11b, FcgRIII, FcgRIV, and C5aR1, or in chemotaxis towards C5a and LTB4. The specific role of MPO in the inhibition of ROS and formation of NETs could be confirmed by treating PMN from WT mice, before stimulation with IC, with PF-1355, a mechanism-based inhibitor of MPO.

Taken together, our results provide evidence that MPO plays a crucial role in EBA. The mode of action may be attributed to the inhibition of ROS release and LTB4, and NET formation, two disease driving forces, in response to IC.

P111 | Desmoglein 3 peptides-conjugated nanoparticles as a novel tool for tolerance induction in a preclinical pemphigus vugaris (PV) mouse model

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PV is a severe autoimmune disorder of mucous membranes and skin mainly caused by IgG autoantibodies against desmoglein (Dsg) 3. Pathogenesis depends on recognition of Dsg3 epitopes by autoreactive T cells, often restricted by HLADRB1* 04:02. Aiming at inducing antigen-specific immune tolerance, peptides representing HLA-DRB1*04:02 restricted T cell epitopes were coupled to nanosized Topas particles (TPCs).

We demonstrated the potency of TPCs in vitro to induce proliferation and cytokine release of T cells of mice transgenic for human HLA-DRB1*04:02/CD4 interaction that were previously immunized with human Dsg3. In vivo efficacy of the Dsg3 epitope-loaded TPCs was assessed by immunization of transgenic mice twice with human Dsg3 at days d0 and d14 and intravenous treatment with a mix of TPCs one day prior to each immunization. At d28 we could demonstrate a significant and concentration-dependent reduction of Dsg3-specifig IgG titers in animals treated with TPCs compared to controls. We further analyzed underlying immunological mechanisms by single Dsg3 immunization at d0 and TPC treatment at d-1. At d7, we found that TPC application compared to empty particle control induced an upregulation of FoxP3+ regulatory T cells primarily in the spleen.

Our data suggest that in Dsg3-immunized HLA transgenic mice the applied TPCmediated tolerance induction protocol leads to an early increase in potentially protective Treg cells, which might have an impact on B cell functions resulting in a reduction of anti-Dsg3 IgG antibody titers. These promising results suggest that peptide-loaded TPCs mightrestore immunological tolerance in PV via modulation of autoreactive T cells.

P112 | Quality-controlled characterization of an EC5 specific monoclonal antibody against Desmoglein 3 as a standardized tool for preclinical pemphigus analysis

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Pemphigus vulgaris (PV) is a life-threatening autoimmune blistering disease that is caused mainly by IgG autoantibodies (auto-Abs) against the cadherin-type adhesion molecule desmoglein (Dsg) 3. Binding of pathogenic Dsg3-specific auto-Abs is Dsg3 epitope-specific leading, amongst others, to signalling involved in pathogenic events such as Dsg3 depletion. As crucial tools in PV characterization and treatment development, a very limited number of antibodies, such as AK23, is applied by the autoimmune bullous disease community. Previously, we have introduced a novel EC5-binding antibody labelled 2G4. Due to its pathogenicity in-vitro and in-vivo, we consider 2G4 a powerful tool for numerous PV related analysis and research techniques. With each batch production, however, we found slight variations in pathogenicity. In order to implement proper scientific reliability, we thrived for a quality controlled production and verification process that allows I) a continuous quality improvement, and II) a verified and comprehensible overall quality with regards to pathogenic antigen-specific binding in a variety of pemphigus assays for each batch production. Finally, a work-flow

based on a standard operating procedure was established were we are able to verify purity and ex-vivo binding capacity (SDS-page), direct and indirect immunofluorescence) as primary parameters. Additionally, size analysis (mass-spectrometry) and ex-vivo pathogenicity (dispase based dissociation) can be applied.

Pemphigus related research relies on reliable tools such as Dsg-specific antibodies, quality assurance is eminent for good and reproducible scientific practice. We here present an extensive point-by-point quality controlled IgG production protocol which will serve as a basis for comparable 2G4 antibody batch release.

Pemphigus related analysis relies on respective positive control tools such as specific antibodies, quality assurance is eminent for good and reproducible scientific practice. We here present an extensive point-by-point quality controlled IgG production protocol which will serve as a basis for comparable 2g4 antibody batch release.

P113 | A Th2-Immunocompetent and Sensory Innervated Full-Thickness Skin Model for Neurocutaneous Inflammation in Atopic Dermatitis

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Itch is a leading symptom and causes a high burden of disease in patients with chronic inflammatory skin disorders. Atopic dermatitis (AD), the most common of these conditions, is characterized by eczematous lesions, dry skin and severe pruritus, particularly mediated by proinflammatory T helper 2 (Th2) cells. Type 2 cytokines including IL-4 and IL-13 can directly activate sensory neurons and thus contribute to the (itch) sensation. Although neuropeptides secreted by activated sensory neurons are known to enhance neurocutaneous inflammation, the precise immunological interplay between Th2 cells, sensory neurons, and keratinocytes remains elusive. Suitable animal or in vitro models to study neurocutaneous inflammation in atopic dermatitis are lacking. We have established here a primary cell-based, Th2-immunocompetent and sensory innervated full-thickness skin model of atopic dermatitis to investigate therapy-relevant signaling pathways.

The commercially available Phenion® full-thickness skin model is based on primary human keratinocytes seeded onto a fibroblast collagen matrix, with epidermis formation occurring during a 10-day differentiation period at the air-liquid interface. Expression analysis showed stable KRT10 expression for up to 24 days. As detected by PCR analysis, co-stimulation of the full-thickness skin model with 10 ng/ml IL-4 and IL-13 resulted in a 70.5% 10.8% (mean SD) decrease in KRT10 and 70.1% 21.4% decrease in filaggrin expression compared with the unstimulated control. The decreased filaggrin expression was also confirmed by immunohistochemistry (IHC). As expected, treatment with the IL-4Rα-directed monoclonal antibody dupilumab reversed this effect, supporting the feasibility of the full-thickness model for drug testing.

To mimic immune responses in this model, primary human CD4+ T cells were isolated from peripheral mononuclear blood cells and polarized towards Th2 cells in vitro, verified by flow cytometric analysis of the transcription factor GATA3 and IL-4 expression. Successful integration of Th2 cells in the full-thickness skin model was confirmed by IHC staining of CD3 and flow cytometric detection of CD4+ cells. Human induced pluripotent stem cells (hiPSC) were differentiated into sensory neurons in vitro according to a published protocol by Muller et al. (Acta biomaterialia, 2018), and their integration into the full-thickness skin model was demonstrated by IHC staining for β3-tubulin.

Taken together, we propose a novel testing platform to study the effects of sensory neurons and Th2 cells on neurocutaneous inflammation in atopic dermatitis.

P114 | Neutrophils show rapid catecholamine trafficking

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Neutrophils are key effector cells of the innate immune system and essential to anti-microbial defense. While previous research has mostly focused on the regulation of immune responses by classical mediators such as chemokines, recent discoveries have highlighted the role of small molecular signalling molecules such as catecholaminergic neurotransmitters in the peripheral immune system. Here, we show that neutrophils are able to rapidly uptake, package and traffic fluorescent false neurotransmitters (FFNs) and catecholamines, including dopamine, via catecholamine-specific machinery. Furthermore, we discovered that serotonin, an important neurotransmitter predominantly released by activated platelets, induced receptor-mediated exocytosis of dopamine containing vesicles via Ca2+ mobilization. Spatiotemporal release of catecholamines from neutrophils was detected by a novel fluorescent carbon-nanotube based sensor with high spatiotemporal resolution which revealed ongoing catecholamine-exocytosis over the course of several minutes. Additionally, we provide evidence that dopamine limits neutrophil activation and formation of neutrophil extracellular traps (NETs), establishing a novel paracrine signalling pathway and creating the rationale for a context-dependent, negative feedback loop in neutrophil biology.

P115 | The distribution of putative skin autoantigens is not limited to a specific type of inflammatory skin disease

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Inflammatory skin diseases (ISD) are characterized by a dysregulation of the normal immune response leading to inflammatory processes. In addition to known trigger factors such as allergens, microorganisms, drugs, diet or stress, autoantigens are also suspected triggers and have been proposed as potential key factors in the pathogenesis of ISD. In the last decades several human-skin derived autoantigens have been described, that are recognized by specific T cells and trigger their activation, e.g., KRT17, LL37, and ADAMTSL5 for psoriasis, BP180 and DSG1/3 for pemphigus and lichen planus (LP), or a-NAC for atopic dermatitis (AD).

To gain further insight into the presence and distribution of autoantigens in different subtypes of ISD, we established a comprehensive autoreactivity map for a total of 57 patients with psoriasis (n=15), AD (n=10), LP (n=10), pityriasis rubra pilaris (PRP, n=9), or healthy controls (n=13). To this end, patients' PBMCs were exposed to 8 autoantigens so far described in psoriasis (KRT17, LL37, ADAMTSL5, PA2G4, SERPINB3), AD (a-NAC), or LP (BP180, DSG-3), as well as to keratinocyte debris (KD), and proliferation rates against these antigens were analyzed by FACS using CFSE-staining. Finally, these autoantigen-specific proliferation rates were correlated with the corresponding patient-specific disease scores and durations to analyze the impact of these autoantigens on pathogenesis.

Among patients, the most frequent T cell reactivity was observed against PA2G4 (32/57, 56%) followed by ADAMTSL5 (27/57, 47%), KRT17 and BP180 (both 26/57, 46%). T cells from psoriasis, LP, but also healthy donors showed the highest, AD an intermediate, and PRP the lowest autoreactivity. The total number of positive autoantigens per patient was highest in psoriasis, followed by LP, AD and PRP, but did not correlate with disease duration, except for AD. In addition, the relative proliferation rate of psoriatic autoantigens KRT17, ADAMTSL5, LL37 and BP180 correlated positively with disease severity (PASI score) in psoriasis patients. However, disease-related autoantigens for psoriasis, LP, or AD described in the literature were not restricted to this defined ISD subtype in this cohort and were also frequently present in other ISD subtypes and healthy controls.

In conclusion, the distribution of putative skin autoantigens does not appear to be restricted to a specific type of ISD, indicating an epiphenomenon at lesion sites rather than a direct cause of the elevated immune response in ISD.

P116 (OP04/02) | Non-canonical Ki-67 accumulation in neutrophils regulates PAD4 activity and neutrophil extracellular trap formation

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Neutrophils are the most abundant white blood cell in humans and are vital to the innate immune response. Within their arsenal of defense mechanisms is the ability to form extracellular traps (NETs), which are composed of expelled chromatin mixed with histones and antimicrobial proteins. The formation of these NETs are critical to controlling infection, but can cause significant collateral damage to the host when dysregulated. As such, they are involved in a number of inflammatory conditions including psoriasis and systemic lupus erythematosus. Understanding the regulation of NET formation is therefore of high importance. Despite being post-mitotic cells, neutrophils require active cyclin-dependent kinases 4 and 6 (CDK4/6) to form NETs and reactivate expression of the highly disordered protein Ki-67, typically used as a marker of cell proliferation, upon stimulation. Here, we aimed to elucidate the role of Ki-67 in NET formation.

After sequence analysis of Ki-67, we found striking similarity to the residue composition within histone tails, the target substrate of the NETotic protein peptidyl arginine deiminase 4 (PAD4). We therefore hypothesized that Ki-67 could interact with PAD4 and its accumulation in the nucleus acts as a mechanism to delay PAD4 access to histone tails. In a cell-free recombinant protein system, we were able to show the direct citrullination of Ki-67 in the presence of active PAD4. We performed proximity ligation assays and co-immunoprecipitation to demonstrate the nanoscale proximity and direct interaction of Ki-67 and PAD4 in primary neutrophils and explored the effects of Ki-67 depletion in both a knockout mouse and a translationinhibited primary cell model. In both Ki-67-deficient models, the rate of NETosis and average nuclear areas were significantly increased. In the translation-inhibited model, chromatin velocity and histone H3 citrullination were significantly increased compared to control cells, suggesting that the difference in inhibited cells was driven by PAD4-mediated chromatin decondensation. Taken together, these results suggest that non-canonical Ki-67 serves as a novel PAD4 substrate in this context to regulate PAD4 activity and essentially slow the rate of NET formation by restricting access to histones. In a broader context, these interactions demonstrate that proteinprotein interaction based on biophysical interactions may serve to govern biological processes such as NETosis.

P117 (OP05/01) | Characterization of the Stromal Compartment of the Skin in Homeostasis and Obesity

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Obesity is known to have adverse effects on skin physiology. Dietary supplementation with the arylhydrocarbon receptor (AhR) ligand precursor indole-3- carbinole (I3C) protects from diet-induced obesity (DIO), it is however unclear whether this improves skin health as well. The AhR is a ligand-activated transcription factor that is responsible for xenobiotic detoxification by inducing three Cytochrome P450 (Cyp) enzymes, CYP1a1, CYP1a2 and CYP1b1. Its ability to act as a transcription factor is regulated by the AhR repressor (AhRR). In this project, we aimed to understand the effect of DIO and AhR signaling on skin physiology.

Skin samples of male WT, AhR-KO and AhRR-KO mice 14 weeks after feeding a HFD with and without I3C supplementation were characterized by H&E staining, IF staining, qPCR, and ELISA. WT animals showed an increase in dermal white adipose tissue (dWAT) thickness in the back skin after HFD feeding as well as an increase in adipogenesis. This was not the case for AhR or AhRR deficient mice. The expression of the antimicrobial peptide Camp, as well as AhR and its target genes were downregulated. To analyze how a longer exposure to HFD influences the skin, we analyzed mice that underwent a one-year feeding period. In line with our findings described above, expansion of the dWAT was even more pronounced after a longer exposure to HFD.

To enable us to study the effect of DIO on the stromal compartment of the skin, we developed a gating strategy for flow cytometry to define these skin cells. With AhRR/ EGFP reporter mice we analyzed EGFP expression to monitor AhR signalling. We were able to see differences in EGFP levels in distinct stromal cell populations, like preadipocytes, or reticular and hypodermal fibroblasts, comparing back and ear skin.

In summary, HFD induced dWAT expansion is dependent on AhR signalling. Deficiency of AhR or AhRR protects from an increase of the dWAT thickness. However, I3C supplementation only shows minor effects.

P118 | Early distinct immune responses in COVID-19 vaccine-breakthrough versus non-vaccinated patients

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Background: COVID-19 patients show a versatile range of severity from asymptomatic to critical conditions requiring hospitalization. SARS-CoV-2 vaccination reduces the probability for severe disease. However, the correlates of immune protection against severe disease still need to be clarified as a function of vaccination status. We investigated multiple dimensions of the immune response, including single-cell proteomics, in vaccine-breakthrough (VB) versus non-vaccinated (NV) patients, early after becoming SC2 positive, in correlation with severity.

Materials and Methods: Patients (N=270) were recruited at their first SC2 positive test or early after, and four samples were taken within the first month. Serum cytokine levels were measured by ultra-sensitive ELISA (Simoa, Quanterix). PBMCs were analyzed by singe-cell intra-cellular-staining (ICS) cytometry, Elispot, and single-cell CyTOF, before/after stimulation with Spike and Nucleocapsid peptide pools.

Results: In NV, two cytokine combinations accurately (>95%) predicted either symptom severity, mainly by type-I-interferon and IL-17, or risk of hospitalization, by the ratio of type-I-interferon to inflammatory cytokine levels.

This is different for NV and VB infections in non-hospitalized patients. The cytokine profile in VB was significantly skewed to that of asymptomatic NV.

Asymptomatics show a significantly higher frequency of Th1-related cytokine expression and a higher level of poly-functional CD4 T-cells expressing multiple cytokines.

VB asymptomatic patients exhibit an early significantly higher antibody response against the Spike antigen, in combination with a significantly stronger Spike-specific CD4 and CD8 T-cell response.

Interestingly, antibody response and Th2-related CD4 expression were not higher in NV asymptomatic patients.

Conclusions: COVID-19 severity can be accurately predicted in NV and VB as early as seven days post symptoms, which is important for guiding personalized treatment. Lower disease severity in VB is associated with a different cytokine profile than in NV patients, in correlation with SC2-specific T-cell response, which is important for guiding future vaccine development.

A potent anti-nucleocapsid CD4 Th1 response, associated with lower viral loads and lower inflammatory cytokine levels, characterizes the

asymptomatic disease course. More severe patients have less potent SC2-specific T-cell response, higher viral loads, and higher levels of inflammatory cytokines, apparently produced by monocytes rather than particular T-cells.

Keywords: SARS-CoV-2, Prediction, Inflammatory Cytokines, Type-I Interferon, Single Cell Analysis

P119 | Targeted systemic treatment modulates the relative abundance of staphylococcus aureus in patients with atopic dermatitis

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Background: Significant innovation has been achieved in treating inflammatory skin diseases like atopic dermatitis (AD) in recent years. However, the skin microbiome's role in the microbial-immunological interplay and its significance in the pathophysiological network AD still needs further elucidation.

Methods: To investigate the effect of local and systemic therapeutics on the human skin microbiome in patients with AD, we collected and analysed data and samples within the context of the ProRaD study at Augsburg and Bonn between 2017 and 2019. In total, 1077 skin microbiome swabs and medication data from 462 subjects were analysed and evaluated in the cross-sectional study design.

The patients' skin microbiome was assessed with amplicon-based 16S-rRNA nextgeneration sequencing of the V1-V3 region and bioinformatic annotation to species level with AnnotIEM. The current therapeutic regime was documented and the patients were clustered into treatment groups according to the recommendations of the EuroGuiDerm Guidelines.

Results: We could confirm a strong correlation between skin condition and the frequency of Staphylococcus aureus in skin lesions of AD patients. However, we observed no association between relative S. aureus frequency and age or sex.

In order to eliminate skin condition as a confounder, separate investigations of the skin microbiome were conducted for mild, moderate and severe disease courses. In patients with moderate AD, we found a significantly lower relative abundance of S. aureus in those receiving systemic treatment compared to those receiving topical treatment only.

Furthermore, in subjects undergoing systemic treatment, we found a trend towards differences in dependency on the active ingredient administered during systemic AD treatment. Specifically, the average relative frequency of S. aureus appeared to be lesser in participants receiving dupilumab than that of participants receiving conventional, systemic immunosuppressive therapy.

Conclusion: In our pilot study we observed and described potential correlations between AD treatment and microbiome changes within the patient collective of the ProRaD cohort. Our pilot study proves the relevance of specific immunological processes in the immunological microbial interplay in AD. However, exploring specific immunomodulatory substances and their influence on the skin barrier bears further potential for a more profound understanding of the host-immunity and skinmicrobiome interplay.

P120 | Revitalizing Treg function in Epidermolysis bullosa acquisita: a promising therapeutic insight

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Pemphigoid diseases (PD) represent a subset of autoimmune dermatoses characterized by chronic inflammation of the skin or mucous membranes, often resulting in severe blistering. One specific form is Epidermolysis bullosa acquisita (EBA), which involves the production of autoantibodies against type VII collagen (COL7). A large body of evidence suggests that neutrophils are among the key effector cells of tissue destruction. Interestingly, regulatory T cells (Tregs), among others, orchestrate the recruitment and activation of these cells. Notwithstanding the fact that Treg populations have been shown to be altered in PDs, the contribution of Tregs during the PD effector phase has been unsatisfactorily investigated. Consequently, further in-depth studies are needed to better understand the role of Tregs in detail.

To investigate the potential therapeutic impact and role of Tregs in PDs, adoptively transferred freshly isolated Tregs labeled with CFSE from healthy mice were administered intravenously one day prior to disease induction utilizing the antibody transfer-induced mouse model of EBA. A reduction in clinical disease manifestation of up to 50 % was observed in this group compared to the untreated mice group on the last day of the experiment. Also, CFSE-labeled transferred Tregs were particularly present in the inflamed skin. Furthermore, the number of Tregs in the skin following adoptive transfer was significantly higher than that in the control group, and there was a significant reduction in the number of neutrophils. Additionally, in vitro experiments revealed a decreased functional capacity in of Tregs from EBAinduced mice on neutrophils and T effector cells. However, an increase in Tregs was observed in the lesional skin compared to the control skin. These findings suggest that Tregs may lose their function in EBA, similar to other autoimmune diseases.

To further deepen our understanding of the function of Tregs in PDs, we also performed bulk and single cell RNA analysis of lesional skin and Tregs isolated from lesional skin and lymph nodes of mice with experimental EBA. Tregs from lymph nodes showed no differences between EBA and control groups. However, Tregs isolated from

lesional skin differed significantly in their gene expression profile from Tregs from healthy control skin. This suggests that Tregs in EBA lose their repressive function only after migrating into the skin. In particular, gene expression related to T cell receptor, Th17 and Th1/Th2 differentiation was significantly altered in Tregs from lesional skin. Therefore, we hypothesize that there is an instable phenotype of exhausted Tregs in EBA.

In summary, Tregs lose their suppressive function only after migrating into the skin in EBA, but therapy with adoptive Treg transfer prior to EBA induction is able to rescue this function and to reduce the clinical disease manifestation significantly.

P121 | Kinome analysis reveals potential therapeutic targets in pemphigoid diseases

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Pemphigoid diseases (PD) are subepidermal blistering diseases characterized by autoantibodies targeting the dermal-epidermal junction zone (DEJZ). The binding of autoantibodies (Aab) to the DEJZ activates the complement system, leading to the deposition of C3 and C5 along the basement membrane zone, giving rise to a local inflammatory milieu. As a result, various immune cells, primarily eosinophils, neutrophils, macrophages, and T-cells, infiltrate the upper dermis. The infiltration is followed by the release of specific proteases, cytokines, and reactive oxygen species, causing the desintegration of the basement membrane zone and the formation of subepidermal blister. Despite these insights, the precise signaling cascades activated in keratinocytes following Aab binding in PD remain unknown. However, in vitro stimulation of keratinocytes with anti-DEJZ Aab indicates their local contribution and amplification of inflammation in PD, due to the release of inflammatory cytokines.

Bullous pemphigoid (BP) is the most common PD caused by Aab against the hemidesmosomal proteins BP180 and/or BP230. BP manifests in the skin with blisters, erosions and erythema, leading to severe pruritus and a highly reduced quality of life. Epidermolysis bullosa acquisita (EBA), a less frequent PD, is induced by Aabs against collagen VII, a major component of anchoring fibrils in the DEJZ. The skin of patients with EBA shows an increased vulnerability, where blisters and erosions are mostly seen on mechanically stressed areas. The current treatment of both entities of PD involves mainly immunosuppression with corticosteroids or other immunosuppressants with potentially severe adverse events, resulting in a high medical demand of new targeted therapies with less adverse events.

Addressing this aim, insights into the kinome of keratinocytes stimulated with BP or EBA Aabs could contribute to the understanding of the underlying pathomechanisms in PD and may lead to new therapeutic targets. In our study, we detected kinase activities with a multiplex kinome analysis of keratinocytes stimulated with BP or EBA Aab from immunoapheresis material using the PamGene technology. Keratinocytes stimulated with normal human immunoglobulin G served as negative controls. By analyzing overall tyrosine and serine/threonine kinase activities within protein lysates of the stimulated keratinocytes on peptide microarrays, we could identify distinct signaling patterns in keratinocytes stimulated with BP or EBA Aab. After analyzing the signaling patterns, we selected kinase inhibitors to assess the suitability of the kinases as potential therapeutic targets. These kinase inhibitors were validated on keratinocytes stimulated with BP or EBA Aab. To access their anti-inflammatory efficacy, we measured the extent of interleukin-8 and C5a release in the cell culture supernatants.

In conclusion, the multiplex kinase analysis of keratinocytes stimulated with BP or EBA antibodies has provided valuable insights into the keratinocytes signaling cascades in PD. Moreover, the validation of their potential as therapeutic targets may provide novel leads for the development of new targeted treatment approaches.

P122 (OP05/05) | Increased expression of the Th17-associated cytokines IL-17 and IL-23 in inflamed skin of Darier disease patients as novel therapeutic targets

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Darier disease (DD) is a rare autosomal dominant inherited multiorgan disorder associated with mutations in the ATP2A2 gene encoding the sarcoendoplasmic reticulum Ca2+ ATPase isoform 2 (SERCA2). The skin is the most commonly affected organ, characterized by keratotic papules and malodorous plaques. Therapeutic options are limited and inadequate for the long-term management of this chronic disease with recurrent severe bacterial and viral infections of the skin. Using single cell RNA sequencing and NanoString technology, we performed highthroughput immunoprofiling of genes expressed in lesional skin of six DD patients. Gene set enrichment analysis of the gene expression data revealed that IL-17- signaling was enhanced in the skin of DD patients compared with the skin of healthy controls (HC). Based on these results, we examined Th17-, Th1-, and Th2-related cytokines in DD patients by qRT-PCR

and found significantly increased IL17A expression compared to HC skin. Overexpression of Th17-related cytokines (IL17A, IL17F and IL22) was confirmed at the protein level using Legendplex analysis of skin sections. Due to these results we administered monoclonal antibodies targeting the overexpressed cytokine to three treatment-refractory patients and observed a clinical improvement of skin manifestations. We demonstrate enhanced expression of Th-17- related genes in DD and might provide new options for the long-term management of skin inflammation in these patients.

P123 | Bullous pemphigoid induced by IgG targeting murine type XVII collagen non-NC15A extracellular domains is driven by Fc gamma receptor-mediated effector mechanisms and ameliorates with neonatal Fc receptor blockade

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Bullous pemphigoid (BP) is an autoimmune blistering disease characterized by autoantibodies targeting type XVII collagen (Col17, also known as BP180). The noncollagenous 16A (NC16A) ectodomain represents the major immunodominant site and the pathogenic role of antibodies directed against human NC16A and its murine homologue NC15A has been shown in various experimental models. To demonstrate the pathogenic potential of additional extracellular targets of Col17 downstream of murine NC15A, we recently established a BP mouse model by transfer of rabbit IgG against a murine fusion peptide consisting of the Col17 NC14-1 domains outside NC15A, which led to the development of erythematous skin lesions and erosions. Disease activity was accompanied by IgG/ C3 deposits along the dermal-epidermal junction and subepidermal blistering with inflammatory infiltrates in the upper dermis. To investigate the pathogenic importance of Fc gamma receptors in the BP mouse model, anti-NC14-1 IgG was injected repeatedly over 12 days in adult Fc gamma receptor-deficient and C57BL/6J wildtype mice. At Day 12, knock-out vs. wildtype mice showed a significant reduction in the affected body surface area ($p<0.0001$) accompanied by a reduction in skin histological signs ($p<0.01$) and changes in the lesional inflammatory cell infiltrate. In addition, pharmacological modulation of experimental BP by inhibition of the neonatal Fc receptor (an atypical Fc gamma receptor regulating IgG homeostasis) with the murine Fc fragment IgG2c-ABDEG, a derivative of efgartigimod, reduced (i) anti-NC14-1 IgG serum levels, (ii) binding of IgG and C3 along the dermal-epidermal junction, and (iii) skin inflammation compared with isotype-treated controls. These data demonstrate that pathogenic effects of IgG targeting the Col17 domain outside NC15A are partly attributable to antibody-mediated Fc gamma receptor effector mechanisms while inhibition of the neonatal Fc receptor is a potential treatment option for BP and is being evaluated in ongoing clinical trial.

P124 | T Cell Receptor Repertoire Analysis During the Pathogenesis of the Autoimmune Skin Blistering Disease Epidermolysis Bullosa Acquisita

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Epidermolysis bullosa acquisita (EBA) is an organ-specific autoimmune disease and associated with tissue lesions and subepidermal blisters. The pathogenesis of EBA involves the production of autoantibodies targeting type VII collagen, a structural element of the skin's dermal-epidermal junction. To study the pathophysiology, an experimental model has been established by immunizing mice with recombinantly produced domains of murine type VII collagen (mCol7c) emulsified in adjuvant. It has been suggested that the initiation of the disease depends on the formation of autoreactive T cells, which lose the tolerance to the self-antigen mCOL7c. In previous experiments, it has been demonstrated that pretreatment with xenogenic proteins such as Ovalbumin can completely suppress EBA pathogenesis. Surprisingly, mCol7-specific IgGs, responsible for disease development, are still found at the dermal-epidermal junction of inflamed lesions suggesting that these specific IgGs are not directly affected by this pre-treatment. Thus, the mechanism behind this Ovalbumin pre-treatment's inhibitory effect on EBA development remains unknown. Based on these findings, we hypothesized that Ovalbumin pre-treatment might hinder the formation of dominant autoreactive T cell clonotypes by introducing more clonal competition. Investigating this hypothesis, we performed next-generation sequencing of T C cell Receptor β after RNA isolation of the activated lymph nodes (Alns). Our results indicate that the dominant T cell clonotypes overlap between all Alns in the diseased mice as well as the Ovalbumin pre-treated (healthy) mice, Interestingly, we observed that this overlap is higher in the EBA group. This data leads to the hypothesis that not only the formation of dominant T cell clonotypes but also the diversity of the locally present T cell clonotypes impact the pathogenesis of EBA.

In conclusion, our data support the notion that pre-treatment with a foreign protein can hinder the development of an autoimmune disease by influencing the diversity of the T cell receptor repertoire.

P125 | Lebrikizumab reduces systemic inflammation in serum of patients with moderate-to-severe atopic dermatitis

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Lebrikizumab is an interleukin (IL)-13 inhibitor that has completed phase 3 studies. It demonstrated statistical superiority vs. placebo in patients with moderate-to-severe atopic dermatitis (AD) across all primary and key secondary endpoints at week 4 and week 16 of ADvocate1 (NCT04146363) and ADvocate2 (NCT04178967). The objective of this analysis is to determine lebrikizumab's impact on AD-relevant serum biomarkers in patients from these studies through week 16.

Protein biomarkers were determined in available serum samples from patients receiving lebrikizumab 250 mg every 2 weeks (n=72) or placebo (n=36) from both ADvocate1 and ADvocate2. Pre- and post-treatment biomarkers were compared to healthy controls (HC, n=30) which were age, sex, race, and ethnically matched. The analysis included biomarkers known to be elevated in AD. IL-13 was only measured at baseline because of lebrikizumab's known interference with IL-13 measurement.

At baseline, CCL13, CCL17, CCL22, total IgE, IL-5, IL-13, and periostin were elevated in patients with AD ($p<0.001$) with IL-13 measuring approximately 7-fold greater in the AD population vs. HC. In patients with AD, IL-4, CCL11, CXCL10, CCL2, and CCL4 were not elevated at baseline vs. HC.

At week 4, lebrikizumab significantly reduced levels of the key type 2 biomarkers, CCL13, CCL17, and periostin vs. placebo ($p<0.01$) with a trend toward levels consistent with HC (within 1.5-fold). At week 16, CCL13, CCL17, and periostin levels remained consistent with HC levels with CCL13 and periostin retaining statistically significant reductions vs. placebo ($p<0.01$).

In sum, selective targeting of IL-13 with lebrikizumab monotherapy reduced known molecular biomarkers of systemic type 2 inflammation in patients with moderate-tosevere AD.

P126 | Lebrikizumab treatment results in rapid improvement of atopic dermatitis disease cytokines and pathways

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Lebrikizumab is a monoclonal antibody specifically targeting interleukin (IL)-13. It demonstrated statistical superiority vs. placebo in patients with moderate-to-severe atopic dermatitis (AD) across all primary and key secondary endpoints at week 4 and week 16 of ADvocate1 (NCT04146363) and ADvocate2 (NCT04178967). The objective of this analysis is to determine the biological pathways by which lebrikizumab treatment positively impacts clinical severity measures in patients with AD by investigating changes in serum proteins using the Olink® Explore 3072 biomarker panel.

Protein biomarkers were determined in available serum samples from a subset of ADvocate1 and ADvocate2 patients who consented to biomarker sampling. Patients were dosed with lebrikizumab 250 mg every 2 weeks (n=72) or placebo (n=36) and were compared to age-, sex-, race-, and ethnically-matched healthy controls (HC, n=29). The analysis included biomarkers that were detected in at least 25% of patients. A linear model R package (limma) compared biomarker changes in patients treated with lebrikizumab vs. placebo from baseline to weeks 4 and 16. Gene set enrichment analysis was performed using a curated pathway and protein signature database for AD.

The Olink biomarker data revealed that, following lebrikizumab treatment, CCL26 (eotaxin-3) was significantly reduced from baseline as early as week 4 and progressively to week 16. Several AD-related pathways were significantly changed during lebrikizumab treatment. Additionally, the level of biomarkers in lebrikizumabtreated AD patients approached HC levels as early as week 4 and continuously to week 16. This trend was not seen with placebo-treated patients.

In patients with AD, selective targeting of IL-13 with lebrikizumab treatment rapidly interrupts and normalizes several biomarker pathways toward healthy control levels. Data analysis across the curated pathways demonstrated changes in both serum immune response related protein signatures as well as skin cell specific signatures from keratinocytes and fibroblasts suggesting lebrikizumab treatment improved multiple facets of disease activity.

P127 (OP01/03) | ZBP1 and Mitochondrial Z-DNA Drive Autoimmune Photosensitivity

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Background: Autoimmune photosensitivity is observed in type I Interferon (IFN) mediated diseases such as systemic and cutaneous lupus erythematosus (SLE/ CLE) and dermatomyositis. We

previously identified a type I IFN-rich environment in nonlesional lupus skin that leads to inflammatory education of myeloid cells. Type I IFN has been described as an activator of UV-induced immune responses, but how a chronic IFN-high environment drives photosensitivity is not understood. Mitochondrial DNA has been identified as a source of IFN responses via activation of cGAS-STING in diverse cells of SLE. Here, we investigated how UV light and type I IFN exposure impact mitochondrial stress and Z-DNA formation, a left-handed dsDNA primarily localized in mitochondria which leads to type I IFN production through activation of cGAS.

Methods: Confocal microscopy of primary keratinocytes (KCs) from healthy controls and SLE patients and N/TERT immortalized KCs was performed to assess mitochondrial dynamics and cytosolic Z-DNA formation after UV exposure and IFN-α treatment. qPCR and single cell RNA sequencing was used to assess gene expression. Tissue immunofluorescence was used for protein expression of ZBP1. Transfection of Z-DNA was performed in primary KCs and N/TERTs to assess downstream signaling compared to B-DNA. shRNA-mediated knockdown of ZBP1 was performed in N/TERTs. We generated a GFP-ZBP1-FLAG tagged KC cell line in N/TERTs and compared it to a NTERT line expressing GFP alone.

Results: After UV light exposure, KCs showed significantly upregulated gene expression of IFNB, IFNK, IFNL, MX1 and OASL. This upregulation was significantly inhibited by preincubation with the mitochondrially-targeted antioxidant mitoTEMPO (MT), indicating mitochondrial reactive oxygen species-dependent IFN responses. Additionally, mitochondria showed significant fragmentation after UV light that was associated with cytosolic Z-DNA accumulation. Strikingly, this accumulation was enhanced with IFN incubation leading to large Z-DNA puncta within the cytosol. Primary SLE KCs exhibit cytosolic Z-DNA at baseline and showed strong cytosolic Z-DNA accumulation after UV exposure. Cytosolic Z-DNA accumulation and UVinduced ISG expression was prevented by MT in SLE KCs. Importantly, ZBP1, the cytosolic sensor of Z-DNA, is induced by IFN-α and upregulated in nonlesional and lesional SLE, childhood SLE and juvenile and adult dermatomyositis skin biopsies but is not detectable in healthy control biopsies. Confocal analysis showed colocalization of Z-DNA with ZBP1 and cGAS after IFN+UVB exposure. Mechanistic studies revealed that Z-DNA promotes a stronger type I IFN immune response compared to B-DNA through activation of the cGAS-STING pathway. Knockdown of ZBP1 in NTERTs attenuated ISG expression after UVB in an IFN-high environment. Overexpression of ZBP1 results in a lupus-like phenotype of KCs with a spontaneous IFN signature.

Conclusion: Our data indicate that type I IFN priming, coupled with UV light exposure, results in mitochondrial stress that leads to increased mitochondrial Z-DNA formation and cytosolic Z-DNA release. Cytosolic Z-DNA interacts with ZBP1 and cGAS to activate STING-dependent IFN upregulation. Collectively, we describe a new pathway of mitochondrial Z-DNA sensing by ZBP1 that drives and sustains IFN responses in KCs, giving further insight into autoimmune photosensitivity.

P128 (OP02/04) | Distinct roles for barrier disruption and S. aureus provoking IL-13 and IL-4 to mount a type 2 immune response in a preclinical model for atopic dermatitis

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Atopic dermatitis (AD) is a chronic inflammatory skin disease typically characterized by skin barrier defects and microbial dysbiosis. Clinical trials and patient data demonstrated the central role of type 2 cytokines for AD and therapeutically targeting type 2 cytokines re-established barrier function and cutaneous microbial composition. However, how both barrier disruption and dysbiosis drive AD pathogenesis and the underlying type 2 inflammation is much less understood. To disclose the impact of barrier impairment and microbial dysbiosis on type 2 inflammation in AD, we set up a new animal model which includes barrier disruption by repeated tape stripping (TS) and forced dysbiosis by application of Staphylococcus aureus (S. aureus). Interestingly, barrier disruption alone already induced a microbial dysbiosis, favoring the predominance of S. aureus. This was accompanied by a downregulation of skin barrier proteins, upregulation of proinflammatory cytokines and an increased transepidermal water loss (TEWL) like in AD patients. In contrast, cutaneous application of S. aureus induced an upregulation of barrier proteins and early type 2 mediators including IL-33 and TSLP, indicating different roles of barrier impairment and bacterial dysbiosis in the initiation phase of AD. As expected, combining barrier disruption and dysbiosis resulted in a worsening of the clinical picture and functional data such as high TEWL and increased epidermal proliferation. To assess early induction of type 2 cytokines we applied our model to IL-4 and IL-13 reporter mice. Here, we detected an increased infiltration of IL-13-expressing immune cells in the skin, indicating that IL-13 acts as an effector cytokine already during the initiation phase of inflammation. At later stages of inflammation, bacterial dysbiosis shaped the innate immune axis and induced high amounts of IL-13+ ILC2s in the skin, independent of barrier damage. In contrast, the induction of IL-4+ T helper cells in skin and skin-draining lymph nodes needed stronger signals as it depended on the combined effects of S. aureus and barrier disruption. This indicates that in the early phase of type 2 and AD inflammation, barrier disruption and bacterial dysbiosis play distinct roles to induce first IL-13 and consecutively IL-4. In summary, barrier impairment and bacterial dysbiosis differently modulate the establishment of the type 2 response but join forces to manifest the inflammation.

P129 | Involvement of Alternative C5-Convertases in Inducing Skin Inflammation in Experimental Pemphigoid Diseases

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Pemphigoid diseases (PDs) represent a group of autoimmune blistering disorders that have intrigued dermatologists and immunologists for decades. These conditions are characterized by the autoantibodies targeting critical structural proteins in the skin surface-close mucosal tissue, resulting in the detachment of the epithelium from the underlying layers. The presence of linear IgG and C3 deposits serves as a diagnostic hallmark for PD. Previous research has identified inhibition of the C5/C5aR1 axis as a central factor in driving skin inflammation in experimental murine PD. Nonetheless, the significance of C3 in PD remained inconclusive. In ourstudy, we employed antibody transfer-induced PD models in mice lacking C3 and could show that experimental PD, including epidermolysis bullosa acquisita (EBA), bullous pemphigoid (BP), and mucous membrane pemphigoid (MMP), manifests independently of C3. This suggests that C5a generation occurs proteolytically independent of C3 in these model. Subsequently, we examined the involvement of alternative C5-convertases using the antibody transfer-induced EBA mouse model. Blocking thrombin resulted in a notable reduction in clinical disease severity although the reduction was less pronounced compared to mice lacking the C5/ C5aR1 axis, indicating the likely existence of additional alternative pathways for C5a production. Further experiments exploring the contribution of thrombin in established MMP and BP mouse models confirmed the requirement of thrombin in MMP induction. In summary, our findings provide compelling evidence that skin inflammation in experimental PD can occur independently of C3 and suggest that thrombin plays a functionally relevant role in C5a generation and antibody mediated tissue destruction.

P130 | A systematic review of nicotine-induced changes in neutrophilic granulocytes: possible implication to hidradenitis suppurativa

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Background: Neutrophilic granulocytes (neutrophils) play a role in the pathogenesis of many chronic-inflammatory diseases (CIDs) including the skin diseases hidradenitis suppurativa (HS) and palmoplantar pustulosis (PPP). Smoking is a major co-factor of many CIDs, and over 70% of HS and PPP patients are current or exsmokers. In order to shed light into the evident connection between smoking and neutrophil-driven CIDs, we performed a systematic revision and critical evaluation of published results on the molecular and functional changes in neutrophils induced by nicotine.

Objective: We evaluated the direct influence of nicotine on human neutrophil functions, specifically on cell death/ damage, apoptosis, chemotaxis, general motility, adhesion molecule expression, eicosanoid synthesis, cytokine/ chemokine expression, formation of neutrophil extracellular traps (NETs), phagocytosis, generation of reactive oxygen species (ROS), net antimicrobial activity, and enzyme release.

Methods: The review was conducted according to the PRISMA guidelines and a literature search was performed in the databases NCBI Pubmed® and Web of ScienceTM in February 2023. Inclusion criteria comprised English written research articles, showing in vitro studies on the direct impact of nicotine on the specified human neutrophil functions. Of the 532 originally identified articles, data from 34 articles were finally compiled after several evaluation steps.

Results: The nicotine concentrations used in the retrieved studies greatly varied between 0.5 nmol/l and 20 mmol/l. While at high concentrations, above ~3 mmol/ l, nicotine started to be cytotoxic to neutrophils, concentrations typically achieved in blood of smokers (in the nmol/l range) applied for long exposure times (24-72h) supported the survival of neutrophils. Smoking-relevant concentrations of nicotine seem to increase the chemotaxis of neutrophils towards several chemo-attractants but reduced their integrin expression. Moreover, nicotine elevated the production of lipocalin-2, CXCL8, leukotriene B4 and prostaglandin E2 by these cells, assumingly leading to the attraction of further neutrophils to the tissue. In contrast, there was no effect on classical pro- (TNF-alpha, IL-1-beta) and anti-inflammatory cytokines (IL-10). Neutrophils are important players in the early antimicrobial defense, once they arrive in the tissue. Based on the current literature findings, the phagocytic function assessed by the uptake of microspheres, bacteria and immune globulineobsonized sheep erythrocytes and the antimicrobial activity, proven by the death of some bacteria species after neutrophil encounter, was reduced by nicotine. Another way neutrophils kill bacteria is the formation of NETs, which was found to be elevated in nicotine-induced neutrophils. No clear impact was found on ROS formation and release of the enzymes beta-glucuronidase, lysozyme and myeloperoxidase. The studies varied in methodological aspects, making it necessary to expand studies to gain clearer insights.

Conclusion: The systematic review revealed that nicotine seems to support the presence of neutrophils in the tissue, while reducing their antimicrobial functions. This suggests that, via influencing neutrophils, nicotine contributes to the pathogenesis of CIDs.

P131 | Investigation of immune-cell metabolism in human skin using flow cytometry

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Cellular metabolism and function are fundamentally intertwined. Thus, immune cell metabolism has become a key interest in biomedical research. Nevertheless, immune cell metabolism in human skin remains underinvestigated. In this project, we use a recently developed, flow-cytometry-based method, called SCENITH (Single Cell ENergetic metabolism by profiling Translation inHibition), to study immune cell metabolism in human skin. Almost 50% of the energy cells produce is immediately consumed in protein synthesis. Consequently, protein synthesis directly correlates with overall ATP cellular levels and therefore metabolic activity. SCENITH measures protein synthesis in cells incubated with inhibitors of specific metabolic pathways to determine cellular-metabolic dependencies and capacities. To measure protein synthesis the method relies on the incorporation of puromycin into nascent proteins. Precisely, cells are treated with puromycin and its incorporation into proteins is then measured by flow cytometry via a conjugated anti-puromycinantibody. To establish SCENITH for skin immune cells, we generated single-cell suspensions from human skin punch biopsies by combining mechanical dissociation with enzymatic degradation. We included patients with different skin pathologies. In pilot experiments, we conducted SCENITH on immune cells from inflammatory and malignant skin conditions, such as granulomatous skin inflammation and keratinocyte-derived skin cancer. We find that SCENITH is a valuable technique to profile energy metabolism from multiple skin cell subsets ex vivo with single-cell resolution.

P132 | Glucocorticoids and their influence on monocytes phagocytosis

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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. 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 show that the ability of monocytes to phagocytosis is significantly improve by GC treatment. We stimulated human monocytes for 48h with GC and subsequently analyzed their phagocytic activity for different time points. We could demonstrate that GCs exhibit pro-phagocytic effects in monocytes resulting in enhanced uptake of latex beads as well as pathogens like Leishmania major and Bacteria and that this uptake happens during the first 15min of incubation. Interestingly, in addition to pathogens and latex beads we could also observe that carboxylate modified latex beads (mimicking apoptotic cells) or granulocytes are increasingly taken up by monocytes stimulated with GCs. However, not only the phagocytic activity of monocytes is enhanced by GCs, but also their ability to eliminate pathogens. These data indicate that GC-treated monocytes are important for the resolution of inflammation but at the same time are still capable to neutralize pathogens. Our next steps are to identify the molecular mechanisms involved in this enhanced phagocytosis as well as the improved elimination of pathogens by GCtreated monocytes. Thus, we reveal a new strategy of GCs -possibly as cell therapyto fight inflammation without increasing the risk of infection.

P133 (OP03/01) | Type 2-mediated metabolic reprogramming regulates the macrophagefibroblast crosstalk in skin repair

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Type 2 inflammation is a core driver of tissue repair, however, if sustained it also contributes to the development of pathological tissue fibrosis. Hence, pharmacological blocking of type 2 cytokine-signalling provides a promising strategy to prevent fibrosis. Yet, despite the relevance, molecular features driving type 2- mediated repair and fibrosis remain largely unknown. Macrophages (MFs) play a pivotal role in wound healing and their dysfunction abrogates repair and promotes tissue fibrosis. The mechanistic basis of type 2-activated MFs in repair and tissue fibrosis is unclear. Here we addressed the question whether type 2-elicited oxidative metabolism (OXPHOS) in wound MFs contributes to their pro-fibrotic function. In this project, we leveraged novel genetic resources (including conditional mouse models, multiomic datasets) to tackle the regulation and function of OXPHOS in wound MFs. By generating mice with myeloid cell-specific IL-4Ra deletion, we identified type 2 signalling as critical regulator of OXPHOS and expression of pro-fibrotic mediators specifically in late-phase wound MFs. To investigate the functional impact of OXPHOS on wound MF activation, we generated mice with myeloid cell-specific Cox10 deletion (deletion of complex IV of the electron transport chain) (Cox10MKO mice). Skin wound healing in Cox10MKO mice was severely impaired. We showed by enzymatic double staining of complexes II and IV of the

electron transport chain that OXPHOS-deficient macrophages in Cox10MKO mice accumulated in defined areas of the wound environment and created defined metabolic niches at the wound site. Interestingly, within these metabolic niches myofibroblast differentiation was virtually abolished and granulation tissue formation was impaired. Transcriptomic profiling of Cox10-deficient MFs revealed that OXPHOS is required to promote the switch from inflammatory towards repair stage. OXPHOS-defective wound MFs also failed to downregulate inflammatory gene expression (Il1b, Nos2) and to induce pro-repair genes (Retnla, Mrc1). Moreover, analysis of wound cell suspensions by flow cytometry revealed significantly lower relative numbers of CD301b+ reparative wound MFs in Cox10MKO compared to control mice. Proteomic analysis of co-cultured repair-activated macrophages (in OXPHOS mode) with fibroblasts identified macrophage-secreted pro-repair mediators that induced myofibroblast differentiation. Collectively, our findings identified a novel role of IL-4Ra-induced OXPHOS in directing the macrophage-fibroblast crosstalk in skin repair. We show that OXPHOS in wound MFs is required for myofibroblast differentiation by balancing both downregulation of potent early-phase pro-inflammatory mediators and induction of pro-fibrotic late-phase repair programs. Our findings reveal a mechanistic basis for repurposing type 2 blockers to target OXPHOS in wound MFs and to prevent tissue fibrosis.

P134 | The role of GPR31, the 12(S)-HETE receptor, in psoriasiform dermatitis

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Psoriasis is a chronic inflammatory disease with a worldwide prevalence of 2-3%, primarily affecting the skin and characterized by hyperproliferation of keratinocytes. The presence of T lymphocytes, neutrophils, macrophages, dendritic cells (DC), and platelets make psoriasis a disease with an inflammatory response alongside the hyperproliferation. Psoriatic skin lesions indicate a marked increase in lipid mediator 12-(S)-hydroxy-5,8,10,14-eicosatetraenoic acid (12(S)-HETE). In mice, 12(S)-HETE is biosynthesized from arachidonic acid due to 12/15-lipoxygenase and recognized by GPR31. In this study, we aimed to understand the role of GPR31, the 12(S)-HETE receptor, in disease progression using in vivo and in vitro models.

To investigate the pathophysiological role of GPR31 in psoriasis, a series of experiments using Gpr31b-/- mice and their littermates in three in vivo models for psoriasiform dermatitis, and in vitro psoriasis models using primary keratinocytes (KC) cells were conducted. In vivo, while WT mice displayed clinical signs of psoriasis upon topical application of Aldara on shaved back skin, Gpr31b-/- mice, in comparison, displayed significantly higher disease scores. Specifically, Gpr31b-/- mice presented with higher desquamation scores with significant differences to WT littermates occurring starting on day 4 after the induction of the disease. Though not significant, analysis of the skin from Gpr31b-/- and WT mice treated with Aldara revealed increased epidermal thickness of skin measured by microscopic analysis, and increased relative gene expression of Il1b and Il23. Topical treatment of mouse ears with TPA, and subcutaneous injection of IL-23 into the ears of mice did not show any significant differences in epidermal thickness measurements between WT and Gpr31b-/- mice. To ascertain the specific role of GPR31 in disease progression, we analyzed the function of DC and primary keratinocytes in the absence of GPR31b. CD86 expression on DC upon 24 h stimulation with imiquimod (IMQ), the active component of Aldara, was lowered in cells from Gpr31b-/- mice when compared to WT. Despite lower activation of CD86 on IMQ stimulated Gpr31b-/- DC, there was no difference in the production of pro-inflammatory cytokines TNF-α and IL-10 when compared to that from WT DC. To investigate the role of GPR31b on keratinocytes, primary keratinocytes (KC) were cultured from tail skin on Gpr31b-/- and WT mice and stimulated with 5 ng M5 (IL-17A, IL-22, IL-1 α, TNFα and Oncostatin M). Preliminary results indicate lowered relative gene expression of Cxcl1 and Cxcl2 in primary KC from Gpr31b-/- mice when compared to WT mice when stimulated with M5 for 48 h. Taken together, both in vivo and in vitro results indicate that the role of GPR31 is dependent on the stimulated pathway and might be specific to each of the disease models and cell types investigated. The intricate dynamics of psoriasis warrant a thorough investigation of the precise involvement of GPR31 in psoriasiform dermatitis.

P135 | Regulation of the inflammatory response during wound healing: why macrophage cell death matters

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Inflammation in the early stage of wound healing is critical for physiological repair. However, the extent and length of the inflammatory phase requires timely regulation, as dysregulations are linked to inflammatory skin diseases and impaired wound healing. Macrophages are key players in wound healing, participating both in the mounting of the inflammatory response in the early stage, and in its resolution in the late stage of wound healing. Despite their ability to undergo a phenotypic switch from pro-inflammatory to anti-inflammatory, the overall number of macrophages following the inflammatory phase reduces significantly, in part through cell death. Yet, the mode of cell death and the impact of different cell death modalities on tissue morphology remain elusive.

To address these questions, we explored the role of macrophage-specific FADD mediated cell death on RIPK3-/- background (Faddfl/flRipk3-/-Cx3Cr1Cre; FaddMKORIPK3-/-) in a mechanical skin injury

model in mice. Analysis revealed that the combined inhibition of RIPK3-mediated necroptosis and FADD-Caspase-8- mediated apoptosis in macrophages significantly impairs wound healing. Delayed wound healing was characterized by i)reduced granulation tissue formation, ii) reduced myofibroblast differentiation, iii) increased number of neutrophils (7- AAD-CD45+CD11b+Ly6G-F4/80+), and iiii) increased number of inflammatory macrophages (7-AAD-CD45+CD11b+F4/80+Ly6Chigh), notably prominent in the mid-phase of repair. Interestingly, combining flow cytometry analysis with our previously published single cell sequencing data of wound macrophages, we revealed a substantially higher expression of Fadd and Ripk3 particularly in the Ly6Chigh inflammatory macrophage subpopulation. These findings suggest a wound macrophage subpopulation specific differential sensitivity for apoptosis and necroptosis. Mechanistically, we found a significantly upregulated expression of TNF in wound macrophages isolated from FaddMKORIPK3-/- mice, suggesting that the overproduction of inflammatory cytokines could cause the impaired healing. To evaluate the contribution of TNF signaling to the delayed wound healing in FaddMKORIPK3-/- mice, we subjected the mutants to a pharmacological inhibition of TNF using Etanercept. Our analysis revealed a significantly improved wound healing and a reduced number of inflammatory wound macrophages following TNF blockade, and we hypothesize that the impaired wound healing in FaddMKORIPK3-/- mice is at least partially TNF-driven. TNF inhibits the phenotypic switch from inflammatory towards pro-repair macrophages. Our hypothesis is supported by a significantly increased Ly6Clow/medium wound macrophages population, and an increased expression of pro-repair programs in wound macrophages in FaddMKORIPK3-/- mice treated with Etanercept.

Overall, our findings establish macrophage extrinsic apoptosis as a contributing mechanism in the resolution of inflammation during skin wound healing. Our findings suggest that different macrophage sub-populations do not only have a distinct function in repair, but also a defined fate through a specific mode of cell death.

P136 | Comparison of different keratinocyte cell line models for NLRP1 inflammasome activation

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The NLRP1 inflammasome is the most relevant inflammasome in human keratinocytes. It plays a crucial role in the regulation of innate immunity. Upon stimulation, the NLRP1 inflammasome can activate caspase-1, which is involved in IL-1β processing and secretion as well as for the execution of the pyroptotic cell death. Here, we compared four different human keratinocyte cell lines regarding NLRP1 activation and function. Two different stimuli of NLRP1 activation, namely UVB irradiation and a talabostat (Val-boroPro, VbP, or PT-100) were chosen. We directly compared the NLRP1 activation of HaCaT immortalized keratinocytes, the recently characterized HaSKpw immortalized keratinocytes, NHEK/SVTERT3-5 immortalized keratinocytes, and primary keratinocytes. The effect of both NLRP1 inducers on the cell viability and on the modification of key NLRP1 molecules by crystal violet staining, FACS analysis and western blotting were investigated. The main advantages of immortalized keratinocyte cell lines compared to primary cells are the cost effectiveness, the possibility to generate reproducible results, easier gene manipulation, and unlimited growth. However, their disadvantages are changed characteristics such as reduced sensitivity to cell death, changes in gene expression patterns, and modifications in signaling pathways. Conversely, primary keratinocytes are particularly advantageous, as they are isolated directly from healthy skin and retain the morphological and functional properties of the original tissue.

Here, we demonstrate that stimulation of primary keratinocytes with talabostat for a duration of 24 hours results in increased cell death. In contrast, under the same conditions, no enhanced cell death is observed in the three immortalized keratinocyte cell lines after up to 72 hours of talabostat treatment. However, UVB irradiation of all four cell types results in a significant increase of cell death, suggesting that not only the NLRP1-induced pyroptosis might be triggered, but also other forms of cell death, such as apoptosis and necroptosis are as well activated. Altogether, these data confirm that, in the progress of UVB radiation or talabostat treatment, primary keratinocytes are more sensitive to cell death than passaged keratinocyte cell lines.

Importantly, the expression of mature IL-1β and caspase-1 p20 in the supernatant of talabostat stimulated-primary keratinocytes is clearly detectable after 12 hours of stimulation. In contrast, no secretion of IL-1β and caspase-1 p20 is present in the supernatant of HaCaT, HaSKpw and NHEK/SVTERT3-5 after up to 48 hours of talabostat stimulation. In contrast to talabostat treatment, UVB-induced inflammasome activation of HaSKpw cells results in the secretion of low but detectable amounts of secreted IL-1β and caspase-1 p20. Moreover, upon UVB irradiation of HaCaT and NHEK/SVTERT3-5, no processed caspase-1 and IL-1β are found by western blotting. These differences show that the primary keratinocytes are the model of choice when NLRP1 activation and function are analyzed. Despite the limitations related mainly to the short lifetime and more challenging gene manipulation, the achieved detection of NLRP1 activation in primary keratinocytes was of the highest quality, compared to the analyzed immortalized cell lines.

P137 | IL-15 neutralization results in the prevention/amelioration of skin barrier defects and pro-inflammatory environment in AD lesions experimentally induced in human xenotransplants

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Atopic Dermatitis (AD) is a common inflammatory skin disease characterized by skin eczema, pruritus, scaling, and dry skin. In particular, Th2 cytokines, such as IL-4, and IL-13, have been described to play a major role in inducing the inflammatory cascades in AD. In contrast, little is known about the contribution of the proinflammatory cytokine interleukin-15 (IL-15) in AD, although high IL-15 expression levels are reported in AD lesions. The pleiotropic functions of IL-15 on the immune system, e.g controlling activation of T-, B-, NK- and generation of tissue resident memory T cells, and on keratinocytes, e.g. regulating proliferation and apoptosis, further support its involvement in AD development. In this study, we aimed at investigating whether IL-15 signaling contributes to AD pathogenesis by using our previously established AD humanized mouse model. Therefore, skin from (n=3) healthy donors was xenotransplanted onto SCID mice and injected with autologous PBMCs, which were in vitro prestimulated with IL-4/IL-2/LPS. As expected, AD lesions developed in human skin grafts within two weeks after injection of the PBMCs. AD development was also confirmed by the enhanced epidermal thickness and reduced filaggrin expression, indicating barrier defects, and the Th2-prominent pro-inflammatory environment, along with Th1/17 cytokines contribution. Importantly, AD lesions were characterized by significantly higher expression of epidermal IL-15 and IL-15 receptor (IL-15RA), and increased IL-15+ and IL-15RA+ cell numbers in the papillary dermis compared to control xenotransplants. Thus, induction of AD lesions in the xenotransplants recapitulated the abnormal IL-15 expression reported in human AD. To further investigate the role of IL-15 in lesion development, we injected mice prophylactically or therapeutically with a novel monoclonal antibody directed against IL-15 (CALY-002), while Dupilumab (Dupi) was used as positive control, and control mice received corresponding IgG isotypes. Inhibition of IL-15 by CALY-002 prevented/improved clinical macroscopical disease signs. Xenotransplants of mice receiving CALY-002 showed significantly reduced epidermal thickening, epidermal keratinocyte proliferation, and increased filaggrin expression, similar to mice treated with Dupi. Moreover, treatment with both, CALY-002 and Dupi, significantly prevented/rescued from the increase of IFNy+, IL-15+, and IL-22+ cell number in PBMC-injected xenotransplants. Instead, CALY-002 failed to modulate the numbers of IL-17+ cells, IL-4+, and CD3+ cells, which were reduced in skin grafts of mice treated with Dupi. Lastly, nanostring analysis was performed on xenografts under CALY-002 and Dupi administration. Inhibition of IL-15 by CALY-002 resulted into the prevention/rescue of an exhaustion phenotype in the CD8+ Tcell pool, enrichment of AD-relevant genes (e.g. S100A8/A9, IL4R, DEFB4A) and the differential regulation of genes associated with T-cell, T-helper cells or B-cell activities. Similar results were observed under Dupi treatment. Taken together, selective inhibition of IL-15 signaling by CALY-002 led to barrier reinforcement and amelioration of the pro-inflammatory phenotype in experimentally induced AD lesions. Thus, our data support the hypothesis that IL-15 signaling plays a critical role in human AD and encourage further (pre)-clinical exploration of CALY-002 as novel treatment strategy for AD management.

P138 | VSNL1 - a novel factor in keratinocytes that promotes psoriatic skin inflammation

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Non-communicable inflammatory skin diseases (ncISD) are a heterogeneous group of several hundred skin disorders which pose a major health burden world-wide due to their high incidence and the associated severe impairment of quality of life. Over the last decade several highly effective therapies for the treatment of ncISD have been approved, most of them targeting T-cell derived cytokines. However, there is still a need for new therapeutic targets as primary and secondary loss of efficacy occurs frequently. Visinin Like 1 (VSNL1) belongs to a neuronal calcium sensor protein subfamily, that has been so far mainly examined in a neurological context. New findings, however, suggested an additional role in various nonnervous tissues. In this study, we aim to shed light on the role of VSNL1 in ncISD, focusing on lichen planus (type 1), atopic dermatitis (type 2) and psoriasis (type 3) as representative diseases. Patient data from bulk, single cell and spatial RNA sequencing, as well as immunohistochemical staining of skin biopsies, revealed that VSNL1 expression is highly specific to keratinocytes located in the basal layers of the epidermis and significantly upregulated in lesional compared to nonlesional skin of all three disorders, with highest expression in psoriasis. Accordingly, VSNL1 expression correlated with clinical attributes of all three disease groups, but particularly with those of psoriasis (e.g. acanthosis and neutrophils). VSNL1 knockout (KO) keratinocytes were generated to investigate the functional effects on skin inflammation in vitro. In reconstructed human epidermis (RHE) models, acanthosis was less pronounced in the VSNL1 KO compared to the wildtype. Consistent with this, a reduced proliferative capacity of VSNL1 KO was observed in scratch assays, as well as a downregulation of proliferation and differentiation genes. In addition, the expression of inflammatory markers, particularly CXCL8, was downregulated in VSNL1 KO, again suggesting a pivotal role in psoriasis. In summary,

we demonstrated that VSNL1 is highly expressed in the epidermis of several ncISD and regulates proliferation and differentiation as well as the release of inflammatory cytokines of keratinocytes. Thus, targeting VSNL1 holds great potential for the treatment of ncISD, particularly with regard to psoriasis.

P139 (OP02/03) | C-type lectin receptor Dectin-2 suppresses melanoma metastases

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Despite recent advances in targeted and immune-related therapeutic strategies, melanoma remains one of the leading cancers in terms of average years of life lost per death. C-type lectin-like receptors (CLR) are innate pattern recognition receptors that recognize conserved pathogen-derived molecules and initiate rapid inflammatory responses as a first line of defense against infections. Dectin-2 (mouse Clec4n, human alias CLEC6A) is an activating transmembrane CLR that is classically activated by α-mannan structures of fungi. Being expressed on myeloid cells including macrophages and dendritic cells, Dectin-2 can mediate enhanced phagocytosis and proinflammatory cytokine production upon activation. Previous studies provided first evidence that Dectin-2 mediates phagocytosis of cancer cells by Kupffer cells and suppresses liver metastases. However, the role of Dectin-2 in anti-melanoma immune responses still remains largely elusive.

We aimed to characterize the effects of Dectin-2 in an in vivo melanoma lung metastasis model by intravenously injecting B16F10 melanoma cells into Dectin-2 knock-out (Clec4n-/-) and wildtype littermate controls. Loss of Dectin-2 led to far higher metastatic melanoma burden in the lung based on increased metastatic nodi on the lung surface and further quantified by elevated melanoma-specific TRP-2 mRNA expression. To evaluate the characteristics and functionality of Clec4n-/- innate effector cells, we performed in vitro cocultures of B16F10 melanoma cells and isolated NK cells using a flow cytometry-based killing readout. NK cells lacking Dectin-2 expression tended to have a lowered intrinsic cytotoxicity against B16F10 melanoma cells. In line, the degranulation capacity of Dectin-2 deficient NK cells was impaired upon contact with B16F10 melanoma cells in comparison to wildtype NK cells. Upon addition of alveolar macrophages (AlvM) to the melanoma coculture setting, NK cell mediated anti-tumor effects were even more pronounced. We observed decreased tumor cell cytotoxicity of NK cells interacting with AlvM in Clec4n-/- mice, highlighting that the interaction of NK cells with AlvM during tumor cell killing may be impaired by loss of Dectin-2. We next analyzed publicly available human gene expression data of the Cancer Genome Atlas (TCGA). Metastatic melanoma patients with low intratumoral Dectin-2 expression have reduced 10-year overall survival rates compared to patients with above median Dectin-2 expression.

Collectively, our results reveal that Dectin-2 is suppressing melanoma metastases in both mice and humans. The anti-tumor effects of Dectin-2 likely rely on an interplay of various cells of the innate immune system, including altered cytotoxic properties of NK cells and their interaction with tissue-resident macrophages.

P140 | The role of innate immune stimulation for BAFF induction in cutaneous lupus erythematosus

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Background: Lupus erythematosus (LE) is a chronic autoimmune disease with diverse clinical manifestations and a complex pathogenesis that is not completely understood. However, research has shown that monogenic forms of LE are causally linked to heightened levels of type 1 interferon (type 1 IFN), which are activated by the cell-intrinsic cyclic GMP-AMP synthase (cGAS) and stimulator of interferon genes (STING) pathway.

Another important molecule in the pathogenesis of LE is the B-cell activating factor (BAFF). It plays a pivotal role in activating B-cells and is expressed predominantly in innate immune cells, but also in tissue-resident keratinocytes. Notably, BAFF levels are elevated in cutaneous lesions, which is a characteristic trait of lupus.

Objective: To investigate the expression of BAFF in the skin cells of multifactorial lupus patients, assess its potential modulation by lupus triggers such as photosensitivity, cold weather and, viral infections, and evaluate its correlation with type I IFN.

Methods: Skin fibroblasts and keratinocytes were isolated from LE patients and healthy controls. The expression of BAFF and other interferon stimulated genes (ISGs) was measured using reverse transcription quantitative PCR (RT-qPCR). To simulate lupus triggers, cells were exposed to UV light (photosensitivity), cultured at 25°C (cold weather), and stimulated with nucleic acids (viral infection). The effects of these triggers were analysed using RT-qPCR and multiplex immunoassays. To investigate the role of the cGAS-STING pathway in BAFF expression, small interfering RNAs (siRNAs) targeting the pathway were transfected into LE cells. Additionally, transcriptome sequencing was performed on a set of LE and control fibroblasts and keratinocytes to identify differentially expressed genes.

Results: Cultured LE human fibroblasts (hFBs) had elevated levels of BAFF mRNA compared to healthy controls, as measured by RT-qPCR. In irradiated LE fibroblasts, BAFF mRNA expression was significantly higher than in native LE fibroblasts. The induction of BAFF mRNA expression in irradiated LE fibroblasts was also significantly higher than in irradiated control fibroblasts. Nucleic acid stimulation with poly dA:dT, a potent activator of cGAS, significantly upregulated BAFF levels in LE fibroblasts, whereas poly I:C did not produce the same effect. Transcriptome sequencing analysis showed upregulated IFN-associated gene expression in LE fibroblasts and keratinocytes compared to controls. IFN stimulated genes (ISGs) such as

IFI27, IFI44, ISG15, Mx1, OAS2, and IFN-κ were upregulated in lupus keratinocytes in comparison to control cells. Interestingly, sequencing analysis also revealed that two inflammasome-associated genes, NLRP2 and NLRP7, were differentially expressed in irradiated lupus keratinocytes.

Conclusion: These results demonstrate that BAFF expression is elevated in lupus fibroblasts and keratinocytes, and that it may be regulated by the cGAS-STING pathway. Additionally, the protein can be triggered by UV-exposure suggesting a role in UVinduced lupus flares.

P141 | The LPS Induced Adaptive Response of Old ABCB5+ MSCs Failed to Elicit Microbicidal Neutrophil Functions to Control Gram Negative Bacteria

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Previously, we showed that skin-derived ABCB5+mesenchymal stem cells (MSCs) upon exposure with infection mimicking lipopolysaccharide (LPS) fundamentally shift their transcriptome with high expression and the release of neutrophil activating chemokines. This adaptive response resulted in a significant increase in neutrophil expulsed DNA traps (NETs) and proteolytic enzymes which guarantees the defense 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 signaling, among others, are highly upregulated, while innate immune signaling 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, phagocytosis and, as a preliminary result, also the release of neutrophil recruiting and activating factors (GCP-2, IL-8, GDF-15), were 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 co-cultured with neutrophils abrogates the strong microbicidal effects. 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.

P142 | Unraveling the role of CD64+ DC population in melanoma

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Dendritic cells (DC) are potent antigen-presenting cells initiating immune responses and bridging the gap between innate and adaptive immunity. The conventional DC (cDC) subsets cDC1 and cDC2 contribute to anti-cancer immunity by inducing T cell responses. While investigating DC subtypes in the transplantable melanoma mouse models B16.OVA and D4M.3A we identified a prominent DC population in tumors and lymph nodes differing from cDC by the expression of the FcgRI/CD64, which was claimed to be a marker for monocytes and macrophages. While a similar DC subset was characterized in models of inflammation, its existence in transplantable melanoma mouse models has not yet been described.

To examine these cells we utilized our novel multiplex 26-marker flow cytometry panel, which allows us to discriminate the DC compartment from other myeloid cells and we learned that CD64+ DC share phenotypic characteristics with cDC2. Moreover, migratory CD64+ DC (MHC-IIhigh CCR7+) showed high levels of the DC costimulatory molecule CD40, indicating their potential involvement in the induction of T cell responses. The expression of the co-inhibitory receptors PD-L1 and PD-L2 on CD64+ DC was similar to cDC2. To determine their pre-DC origin we used the Zbtb46-GFP reporter mouse model. We observed that CD64+ tumorinfiltrating DC and lymph node resident CD64+ DC were just partly pre-DC lineage derived, however, all migratory CD64+ DC expressed the Zbtb46

transcription factor indicating their pre-DC origin. In future, we aim to investigate CD64+ DC developmental fate and understand whether they are a separate DC subtype in regards to functional aspects. Moreover, we will unravel their role in the induction of T cell responses against melanoma as migratory CD64+ DC displayed an activated phenotype but co-expressed inhibitory receptors. Gaining more profound knowledge of this cell population's biology might identify them as possible targets for DC-based personalized cancer therapy.

P143 | The influence of indisulam on human immune effector cells: Is a combination with immunotherapy feasible?

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Background: Immunotherapy has great potential in cancer control. The effectiveness of immunotherapy correlates with a high tumor mutational burden, leading to the presentation of many neo-antigens on the tumor surface and triggering a tumortargeted cellular immune response. Neo-antigens derive not only from somatic mutations but can also be caused by alternative RNA splicing. Indisulam (E7070) is a chloro-indolyl sulfonamide cytostatic anti-cancer agent that causes ubiquitination and proteasome-mediated degradation of the mRNA splicing factor RBM39 (RNAbinding motif protein 39). As a modulator of pre-mRNA splicing, treatment with indisulam can generate aberrantly spliced neo-antigens, enabling an immunologic anti-tumor activity. Indisulam monotherapy led to variable outcomes in patients with metastatic melanoma. However, based on the idea of inducing alternative RNA splicing, combining indisulam with immunotherapy is expected to be a promising therapeutic approach to improve and extend the effectiveness of immunotherapy in cancer control. In this context, it is necessary to first exclude any function-impairing effects of indisulam on the immune system.

Objective: This study aims to provide insights into the feasibility of a potential future combination of indisulam with immunotherapy.

Material & Methods: In this perspective, we investigated the influence of indisulam on ex vivo isolated T cells and monocyte-derived dendritic cells (moDCs) from healthy donors. Different concentrations of indisulam ranging from 0.6 µM to 160 µM were tested. Assays were measured and evaluated using flow cytometry. We examined: i) The impact of indisulam on the non-specific activation of CD4+ and CD8+ T cells by CD3-crosslinking and the antigen-specific activation of TCR-transfected CD4+ and CD8+ T cells by peptide-loaded target cells. Hence, the surface expression of activation markers and the cytokine secretion were analyzed. ii) The effect of indisulam on the maturation process of moDCs regarding changes in the maturation marker profile. iii) The influence of indisulam on antigen-specific CD8+ T cell priming determined by the percentage of antigen-specific CD8+ T cells after co-incubation with autologous moDCs loaded with the tumor antigen MelanA.

Results: Our data indicate that high levels of indisulam inhibit the upregulation of the activation markers CD69 and CD25 on both CD4+ and CD8+ T cells after nonspecific and antigen-specific activation. The cytokine secretion also seems to be impaired at higher indisulam concentrations. In general, antigen-specific activation is less affected by indisulam than non-specific activation. In addition, our data show that high levels of indisulam inhibit the expression of several maturation markers on moDCs. Indisulam also appears to suppress antigen-specific priming of CD8+ T cells. Evaluations indicate that high inhibitor concentrations cause a reduction in lymphocyte size without a relevant increase in cell death.

Conclusion: In summary, we demonstrate dose-dependent effects of indisulam on T cells and moDCs. Our data show that various functions of the immune system are differently sensitive to indisulam, which should be considered in possible future combinations of this agent with immunotherapy.

P144 | Multi-omics analysis enables machine learning based differentiation of clinical mimickers in lichenoid skin diseases

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Lichenoid skin diseases belong to the large and heterogeneous group of cutaneous inflammatory disorders. The two major entities of lichenoid dermatoses are lichenoid drug eruptions (LDE) and lichen planus (LP). Given their clinical and histological similarities, differentiation between them is challenging and has consequences for patient management. Molecular disease signatures hold the promise to uncover novel biological insights, providing a basis for more accurate diagnosis and therapy. To date, most studies applying omics technologies have focused on transcriptomic and genomic analysis, mainly in the field of medical oncology. In contrast, mass spectrometry (MS) -based proteomic integration for inflammatory conditions has rarely been performed. In this study, we conducted a multi-omic analysis, including MS-based proteomics

and targeted transcriptomics (624 genes; inflammatory panel), on paraffin-embedded tissue sections from 13 patients diagnosed with LP and 23 with LDE. Among the LDE group, 12 patients suffered from classical and 11 from checkpoint inhibitor-induced LDE. MS-based proteomics identified a total of 8094 proteins among all groups. Biological pathway analysis revealed a robust enrichment of the interleukin-1 family in lichenoid dermatoses, which was confirmed on a transcriptional and immunohistochemical level. This finding is novel and provides a rationale for further investigation of interleukin-1 antagonism in lichenoid dermatoses. Machine learning approaches can be useful to select important features out of a high dimensional matrix like our multi-omics data and consequently use those for predictive purpose. We applied a random forest algorithm on the pooled transcriptome and proteome dataset with 5-fold cross-validation and achieved a predictive accuracy of 0.63 to 0.8 ROC-AUC (Receiver Operating Characteristics - Area Under Curve) in distinguishing LP and LDE. When focusing on the 13 to 15 top-ranked proteins/transcripts obtained by random forest, the predictive accuracy reached an ROC-AUC score of at least 0.99. In particular, unsupervised hierarchical clustering based the 13 top-ranked proteins/transcripts (BCL3, STAT3, FAS, LILRB2, CFDP1, PPIE, AGFG1, CHUK, PTK2, CASP3, GGT1, OMD, MOB4) fully separated the clinical mimickers LP and classic LDE. In summary, transcriptomics and proteomics combined with machine learning algorithms identified a subset of proteins and transcripts that effectively distinguish skin diseases with virtually identical clinical presentation and histological features. While these results need to be validated in independent cohorts, they highlight the potential diagnostic power of multi-omic analysis in cutaneous inflammatory disorders for better disease stratification.

P145 | Impact of different contact allergens on a full-thickness human skin model including primary immune cells

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Allergic contact dermatitis is a delayed hypersensitivity reaction of the skin to an external substance. Repeated exposure to the allergen is usually required, and penetration of the allergen through the epidermal barrier triggers a primarily Tcell- mediated immune cascade that is initiated by antigen presenting cells. Since the EU's ban on animal experiments for testing the compatibility of cosmetics and consumer products in 2013, enormous efforts have been made to identify alternative test methods. Immunocompetent full-thickness skin models are arguably reasonable replacements.

We successfully integrated primary immune cells into an organotypic 3D skin model generated from primary fibroblasts and keratinocytes of the same donor. Proper differentiation of the organoids was confirmed by orthotopic expression of filaggrin, involucrin, hornerin, and loricrin. Different methods of integrating pre-differentiated Langerhans cells (LCs) and untreated peripheral blood mononuclear cells (PBMCs) were performed. Immunohistochemistry detecting CD3, CD14, CD20, CD45, and CD68 verified the correct localization of the immune cells within the skin equivalents. Analysis of isolated single cells from the skin model identified different immune cell subpopulations in the epidermis and dermis. Application of the contact allergens dinitrochlorobenzene (DNCB) and nickel sulfate (NiSO4) onto the epidermis induced robust activation of LCs and T cells as analyzed by qPCR and flow cytometry (CD80, CD86, CD69 expression; multiple cytokines and chemokines).

In the future, DCs from contact allergy patients will be integrated into the skin model treated with their known contact allergens. In this way, this immunocompetent skin equivalent will likely contribute to personalized allergologic diagnostics.

P146 | DEC205, a receptor characterizing non-lymphoid dendritic cells also mediates endocytosis in brain endothelial cells

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The antigen uptake receptor DEC205 was originally described in langerhans cells in skin. Within the intracellular domain we characterized three defined sequences, mediating (a) the uptake of ligands, (b) the targeting to deeper endosomal compartments and (c) the recycling back to the surface of cells. This makes DEC205 a highly specialized receptor for absorptive endocytosis. When testing other cell types for DEC205 expression, we found that among all non-leukocyte populations only brain endothelial cells (ECs), as indicated by co labeling PECAM-1 (CD31), react with the DEC205 antibody. We therefore further tested a cellular model for ECs, the endothelial cell line bEnd.3 that is derived from mouse brain. Here, similar to brain sections, we found expression of DEC205 by PCR and FACS staining. According to our results obtained in LCs, showing that DEC205 facilitates receptor mediated endocytosis, we hypothesize that DEC205 analogously serves as transporter for nutrients and/or antigens in ECs. Therefore, we started experiments by incubating monolayers of the DEC205+ EC line bEnd.3 with fluorescently labeled anti-DEC205 (αDEC-PE) antibodies and followed the uptake and fate of the antibodies by live cell imaging. We revealed a rapid uptake of αDEC-PE which targets deeper (LAMP1+) cellular compartments that differ from transferrin-receptor + (CD71) vesicles. Within 48h αDEC-PE is degraded, whereas CD71+ vesicles targeted by respective anti-CD71 specific antibodies are still loaded. After incubation with cytokines, mimicking EC activation, LAMP1+ αDEC-PE+ vesicles also express MHC class II molecules, which may be able to activate CD4+ T cells. Thus, DEC205 may serve as "linker" molecule connecting the function of ECs with those of the immune system.

P147 | Induction of antigen-specific tolerance by targeting the endocytic receptor DEC205 with artificial liposomes

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Extracellular vesicles (EVs) are membrane-bound structures which originate from the endosomal system or plasma membrane. After being released to the extracellular environment, EVs play a significant role in communicating between cells and can regulate the biological pathways in recipient cells. Among EVs, exosomes are the smallest vesicles. They exert biological function by transporting bioactive molecules such as lipids, proteins, mRNAs, and microRNAs between cells. However, natural extracellular vesicles are limited by lack of control over their composition and scalability. So, we try to synthesize liposome-based artificial EVs to encapsulate and deliver substances that can regulate immune responses. DEC205 is an antigen presenting receptor which is mainly expressed on the surface of dendritic cells (DCs). By surface modification of liposomes with anti-DEC antibodies, liposomes may target DCs and transport the encapsulated substances to DCs efficiently.

According to the previous in vitro work, liposomes with encapsulated miRNA and surface tetraspanins typical of EVs showed therapeutic effects on skin wound healing. We further analyzed the in vivo effect of liposomes that specifically mimic mesenchymal stem cell extracellular vesicles(MSC EV) in the contact hypersensitivity (CHS) model, as MSC EVs are known for their immune-modulatory effects. After sensitization of the mice on the abdominal skin by 1-Fluoro-2,4-dinitrobenzene (DNFB), we treated them daily with liposomes decorated with MSC EV-typical CD proteins before challenging. Then we measured the ear thickness and isolated cells from ear skin for FACS. In this in vivo experiment, we were unable to define a clear difference in ear thickness. However, less infiltration by CD8+ T cells can be detected after treatment with liposomes. To improve the efficacy of the liposome treatment, we will next modify the liposome surface by adding anti-DEC antibodies to target exclusively DCs in skin directly. Moreover, we will test which cargo encapsulated in the liposomes will be most potent to either suppress or to stimulate immune responses.

P148 | Deciphering the CD4+ T cell compartment in pemphigus

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Pemphigus is a severe blistering disorder of skin and mucosa characterized by autoantibodies against desmosomal proteins of the skin. Here the T / B cell crosstalk is crucial in the disease pathogenesis. In this on-going study, we aimed to perform a deep immunophenotyping of the major CD4+ T cell subsets: T helper (Th), T follicular helper (Tfh), T regulatory (Treg) and T follicular regulatory (Tfr) cells. We initially compared frequencies of these four subsets in a large group of pemphigus patients (n=63) compared to age and sex matched healthy individuals (HI, n=19) by multicolor flow cytometry. To further characterize the phenotype of these cells, intracellular production of cytokines (interferon (IFN)-γ, interleukin (IL)-17, IL-10, IL-2 and IL-4) and co-stimulatory/inhibitor receptors (OX40, CTLA-4, TIGIT, TIM-3, PD-1 and LAG-3) were analyzed (n=21 for pemphigus, n=10 for HI). We then focused on the suppressive role of Treg and Tfr cells and performed a T cell suppression assay by using carboxyfluorescein succinimidyl ester (CFSE) staining (n=14 for pemphigus, n=8 for HI). We did not detect major differences in the distribution of Th, Tfh, Treg and Tfr cells when comparing relative frequencies between HI and pemphigus patients. Importantly, intracellular cytokine expression showed differences between pemphigus and HI. Th cells presented a higher expression of pro-inflammatory cytokines and a lower expression of IL-10 in the pemphigus group, while Tfh cells showed a higher expression of IL-17 and IL-2, arguing for a more inflammatory phenotype of these cells in pemphigus. In the regulatory cell groups, pemphigus patients showed higher expression of IFN-γ in Tregs and lower expression in Tfr cells compared to HI. Tfr cells showed higher expression of IL-17 in pemphigus compared to HI. When analyzing surface receptors, PD-1 expression was much lower in all four cell populations in pemphigus. Inhibitory receptors such as as TIM-3 and LAG-3 showed lower expression in Th, Tfh and Treg cells. Proliferation assays indicated that Treg and Tfr cells have a reduced but not statistically significant suppressive capacity in pemphigus patients compared to HI. Taken together, our data show an altered CD4+ T cell phenotype and function in pemphigus patients compared to HI.

P150 | Multiplexed analysis of mast cells within healthy human skin tissues by imaging mass cytometry reveals heterologous expression of proteases

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Introduction: Imaging Mass Cytometry (IMC) is a high-dimensional tissue imaging system that allows antibody-based comprehensive investigation of single cells in formalin-fixed paraffin-embedded (FFPE) tissue within their spatial environment. As of now, the multiplexed immuneprofiling of mast cells (MCs) within healthy human skin has not been established. A panel of rare-earth metal-tagged antibodies was used to stain healthy human skin tissue as proof of concept for investigation of differential protein expression patterns in MCs and explore potential intra-tissue heterogeneity.

Material and Methods: Here, we performed proof-of-concept multiplexed imaging of selected markers, as part of the development of a 29-antibody IMC panel for simultaneous staining of FFPE human skin tissues, which will comprise a variety of MC-expressed targets, including receptors involved in mast cell activation, survival and inhibition (FceR1a, CD117, MRGPRX2, CD63, CD88, ST2, TLR3, OX40L, CD95L, CXCR1, CXCR2, CXCR3, Siglec-3, Siglec-6, Siglec-8, CD300a, and CD200R), proteases (Chymase, Tryptase, Cathepsin G, Granzyme B, and Carboxypeptidase A3). The final panel will also include markers for nucleus (Histone 3), proliferation (Ki67), blood and lymphatic vessels (CD31 and LYVE-1), nerve endings (PGP9.5), sweat gland (CD66a), and tissue resident immune cells (CD68).
Results: Skin MCs showed considerable protein expression diversity and reside primarily in proximity to blood and lymphatic vessels and hair follicles. In healthy human skin, most MCs co-expressed of FceR1a, Siglec-6, and Cathepsin G. However, a small population of MCs were only positive for FceR1a, or double-positive for FceR1a and Siglec-6. In addition, the majority of dermal MCs showed co-expression of CD117, chymase, CD63, CD68 and granzyme B. However, all MCs investigated were negative for Ki67.
Conclusion: IMC allows for the characterization of MCs in human skin with a multiparametric single-cell approach. Human skin MCs display a distinct spatial distribution and non-proliferative state. Our findings provide proof of concept that IMC can help to characterize skin MC populations in healthy human skin. Further studies are needed to expand the scope of markers and application to the skin of patients with chronic inflammatory conditions.

P151 | Higher IL-10+ T cell and Treg cell counts in psoriatic skin are associated with super-response to guselkumab: Data from the Phase 3 GUIDE trial

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Interleukin-10 (IL-10) and regulatory T (Treg) cells are immunosuppressive mediators with key roles in autoimmunity. Their increased presence is believed to counterregulate pathogenic tissue resident memory (TRM) and Th17 cells in psoriasis (PSO). The GUIDE study (NCTO3818035) aimed to determine if guselkumab (GUS)- treated plaque-PSO patients who achieved 'super-response' (SRe; PASI=0 at week [W] 20 and W28) show immunological differences in skin T cell composition versus non-SRe (nSRe) based on flow cytometry analysis of non-lesional (NL; W0) and lesional (L; W0, 4, 28, 68) skin cells (63 patients).

IL-10+ T cell counts were higher in both NL and L skin at baseline in SRe versus nSRe (SRe to nSRe ratio of 3.1:1 and 3.0:1, respectively, $p<0.05$). This trend was maintained in L skin after GUS treatment, with higher IL-10+ T cell counts seen in SRe at W4 and W28 (SRe to nSRe ratio of 4.6:1 and 4.4:1 respectively; $p<0.05$). Also, Treg cell (CD4+CD25+FoxP3+) counts were higher in SRe at W4 and W28 (SRe to nSRe ratio of 3.1:1 and 2.9:1 respectively, $p<0.05$). Further analysis of effector T cell subsets showed that CD8+ TRM and IL-17A+ T cell counts in L skin were reduced by GUS up to W68. Normalization of these T cell populations in L skin to NL skin levels appeared to be earlier in SRe (W28) than nSRe (W68).
These results suggest clinical super-response may be characterized by higher IL-10+ T cell and Treg cell counts, and faster normalization of CD8+ TRM and IL-17A+ T cell counts. Thus, higher IL-10+ T cell and Treg cell counts may be predictive biomarkers for better response to GUS in PSO.

P152 | Safety, tolerability and pharmacokinetics of TPM203, a tolerizing Topas particle mixture in pemphigus vulgaris: preliminary results from a phase 1, first in man study (EudraCT Number: 2019-001727-12)

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Pemphigus vulgaris (PV) is an autoimmune disorder of the mucous membranes and skin caused by IgG autoantibodies against desmoglein (Dsg) 3 and Dsg1. Growing evidence shows that in PV the production of the pathogenic autoantibodies is largely dependent on the presence of autoreactive T cells.
T cell recognition of a set of epitopes of Dsg3 is restricted by the HLA class II allele HLA-DRB1*04:02, which is prevalent in PV. TPM203 is a mixture of 4 different Topas Particle Conjugates (TPCs), each coupled to a distinct Dsg3 T cell epitope, shown to induce tolerance against Dsg3 in an HLA-DRB1*0402-transgenic mouse model of PV. We report data from the single ascending dose part of a phase 1, open label study in which four cohorts of three patients each received TPM203 at 0.12, 0.36, 1.2 and 3.6 μmol of total TPC-bound peptide and were followed-up for 12 weeks. Patients had to be in complete clinical remission or with low to moderate clinical disease activity and positive for anti-Dsg3 antibodies as well as for peripheral blood CD4+ T cells specific for at least one of the Dsg3 peptides present in TPM203. No immunosuppressive/ immunomodulatory treatment were allowed, other than ≤10 mg/d of prednisone equivalent.

After a single TPM203 administration, 11 out of 12 patients reported at least one adverse event (AE). No serious AE nor TPM203-related AE leading to discontinuation was reported. Three PV related AEs (lip erosion, oral mucosal erosion and oral blood blister) were reported as mild events. Worsening of PV was reported in 2 additional patients following COVID vaccination (one mild and one moderate). During the study, no clinically relevant changes in laboratory parameters were observed.

After TPM203 infusion, detectable levels of the different TPCs could only be found in patients at the 2 highest doses (from 0.5 h up to 2.5 hours post administration for the highest administered dose). As to exploratory pharmacodynamic parameters, when pooling all 12 patients, a trend toward a decrease in anti-Dsg3 IgG was found in patients receiving the first 3 dose levels. The phenotypical analyses of T and B cells revealed the following trends: decrease (% change, 95% CI) in CD27+ memory B cells, increase (% change, 95% CI) in TH2 and TFH2, decrease (% change, 95% CI) in TH17 cell compartment. Furthermore, a trend towards an increase in Tregs was also observed. Interestingly, a concomitant decrease of memory T cells and Th cells, especially Th17, Th17.1 and Th1 cells, with diminished IgG autoantibody levels was observed in 5/12 patients.

In conclusion, escalating single doses of TPM203 were safe and well tolerated in PV patients with no or low/moderate disease activity. Despite the limitations of the study due to the small number of patients and the short observation period, these preliminary data could indicate that this approach might modulate the immune response in PV patients by increasing Treg cells and decreasing Th17 and memory B cells.

P153 | Vascular damage and neurogenic inflammation in Erythema migrans of the skin.

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The most common manifestation of tick-borne Lyme disease is Erythema migrans (EM), caused by Borrelia burgdorferi spirochetes infecting the skin. The EM characteristically presents as an erythematous, targetoid-shaped rash and may lead to chronic skin inflammation and localized neurologic manifestations if untreated.

This research provides insight into the dissemination and translocation of B. burgdorferi in EM and analyses their interaction with local, dermal vessels and sensory nerve fibers. Von Willebrand factor (vWF) serves as a marker for potential endothelial dysfunction due to borrelia infection and the neuropeptide Calcitonin Gene-Related Peptide (CGRP) is used to determine neurogenic inflammation and systemic neuropeptide-immune cell interactions in EM.

Our findings suggest higher B. burgdorferi loads within the lesional center than border. The vast majority is located in proximity to endothelial vessel cells, which were found to express decreased amounts of vWF, indicating vascular dysfunction. Schwann cells of dermal nerve fibers colocalizing with B. burgdorferi were identified with increased CGRP expression compared to B. burgdorferi negative Schwann cells. Comparing lesional skin and healthy skin in single-cell RNAseq analyses higher CGRP-receptor CALCRL expression was found in lesional skin, particularly in lymphocytes, dendritic cells and fibroblasts.

Our findings indicate dermal B. burgdorferi translocation from the central tick bite site towards the EM border and systemic distribution via vessels with associated endothelial dysfunction. Additionally, neuropeptide release by Schwann cells colocalized with B. burgdorferi and increased CALCRL expression in immune cells implies neurogenic modulation of the local inflammatory response to B. burgdorferi infection.

P154 | The metabolite sensing Hca2/Gpr109a receptor as a regulator of skin and brain inflammation

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Chronic inflammatory skin diseases such as atopic dermatitis (AD) and psoriasis are associated with a high co-morbidity of neuropsychiatric disorders like anxiety and depression, suggesting a link between pathophysiological processes in the skin and the brain. G-protein coupled receptors, such as the Hydroxy-Carboxylic Acid Receptor (HCA2R), expressed on adipocytes, keratinocytes, immune cells and microglia and its ligands butyrate and beta-hydroxybutyrate might act as a potential link between host metabolism, inflammation and behavior.

In previous experiments, we demonstrated that Hca2-/- animals display increased contact allergic inflammation in the skin. To further assess the potential role of Hca2 receptors in the "skin-brain-axis", wild type and Hca2-/- mice were exposed to the DNFB-induced model of chronic AD-like skin inflammation. Then we examined the prefrontal cortex, important for cognitive control functions as well as the dorsal and ventral hippocampus, important for learning, memory and anxiety in naïve mice and animals with skin inflammation. Gene expression analysis by qPCR revealed that IFNy and Ccl8 already were increased in the hippocampus of naïve Hcar2-/- mice. Inflammation-naïve HCA2-/- mice also displayed enhanced expression levels of IL4 in the dorsal hippocampus. In AD-like inflammation

IFNy, Ccl8 and IL-4 significantly increased in the prefrontal cortex and hippocampus of Hcar2-/- mice in comparison to WT animals. In a next set of experiments naïve mice and animals with AD-like inflammation were exposed to behavioral tests to assess the effect of Hca2 signaling on anxiety-like behavior and fear memory. The assessment of emotion-related behavior in naïve Hca2-/- mice revealed a significant altered anxiety-like behavior and generalized tone-dependent fear memory in comparison to naïve wild type mice pointing to hippocampal dysfunction. Under chronic inflammatory conditions increased anxiety-like behavior and fear memory was higher in Hca2-/- mice but did not reach significance when compared to wild type animals. Further analyses of inflamed brain tissue and serum cortisol levels are currently ongoing.

Together, our first results indicate a modulation of proinflammatory responses to peripheral skin stimuli by the HCA2 receptors. HCA2 receptor deficiency may lead to altered glial function, thereby affecting inflammatory responses in brain areas important for emotion and memory. Ultimately, the HCA2 receptor may provide an interesting target for treatment options of anxiety-related co-morbidities of skin inflammations.

P155 | Neoadjuvant immune checkpoint inhibition protects from hepatic melanoma metastasis by mounting an enhanced anti-tumoral Th1 immune response

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Despite immune checkpoint inhibition (ICI) stage III and IV melanoma patients often suffer from progressive disease. Notably, liver metastasis correlates with low overall survival rates in response to ICI. Here, we evaluated whether a neoadjuvant ICI treatment regimen improved therapy response and could protect against development of liver metastasis.

Combined ICI (anti-PD-1/anti-CTLA-4) was applied in murine models of hepatic melanoma metastasis using adjuvant or neoadjuvant treatment regimens. Immune cell composition in the liver, blood and cutaneous melanomas was comparatively analyzed by immunofluorescence and flow cytometry. Cytokines were measured by bead-based multiplex cytokine assays and in situ hybridization. Currently, we are analyzing scRNA-Seq of T cells from the primary tumor, the blood and the liver.

Neoadjuvant ICI demonstrated an improved clinical response rate and a stronger reduction in hepatic metastasis in comparison to adjuvant ICI. This superior response was related to an expansion of T cells at the primary site, the peripheral blood and the liver. Notably, CD4+ T bet+ Th1 cells were strongly increased, while CD4+ Gata3+ Th2 cells were decreased when employing the neoadjuvant as compared to the adjuvant treatment regimen. Additionally, the analysis of hepatic cytokines showed lower levels of Th2-associated IL-4 and of IL-15. Increased frequencies of shared T cell receptors among CD8+ cytotoxic, CD8+ proliferating or regulatory T cell clusters in the cutaneous melanomas and the livers were seen so far in preliminary analyses of the scRNA-Seq data.

Overall, our data demonstrate that neoadjuvant ICI superiorly protects against hepatic melanoma metastasis. This appears to be mediated by a shift towards an anti-tumoral Th1 immune response in the liver while adjuvant ICI was associated with a pro tumoral Th2 immune profile. Therefore, neoadjuvant ICI is a highly appealing therapeutic option for cutaneous melanoma to prevent development of organ-specific therapy resistance mechanisms.

P156 | Circadian Rhythmicity of Immune Cells in Systemic Lupus Erythematosus

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The autoimmune disease systemic lupus erythematosus (SLE) is characterized by a variety of symptoms such as the butterfly rash on the face or kidney inflammation, which appear in recurring flares. The disease development and its triggers are mostly uncharacterized. The circadian rhythm is a vital aspect, guiding all of the body's functions. The interactions of various genes and proteins form interlocked feedbackloops, coordinating their transcription and repression in a timeframe of about 24 hours (h). Therefore, the master clock, called suprachiasmatic nucleus, is located in the brain and its signals synchronize peripheral clocks throughout all organs and cells in the body. Additionally, extrinsic factors such as light and food-intake are able to support or disturb this rhythm. It is known that this kind of disturbance can cause or worsen diseases. Shift work is one of the main life-style factors that force people to act against their natural timings. Its deleterious effects have been studied with regard to cancer, overweight and much more. However, it is not clear how shift work is able to trigger autoimmunity on a cellular level. We profiled the 24-hour rhythms of different immune cell populations of blood and four organs of C57BL/6 wild type mice. Moreover, different pre-disease states of SLE are investigated in NZM2410 and SLE1,2,3 mice, mouse lines that are already known to replicate the autoimmune disease well. Hemograms as well as flowcytometry

data of T cells describe changes during disease progression. In future experiments, this will be compared to simulated shift work in the named mouse lines. Moreover, initial interferon responses in keratinocytes after UV light exposure are known. However, in these lupus prone mouse lines no skin involvement was described so far. In upcoming experiments, we will expose skin of these mice to UV light and investigate the changes upon the exposure by RNA sequencing and immunohistochemistry. Keratinocytes might be the initiators of SLE flares by the release of interferon which in turn activates dendritic cells. These experiments might show how the interplay of UV light and skin influence the pre-disease state and act as a trigger in SLE, even in the mouse models.

P157 (OP06/03) | Direct binding of complement components to immunodominant skin antigens: a key step in early pathogenesis of autoimmune skin diseases?

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Bullous pemphigoid (BP) is characterized by autoantibodies (abs) that bind to BP180, promoting complement (C) fixation, inflammation, and blister formation. In contrast to the rather detailed understanding of the effector phase, mechanisms enabling the emergence of self-directed abs remain less understood. Insights into these early steps in immunopathogenesis may identify new avenues for treatment for this fragile patient group. Incubating recombinant BP180-NC16A antigen (ag) with a C source we performed IP and SDS-PAGE. This resulted in the appearance of novel bands that were subjected to mass spectrometry, identifying C3b alpha' bound to the ag. Our results point toward direct opsonization of the autoag with activated C component 3 (C3) fragments, in the absence of abs. This was validated by 3D confocal microscopy on G3D biopsies stained for both BP180 and C3. To investigate conditions that foster this spontaneous activation and deposition of C3, we incubated 3D-cultured keratinocytes with various stimuli (TNF alpha, IL-6, S. aureus) and assessed gene expression of C pathways proteins by qPCR and RNAseq. We found an upregulation in genes associated with the classical (C1s, C1R, C2, C3) lectin (C2, MASP1, COLEC10, COLEC11, C3) and the alternative pathway (CFB, CFP, C3) as well as ITGB2 (receptor of iC3b) and complement inhibitory genes (CR1, CSMD2, C4BPB, CFI, CD55, CLU, CD59, CFH) but not in those of the terminal pathway. Against the well-documented immunogenicity-boosting effect of C3-derived fragments (when bound to protein ags of infectious origin), we interpret our findings to represent a previously underexplored key step in the early immunopathogenesis of BP. During local inflammatory or infective episodes, or for genetic or age-related reasons, these processes that are normally kept in check by complement regulatory or inhibitory mechanisms, may lose their protective capacity, leading to C3 activation and direct covalent binding of C3b to autoantigens. This may increase the normally low immunogenic potential of BP180 in vivo, possibly to a point where tolerance cannot be maintained, leading to the emergence of abs. We propose that the binding of complement factors to skin autoantigens may constitute one of the earliest processes in the pathogenesis of autoimmune skin blistering diseases such as BP, mucous membrane pemphigoid or epidermolysis bullosa acquisita. Our evidence warrants further exploration of its relevance and contribution in developing skin autoimmunity.

P158 | NK cells are activated and possess immunoregulatory functions in patients with pemphigus vulgaris

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Pemphigus vulgaris (PV) is an acquired autoimmune bullous disease which results from loss of desmosomal cell-cell adhesion caused by IgG autoantibodies against desmoglein (Dsg) 1 and Dsg3. The role of the adaptive immune system in PV is well-established. However, little is known about the immunoregulatory function of innate immune cells, including Natural Killer (NK) cells, which possess the capacity to kill target cells and secrete pro-inflammatory cytokines. Here, we aimed to comprehensively characterise the NK cell phenotype and function in peripheral blood and skin lesions from PV patients compared to healthy controls (HC) using multiparametric flow cytometry.

Detailed phenotypic analysis showed that both the CD56dim and the CD56bright NK cells were activated in PV patients as indicated by CD69 and Ki67 expression. A higher expression of inhibitory CD39 was detected on CD56bright NK cells and of granzyme B in CD56dim NK cells, suggesting a more regulatory function of CD56bright NK cells and an increased cytotoxic potential of CD56dim NK cells in PV patients compared to HC. Frequencies of Killer cell immunoglobulin-like receptor- (KIR-) positive CD56dim NK cells correlated negatively with T follicular helper (Tfh) cells in HC, but not in PV patients. Moreover, NK cells from PV skin lesions expressed higher levels of NKG2A and CD39 as well as less perforin and granzyme B compared to NK cells from HC skin, confirming a more inhibited phenotype of NK cells in PV skin lesions. Peripheral blood NK cells from PV patients retained their functional capacity

upon target cell stimulation, but CD56dim NK cells degranulated less and produced less MIP1b upon cytokine priming than NK cells from HC. To investigate whether NK cells might mediate killing of Dsg-3-specific T cells, PBMCs from PV patients and HC were stimulated with Dsg3-overlapping peptide pools. The collected supernatants from PV patients, but not from HC, contained increased levels of perforin, granzyme B, IL-18, TNFa, IL-4, IL-10 and sCD40, indicating increased killing activity and the presence of pro-and anti-inflammatory cytokines. Finally, we found negative correlations between Dsg-3-specific (CD154+CD69+) CD4+ T cells and the expression of perforin and granzyme B in NK cells in PV but not HC, which may indicate cytotoxic molecule-dependent killing of pathogenic T cells.

Taken together, our results suggest an immunoregulatory role of NK cells in PV and identify them as another cell type important for the regulation of autoreactive T cells.

P159 | Bullous pemphigoid induced by immune checkpoint inhibitors - an issue of relevance?

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Bullous pemphigoid is the most common autoimmune bullous skin disease of man. Target antigens, major pathogenic aspects, clinical picture and therapeutic approaches are well-described. Yet, inducing factors are largely unknown, though the rising incidence among elderly indicates an immunological background. Recently, an increasing number of BP induced in patients undergoing immune checkpoint inhibition (ICI) for malignant melanoma was reported. Therefore, 52 melanoma patients were serologically monitored prior and during ICI treatment with a least two serum samples using indirect immunofluorescence (IIF) on cyrosections of monkey esophagus and ELISA for circulating antibodies against BP180 and BP230. The mean age was 61.6 years with 55% male patients. 73% patients suffered from stage IV, 25% from stage III and 2% stage from II melanoma. 71% patients were treated with ipilimumab monotherapy or in combination with PD-1 inhibitors, 30% with PD-1 inhibitors only. No patient developed any clinical manifestions of BP including erythema, eczema, urticarial lesions, blisters or itching. Two patients had slightly increased anti-BP180 at month 2 of ICI treatment at 12 and 21 IU/ml (cut-off 10) with negative IIF, respectively. In one patient anti-BP230 were slightly increased. Whereas 10 patients had faint linear deposits of IgG along the basement membrane zone in IIF, none of them was positive in ELISA. Among this cohort of melanoma patients undergoing ICI treatment, no relevant and specific immunoserological parameters indicative of BP could be detected. The scenario of pre-existing BP antibodies with manifesting clinical picture induced by ICI or primary induction of BP antibodies by ICI has been proposed, yet is not supported by this study. The incidence of BP, even only immunoserologically positive seems low. This is in line with the observation in our center that only five among approximately 750 patients receiving ICI developed clinically manifest BP. Indeed, accumulating evidence indicates the incidence of ICI-induced BP to be at 1% of ICI-patients with specific clinical manifestations, clinical course and therapeutic response when compared to general BP. Still, physicians need to stay alert to detect early and minimal clinical presentations of BP like erythema, eczema and itching during ICI treatment and should quickly initiate ample diagnostic procedures and therapy.

P160 | Importance of pre-clinical validation of pharmacological targets in pemphigoid diseases

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Pemphigoid diseases are characterized by the formation of autoantibodies, which, upon tissue deposition and the consequential inflammation, cause (sub-) epidermal blistering of the skin. Based on the technological progress and available bioinformatic approaches, an increasing number of genes are identified, which are thought to have disease-regulatory functions. In this study, we focused on the pre-clinical validation of such candidate genes. To achieve this, we implemented a stepwise approach by integrating both in vitro and in vivo assays. Briefly, mixed lineage kinase domain like pseudokinase (Mlkl) plays a critical role in TNF-a induced necroptosis. The cell-surface receptor CD93 is important for intercellular adhesion and the triggering receptor expressed on myeloid cells 1/3 (Trem1/3) is a key component in the development of septic shock. Additionally, all three candidate genes have previously been implicated to influence autoimmune diseases.

We first looked at the expression of these three genes in lesional skin from wildtype mice with antibody-transfer induced epidermolysis bullosa acquisita (EBA). All three candidate genes were overexpressed, when compared to nonlesional skin. However, ex vivo, the capacity of neutrophils to produce reactive oxygen species upon immune-complex stimulation was not altered in any of the knockout strains. Further, we looked at the impact of the respective knockouts on disease development in the antibody-transfer induced EBA mouse model. Disease development was unchanged in the Mlkl and Cd93 knockout mouse strains. However, we obtained a delayed disease progression in the Trem1/3 knockout strain, and notably, this effect was sex-dependent, with a more pronounced impact in male mice.

In summary, despite the overexpression of all three candidate genes in lesional skin, only one candidate gene, Trem1/3, could be validated in vivo. The absence of Trem1/3 resulted in a delayed disease progression in EBA, and this effect exhibited a sex-dependent

pattern. Given that therapeutics targeting TREM1 are already under development, evaluation of their potential efficacy in autoimmune blistering diseases should be considered.

P161 (OP02/02) | Impact of mitochondrial stress in the pathogenesis of dermatomyositis

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Dermatomyositis (DM) is a rare autoimmune disease characterized by 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. While the exact molecular pathways of DM pathogenesis remain to be elucidated, the activation of innate immune sensors by self-nucleic acids is a relevant trigger for type I IFNdriven inflammation and cutaneous autoimmunity. Here we asked whether innate sensing of self-nucleic acids is involved in the induction of type I IFN stimulated genes (ISG) in fibroblasts of patients with DM. Importantly we found by RT-PCR and RNA sequencing that fibroblasts isolated from skin biopsies of patients maintain an elevated ISG signature in culture.

Gene Set Enrichment Analysis further demonstrated a significant downregulation of pathways involved in oxidative phosphorylation. In particular, the gene expression of mitochondrial components complexes I and IV was decreased.

We therefore hypothesized that impaired mitochondrial function might be a relevant trigger factor for ISG upregulation. To investigate a possible link between mitochondrial dysfunction and inflammation, we compared mitochondrial function between fibroblasts from DM patients and healthy controls using the Seahorse Assay and found that both basal oxygen consumption rate and ATP production were significantly reduced in patient cells. This was consistent with analysis using MitoTracker Green(TM) or Mitosox(TM) labeled fibroblasts by FACS, which demonstrated significant upregulation of reactive oxygen species in DM fibroblasts compared to healthy controls as a sign of mitochondrial stress. Such mitochondrial deterioration may be associated in low level mitochondrial DNA release. To test whether mitochondrial self-DNA might be a trigger factor for ISG induction in DM, we analyzed the levels of mitochondrial DNA (mtDNA) in the cytosol of fibroblasts from patients and controls. Interestingly RT-PCR of several mitochondrial genes revealed a significantly increased abundance of mtDNA in cytosolic fractions of DM fibroblasts compared to healthy controls. To prove that the increased presence of cytosolic mtDNA may be causal for inflammation in DM, we performed mtDNA depletion with 2',3'-Didesoxycytidin (= ddC) for 9 days. This led to a significant downregulation of ISGs in DM fibroblasts while the normal ISG response to G3-YSD (DNA) or pily I:C (RNA) used as viral mimicry was maintained. These data clearly indicate that mtDNA is a trigger factor for ISG upregulation in DM fibroblasts. To further assess the mechanism of sensing and to evaluate a potential therapeutic intervention we performed si RNA mediated downregulation of the stimulator of interferon genes (STING) which significantly decreased the ISG expression in patient fibroblasts. Given the essential role of STING in the cytosolic DNA sensing pathway these data link mitochondrial deterioration with mtDNA release and cell intrinsic ISG induction in DM fibroblasts. This chronic activation of the type I IFN pathway can enhance and maintain autoimmunity. The normalization of the IFN signature upon STING reduction suggests a new therapeutic interventional strategy for DM that is currently often refractory to conventional treatment.

P162 | Characterization of pemphigus patients by plasma-derived extracellular vesicles

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Pemphigus is an autoimmune blistering disease characterized by the production of autoantibodies targeting the cell surface adhesion proteins desmoglein (Dsg)3 and Dsg1, both crucial components of desmosomes in skin and mucous membranes. The generation of Dsg-specific, pathogenic IgG autoantibodies requires the complex interplay of different cell types, including e.g. autoreactive T and B cell subsets. Moreover, small extracellular vesicles (sEVs) have gained more and more attention in recent years for their role in cell-cell communication and their potential as diagnostic biomarkers.

In the present study, we have investigated the surface protein composition of plasma sEVs in patients with pemphigus vulgaris (PV) to get insights into their cellular origin and to explore the potential correlation with clinical parameters and therapeutic interventions. Therefore, plasma samples were collected from PV patients with active disease (n=28), patients in remission (n=16), and healthy control subjects (HC; n=15). Surface protein profiles of sEVs were analyzed using the MACSplex Exosome kit and machine learning approaches. Our findings revealed differential expression of several sEV surface proteins between active PV patients and both patients in remission and HC. Principal component analysis highlighted the ability of these differentially expressed sEV markers to effectively distinguish PV patients based on clinical manifestation. Ensemble Feature Selection identified CD24, CD63, CD326, and CD8 as pivotal markers for discriminating active PV patients from those in remission. Receiver operating characteristic analysis underscored their diagnostic

potential, with especially CD63 demonstrating a good performance (AUC = 0.7902). Furthermore, our study revealed that therapeutic interventions, i.e. prednisolone or rituximab treatment, were associated with an altered surface protein composition on sEVs.

In conclusion, our findings highlight the potential of plasma sEV surface proteins as diagnostic markers for distinguishing active PV from PV patients in remission and HC. In addition, the identified markers may prove valuable for disease monitoring and assessment of treatment responses, thus providing both new insights into the complex pathophysiology of pemphigus and indications for personalized therapeutic strategies.

P163 | Gliptin-induced bullous pemphigoid: characterization of patient autoantibody profiles and direct effects on adaptive immune cells

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Dipeptidyl peptidase 4 inhibitors (DPP4i), commonly known as gliptins, constitute a class of oral antidiabetics used for managing type 2 diabetes mellitus (T2DM). A noteworthy adverse effect associated with DPP4i treatment is the development of bullous pemphigoid (BP), the most prevalent of autoimmune blistering diseases (AIBDs). In BP, the generation of autoantibodies targeting the BP180 domain within human COLXVII (COL17) and or BP230, results in severe blistering and chronic inflammation of the skin.

The exact cellular mechanisms underlying gliptin-induced BP, as well as the molecular distinctions between spontaneous BP and gliptin-induced BP, remain unclear. Previous research, conducted by us and others, has highlighted a more severe blistering manifestation in BP patients linked to DPP4i treatment compared to those without DPP4i involvement. Paradoxically, the levels of anti-BP180 IgG antibodies were lower in DPP4i-associated BP patients.

To corroborate our prior findings, we explored the direct effects of four gliptins (alogliptin, saxagliptin, sitagliptin, vildagliptin) on human immune cells and keratinocytes. Regarding cells of adaptive immunity, we observed that various gliptins inhibited B or T-cell proliferation in a concentration-dependent manner in vitro, with alogliptin suppressing the proliferation of both cell types. Likewise, these same gliptins affecting adaptive immune cell populations also dampened the release of reactive oxygen species (ROS) from neutrophils. Moreover, all tested gliptins reduced cytokine release from cultured keratinocytes. Notably, this reduction did not extend to components of the complement system, known to drive local inflammation in pemphigoid diseases by recruiting neutrophils.

Additionally, we conducted a comprehensive analysis of serum samples obtained from patients diagnosed with (1) BP, (2) BP coexisting with T2DM, and (3) T2DM managed with DPP4i inhibitors which induced BP. This investigation aimed to delve deeply into the autoantibody response associated with these conditions.

Specifically, we investigated the distribution of specific IgG subclasses, glycosylation patterns of both IgG and IgM, as well as the levels of total IgG and IgM. In functional assays, the complement-fixing abilities and the degree of immune-complex-induced neutrophil activation was assessed. Presence of both BP and T2DM led to alterations in IgG subclass distribution and complement-fixing capacities. Notably, sera from patients with BP, T2DM, and gliptin treatment exhibited heightened ROS release compared to those with BP alone or BP coexisting with T2DM. In essence, our results suggest that the increased occurrence of blisters in gliptin-treated patients may be attributed to enhanced ROS release.

P164 | Immunoglobulin therapy suppresses murine acute contact hypersensitivity similar to CTLA4Ig fusion protein treatment

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Introduction: Immunoglobulin therapy is widely used in the clinics for treatment of several chronic inflammatory conditions including autoimmune diseases such as dermatomyositis and multiple sclerosis. Here we followed the therapeutic effects of recombinant human immunoglobulin G1 (hIgG1) therapy (mimicking clinically applied immune globulin treatment) and of recombinant mouse CTLA4Ig fusion protein therapy (clinically approved for treatment of psoriasis arthritis) on acute and chronic contact hypersensitivity (CHS). In addition, we monitored migration of CD4+ T cell non-invasively in vivo with positron emission tomography (PET)/magnetic resonance imaging (MRI).

Methods: One day before sensitization with 0.2% DNFB female C57BL/6J mice underwent CTLA4Ig-FP ((a) 1 mg/kg, (b) 3 mg/kg, (c) 25 mg/kg or (d) hIgG1 (25 mg/ kg) treatment (n = 6). Five days after sensitization mice were challenged (ch) with 0.2% DNFB at the right ear to elicit acute CHS, while the contralateral left ear served as healthy control. To induce chronic CHS mice were challenged every 48h up to 5 times at the right ear. Mice with chronic CHS underwent a second round of treatment (a-d) one day after the 1st ch. Four cohorts of experimental mice were studied to follow the early

and the recovery phase of acute and chronic CHS upon the four different treatment protocols (a-d): 1) early phase (48h after 1st ch); 2) recovery phase of acute CHS (96h after 1st ch); 3) early phase (48h after 5th ch); 4) recovery of phase of chronic CHS (96h after 5th ch). We determined ear swelling responses every 24h and monitored CD4+ T cell migration into the inflamed ears non-invasively in vivo with 89Zr-DFO-CD4-minibody (IAB46) PET/MR imaging. For ex vivo cross validation we conducted histological (H&E) and CD4 immunohistochemistry (IHC) of the inflamed ears.

Results: Treatment with 25 mg/kg CTLA4Ig revealed the strongest therapeutic effect reflected by reduction in the ear swelling response during acute and chronic CHS (early and recovery phase) in comparison to 1 and 3 mg/kg CTLA4Ig-FP treatment. In line with this, in vivo 89Zr-DFO-CD4-minibody PET/MRI and ex vivo histopathological and CD4-IHC analysis of inflamed ears confirmed a significant reduction in inflammation and CD4+ T cells infiltrate in inflamed ears in the 25 mg/kg of CTLA4Ig treatment group in comparison to the 1 and 3 mg/kg CTLA4Ig treatment groups during acute and chronic CHS (early and recovery phase). Treatment with 25 mg/kg hIgG1 yielded a similar efficient reduction in ear swelling response, inflammation and migration of CD4+ T cell into the inflamed ears during acute CHS (early and recovery phase) but not chronic CHS, approving a remarkable antiinflammatory effect of hIgG1 treatment during acute T cell driven inflammation.

Conclusion: Treatment with 25 mg/kg hIgG1 successfully suppressed early and recovery phase of acute CHS similar to 25 mg/kg CTLA4Ig treatment but failed to limit chronic CHS. In sharp contrast, treatment with 25 mg/kg CTLA4Ig was efficient in inhibition of both, acute and chronic CHS. Thus, hIgG1 treatment is efficient in inhibition ears swelling, inflammation and immigration of CD4+ T cells into inflamed ears during acute T cell driven CHS indicating that Immunoglobulin therapy in the clinical setting might be more efficient during acute T cell driven autoimmune diseases.

P165 | Gamma delta T cells boost blister formation during PD-1 immune checkpoint therapy in pemphigoid disease.

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Checkpoint-inhibitory molecules are regulators of T cell driven immune responses and prevent tissue damage by limiting immune cell activation. Immunotherapy with checkpoint inhibitors activates immune cells to unleash immune responses and has become a mainstay in treating several types of cancer. As a new class side effect immune related adverse events (irAE) are observed in >50% of treated patients. PD-L1/PD-1 inhibitors elicit in 1% of patients a specific IgG autoantibody- and granulocyte-driven inflammatory disease of the skin, namely bullous pemphigoid (BP). Similar to autoimmune BP patients, most of the affected irAE-BP patients are elderly. It seems likely that autoantibodies targeting hemidesmosomal skin proteins may circulate in some older cancer patients putting them at risk for BP and interruption of the PD-L1/PD-1 axis during immune checkpoint therapy eventually pushes the threshold to develop BP.

To investigate a potential role of PD-L1/PD-1 in the antibody driven effector phase of BP, we used the anti-collagen VII antibody transfer mouse model of BP-like epidermolysis bullosa acquisita (EBA). In this model, both pharmacologic and genetic PD-1 deficiency resulted in more severe skin lesions compared to isotype-treated and wild-type littermate controls, respectively. We found an increased infiltration of neutrophils by flow cytometry and detected elevated levels of interleukin (IL)-2, IL-4 and interferon gamma expression in lesional skin by qPCR during PD-1 deficient inflammation, reflecting a stronger inflammatory response. However, the composition of the immune infiltrate did not change drastically. Single-cell (sc)RNA sequencing and flow cytometry revealed PD-1 expression restricted to NK-T cells, CD4+ T cells and gamma delta (γδ)T cells in lesional skin. While NK/NK-T cell deficiency did not affect the observed worsened phenotype under PD-1 inhibition, Tcrd-/- mice lacking γδT cells were protected from an aggravated disease if the PD-L1/PD-1 axis was blocked. Because the difference between Tcrd-/- and WT mice was visible as early as day seven after disease induction, disinhibited skin-resident γδT cells may play a central role in paving the way for granulocytes to enter the skin during the effector phase of BP. This is supported by ScRNA sequencing data showing that at day seven PD-1 deficiency has already lead to an increased expression of proinflammatory cytokines and chemokines in lesional skin, which are known to attract granulocytes and monocytes. ScRNA sequencing data also showed broad expression of PD-L1 in dendritic cells, granulocytes, monocytes, and macrophages as well as several T cell subsets, providing the ligand for PD-1+ γδT cells. Treatment of mice by administration of PD-L1 fused to a Fc-protein to enhance PD-1 signaling failed to significantly lower disease activity compared to isotype control treated mice, indicating that the degree of PD-1 related immunosuppression could not be raised.

Collectively, these findings demonstrate that skin resident γδT cells are involved in the granulocyte driven effector phase of pemphigoid diseases and boost inflammation during blockade of the PD-L1/PD-1 axis triggering blister formation in predisposed skin.

P166 | Establishing a human 3D skin model for pemphigus

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Pemphigus diseases are rare but serious, IgG-mediated, autoimmune dermatoses characterized by the formation of painful, itchy blisters

and erosions of the skin and mucous membranes. The blisters result from a dysfunction of cell-cell adhesion where autoantibodies are directed against desmoglein (Dsg) 1 and/or Dsg 3 forming desmosomes to connect the epidermal keratinocytes. The loss of function by binding autoantibodies, is accompanied by intraepithelial acantholysis.

The aim of this project was to create an in vitro model for pemphigus diseases to further understand the pathophysiology to prospectively find better treatment options. For this purpose, keratinocytes and fibroblasts were isolated from elective plastic surgery specimens. In an already established procedure, we created human 3D skin models with the previously isolated cells. After the 3D skin was completely cultivated, we added the single chain variable fragment (scFv) PX43, targeting Dsg 1 and 3 and imitating the pathophysiology of pemphigus vulgaris. PX43 was added in 3 different application routes: to the medium, topically applied to the skin and injected into the skin. Afterwards the skin was incubated for 24 hours. The negative control for each application route was performed using IVIG.

For evaluation, haematoxylin and eosin stained sections were analysed, Dsg 1/ Dsg 3 immunofluorescence stainings and a direct immunofluorescence for PX43 were performed qualitatively. We can observe that PX43 leads to acantholysis in the upper epidermis. It is not equivalent to the human skin organ culture model for pemphigus vulgaris. The positive control where we put PX43 into the medium reacted with a destruction of the cell adhesion compared to the negative controls. Our results suggest that the 3D skin model is a promising preclinical tool for the evaluation of pemphigus therapies.

P167 | Immune complex induced cell migration and cytokine production of nonclassical monocytes are defined by FcγRIII-genotype and IgG glycosylation

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The excessive formation and deposition of immune complexes (ICs) are hallmarks of several autoimmune diseases. We recently described homing of nonclassical monocytes (ncMo) to vascular immune complexes in lupus erythematosus. ICengagement induced a proinflammatory response with production of TNFα and CXCL2 to induce vascular inflammation and recruitment of neutrophils. Most interestingly, we observed an IC-induced highly directed migratory response being specific for ncMo. The current study aimed to gain deeper insights into the role of the binding affinity of ICs. The quantification of haptokinesis was done by live imaging. We analyzed the cytokines released during cell migration on different concentrations of immobilized immunoglobulins using a bead-based multiplex assay for flow cytometers. Initially, we examined the influence of Fc gamma receptor IIIa polymorphism with either valine (V) or phenylalanine (F) on position 158 by genotyping ncMos prior imaging. Our findings revealed that a significantly higher percentage of ncMo with the polymorphism VV adhered to immobilized ICs while the average migration distance was reduced compared to those with the FF and VF polymorphisms. Furthermore, we explored the impact of glycosylation patterns of immobilized antibodies on cell migration. Firstly, human immobilized intravenous immunoglobulins (IVIGs) were treated with rapid PNGase F for complete deglycosylation, resulting in a significant reduction of cell adhesion but increase in speed and pathlength of migrating cells. Secondly, we investigated the effect of antibody afucosylation on ncMo activation. Afucosylated IgG is regarded as highly proinflammatory. In this regard, we compared the therapeutic antibody Rituximab to Obinutuzumab, the latter which is known for non-fucosylated sugars on the Fc-portion. At low antibody concentrations, more ncMo adhered to immobilized Obinutuzumab but migrated shorter distances compared to Rituximab. This suggests a potentially enhanced binding affinity of ncMo for afucosylated ICs. The analysis of the supernatant revealed an IC concentration-dependent secretion of proinflammatory cytokines such as IL-1β and TNFα, highlighting the inflammatory response of ncMo after stimulation with ICs. These findings indicate an important role of ncMo in IC-mediated autoimmune diseases like lupus erythematosus. In summary, we demonstrated that high affinity interaction of ncMo with ICs due to expression of CD16 polymorphism VV, or interaction with afucosylated IgG influence cell adhesion and thereby migratory response and cytokine production. These results help our understanding of the proinflammatory function of ncMo in IC induced inflammation.

P168 | A subsets of skin resident CD8+ T cells protects organ transplant receivers from skin cancer development

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Organ transplant receivers (OTRs) are at 100-fold higher risk of developing keratinocyte cancers (KC), probably due to the long-term systemic immunosuppressive therapy (SIT) to prevent graft rejection. Interestingly, not all patients develop KC in the post-transplant period (PTP). Moreover, OTRs develop more HPV-related squamous cell carcinomas (SCC) compared to basal cell carcinomas (BCC), inverting the SCC:BCC ratio of the general ageing population. Such clinical observations point to the pre-existence of protective patients- and skinspecific immune players in some OTRs.

To find out what confers cancer protection in some patients, we compared 10 OTRs with of >10 KC in the PTP, with OTRs patients that had no diagnosis of cancer. Patients were matched for age, gender, length of PTP and immunosuppressive drugs. We performed flow cytometry, tissue FAXS, RNA-seq and single-cell RNASeq matched with T-cell receptor (TCR) analysis of skin cells and PBMCs. We observed less skin CD4+ T cells are in OTRs compared to HC independent of the KC prevalence, which might reflect the general effects of SIT. In OTRs that are protected from KC, we identified increased numbers of skin CD8+ T cells with higher levels of CXCR3

and CD56. Single-cell RNA sequencing revealed increased CD8+T GZM+ NK-Like cells in the skin of patients protected from KC.
Further analysis will help identifying the origin and function of cancer-protective T cells and the molecular mechanisms that might help to enhance tissue-specific anticancer defenses in this high-risk group.

P169 | TSLP instructs dendritic cells to prime IL-31 producing TH2 cells promoting pruritus and granulocytic inflammation

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During recent years, we witnessed that IL-31 receptor signalling plays a crucial role during pruritic skin inflammation. Although it has recently been shown that therapeutic targeting of this signalling pathway is highly effective in controlling pruritus and skin inflammation, the exact cellular sources of IL-31 remain largely elusive. In this study, we aimed at characterizing the dominant cell source of IL-31 and unravel its upstream differentiation conditions as well as defining the cell phenotype. CD4+CD45RO+CCR7-CD62L- effector memory T cells represent the dominant IL-31 producing leukocyte subset within the circulation in healthy donors. Atopic dermatitis (AD) patients demonstrate an increased frequencies of IL-31+ T cells compared to healthy volunteers. In activated CD4+ T cells, the majority of IL-31+ cells did not co-express signature cytokines such as IL-4, IL-13 or IFN-γ, but demonstrated the skin-homing marker CLA and the chemokine receptors CCR4 and CCR10. RNA-Sequencing analyses of TH1, TH2 and TH17 T cell clones confirmed the predominant expression of IL31 transcripts by TH2 cells. Temporal co-expression networks as well as single cell RNA sequencing confirmed that IL31-expressing T cells co-express IL13, IL3 and CSF2. Moreover, IL31+ T cell transcriptomes confirmed the unique phenotype of this GATA3-expressing TH2 subset. To unravel upstream differentiation pathways, we analyzed 82 different conditions of dendritic cell-driven T cell priming and observed that only TSLP-stimulated blood-derived CD11c+ dendritic cells differentiated T cells in the direction of IL-31 producing T cells. Moreover, this T cell subset also co-expressed GM-CSF suggesting a 'pathogenic' effector memory T cell phenotype. Taken together, the keratinocyte-derived alarmin TSLP instructs dendritic cells to prime IL-31 producing T cells representing a unique subset of effector memory type 2 T cells that initiate pruritus and support granulocytic inflammation within the skin.

Infectious Diseases

P170 | Transcriptional profiling of Staphylococcus aureus during the transition from asymptomatic nasal colonization to skin infection in atopic dermatitis

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Staphylococcus aureus interacts with its human host in two ways: As a harmless member of the microbiota or as a pathogen once the epithelial barrier is compromised. While colonization of its primary reservoir - the human nose - is asymptomatic, nasal colonization is associated with an increased risk of infection and it is usually the nasal S. aureus strain of the individual that causes infection. The bacterium is generally part of the nasal, but not healthy skin microbiota. However, the prevalence of S. aureus carriage in patients with atopic dermatitis (AD) is ~70% on lesional skin and increases with disease severity. S. aureus possesses several wellstudied virulence factors, but their exact role in vivo, especially in the transition from asymptomatic colonization to symptomatic colonization / infection, is yet incompletely understood.
Therefore, to identify genes involved in the reprogramming the virulence arsenal of S. aureus, we took nasal swabs from AD patients and simultaneously from the skin and performed direct transcript analysis by quantitative real-time PCR without subculturing the bacteria.
In general, the expression pattern of the genes studied was very similar in both habitats. For example, genes encoding adhesion molecules (ClfB, FnbA and WTA) were highly transcribed in both nose and skin. Interestingly, the expression of the α-toxin (Hla) as well as the phenol-soluble modulins (PSMs) was low in the nose of all analyzed patients, whereas the transcription was increased on the skin of some AD patients. These observations are consistent with the expression of the global virulence regulator Agr, which positively regulates these genes. The strong Agr activity observed in some patients on the skin but not in the nose suggests that this peptide quorum sensing system and its target genes may be involved in the progression of the disease as well as in the transition from asymptomatic colonization to infection. In summary, to the best of our knowledge, this is the first time to proof the activity of the global virulence regulator Agr in an authentic human environment.

P171 | Regulation of necroptosis via HSV-1 infections on human keratinocytes

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Herpes Simplex Viruses (HSV-1 and 2) are members of the alpha-herpesvirus family and are highly prevalent DNA-viruses leading to frequent painful vesicular lesions in orolabial- and genital mucosa, causing a huge worldwide disease burden. During HSV-1 infections, keratinocyte cell death is supposed to be induced by apoptosis or autophagy. Our studies demonstrate the relevance of keratinocyte inflammatory cell death by necroptosis in the pathogenesis of HSV-1 skin infections. Furthermore, we provide evidence that high viral burden opposes viral clearance by inhibiting necroptosis and in this context identify a druggable target.

HSV-1 infection causes T cell activation and interferon gamma (IFNγ) production. IFNγ induces de novo expression of Z-DNA binding protein 1 (ZBP1) through the JAK/STAT signaling pathway in human keratinocytes. By activating nucleic acid sensor ZBP1 through HSV-1 DNA, we were able to detect the phosphorylation of receptor interacting protein kinase 3 (RIPK3) and of the mixed lineage kinase domain-like protein (MLKL). Phosphorylated MLKL is known to oligomerize forming membrane penetrating pores, the final step of necroptosis activation. Through immunohistochemical and immunofluorescence staining from patient's skin biopsies, we detected a zonal distribution of IFNγ-signaling and necroptotic mediators, with a maximal detection in the vicinity of uninfected keratinocytes.

Interestingly, we could demonstrate that high viral titers inhibited keratinocyte necroptosis. In these experiments, IFNγ-Receptor 1 (IFNγR1) expression was downregulated and subsequently IFNγ-induced STAT-signaling was absent . Searching for a cause of IFNγR1 downregulation, we discovered that inhibiting casein kinase 1 (CK-1) reduced expression of infectious HSV-1 as determined by plaque assay and thereby prevented IFNγR1 degradation.

Taken together, our studies: I) unravel the importance of IFNγ-mediated necroptosis in HSV-1 infected human keratinocytes, II) demonstrate the inhibition of necroptotic cell death by high viral titers, III) identify the virus induced downregulation of IFNγR1 degradation, and IV) identify CK-1 as regulator of viral protein expression and IFNγR1 degradation.

Therefore, inhibition of CK-1, thereby securing IFNγR1 expression and signaling, may provide a therapeutic approach for treating vesicular lesions after HSV1/2- infection of the skin.

P172 | Azole resistance assays indicate that Trichophyton schönleinii is azole sensitive compared to closely related T. quinckeanum subtypes, which is accompanied by variability of sterol-14α demethylase Erg11A protein sequences

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Introduction: Trichophyton (T.) quinckeanum, the causative agent of mouse favus, and the anthropophilic T. schönleinii show a high degree of similarity in phylogenetic analyses.1 The new T. quinckeanum subtype2 demonstrates increased itraconazole resistance and all T. quinckeanum subtypes show high resistance to fluconazole. The morphological peculiarity of T. schönleinii is the suppression of microconidia formation, accompanied by mycelium-forming 'stag antler' hyphae. Therefore, resistance assays like microplate laser nephelometry3 based on microconidia solutions were not suitable.

Objectives: A plate assay was established to measure azole resistance. DNA sequence comparison were obtained from the sterol-14α demethylases Erg11A and Erg11B genes4 as well as the squalene epoxidase gene Erg1.

Results: T. schönleinii isolates were grown on plates with a fluconazole concentration that is the power of ten lower than growth of both T. quinckeanum subtypes. They differ from the resistant T. quinckeanum subtypes in having a 100-fold reduced itraconazole tolerance. Interestingly, the T. mentagrophytes strain ATCC 46950, which also forms 'stag antler' hyphae, was the most sensitive isolate against fluconazole and itraconazole.

Sequence comparison between T. schönleinii and T. quinckeanum showed an identical Erg11B and Erg1 sequence, whereas variability was detected in Erg11A. Comparing T. schönleinii with both T. quinckeanum subtypes, four amino acid substitutions were . The Pro215His mutation is part of the F-F' motif, which is important for protein function. The Erg11A nucleotide sequence of T. schönleinii was more closely related to the sequence of the neotype1 T. quinckeanum IHEM 13697 reference strain.

Conclusion: Increased azole resistances of T. quinckeanum compared to T. schönleinii may be the result of azoles used extensively in agriculture.

1. Hoog de et al. (2017) Mycopathol 182:5-31; 2. Gregersen et al. Hautarzt (2021) 72:847-845; 3. Burmester et al. Mycoses (2020) 63:1175-1180; 4. Burmester et al. Mycoses (2022) 67:97-102

P173 | Low doses of fluconazole or voriconazole induce large increases of sterol 14α demethylase Erg11A and multidrug resistance transporter MDR3 transcripts in several T. indotineae isolates

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Introduction: Trichophyton (T.) indotineae is an emerging human pathogen that often shows multiple resistance to antifungal drugs like

terbinafine or azoles.1,2 Terbinafine resistance is the result of a point mutation in the squalene epoxidase gene Erg1, resulting in amino acid exchanges at specific positions.1,2 Point mutations in Erg11B associated with amino acid exchanges were also found in T. indotineae isolates.3 These strains show increased resistance against one specific azole but not a multiresistance to several azoles. Azole multidrug resistant strains belong to Erg1A448T mutants in approx. 50% of isolates, whereas terbinafine resistance is not significantly increased.4

Objectives: To elucidate further resistance mechanisms, qPCR analyses were performed for selected putative resistance genes such as MDR and MFS transporter as well as ergosterol biosynthesis genes.

Results: Comparison of resistant and sensitive Erg1A448T mutant strains showed a strong up regulation of Erg11A and MDR3 by the sensitive strain, if fluconazole or voriconazole was added to the medium at concentrations below significant growth inhibition. A similar phenomenon was observed in other T. indotineae strains that do not carry the specific Erg1 mutation. Interestingly, the multidrug-resistant Erg1A448T mutant strain did not respond to voriconazole and to a lesser extent to fluconazole. An increase in Erg11B transcription was also observed, but always at a lower level. Under untreated growth conditions, Erg11B transcript levels exhibited a higher level compared to Erg11A.

Conclusion: The resistance mechanism of the ERG1A448T mutant strain is independent of MDR3 and Erg11A transcript regulation.

1. Singh et al. Mycoses (2018) 61:477-484; 2. Ebert et al. Mycoses (2020) 63: 717-28.; 3. Burmester et al. Mycoses (2022) 67:97-102; 4. Burmester et al. Mycoses (2020) 63:1175-1180

P174 | Expression of MIF receptor CD74 is associated with the expansion and differentiation of cytotoxic CD8+ T cells in COVID-19 patients

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The importance of cytotoxic CD8+ T cells as mediators of protective immunity in viral infections has been known for decades. In spite of their beneficial role, T cellmediated cytotoxicity can result in severe inflammation and pathologies of the infected organ.

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection induces a vigorous T cell response including the expansion of cytotoxic T lymphocytes, thereby leading to the elimination of SARS-CoV-2 infected lung epithelium, but also enhancing pneumonia symptoms. To prevent tissue damage, tight regulation of the cytotoxic T cell response is required. Many factors and mechanisms affecting the functionality of T cells during infection have been intensively studied, but multiple components still remain elusive. One molecule that might be of special interest in this process is the Macrophage Migration Inhibitory Factor (MIF), which has been shown to be a biomarker for severe clinical courses and fatal outcomes in COVID-19 patients.

To date, the effect of MIF signaling on cytotoxic T cells in COVID-19 patients has not been characterized. In order to investigate a potential regulatory effect of the proinflammatory cytokine MIF on this T cell population in SARS-CoV-2 infection, we sought to describe the expression of MIF receptor molecule CD74 as well as its coreceptors CD44, CXCR2, and CXCR4 to reveal the susceptibility of CD8+ T cells to MIF along with studying its effects on T cell proliferation and the cytotoxic potential.

Our study demonstrated greater populations of CD74+ CD8+ T cells especially in COVID-19 patients suffering from a severe disease course. A differentiation analysis of CD8+ T lymphocytes revealed an expansion of effector memory and effector CD8+ T cells in COVID-19. Interestingly, the disease was associated with a decrease in the expression of CD74 on terminally differentiated effector CD8+ T cells. One week after hospital admission, CD74-expressing T cells with a central memory, effector memory or effector phenotype expanded. These cells also expressed the co-receptor molecules CXCR2 and CXCR4, which are crucial for intracellular signaling through MIF receptor CD74. The enhanced expression of CD74 on different CD8+ T cell subpopulations was also associated with increased levels of circulating MIF in the plasma of COVID-19 patients. Further, we showed that MIF receptor-expressing T cells proliferated extensively and increased the production of cytotoxic molecules.

Our findings indicate that MIF signaling might play an important role in the functional regulation of CD8+ T cell responses during COVID-19. Further investigation into the interaction of the MIF system and cytotoxic potential of effector CD8+ T cells in COVID-19 will shed light on possibly interesting targets for novel therapies.

P175 (OP01/05) | ICER expression on NKp46-positive NK cells/ILC1 controls immunity to cutaneous leishmaniasis

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Inducible cAMP early repressor (ICER) belongs to a group of proteins that act as transcription repressor and binds to cAMP responsive elements such as CREM and CREB. ICER thus attenuates

cellular responses in various physiological processes; we have recently shown that expression of ICER in melanoma-associated macrophages (MΦ) led to their polarization toward a non-inflammatory M2 phenotype and promoted tumor growth. Skin infection by Leishmania major, a protozoan parasite, induces Th1/Tc1-dominated immunity in immune-competent humans and resistant mice, while in immune-compromised patients and BALB/c mice, a Th2/Treg/Th17-dominated immune response dominates supporting progressive disease. We now studied the role of ICER for disease outcome in cutaneous leishmaniasis. First, using ICER-deficient C57BL/6 mice we observed that lesions of ICER-deficient mice were significantly, almost 2-fold larger and lesion resolution was delayed compared to wild type animals. Since ICER is produced by a variety of immune cells, we next assessed cell-specific expression of ICER. Interestingly, mice with ICER deletion in T cells (CD4-Cre) or MΦ (LyM-Cre) did not exhibit a change in disease course as compared to control mice. In contrast, mice that lack ICER in NK cells and innate lymphoid cells type 1 (ILC1) utilizing NKp46-Cre mice phenocopied the worsened disease outcome as mice lacking ICER in the whole body. In line, parasite burdens displayed higher number of parasites in lesions in week 6. Lesions harbored fewer ILC1 (but not NK cells), as well as CD4+ and CD8+ T cells and the frequency of IFNγ-positive ILC1 (and not NK or CD4/8+ T cells) in lesions was lower compared to Cre-negative controls. This is in line with data obtained with NKp46+Cre x DTA mice genetically devoid of NK cells and ILC1, which after L. major infection exhibited increased pathology and reduced IFNγ expression associated with delayed recovery. In contrast, in wild type mice, both the numbers of IFNγ-producing NK cells and ILC1 strongly increase early after infection, which appears to be important for early parasite control. In summary, our data shows that ICER expression on ILC1 (and less relevant on NK cells) promotes the recruitment of IFNγ+ ILC1 to skin in Leishmania infections, which is important for the development of protective immunity against this important pathogen.

X. Liu, B. Lorenz, D. Lukas and M. Reibetanz contributed equally

Pharmacology

P176 | MRGPRX2 Small Molecule Antagonists Potently Inhibit Agonist-Induced Skin Mast Cell Degranulation

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Rationale: Mas-Related G-Protein Coupled Receptor X2 (MRGPRX2) is a promiscuous receptor on mast cells that mediates IgE-independent degranulation in response to many structurally diverse compounds and has been implicated in multiple mast cell-mediated disorders, including chronic urticaria and atopic dermatitis. We investigated whether small molecule MRGPRX2 antagonists inhibit skin mast cell degranulation both in vivo and ex vivo across multiple species.

Methods: MRGPRX2 antagonist pharmacology was characterized in vitro using cell lines overexpressing human MRGPRX2 as well as mouse and canine orthologs, LAD2 mast cells, peripheral stem cell-derived mast cells, and isolated human skin mast cells. In vivo skin mast cell degranulation was evaluated in MRGPRX2 knockin (KI) transgenic mice and beagle dogs by assessment of agonist-induced skin vascular permeability and erythema development, respectively. Ex vivo skin mast cell degranulation was evaluated by microdialysis and assessment of histamine release in human skin samples.

Results: MRGPRX2 antagonists potently inhibited agonist-induced MRGPRX2 activation in vitro across species, and inhibited agonist-induced mast cell degranulation in all mast cell types tested. Orally administered antagonists demonstrated excellent in vivo efficacy to inhibit agonist-induced degranulation in both MRGPRX2 KI mice and dogs. In addition, MRGPRX2 antagonists dosedependently inhibited agonist-induced degranulation in ex vivo human skin.

Conclusions: MRGPRX2 small molecule antagonists potently inhibited agonistinduced skin mast cell degranulation in vitro and in vivo in multiple species as well as ex vivo human skin, supporting potential therapeutic utility as a novel treatment for mast cell-mediated diseases.

Photobiology

P177 (OP04/01) | Mitochondrial dysfunction in Xeroderma pigmentosum type A (XPA) causes UV-induced collapse of proteostasis due to lack of ATP

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Xeroderma pigmentosum type A (XPA), an inherited disease characterized by UV hypersensitivity, high skin cancer risk and premature aging, is thought to be caused by defective repair of nuclear DNA. Recent studies, however, suggest that XPA proteins might have functions beyond nuclear DNA repair. Here we show - by analyzing purified mitochondrial fractions from primary human dermal fibroblasts (HDF) - that XPA proteins are located inside the mitochondria. In particular, mitochondrial XPA content was increased when HDF were either irradiated with UVB or treated with the pro-oxidant menadione suggesting that XPA might be important for repairing damage inside the mitochondria and protecting their integrity. Accordingly, sequencing of mitochondrial DNA (mtDNA) of HDF obtained from XPA patients (XPA HDF) revealed an elevated load of mutations as compared with healthy HDF, which was further increased by UVB irradiation. These data suggest that XPA is not only involved in the repair of nuclear DNA but also participates in the repair of mtDNA. The pivotal role of XPA in maintaining mitochondrial

function was also reflected by a RNA-Seq transcriptome analysis showing that "Mitochondrial Gene Expression" and "Mitochondrial Translation" were among the most severely suppressed biological processes in UVB-irradiated XPA HDF. To assess potential consequences for mitochondrial function, we next measured the cellular ATP production rate. We found that unirradiated XPA HDF had a higher total ATP production rate than healthy HDF, which was due to increased mitochondrial but not glycolytic ATP production rate. If cells were stressed by UVB irradiation, mitochondrial ATP production rate in XPA HDF was reduced by more than 50% while it remained stable in normal HDF. Thus, XPA HDF have an increased ATP demand, which in unstressed cells can still be met by their mitochondrial function. Upon irradiation, however, compensation fails and XPA HDF become energy deficient. As the chaperone HSP90 serves as an ATP sensor which stabilizes proteins in an ATP-dependent manner, we next analyzed the abundance of HSP90 client proteins. HSP90 levels remained constant, but a marked loss of ErbB2, EGFR, STAT3 and SIRT1 was detected in irradiated XPA HDF when compared to healthy HDF, reflecting collapse of proteostasis. Our results indicate that XPA proteins are present in mitochondria where they maintain integrity of mtDNA and mitochondrial function to ensure cellular ATP supply and thereby prevent collapse of proteostasis, a wellknown driver of aging.

P178 | Mice with HIF-1a/AHR-double deficient keratinocytes share similarities with UVB-exposed wildtype littermates

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In keratinocytes, the structurally related Per-ARNT-Sim transcription factors HIF-1a (Hypoxia-Inducible Factor 1a) and AHR (Aryl Hydrocarbon Receptor) both act as UVB-responsive sensor proteins but are also relevant in homeostatic as well as other inflammatory settings. They dimerize with their shared co-factor ARNT (AHR Nuclear Translocator), but activate distinct sets of target genes to orchestrate cellular metabolism, immune responses, xenobiotic response and DNA repair.

The interplay resp. combined function of HIF-1a and AHR in keratinocytes for the regulation of skin functions has hardly been addressed despite potentially high relevance for functional outcomes. To investigate their relationship, we bred Crelox- mediated conditional keratinocyte-specific HIF-1a/AHR double knock-out mice ("DcKO") and also generated human single and double-KO HaCaT keratinocytes for HIF-1a and AHR through the CRISPR-Cas9 method.

The skin of naïve DcKO mice, as compared to WT and single cKO mice, showed several features which would be expected after UVB exposure in (wildtype) animals: these comprise visible hyperpigmentation of ears and tails mirrored by increased tyrosinase expression, mild epidermal thickening and significantly fewer Langerhans cells (LC) with longer dendrite expansion, indicating altered activity compared to controls. Accordingly, FoxP3+ Treg frequency was higher in skin-draining lymph nodes and upon ex vivo stimulation secreted more IL-10, similar to a state of UVBinduced immunosuppression.

In line, initial single-cell RNA sequencing analysis of murine whole skin indicated that in DcKO skin, signatures for multiple immune processes were significantly downregulated in the designated LC cluster compared to WT, including "responses to stimuli" and "regulation of leukocyte chemotaxis and migration". First cytokine array results of HaCaT cells indicate that in double-KO cells, multiple proteins were differentially expressed which are known to modulate UVB-related and inflammatory processes, e.g. EGF, IL-12p70, IL-13, TNF, KGF and more, further indicating the potential of double-KO keratinocytes to alter epidermal functions in vivo.

Our data raise the question whether the murine DcKO phenotype exhibits improved protection against UVB and/or is a consequence of constantly increased cellular stress due to the double transcription factor deficiency in keratinocytes. Intriguingly, initial UVB-induced CPD formation appeared lower in DcKO mice compared to WT, most probably due to their epidermal hyperpigmentation and hyperplasia. Therefore, we will next examine the DNA repair capacity in human vs. murine keratinocytes and mouse skin as well as the functionality of Langerhans cells and T cell responses in the DcKO mouse. Further, we want to dissect the mechanism leading to UVB-independent hyperpigmentation in these mice. Taken together, we aim to provide new insight into the interplay of environment-sensing transcription factors in epidermal keratinocytes for the regulation of cutaneous functions.

P179 | Assessment of temperature and humidity induced by a medical device for red light photodynamic therapy

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Photo dynamic therapy (PDT) belongs to the most effective therapies for field cancerization in patients with epithelial premalignant lesions like actinic keratoses. Beside daylight PDT red and blue light PDT devices are used emitting wavelengths of about 635 nm and 400 nm, respectively. In the latter heat and burning sensations are common side effects claimed by patients. Therefore cooling is applied regularly to soothe these effects. However, data whether the heat sensations are due to increase of temperature or rather caused by the 5-aminolevulinic acid dependent phototoxic process itself are sparse. Our study aimed to measure systematically the temperature induced by a red light PDT device depending on the time of irradiation. For the analysis we used the standard medical device BF-RhodoLED by Biofrontera Pharma GmbH emitting radiation with a peak at a wavelength of 635 nm. The temperature was measured in a typical working distance of 5 cm and at fixed timepoints (0, 5, 10 and 15 minutes).

Measurement of air temperature showed constant values at measured timepoints. However, air humidity values measured were decreased significantly after 5, 10 and 15 minutes of 635 nm irradiation. These changes are possibly due to increase of material temperature of the measurement device. The data underline the importance of measurement not only of air temperature but also of surface temperature of irradiated material when analysing medical devices.

P180 | Assessment of Penetration Depth and Cytotoxicity of Titanium Dioxide Nanoparticles on Skin with Barrier Defects

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Titanium dioxide is used as a mineral UV filter in many sunscreens or other cosmetic products to protect the skin from harmful UV exposure. However, the exposure of titanium dioxide nanoparticles to UV radiation in the presence of water leads to the production of reactive oxygen species (ROS) in a process called photocatalysis. The increased production of ROS in turn may lead to increasing damage to cellular macromolecules such as proteins and nucleic acids and can therefore induce damage in the genome.

Several studies have shown that titanium dioxide nanoparticles from sunscreens do not penetrate through the stratum corneum of the epidermis and hence do not get in contact with living cells of intact and healthy skin. However, it remains unclear if defects in the skin barrier due to sunburn, salt water, or diseases such as psoriasis or atopic dermatitis allow titanium dioxide nanoparticles to penetrate deeper into the skin and get close to living and proliferating cells.

Against this background, this project aims to determine the penetration depth and cytotoxicity of titanium dioxide nanoparticles in skin with barrier defects. Therefore, an in vitro three-dimensional skin equivalent model with and without skin barrier defects is established and compared to a psoriasis-like mouse model as well as to lesional and non-lesional human ex vivo skin biopsies of patients with psoriasis. Samples are analyzed with various methods such as total reflection X-ray fluorescence spectroscopy (TXRF), raman spectroscopy as well as cryo-scanning electron microscopy (SEM) to generate penetration depth profiles of titanium dioxide nanoparticles. The photocatalytic activity of the nanoparticles under UV exposure is assessed in vitro. DNA damage and alterations in the epidermal differentiation after exposure to titanium dioxide nanoparticles and UV will be determined by immunohistochemical and immunofluorescence staining as well as quantification of gene expression using RT-qPCR.

Results of this project will contribute to the risk assessment of sunscreens containing titanium dioxide nanoparticles for skin with barrier defects and provide insight into the usability of in vitro skin equivalents as an alternative to mouse models or human ex vivo skin.

P181 | Filtered 222nm UVC irradiation did not induce detectable CPD formation in skin reconstructs even at high and repetitive doses for bacterial killing

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In general, the application of UVC as a disinfection method is capable to combat and kill microbial pathogens. However, due to UVC's potential to induce protein and DNA damage in human cells, leading to proliferation arrest or even cell death or mutagenicity, it is crucial to determine its application safety.

Recently several research groups demonstrated that irradiation with filtered 222nm UVC is a promising strategy to neutralize pathogens, including the corona virus. Such an increased purity of UVC was shown to have less detrimental effects on eukaryotic cells compared to its effects on procaryotes and viruses. Still, although new 222nm UVC-devices have emission peaks in the short-wavelength region of UVC (~222nm), there is still residual "collateral" radiation at wavelengths that could be potentially harmful to human health (>230nm). To exclude potential mutagenic effects on human skin during clinical application, this study investigated the DNA damage in form of cyclobutylpyrimidine dimers (CPD) caused by filtered 222nm UVC on human skin reconstructs. In addition, the antimicrobial capacity of such filtered 222nm UVC was investigated as well.

Human full-thickness skin reconstructs were irradiated with 222nm UVC without and with filters (blocking range of 230nm to 270nm) with different single irradiation doses (100mJ/cm2, 500mJ/cm2) and with repetitive treatment (3x500mJ/cm2) or with UVB (308nm) only. The administered doses had no significant effect on the viability of the skin reconstructs. The treatment with UVB (positive control) and non-filtered UVC irradiation induced a significant amount of CPDs, compared to non-treated samples. When filtered 222nm UVC was applied, the amount of CPD was lower compared to unfiltered UVC treatment and UVB treatment. Repetitive filtered UVC irradiation did not result in accumulation of CPDs compared to UVB treatment. In addition, an antimicrobial effect (99.9% reduction of colony forming units) of such filtered UVC 222nm against E. coli, S. aureus and Candida albicans could be demonstrated. This means that a therapeutic window has been identified in which microorganisms are killed but tissue is still alive and not damaged.

Overall, this study shows both an antimicrobial effect against bacteria and fungi and the protective potential of filters against DNA damage induced by UVC 222nm irradiation, which could give rise to clinical applications in the future. Therefore, it is a simple and relatively easy to apply method that could be implemented in a variety of fields such as means to disinfect hospital rooms, appliances, or even in hand sanitization.

P182 | The role of UV-induced DNA damage, matrix metalloproteases and mi-RNAs in the pathogenesis of cutaneous and systemic lupus erythematosus

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Cutaneous (CLE) and Systemic Lupus erythematosus (SLE) are autoimmune diseases with multifactorial pathogenesis that includes genetic predisposition, environmental triggers, and abnormalities of the innate and adaptive immune response. Clinical manifestations of LE range from mild effects limited to the skin in cutaneous LE (CLE) to serious and possibly life-threatening manifestations found in systemic LE (SLE). CLE can be further subdivided into acute cutaneous lupus erythematosus (ACLE), subacute cutaneous lupus erythematosus (SCLE), and chronic cutaneous lupus erythematosus (including discoid erythematosus (DLE)), LE tumidus (LET), and Chilblain lupus. Ultraviolet (UV) radiation, the most important environmental trigger of LE, is known to cause DNA damage and cell apoptosis, especially in the upper layers of the epidermis. In healthy skin, damaged keratinocytes are cleared effectively without triggering an immune reaction. However, in LE patients, the disposal of damaged cells is impaired, leading to an autoimmune response. Matrix metalloproteases (MMPs) are involved in apoptotic clearance and skin homeostasis by rearranging extracellular matrix and thus enabling cell migration. In addition, many MMPs are also UV-regulated, making them potential players in the development and progression of LE.

Besides MMPs, micro RNAs have also been shown to play an important role in LE pathogenesis. Amongst 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 our current work, we hypothesized that different CLE subtypes and SLE react differently to sun exposure due to clinically observed differences in photosensitivity and manifesting skin lesions. We investigated the amount of DNA damage (CPDs and H2AX) in lesional skin samples from patients with CLE and SLE and compared them to healthy skin and samples from patients with polymorphous light eruption (PLE). Furthermore, we correlated the expression of mir-31 and mir-150 with the expression of the GLUT1 receptor, as well as MMP1 and MMP28. In addition, we have investigated the influence of UV radiation and pyruvate (as an intermediate product of glucose metabolism) on the expression of MMP28 in healthy skin cells.

We found out that MMP28 expression is up-regulated by UVA irradiation. Furthermore, high concentrations of pyruvate, in combination with UVA, have different effects on MMP28 expression in human fibroblasts compared to keratinocytes. MMP28 also showed distinct vertical distribution in Chilblain samples compared to all other LE subtypes. This expression pattern also correlated with decreased GLUT1 expression in the epidermis of the Chilblain patients, which in turn overlapped with high expression of both mir-31 and mir-150. The remaining LE subtypes showed no differences in mir-31 and mir-150 expression compared to normal skin and PLE. As for the GLUT1 expression, all other LE subtypes, except Chilblain LE had epidermal GLUT1 levels similar to normal skin. On the other hand, the dermal expression of GLUT1 was uniformly higher in LE samples than in normal skin. These data present evidence for possible metabolic dysregulation playing a role in the etiology of LE.

Pruritus

P183 (OP01/04) | 4-1BB and its ligand are upregulated by itch-associated cytokines and seem to mediate neuroimmune communication

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Chronic skin inflammation involves the communication of immune cells with cutaneous nerve fibres. Unlike other receptor-ligand pairs of the TNF superfamily, 4-1BB and its ligand, 4 1BBL, are expressed by neuronal as well as immune cells and are upregulated upon inflammation. Worth mentioning that 4-1BB is also enhanced in lesional skin from individuals with Atopic Dermatitis (AD), leading us to hypothesize that 4-1BB/4-1BBL signalling might contribute to neurogenic skin inflammation by mediating neuroimmune communication. To investigate this aspect in detail, we generated transgenic mice overexpressing 4-1BB in basal keratinocytes (K14-4-1BB TG). Interestingly, K14-4-1BB TG mice spontaneously developed a chronic pruritic skin inflammation, histologically resembling human AD. To better understand the underlying cellular and molecular mechanisms we first depleted mast cells and could show that this cell subset was of minor importance during disease progression in TG mice. However, the depletion of CD8+ T cells or the local ablation of peripheral sensory nerve fibres ameliorated pruritus as well as skin inflammation, suggesting that cutaneous 4-1BB/4-1BBL signaling might indeed play a critical role during neuroimmune communication and the development of an AD-like pruritic skin inflammation in K14-4-1BB TG mice. Next, we intended to investigate the impact of 4-1BB/4-1BBL signalling on neuroimmune crosstalk in detail and therefore, performed RNA sequencing analyses. Interestingly, we identified a markedly increased expression of IL-31, thymic stromal lymphopoietin (TSLP), S100A8/A9, as well as IL-4 and IL-13 in lesional compared to non-lesional skin from K14-4-1BB TG mice. To analyse whether these factors were able to modulate the phenotype and function of peripheral nerves or were directly linked to 4-1BB and 4-1BBL, we cultured nerve fibres that were generated from dorsal root ganglia (DRG) in the presence of IL-31, IL-4 + IL-13, TSLP or S100A8/A9. In particular,

treatment with IL-31, IL-4 + IL-13, and TSLP significantly upregulated the expression of 4-1BB and 4-1BBL on mRNA as well as protein level and induced the expression of further pruritus-associated genes such as Nav1.7, Nav1.8, members of the Mas-related G protein-coupled receptor (Mrgpr) family, TRPV1, TRPV4, or TRPA1, thus suggesting a direct link between 4-1BB/4-1BBL signalling, itch and potentially, neurogenic skin inflammation. Finally, to confirm that the 4-1BB/4-1BBL pathway indeed contributes to the neuroimmune crosstalk, we co-cultured DRG-derived neurons that were treated with TSLP to upregulate 4-1BB and 4-1BBL with dendritic cells (DC) or T cells. Subsequently, the interaction of immune cells and neurites was investigated by immunofluorescence staining and we could demonstrate that 4-1BB and 4-1BBL expression was mainly detectable in regions where DC or T cells attached to the neurites. Taken together, our data suggest that 4-1BB/4-1BBL interactions might play an important role in the communication of immune cells and sensory neurons and thus, be critically involved in the development of neurogenic skin inflammation such as AD, which we will investigate in more detail in the future.

P184 | Altered epidermal neuroanatomy and increased local sensitivity driven by scratching in different entities of chronic pruritus

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Scratching is the physiologic response to relieve the sensation of itch. Patients with chronic pruritus (CP) may enter an itch-scratch cycle that implies repetitive mechanical stress and barrier impairment of the epidermis leading to lichenification. In this study, we aimed to investigate the influence of chronic scratching on epidermal neuroanatomy and the response to electrical stimuli in different entities suffering from CP. Therefore, biopsies of patients with atopic dermatitis (AD; n=35), brachioradial pruritus (BRP; n=34) and chronic nodular prurigo (CNPG; n=36) were analyzed in pruritic lichenified (chronically scratched), pruritic non-lichenified and non-pruritic non-lesional skin compared to matched healthy controls.

The intraepidermal nerve fiber density, assessed by the number of nerve fibers crossing the basement membrane, was significantly reduced in all of the three tested skin areas of AD, BRP and CNPG patients compared to healthy controls. Both pruritic areas of AD and BRP patients showed significantly fewer nerve fibers compared to their non-pruritic skin whereas in CNPG the chronically scratched (pruritic lichenified) skin had the lowest fiber count compared with pruritic non-lichenified and not affected skin. Epidermal branching of nerve fibers was most abundant in lichenified skin, especially in BRP patients. The increased branching may increase local neuronal excitability, thereby partially compensating for fiber loss. In each group up to 50% of CP patients responded with itch by electrical stimulation with half sine and sine wave pulses. The maximal electrically induced itch intensities of these responders were significantly increased in the tested areas of patients, especially in pruritic skin, compared with healthy controls.

Our results suggest that chronic scratching reduces intraepidermal nerve fiber density but increases epidermal branching. The combination of fewer, but hyperexcitable fibers in symptomatic skin may facilitate an electrically stimulated "spatial contrast" pattern that is interpreted as itch on a spinal level. Additionally, increased electrically induced itch in non-pruritic skin might be a sign of central sensitization.

P185 | Mechanical stress in pruritus pathogenesis and chronicity

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Patients suffering from chronic pruritus often experience an aggravating itch-scratch cycle. While reflexive scratching provides momentary relief from the itch, extensive scratching can trigger a cascade of mechanisms, including the release of interleukins and damage to the skin barrier due to scratch-induced lesions, ultimately intensifying the itching sensation. In most in vitro models designed to simulate scratching, the conventional approach involves disrupting cells, resulting in a non-physiological release of mediators. To better understand the role of mechanical stress in pruritus pathogenesis, we employed an innovative method to simulate scratching in vitro using a cytostretcher. This approach allows us to apply mechanical stress to cells, mimicking the mechanical conditions of chronic scratching without causing cell damage.

Our study aimed to investigate the impact of mechanical forces on the regulation of genes associated with pruritus in human keratinocytes. Therefore, we treated keratinocytes from healthy donors (NHEK), as well as keratinocytes from patients with chronic pruritus who exhibited pruritic-lichenified skin due to persistent scratching (PL) and unaffected skin (NPNL) from the same patients (n=6), using the cytostretcher. Subsequently, we conducted comprehensive transcriptome analyses based on RNA sequencing and proteome analyses using mass spectrometry.

Our findings unveil a striking connection between mechanical stress and the regulation of fundamental structural components, such as keratins and cytoskeletonassociated genes, like actin. Remarkably, this influence is consistent across all samples, regardless of their

origin. Even more intriguingly, our research has illuminated an unexpected impact on genes associated with neuron projection development e.g. DPYSL3, VIM, CIB3, NDRG4, as well as peripheral nervous system development e.g. SLC5A3. This compelling discovery extends beyond mere gene expression patterns and is in part substantiated by our comprehensive proteome analysis.

These results underscore the far-reaching implications of chronic scratching, a mechanical stressor, on cellular stability and morphology. Furthermore, they suggest a potentially transformative role in the complex processes of epidermal innervation and the regeneration of epidermal nerve fibers. This could have profound implications for the study of both dermatological conditions and neurodegenerative disorders like small fiber neuropathies as often found in pruritic entities, bridging the seemingly disparate realms of skin biology and neurological health.

P186 (OP05/02) | Recovery of altered marker gene expression, intraepidermal neuroanatomy and neurophysiology in pruritic lesional skin of atopic dermatitis patients after treatment with dupilumab

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Th2 cells and ILC2 cells play a crucial role in the development of atopic dermatitis (AD), producing cytokines like IL4, IL13, and IL31. IL4 and IL13 seem to be of major importance in this context, as demonstrated by the remarkable therapeutic success achieved by dupilumab - an antibody targeting the IL4Ralpha chain, shared by both the IL4- and IL13-receptor - in the treatment of patients with AD. Within this study, we aimed at identifying changes in the expression of relevant marker genes, neurophysiology and intraepidermal neuroanatomy before and after treatment with dupilumab in patients with AD.

In a monocentric, open-label study, 49 patients were enrolled, with a mean age of 41.1 years (32; [19-78]) and moderate to severe AD with pruritus lasting >6 weeks, receiving an in-label therapy with dupilumab (300mg s.c., Q2W) for 16 weeks. At baseline (BL) and after 16 weeks (W16), quality of life (via DLQI), anxiety and depression (via HADS), and pruritus intensity in the past 24 h (via NRS) were measured. For analysis of intraepidermal nerve fiber density (IENFD) by PGP9.5 staining, three skin biopsies were taken - two biopsies at BL (pruritic lesional (PL) and non-pruritic, non-lesional (NPNL)) and one at W16 (former pruritic lesional/ healed (FPL)). Furthermore, biopsies were used for analysis of gene expression of relevant key target mediators such as pruritogen receptors (IL4R, IL13RA1, IL13RA2, CRLF2 and its binding partner TSLP) and mediators modulating innervation density (NGF, SEMA3A, IL31). Alloknesis testing (itch induced by innocuous mechanical stimuli) was performed before biopsy via cotton swab/brush strokes at BL PL, NPNL and W16 FPL.

Treatment with dupilumab significantly decreased NRS ([0-10]) from BL (8; [2-10]) to W16 (3; [0-9]; $p < 0.001$), as well as the DLQI ($p < 0.001$; BL: 11, [3-30]; W16: 3, [0-17]), both showing a highly significant correlation with another ($p < 0.001$). We found IENFD reduced at PL and NPNL at BL, improving significantly after 16 weeks of dupilumab treatment (PL: 5.62; [0.75-19.91] vs. FPL: 7.69; [1.13-15.47]; $p = 0.002$). Alloknesis was significantly more frequently detected at BL PL vs. NPNL ($p = 0.021$), improving significantly from BL to W16 (PL vs. FPL, $p = 0.016$). Both IENFD and alloknesis correlated significantly with another at BL PL ($p = 0.019$, $r = 0.365$)). Further, alloknesis showed a significant correlation with NRS at BL NPNL ($p = 0.011$, $r = 0.393$), as well as at W16 FPL ($p = 0.049$, $r = 0.345$). We found increased expression of all analyzed marker genes in BL PL compared to BL NPNL, recovering at W16 FPL. Furthermore, we found a significant positive correlation of NRS with expression of CRLF (BL PL: $p = 0.01$, $r = 0.375$) but not with its agonist TSLP. Additionally, in BL PL skin, IENFD showed negative correlations with SEMA3A ($p = 0.041$; $r = -0.302$) and TSLP ($p = 0.008$, $r = -0.387$).

In sum, treatment of AD patients with dupilumab seems to have not only a clinical beneficial, but neuroanatomical and neurophysiological measurable effect, demonstrating the therapeutic relevance in curing AD patients. In addition, IENFD and marker gene expression may provide corresponding markers for clinical successful treatment.

P187 | Quality of life across patients with chronic pruritus of various etiologies: results of a national research consortium (FOR 2690)

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Introduction: Chronic pruritus (CP, >6 weeks duration) is a frequent complain in the general population and indicator of underlying diseases. The consequence of CP is regularly a reduced quality of life (QoL). The DFG-funded research consortium FOR 2690 "PruSearch"

(https://www.umm.uni-heidelberg.de/prusearch/for-2690/) aims to identify targets and mechanisms in a multidisciplinary and patient-centered approach. In this context, we compared the differences in patient-reported QoL of various patient groups depending on itch intensity using a numerical rating scale (NRS).

Methods: At baseline, patient with CP filled in several digital questionnaires including NRS (in last 24h) and a pruritus-specific 5-item questionnaire on QoL (5PLQ), Hospital Anxiety and Depression Scale (HADS) questionnaire, 12-item Short Form survey (SF-12) on general mental and physical health status, and the State-Trait Anxiety Inventory (STAI) questionnaire. We included patients with atopic dermatitis (AD, n=142), brachioradial pruritus (BRP, n=34), lichen planus (LP, n=27), notalgia paraesthetica (NPE, n=16), neuropathy with itch (NPI, n=21)/with pain (NPP, n=84)/ with pain and itch (NPPI, n=27)/without pain or itch (NP, n=46), psoriasis (PSO, n=57), systemic disease with itch (SD, n=84) and healthy volunteers (HV, n=208). We stratified by high (NRS>3) or low (NRS≤3) itch intensity and performed an ANCOVA test using NRS as covariates.

Results: As expected 5PLQ scores of AD and PSO patients were significantly higher in patients with itch NRS>3. However, independent of itch intensity, patients` 5PLQ scores in AD differed significantly from PSO, BRP, NPI, NPPI and SD patients potentially reflecting differences in itch quality or scratching. Interestingly, NPPI patients had higher scores than NPI and NPP patients indicating that additional pain symptoms reduce QoL in patients with neuropathic itch. Moreover, 5PLQ scores of BRP patients exceeded those of NPI patients indicating that despite the larger spatial extent of symptoms in polyneuropathy patients, QoL is reduced more by localized neuropathy. The SF-12 scores indicated that patients with neuropathic pain report significantly worse physical health than all other CP patients. Besides, HADS anxiety and SF-12 mental health scores of NPP and NP patients were significantly different from SD patients. We did not find significant differences between CP patients in all QoL scores, but significant differences from HV were consistently present.

Conclusion: Pruritus related QoL was most impaired in AD, LP and PSO. Importantly, major differences between subgroups of patients with neuropathic itch exist. In a multidisciplinary fashion, we will analyze in detail the role of different mechanisms in neuropathy leading to either itch, overlap of itch and pain or pure pain symptoms.

Tumor Biology

P188 | Alpha-Melanocyte-stimulating hormone and its impact on MDSC generation in skin cancer

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Skin cancer, including malignant melanoma (MM) and non-melanoma skin cancer (NMSC) like basal cell carcinoma (BCC) and squamous cell carcinoma (SCC), is one of the most common cancers worldwide with an ever-rising prevalence. Various factors contribute to the development of skin cancer among others inflammation. In particular myeloid-derived suppressor cells (MDSC), an immunosuppressive cell subset that develops and expands during cancer and inflammation, are able to control skin cancer progression by suppressing host anti-tumoral immune responses. MDSC have different mechanisms of suppressing anti-tumoral immunity such as blocking the proliferation of CD8+ cytotoxic T lymphocytes (CTL) and enhancing the development of immunosuppressive regulatory T cells (Tregs). Therefore, one way to support cancer therapy is to improve anti-tumoral immunity by inhibiting or reducing MDSC development. Previously, we have shown that the neuropeptide alpha- Melanocyte-stimulating hormone (α-MSH), which is a potent immunomodulatory compound, increases CTL numbers in tumor-bearing mice as well as in patients with NMSC. In a murine carcinogenesis model, α-MSH prevented the expansion of MDSC leading to increased levels of tumor-specific CTL and a reduced tumor growth. The effect was dependent on binding of α-MSH to the melanocortin 1 receptor (MC1R) since α-MSH treated mice lacking a functional MC1R showed regular MDSC counts and CTL levels as well as normal tumor development. It was shown that MC1R ligation by α-MSH suppresses NF-κB activation. However, NF-κB activation (for instance, via the damage-associated molecular pattern (DAMP) proteins S100A8 and S100A9 binding to TLR4) is crucial for MDSC generation in cancer. Since the underlying mechanism in humans is not completely understood, we first investigated the effect of S100A8 and S100A9 on the generation of MDSC from human peripheral blood mononuclear cells (PBMC) in the presence or absence of α- MSH. As expected, S100A8 and A9 proteins induced the conversion of myeloid cells into MDSC, which were predominantly of the monocytic phenotype. Alpha-MSH in contrast, seemed to reduce the generation of total MDSC in PBMC stimulated with GM-CSF and IL-6. Taken together, we could show that α-MSH inhibits conversion of myeloid cells into MDSC in mice and probably also in human PBMC leading us to hypothesize that α-MSH might also be capable of controlling the expansion and function of MDSC from human skin cancer patients, which we will investigate in the future.

P189 | Overexpression of DIRAS1 in mouse skin reduces tumorigenesis during multistage chemical carcinogenesis

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The incidence of non-melanoma and melanoma skin cancers has been increasing steadily over the past years. Currently two to three million people are diagnosed with non-melanoma skin cancers each year.

The GTP-binding protein Di-Ras (DIRAS) family compromises three Ras-related small GTPases (DIRAS1-3) with low intrinsic GTPase activity. DIRAS1 has been reported to function as tumor suppressor in

various cancers including glioblastoma, colorectal cancer and renal cell carcinoma. Furthermore, loss of Diras1 expression has been associated with poor treatment response in HER2+ breast cancer and esophageal squamosa cell cancer. While the exact biological function has not been fully understood in-silico docking studies suggest Diras1 acts as competitive antagonist of other small GTPases and may inhibit the PI3K/AKT as well as the MAPK/ERK pathway.

DIRAS1 is strongly expressed in the cortex, cerebellum, spinal cord, pituitary gland, adrenal gland, testis, and also in skin. Even though DIRAS1 is expressed in squamous cell carcinoma (SCC), its function in cutaneous SCC (cSCC) is still unknown, but it is assumed that DIRAS1 could act as tumor suppressor in cSCC. To investigate the function of DIRAS1 in vivo we have established a transgenic mouse model by overexpressing the murine cDNA of Diras1 under the control of the chicken beta actin promoter (CBA-Diras1). The new generated transgenic mouse line shows no distinct phenotype and histological analysis of the skin have revealed no differences in comparison to control animals. In order to analyze the role of DIRAS1 during skin carcinogenesis, DIRAS1 transgenic mice and controls received a single application of the initiating agent 7,12- dimethylbenz(a)anthracene (DMBA) followed by multiple applications of the promoting agent 12-O-tetra-decanoylphorbol-13- acetate (TPA). Preliminary results show that after 18 weeks past DMBA application Diras1 overexpression decreases tumor incidence and CBA-Diras1 mice also developed less tumors and show decreased tumor burden compared to control litter mates. These findings suggest DIRAS1 acts as tumor suppressor in skin carcinogenesis, making DIRAS1 a potential target for a tumor suppressor therapy in cSCC.

P190 | The Influence of Nras and Trp53 Point Mutations on Melanoma Plasticity and the Tumor Microenvironment

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Oncogenic mutations in the MAPK signaling pathway are pivotal in the progression of melanoma. These genetic alterations not only drive the development of melanoma but also have the potential to influence cell differentiation states, thereby impacting the tumor microenvironment and response to cancer immunotherapies. Here, using the CRISPR-Cas9-based "prime-editing" technology, we introduced humanrelevant oncogenic point mutations in murine B16F1 melanoma cells in order to investigate their effects on melanoma cell plasticity, progression, immune regulation and therapy-resistance.

We have established polyclonal B16F1 Trp53 Y217C and B16F1 Trp53 Y217C Nras Q61K melanoma cell cultures and generated a variety of monoclonal cell lines. Successfully prime-edited melanoma cells were validated by NGS and functionally characterized, e.g. by qRT-PCR or Western Blot analyses to determine cell differentiation states in vitro. Surprisingly, we identified matched-pairs of monoclonal cultures exhibiting either differentiated (MITF high) or dedifferentiated (MITF low) cell states. In vivo, we observed slightly reduced tumor growth, however increased CD45+ immune cell infiltration in dedifferentiated tumors when compared to differentiated melanomas upon subcutaneous implantation into immunocompetent mice, so far. Analyses of tumor tissue and established ex vivo melanoma cell lines in addition confirmed the maintenance of differentiated as well as dedifferentiated cell states compared to in vitro data.

In summary, we have developed innovative human-relevant experimental melanoma models that provide an excellent platform to study genetic as well as phenotypic heterogeneity in melanoma and its effects on immune regulation. In further studies, we will employ immunotherapeutic approaches to explore therapeutic responses and identify mechanisms of resistance in order to identify novel and personalised treatment strategies for melanoma in the future.

P191 | Use of a chick chorioallantoic membrane-based uveal melanoma patientderived xenograft model to assess the therapeutic potential of calcium electroporation in comparison to electrochemotherapy with bleomycin

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Uveal melanoma (UM) is the most commonly diagnosed intraocular tumor in adults. First-line treatment modalities include resection, radiotherapy and enucleation. Despite significant advances in the diagnosis and prognosis, positive long-term survival outcome of patients with UM remains low due to high metastatic and mortality rates. The need of a safe and efficient treatment strategy that prevents further disease progression, increases the survival benefit and improves the quality of life is crucial for UM-patients. The chorioallantoic membrane (CAM) represents a highly vascularised extraembryonic membrane and the CAM assay is a suitable, cost-efficient in vivo platform for cancer research that offers a short experimental period and high reproducibility.

The aim of this study was to evaluate the potential use of calcium electroporation (CaEP) as a novel method for UM treatment therapy in a recently established UM patient derived xenograft (PDX) model based on the CAM assay. The application of short electric pulses increases the permeability of the cell membrane allowing the passage of molecules into the cytosol, where calcium uptake leads to the depletion of adenosine triphosphate, linked to cell death. Similarly, electrochemotherapy (ECT) facilitates the administration of chemotherapeutic agents, such as bleomycin, in combination with high voltage pulses enhancing the cytotoxic effect of the applied agent.

Chicken embryos were grown in ovo. At embryonic day 7 (ED7) fresh tumor fragments obtained from UM patients were engrafted on the CAM with Matrigel. Seven days after implantation treatment

was performed. Accordingly, calcium in a concentration of 5 mM and 10 mM, and 1.0 μg/mL and 2.5 μg/mL bleomycin were prepared. Tumor samples were electroporated with eight rectangular pulses of a 1000 V/cm pulse strength alone or in combination with either calcium or bleomycin. Untreated tumor samples were used as a control. Calcium- or bleomycin-only treatment was included in the analyses. At ED18 the Matrigel grafts were harvested from each embryo. Following, hematoxylin/eosin (HE) staining was applied to assess the cell growth and evaluate the cell morphology. Immunofluorescence was conducted to determine the cell viability and cytotoxicity using characteristic markers. Melan-Mix staining was utilised to detect specific melanoma cells, whereas Ki-67 and BrdU were used for the assessment of cell proliferation. The recognition of neovascularisation was obtained by CD31 and caspase-3 served the visualisation of apoptotic cell formations. Additionally, specific tumor parameters, such as length, width, diameter, circumference, were analysed after photographic documentation during the development and treatment period as well as via digital image analysis.

Qualitative analysis of the HE staining provided evidence of a possible induction of partial disorganisation of the tumor structure with local depletion of cells intratumorally for both combined treatment settings. Additionally, the current results supported the hypothesis, that all applied therapeutic agents showed a reduction of size parameters when combined with EP. Furthermore, a higher dispersion of tumor cells was observed for the calcium-only treatment modality.

This study demonstrated the use of CaEP as a novel treatment modality for UM in a previously established PDX platform based on the CAM assay. The different treatment settings allowed extensive exploration of this method. The application of CaEP may be considered for therapy of small UM. Further investigation in vivo for a better understanding of the CaEP effect on the tumor microenvironment, as well as the design of a new electrode suitable for larger tumors could be proposed as subsequent stepping stones.

P192 | Electroporation in combination with calcium chloride or bleomycin in chick chorioallantoic membrane tumor xenografts from uveal melanoma cell lines as a potential adjuvant treatment modality

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Introduction: The objective of the present study is to establish and optimize an in vivo tumor xenograft model for uveal melanoma (UM) from two different cell lines, based on the chick chorioallantoic membrane (CAM) assay. Furthermore, the effect of electroporation was investigated in combination with calcium chloride and bleomycin in various concentrations in vivo, underlining its feasibility as a new therapeutic modality for uveal melanoma.

Materials and Methods: Fertilized white chicken eggs were incubated at a temperature of 37.5 °C and a humidity of approximately 60-70% to induce embryogenesis. On experimental day five (ED 5) of embryonic development, the CAM was lowered by removing 6-8 mL albumin and on ED 6 a window was cut in the shell of the eggs, exposing the embryonic structures. Tumor pellets with 1x106 cells were generated on ED 7 using UPMM3 and UPMD2 uveal melanoma cell lines. The CAM's surface was gently lacerated using a sharp debridement spoon in the proximity of a blood vessel bifurcation without causing extensive bleeding. A sterile ring was gently tapped on the lacerated periderm, while the tumor pellets were resuspended with 25μl Matrigel, bevor being pipetted in the middle of the ring. The rings were removed on ED 8 from the CAM. 5 or 10mM calcium chloride and 1 or 2,5 μg bleomycin were prepared on ED 14 for injection alone or in combination with electroporation. A control group consisted of untreated tumors. The tumor grafts were positioned between the two ends of a customized electrode and 8 square waves of electric pulses of 1000 Volts/cm strength, 100 μs pulse duration and 5 Hz repetition frequency were subsequently applied. On ED 18 the primary implanted tumors were surgically excised and the embryos were decapitated. The lower CAM was inspected for signs of tumor growth and pigmentation. Moreover, the histological and immunhistochemical examination of the specimens were conducted for the evaluation of size parameters, proliferation, viability, vascularization and apoptosis using haematoxylin/eosin staining as well as Ki67, CD31, Mel-Mix and Caspase-3 fluorescence staining.

Results: A significant decrease of the measured size parameters of the tumor grafts was observed for all tested conditions with a more prominent shrinkage in the group treated with 5mM CaCl and EP for both cell lines. An increase of the apoptotic rate as well as a reduction of the proliferation rate occurred in the groups with 2,5μg Bleo+EP, 5mM CaCl with EP, 10mM CaCl alone and in combination with EP in comparison to the untreated control group. The achieved effect regarding apoptosis, proliferation and vascularization after a treatment with 5mM CaCl with EP was significantly higher than with 1μg Bleomycin with EP. In both tested cell lines, no significant differences could be detected between the conditions with higher concentrations of the two tested agents.

Conclusion: Due to the rarity of UM and its high accompanying metastatic and mortality rate, reliable in vivo models and new treatment pathways are required for the investigation of the tumor's behavior and of potential novel targets. Electroporation remains for the therapeutic regime of ocular tumors unexplored as an adjuvant alternative. The results allow us to propose the CAM-based tumor model as a new tool to evaluate the properties of uveal melanoma tumors in vivo and facilitate the application and the establishment of electroporation as a viable treatment modality for ocular melanoma.

P193 | Melanoma cell-derived extracellular vesicles transport miR-92b-3p and mediates the formation of carcinoma-associated fibroblasts by targeting PTEN

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Background: In melanoma, carcinoma-associated fibroblasts (CAFs) are important cellular components in the tumor microenvironment due to their potential to promote tumor growth and metastatic spread of malignant cells. Tumor cells are able to influence cells in the microenvironment in their favor by direct cell-cell contact, cytokines and by the release of extracellular vesicles (EVs). EVs safely transport whole packages of functional molecules such as proteins and otherwise easily degradable mRNAs and microRNAs (miRNAs) through the extracellular space into other cells (e.g. immune cells and stromal cells). MiRNAs are small non-coding RNAs that regulate protein expression post-transcriptionally. We have evidence that EVs from melanoma cell lines decisively influence the formation of CAFs by transport of miRNAs.
Aim: We investigated if and by which molecular mechanisms miR-92b-3p delivered in EVs from melanoma cells contribute to formation of CAF.
Methods: We isolated EVs from melanoma cell lines and normal melanocytes by ultracentrifugation and size exclusion chromatography, according to MISEV guidelines. We incubated primary normal human dermal fibroblasts (NHDFs) with these EVs and analyzed the biological functions and gene expression profiles of induced CAFs. To identify melanoma specific miRNAs of EVs from melanoma cell lines and EVs from normal melanocytes by next generation sequencing.
Results: Uptake of melanoma cell-derived EVs in vitro by NHDFs induces a CAF-like phenotype, defined by increased expression of CAF marker genes (e.g. IL-8, αSMA and FAP). This was associated with an increase in proliferation, migration and cell contractility. By next generation sequencing, we identified a strong enrichment of miR-92b-3p in melanoma cell-derived EVs compared to EVs derived from normal melanocytes (NHEMs). Correspondingly, we found an accumulation of miR-92b-3p in NHDFs after incubation with melanoma cell-derived EVs. Overexpression of miR-92b-3p mimic in NHDFs resulted in a similar CAF-like phenotype as observed in NHDFs incubated with EVs from melanoma cells. The locked nucleic acids (LNAs) mediated block of miR-92b-3p incorporation into melanoma cell-derived EVs prevented the formation of the before observed CAF-like phenotype by these EVs. To search for possible targets of miR-92b-3p we used databases and identified the tumor suppressor PTEN. Correspondingly, treatment with melanoma cell-derived EVs or overexpressing miR-92b-3p leads to decreased expression of PTEN protein in NHDFs. In addition, siRNA mediated knockdown of PTEN in NHDFs, resulted in an increased expression of CAF markers.
Conclusion: EV mediated delivery of miR-92b-3p from melanoma cells into fibroblasts contributes to the formation of a CAF-like phenotype by targeting PTEN.

P194 | A prospective clinical trial in melanoma patients with bone involvement addressing immunological effects of the RANKL-inhibitor denosumab in combination with dual checkpoint inhibition

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Immune checkpoint inhibitors (ICI) are established in the treatment of melanoma, and the RANKL-blocking antibody denosumab is approved for the prevention of skeletalrelated events due to bone metastases. Recent evidence suggests additional immunomodulatory properties of RANKL inhibition possibly boosting the clinical efficacy of ICI.
To test this hypothesis, we performed a prospective, multicenter, low-intervention clinical trial of unresectable stage IV melanoma patients with bone metastases who received denosumab in parallel with PD1 and CTLA-4 blockade (BONEMET). Therefore, a comprehensive immune monitoring at baseline and 4, 12, and 24 weeks after initiation of therapy was conducted. Specifically, serum concentration of 57 cytokines and phenotypes of circulating T cell populations were measured by electrochemiluminescence and flow cytometry, respectively. Secondary endpoints included tolerability and efficacy. For comparison, biospecimes collected from melanoma patients treated with dual ICI without denosumab at the skin cancer center in Hannover were analyzed accordingly at baseline and 4 weeks after initiation of therapy and served as retrospective reference cohort.
In both the BONEMET (n=16) and the reference cohort (n=18) serum levels of 17 cytokines, including IFNy were significantly increased after 4 weeks of treatment. Patients who received ICI and denosumab showed a significantly higher increase in serum CXCL-13 and a significant decrease in VEGFc compared with the reference cohort. While no changes in T cell composition were observed at 4 weeks, patients in the BONEMET cohort showed a significant decrease in

the peripheral naïve T cell population and an increase in CD8+ effector cells 12 weeks after initiation of therapy. Regarding secondary endpoints, treatment-related adverse events occurred with comparable frequency (93.8% in the BONEMET cohort versus 83.3% in the reference cohort). 7/16 patients (43.8%) in the BONEMET cohort and 8/18 patients in the reference cohort (41.2%) achieved disease control.

In conclusion, denosumab in combination with dual ICI modulates cytokine expression and T-cell composition in peripheral blood. While the downregulation of VEGFc can be interpreted as an indicator of the bone protective effects of denosumab, the upregulation of CXCL-13, a key factor for initiating tertiary lymphoid structures, strengthens the hypothesis that denosumab indeed exerts immunological effects.

P195 | 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 signaling 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 tumor progression and in therapy resistance.

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. In addition, we saw that Girdin and Daple expression was not significantly different between MAPKi sensitive and resistant melanoma cell lines. However, MAPK signalling pathway may be involved in Girdin and Daple regulation after both GEMs expression was altered when using BRAF and MEK inhibitor treatment. This altered expression was also found after treatment with poly(ADP-ribose)-polymerase (PARP) inhibitor indicating the involvement of Girdin and Daple in DNA damage repair.

These preliminary data suggest that overexpression of Girdin and Daple in melanoma metastatic stages may be involved in signal modulation of different pathways promoting malignant traits.

P196 | Exploring primary malignant melanoma with single-cell multiomics: An investigation of cellular crosstalk and immune escape

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Malignant melanoma remains an ongoing challenge in cancer research and treatment due to its complex cellular interactions within the tumor microenvironment. A better understanding of these interactions, especially those allowing tumor cells to escape the immune system, is fundamental for new therapeutic approaches.

Our research employs a multifaceted approach integrating single-cell RNA sequencing (scRNAseq) and single-cell ATAC sequencing (scATACseq) to characterize the transcriptome of different cell populations and their epigenetic chromatin status. Our dataset comprises scRNAseq data from 10 primary melanomas and 3 benign melanocytic nevi, along with scATACseq data from 6 primary melanomas and 2 benign melanocytic nevi. Leveraging advanced bioinformatics tools such as RNA velocity, LIANA (Ligand-Receptor Interaction Analysis), and oposSOM (self-organizing maps machine learning) we dissect the molecular landscape of these early-phased malignancies to gain deeper knowledge on tumor development, gene regulatory mechanisms and tumor heterogeneity.

In recent years treatment approaches experienced a strong shift towards immunotherapies, however, therapy-resistant tumors still pose significant challenges. Our study aspires to reveal novel axes of interaction between tumor cells and lymphocytes potentially influencing tumor survival. This deepened understanding holds promise for the refinement and development of melanoma therapeutics. Therefore, using LIANA, we computationally unravel these cellular interactions and identify promising candidates for extended investigation. Furthermore, we utilized functional assays, including siRNA-mediated knockouts of melanoma-specific targets followed by co-culture with TCR-transgenic Jurkat cells, to investigate the purpose of the predicted cellular interaction with implications for therapeutic strategies.

Besides an elaborative analysis of the transcriptome, our approach also introduces innovation by applying scATACseq to the epigenetic study of melanoma, particularly at its early stages. Notably, most melanoma research focuses on metastatic forms, whereas our project centers around primary melanomas, shedding new light on this underrepresented domain. The inclusion of melanocytic nevi in our analysis provides a unique opportunity to comprehensively characterize early melanoma development, potentially enriching our understanding of melanoma pathogenesis.

In conclusion, our research contributes to a holistic understanding of primary malignant melanoma, emphasizing the critical role of tumor

immunity. It may pave the way for novel therapeutic strategies and underscores the significance of investigating early-stage melanoma.

P197 | Melanoma cells induce tumor promoting properties in astrocytes supporting establishment and outgrowth of brain metastases

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Malignant melanoma is a highly lethal form of skin cancer, particularly dangerous due to its propensity to spread to the brain in patients with progressive disease. Within the tumor microenvironment, melanoma cells engage in intricate interactions with brainresident cells, such as astrocytes. It is already established that melanoma cells that metastasize to the brain undergo significant genetic alterations that facilitate their migration, infiltration into blood vessels, exit from vessels and differentiation. These genetic variations can dictate how tumor cells behave during the metastatic process, particularly when compared to melanoma cells found outside of the brain. When astrocytes come into contact with melanoma cells, they can exhibit pro-metastatic effects by releasing various molecules that influence the tumor microenvironment. However, there is currently limited knowledge regarding the impact that melanoma cells themselves have on astrocytes.

To further investigate how melanoma cells affect astrocytes with regard to astrocytic functions like proliferation, gene expression, and its influence on melanoma cell migration and invasion, we conducted in vitro experiments. Astrocytes were stimulated by exposure to melanoma cell-conditioned medium (MCM) or were cocultured with melanoma cells obtained from the MT/ret mouse model. Subsequently, we conducted immunofluorescent staining using Ki-67 to evaluate astrocytic proliferation. We also assessed alterations in astrocyte gene expression through RT-qPCR analysis. In addition, we performed migration and invasion assays to determine whether astrocytes exerted an influence on melanoma cell migration and invasion using the in vitro xCelligence system.

The findings revealed that when astrocytes came into direct contact with melanoma cells, there was an observed increase in astrocytic proliferation. It was also noted that the secreted molecules from melanoma cells alone were sufficient to influence the gene expression of astrocytes. The stimulation of astrocytes by the melanoma cell secretome caused a significantly reduced mRNA expression of GLT1 in astrocytes, which is known to be downregulated during astrogliosis. Decreased GLT1 expression results in an increased extracellular glutamate concentration, which can be used as a bioenergy substrate by the tumor and therefore could result in an increase of tumor growth. There was also a particular effect on the expression of inflammatory cytokines in by melanoma cell conditioned medium stimulated astrocytes; we detected induced IL-1β production and a downregulation of IL-17 and TNF-α. This modulation suggests that melanoma cells may influence astrocytes to create an inflammatory microenvironment that supports tumor growth. Furthermore, astrocytes were found to enhance melanoma cell migration and invasion, thereby facilitating the development of melanoma brain metastases. In light of these results, it becomes evident that astrocytes play a crucial role in the progression of melanoma brain metastases. Within the tumor microenvironment, melanoma cells have the capability to induce tumor-promoting properties in astrocytes. Vice versa, the astrocytemeditated inflammatory niche could affect the efficacy of cancer immunotherapies in the brain. Thus, identifying potential therapeutic targets related to astrocytic activity could potentially reduce the formation or outgrowth of brain metastases.

P198 | Exploring the Therapeutic Potential of BRAFi+MEKi in NRAS Mutated Melanoma: A Preclinical In Vivo Study Using PDX Models

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New therapeutic approaches such as immunotherapy or small molecule inhibitors has significantly improved the overall survival rate of melanoma patients. However, the response rates of current treatment options are limited, and the emergence of resistance mechanisms to immunotherapies and targeted therapies has become a major challenge in the treatment of melanoma. We and others have shown that the PI3K/AKT pathway is deregulated in 70% of melanomas and plays a key role in the development of these resistance mechanisms.

Therefore, PI3K may be a promising target for specific inhibitor treatment. In particular, the combination of PI3K and MEK inhibitors (PI3Ki and MEKi) that simultaneously target the PI3K/AKT and MAPK pathways may be an effective therapeutic option in metastatic melanoma. As monotherapy, the pan-PI3K inhibitor BKM120 can induce growth inhibition and apoptosis in most of the melanoma models tested, but shows severe side effects in patients. In contrast, the alpha isoform specific inhibitor BYL719 has limited antitumor activity as monotherapy but is considered less toxic.

However, both combination treatments of PI3K and an MEK inhibitor resulted in effective growth inhibition and apoptosis in the cellular melanoma models tested (BRAF mutated and BRAF wild-type), exceeding the effect of MEK inhibition as monotherapy. Initial in ovo test results (on chick chorioallantoic membrane (CAM) showed reduced tumor burden by using BYL719 in combination with the MEKi

trametinib. In vivo results in NSG mice injected with either BRAF mutated or BRAF wildtype cells showed a synergistic effect of the combination in the BRAF wild-type cells. These data suggest that the combination of PI3Ki with MEKi may be a new therapeutic option for BRAF wild-type melanoma patients.

P199 | Exploring ErbB Family Receptor Inhibition: A Novel Therapeutic Approach for Resistant Melanoma Patients

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Melanoma, a highly aggressive form of skin cancer, presents significant challenges in terms of treatment efficacy, especially for patients who are resistant to existing therapeutic options. The ErbB family of receptor tyrosine kinases, including epidermal growth factor receptor (EGFR/ERBB1), and HER3/ERBB3, have been implicated in melanoma progression and resistance to treatment. Melanoma is characterized by genetic heterogeneity and the activation of multiple signaling pathways involved in tumor growth and survival. Despite advances in targeted therapies and immunotherapies, a subset of melanoma patients faces treatment resistance and poor outcomes. The ErbB family of receptors has gained attention in recent years due to its role in promoting melanoma progression, therapy resistance, and metastasis. In previous studies we and others found that increased expression and activation of ErbB receptors is associated with resistance to targeted therapies and immunotherapies and poorer prognosis in melanoma patients.

Our cell lines show a high expression of EGFR, HER3 or both. By targeting the ErbB receptor family with small molecule inhibitors we could show significant effects on tumor cell proliferation and also on the induction of apoptosis. To investigate the treatment in vivo, NSG (NOD.Cg-Prkdcscid Il2rgtm1Wjl/SzJ) mice were subcutaneously injected with the BRAF wildtype melanoma cell line MEWO and treated orally with afatinib (20 mg/kg body weight, daily), neratinib (40 mg/kg body weight, daily) or osimertinib (5 mg/kg body weight, daily). The treatment with afatinib resulted in significantly reduced tumor growth compared to the control and also to a significant increase in survival time.

In conclusion, ErbB family receptor inhibition represents a promising and novel therapeutic strategy for difficult-to-treat melanoma patients. By targeting these receptors and their associated signaling pathways, we may overcome treatment resistance, improve patient outcomes, and advance personalized medicine in melanoma management.

P200 | Elevating MAPK Pathway Suppression with Combinatorial ERK Inhibitors

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The advent of small molecule inhibitors specifically designed for BRAF mutations at V600, along with their downstream target MEK, marked a significant advancement in treating BRAF mutant melanoma. Despite their notable anti-tumor activity and enhanced patient survival, the rapid emergence of resistance poses a significant challenge, limiting their clinical efficacy. Various resistance mechanisms have been documented, with a common feature being the reactivation of the MAPK signaling pathway, primarily mediated by extracellular signal-regulated kinases 1 and 2 (ERK1/2).

This study aimed to assess the potential benefits of the ERK1/2-specific small molecule inhibitor Ravoxertinib (GDC0994) in treating BRAF mutant melanoma cells. Melanoma cell lines with acquired resistance to BRAF inhibitors or the combination of BRAF and MEK inhibitors, along with their respective parental cells, were subjected to testing. Intriguingly, prolonged treatment with the ERK inhibitor substantially reduced melanoma cell growth, demonstrating an apparent independence from sensitivity to BRAF or MEK inhibitors. Additionally, analyses of cell cycle and viability indicated a distinct advantage in adding the ERK1/2 inhibitor to BRAF and/or MEK inhibitors, effectively targeting melanoma cells with BRAF mutations.

P201 | Prediction of durable response upon treatment with immune checkpoint inhibitors in melanoma

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A durable remission even after discontinuation of systemic treatment is the ultimate goal when treating advanced cancer. Only a few patients with an advanced melanoma under treatment with immune checkpoint inhibitors (ICI) achieve this goal. Patients with an excellent response to ICI can electively discontinue treatment, however, experience a relapse in 6-22% of cases. Furthermore, the re-induction of ICI does not always result in a response. On the other hand, long term treatment with ICI is associated with an increased rate of immune related adverse events, leaving the question open which patients can stop treatment with immune checkpoint inhibitors.

To identify patients that are candidates for elective discontinuation of ICI melanoma samples collected prior to ICI treatment were analysed by Nanostring. Durable responders were defined as patients who stopped ICI in the absence of treatment progression and remained relapse-free for a minimal duration of 2 years, while the relapse group consisted of patients who developed a melanoma recurrence upon elective discontinuation of ICI. Gene expression analysis revealed 3 genes (LTBP1, IL6, CXCL8) significantly upregulated in the relapse group while 10 genes (ICOSLG, A2M, MARCO, MMP9, LTB, BLK, SOX11, CD40LG, CCL21, MSA41) were associated with durable response. Except for IL6 and CXCL8 these biomarkers have not been previously associated with enhanced or reduced response to ICI and represent interesting further therapeutic targets. Machine learning algorithms were trained using 80% of the data. Support Vector Machine, Naive Bayes and Random forest had the best accuracy in predicting durable response in the test data set. Immunofluorescence staining of a large tissue microarray containing melanoma samples before initiation of ICI could confirm a prolonged progression free survival under ICI, in patients with an upregulation of RASGRF1 and CCL21 and downregulation of IL6 and LTBP1.
Taken together, our data indicate a set of biomarkers are associated with durable response upon ICI and should be further explored in prospective studies.

P202 | Role of the metabolite sensing Hca2 receptor in skin carcinogenesis

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Recurrent sunburns and the associated inflammatory damage to the skin are a major risk factor for the development of squamous or basal cell carcinoma. The regulation of inflammatory processes in the skin is therefore of central importance to protect the tissue from chronic damage. Recent work has shown that metabolites such as hydroxycarboxylic acids (HCAs), produced by gut and skin microbes and binding to specific GPCRs (HCA1-3), play an important role in the regulation of inflammation. The HCA2 receptor is expressed on keratinocytes and immune cells like neutrophils, dermal dendritic cells and t-cells. HCA2 binds butyrate, niacin (vitamin B3) and beta-hydroxybutyrate (BHB). Recent studies demonstrated a central role of HCA2 signaling for the regulation of inflammation and tumorigenesis e.g. in the intestine. We hypothesize that the HCA2 receptor also has an impact on the development of benign and malignant tumors in the skin.
To evaluate the effect of acute sunburn in Hca2-WT and Hca2-/- mice, a sunburn dose of 3.5 kJ/m2 UVB was applied to the shaved back skin on day 0 and 3 in a short-term skin inflammation model. On day 4, skin and blood were analyzed immunologically and transcriptionally. Comprehensive flow cytometric analyzes revealed significantly increased infiltration of CD45+ cells in the UVB-irradiated skin of Hca2-/- animals compared to Hca2-WT mice. In particular, CD11b+/Ly6C +/Ly6G+ neutrophils, CD11b+/Ly6C+/Ly6G- monocytes, and CD3+ T cells were increased. Consistent with these observations, we detected significantly increased mRNA expression levels of TNFα, CCL2, CCL8, CXCL2 and CXCL10 in Hca2-/- mice. These cytokines are pro-inflammatory mediators secreted in large amounts by neutrophils, monocytes and keratinocytes. In addition, the mRNA level of the transcriptional regulator HOPX, which modulates the terminal differentiation of keratinocytes and functions as an oncogene in the pathogenesis of squamous cell carcinoma also was significantly increased in Hca2-/- mice after acute UVB irradiation. These results suggest a central role for the HCA2 receptor in the regulation of cutaneous inflammation and tumorigenesis. Further experiments including the UVB irradiation of primary keratinocytes cultures and the long-term UVB irradiation of Hca2-WT and Hca2-/- mice are currently running.

P203 | Expression of pH-sensitive TRPC4 in Common Skin Tumors

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TRPCs (transient receptor potential classical or cation channels) play a crucial role in tumor biology, especially in the Ca2+ homeostasis in cancer cells. TRPC4 is a pHsensitive member of this family of proteins. As solid tumors exhibit an inversed pHgradient with lowered extracellular and increased intracellular pH, both contributing to tumor progression, TRPC4 might be a signaling molecule in the altered tumor microenvironment. This is the first study to investigate the expression profiles of TRPC4 in common skin cancers such as basal cell carcinoma (BCC), squa-mous cell carcinoma (SCC), malignant melanoma (MM) and nevus cell nevi (NCN). We found that all SCCs, NCNs, and MMs show positive TRPC4-expression while BCCs do only in about half of the analyzed samples. These data render TRPC4 an immunohistochemical marker to dis-tinguish SCC and BCC, and it also gives rise to future studies investigating the role of TRPC4 in tumor progression, and especially metastasis as BCC very rarely spread and mostly are negative for TRPC4.

P204 | Propagating viral vector-based vaccine platform as potent therapeutic strategy for solid tumor elimination

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Harnessing the immune system to eradicate tumors requires identification and targeting of tumor antigens, including tumor-specific neoantigens and tumorassociated self-antigens also expressed by normal tissue. Tumor-associated antigens are subject to existing immune tolerance, which must be overcome by immunotherapies. However, the precise requirements for inducing self-antigenspecific immune responses are not well defined. Here, we systematically investigate viral-vector-based cancer vaccines encoding a tumor-associated self-antigen (TRP2) for the treatment of established melanomas in preclinical mouse models, alone or in combination with adoptive cell therapy. We reveal that, unlike foreign antigens, tumor-associated antigens require a propagating vector to break tolerance and induce effective antigen-specific CD8+ T-cell responses. Immunization with a propagating vector leads to complete tumor rejection when combined with TRP2-specific T-cell transfer. Importantly, immunization with propagating vectors leads to extended antigen persistence in secondary lymphoid organs, resulting in efficient T-cell priming, which renders previously "cold" tumors open to immune infiltration. Our findings have important implications for the design of next-generation immunotherapies targeting solid cancers utilizing viral vectors and adoptive cell transfer.

P205 | Melanoma development: linking tRNA composition to mRNA expression

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On a molecular level, it has been shown for other types of cancer that the tumor development is associated with changes of the tRNA pools that favor the translation of tumor-promoting transcripts. Therefore, in the present study, we explore the role of tRNA-mRNA coordinated expression as part of a deeper, genetically regulated program that supports malignant tumor growth in melanoma progression. A single human cell contains a pool of approximatly 60 million tRNA molecules encoded by over 800 different genes. As a result, this project aims to detect variances in the tRNA pools between tumor and non-tumor specimen.

In preliminary experiments, we performed tRNA sequencing of a benign melanocytic nevus, a primary melanoma and a cutaneous melanoma metastasis. We then tested the correlation of the abundance of tRNA with different anticodons encoding the same amino acid (isoacceptors) with the codon usage of genes that are highly expressed in either highly proliferative melanomas or benign melanocytic nevi. We found a direct correlation between codon usage of the nevus and melanoma genes and the respective isoacceptors of the tRNAs exists for 9 out of 13 amino acids. A strong correlation was also found when comparing the codon usage with the presence of tRNA anticodons in melanoma samples.

To gain a deeper understanding of the regulatory interplay between tRNA and mRNAs in melanoma, we plan to expand this research by using a novel tRNA-seq technology which includes a mature tRNA isolation method, called long hairpin oligonucleotide based tRNA high throughput sequencing (LOTTE-seq), that will allow us to precisely assess the abundance of tRNA molecules in tissue samples from the different stages of the melanoma progression. Additionally, mRNA expression profiles of these stages will be analyzed to corroborate the correlations between the codon usage of differentially expressed mRNAs and the anticodons of specific tRNA pools. Furthermore, these analyses will be used to identify the top candidates of differentially expressed tRNAs in melanoma development for functional studies.

This possible general mechanism of malignant transformation and cancer progression could help in the identification of important mechanisms of protein translation for a better understanding of melanoma biology that might enable the development of new treatments.

P206 (OP06/01) | Tumor heterogeneity: Extracellular vesicles mediated transport of melanoma cell-derived miR-1246 between subpopulations of melanoma cells enhances the invasive capacity by targeting CCNG2

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Background: During invasion and metastasis, tumor cells must induce various molecular processes to spread from the primary tumor to distant sites or organs. Through intercellular communication among tumor cells, certain properties can be transferred, resulting in improved adaptation to specific microenvironments. One

mechanism for this cooperation is the exchange of genetic information and functional molecules by means of extracellular vesicles (EVs). EVs are nanoparticles surrounded by a lipid membrane that transport a package of functional molecules such as DNA, proteins, RNAs and non-coding RNAs (e.g. miRNAs), which can affect the phenotype and function of recipient cells.

Aim: Investigation of the molecular mechanisms by which EV transported miRNAs contribute to melanoma invasion and metastasis.

Methods and results: By invasion assays, we isolated a highly invasive subpopulation (BLM-HI) of the parental melanoma cell line BLM. Incubation of the less invasive parental BLM cells with conditioned medium of BLM-HI cells resulted in a higher invasive capacity in a 3D spheroid model. Based on these results, we wondered if EVs isolated from the supernatant of the BLM-HI subpopulation would mediate this invasive quality. We found that these EVs indeed increased the invasive ability of parental BLM cells. By next generation sequencing (NGS) we observed a differential gene expression in BLM cells treated with BLM HI-EVs compared to untreated cells. Transcriptome analysis revealed significant upregulations of pathways related to extracellular matrix organization and pathways connected to the hallmark of epithelial-mesenchymal transition (EMT) - a key process for metastasis. These findings support our thesis that EVs and their contents are able to promote invasion and phenotype switching within tumor cell subpopulations.

Since one of the main functions of EVs is the intercellular transport of miRNAs, we analysed and identified differential enrichment for specific miRNAs in EVs derived from the BLM-HI subpopulation compared with parental BLM cells by small RNA sequencing. We found miR-1246 significantly enriched in EVs from BLM-HI cells. Treatment of parental BLM cells with EVs released by BLM-HI resulted in increased intracellular levels of miR-1246. Inhibition of miR-1246 by locked nucleic acids (LNAs) decreases invasion of BLM-HI cells. By a database screen, we found Cyclin G2 (CCNG2), which is known to inhibit EMT, as a putative target of miR-1246. Treatment of BLM cells with EVs derived from BLM-HI cells strongly reduces CCNG2 protein expression, while blocking of miR-1246 by LNAs increases CCNG2 protein level in BLM cells. In public datasets we found CCNG2 expression to be reduced in metastases compared to primary tumors. This is relevant since in several cancers (analysed in PANCANCER dataset TCGA), reduced expression of CCNG2 is associated with worse patient outcome.

Conclusion: We have evidence that miR-1246in EVs derived from melanoma cells, contributes to a pro-invasive phenotype of melanoma cells by targeting CCNG2, which is associated with metastasis and poor patient outcomes.

P207 | Development of a Vδ1 T cell-competent organotypic 3D human skin model with HaCaT cells and SCC13 skin squamous cell carcinoma cells

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Skin immune cells are important for barrier integrity of normal skin, as well as to protect against infections and cancer (i.e., tumor surveillance). Nevertheless, immunocompetent human skin models are rare to date. An important resident immune cell population in the skin are γδ T cells. In murine skin, these resident epidermal γδ T cells are located in the epidermis (also called dendritic epidermal T cells) and are well-studied for their immunosurveillant potential. In human skin, however, γδ T cells - which are mostly of the Vδ1 subtype - are predominantly located in the dermis and data on their role and function are still scarce. So far, the human γδ T cells have been implicated in the cytolytic response against malignant melanoma cells, among other types of solid tumors. However, their role in the protection against cutaneous squamous cell carcinomas (cSCC) remains unknown. Here we report the successful integration of Vδ1 T cells into our human long-term organotypic skin model (fdmOTC), which is based on a fibroblast-derived matrix dermal equivalent (DE) and a HaCaT cells epithelial layer. Vδ1 T cells were isolated from human blood, where they present a very minor fraction (0.2-4%). We established culture conditions allowing the integration of non-preactivated or ex vivo expanded Vδ1 T cells, without compromising the cellular integrity of the fibroblasts and keratinocytes in the fdmOTCs. Though plated on top of the preformed DE, the Vδ1 T cells migrate (nearly exclusively) into the DE in this setting. RNAseq analysis revealed that in the presence of Vδ1 T cells gene expression of both the DE and epithelium changed, with enrichment of leukocyte migration and chemotaxis genes in the DE, and detection of several keratinocyte differentiation genes in the HaCaT epithelium. Indeed, histology demonstrated improved tissue organization and differentiation of the HaCaT epithelia in the presence of the Vδ1 T cells. In models established with SCC13 cells, the presence of the Vδ1 T cells caused reduction of the epithelial cell layer (histology) and a dominance of inflammation-related RNAs in the RNAseq analysis. Together, our results show that integration of this skin-resident T cell population is possible and underline their immunosurveillant potential in skin. Thus, this model will now allow to extend these studies and widening the scope of application to wound healing, infection, and UV-damage analyses.

P208 (OP05/04) | Heparan sulfate regulates angiogenesis and infiltration of immune cells in melanoma

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The composition of the tumor microenvironment defines the malignancies and therapy response of melanoma. Glycosaminoglycans such as heparan sulfate (HS) are major regulators of the tumor microenvironment. The biosynthesis of HS involves the consecutive action of various enzymes. At each modification step only a fraction of the potential substrates are modified resulting in a linear polysaccharide of considerable structural heterogeneity. Structural features of HS, such as chain length, degree of sulfation and pattern of sulfation control the activity and function of various growth factors, cytokines and morphogens such as vascular endothelial growth factor A, CXCL9 or TGF-β. Therefore, HS is fundamental for angiogenesis and the recruitment of immune cells into the tumor tissue. In the present project, we aimed to investigate the impact of melanoma cell produced HS on tumor angiogenesis and the relationship between HS structure and the attraction of neutrophils, T cells and macrophages. To evaluate the pathophysiological relevance of HS heterogeneity, we used CRISPR/Cas9 to modulate the expression of several HS-biosynthesis related enzymes in melanoma cells.

After intradermal injection of these cells into the back skin of mice, we analyzed the impact of the HS structure on the tumor microenvironment by immune fluorescence microscopy. We evaluated the number and spatial localization of blood vessels, Ly6G+ neutrophils, CD8+ T cells and F4/80+CD206+ macrophages using neuronal network assisted image analysis. Our research revealed that complete lack of HS limited angiogenesis within the tumors. Compared to control tumors, we found a significantly decrease in the recruitment of Ly6G+ neutrophils, CD8+ T cells and F4/80+CD206+ macrophages in HS deficient melanoma tissues. Increased HS chain length and decreased 3-O sulfation promoted the infiltration of cytotoxic CD8+ T cells and reduced tumor growth.

Our study provides first insights into the fundamental role of HS-mediated regulation of the melanoma microenvironment. Further analysis will reveal whether the impact of HS on the tumor microenvironment is also associated with the response to immune modulatory drugs such as immune checkpoint inhibitors. In future, identification of tumor supportive patterns in HS biosynthesis may offer novel targets to improve current cancer therapies.

P209 | Selenoprotein O regulates melanoma metastasis through mitochondrial complex II activity

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Selenoprotein O (SELO) represents an evolutionarily conserved enzyme that orchestrates a post-translational protein modification termed AMPylation. Nevertheless, our understanding of the substrates associated with the mammalian SELO homolog and its functional significance in the context of cancer remains limited. In this study, we unveil, for the very first time, the role of SELO in cancer metastasis. In melanoma, elevated SELO expression levels are correlated with reduced overall survival and an increased propensity for metastasis. Using a murine model of melanoma metastasis, absence of SELO substantially reduced the presence of melanoma cells in the bloodstream and their occurrence in distant visceral organs. This attenuation in metastatic potential was subsequently rescued by antioxidant therapy, suggesting that SELO deficiency impedes metastasis by increasing oxidative stress. Mechanistically, SELO AMPylates succinate dehydrogenase subunit A, and SELO-deficient cells respond to increase oxidative stress by enhancing mitochondrial complex II activity. In summary, these findings collectively underscore the role of SELO deficiency in restraining the metastasis of melanoma cells via the regulation of mitochondrial complex II, with potential rescue through antioxidant interventions.

P210 | Combined Skin Cancer Treatment with Cold Gas Plasma and a Chromone Derivative shows Synergistic Efficacy In Vitro and In Vivo

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The potential use of cold gas plasma for cancer treatment alone or in combinational therapies has gained increasing interest. Although progress has been made towards understanding the effects of cold gas plasma on cancer cells, much still needs to be learned, especially concerning combinational therapies. Based on the recent success of small molecule-based targeted skin cancer therapies, we aimed to identify effective combinations of experimental small molecules with cold gas plasma. We confirmed a reduction in cellular metabolic activity, motility, and viability after oxidative stress induced by cold gas plasma in skin cancer cells. After screening an in-house 155-compound library using 3D tumor spheroids and high content imaging two promising chromone derivatives showing synergistic efficacy in combination with cold gas plasma were identified. Treatments of tumor organoids grown in ovo confirmed the principal anti-cancer effect of the selected drugs, especially when combined with cold gas plasma. In a xenograft mouse model, tumor growth was followed using caliper measurements and animal survival. While one of the two compounds (IS112) exerted severe toxicity in vivo, the other (Sm837) resulted in a significant synergistic anti-tumor toxicity at good tolerability. Both compounds reduced proliferation and viability and showed increased oxidative stress as well as DNA double-strand break formation in combination with cold gas plasma in vitro. A principal component analysis of protein phosphorylation profiles confirmed the substantial difference of the combination treatment from the monotherapies. In summary, we identified a novel compound that, combined with topical cold gas plasma-induced oxidative stress, is a promising substance to target skin cancers.

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P211 | Assessment of the synergistic inhibitory efficacy of cold atmospheric plasma in combination with a chromone derivative on melanoma cells

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Recently, much progress has been made in the development of novel melanoma therapies. However, despite game-changing treatment options such as targeted therapies and immune checkpoint inhibition many patients with advanced skin cancer still succumb to their disease. Hence, there remains an urgent need to develop innovative treatment strategies to target melanoma. These innovative strategies should also consider combination therapies as monotherapies often lead to the development of secondary therapy resistance. We have recently identified a novel small molecule (SM837, a chromone derivative) which in combination with cold atmospheric plasma (CAP) treatment showed a striking synergistic anti-melanoma efficacy in vitro, in ovo, and in vivo. CAP is a partially ionized gas containing among others reactive oxygen and nitrogen species, ions, electrons, as well as electromagnetic fields. Against this background, this project aimed to identify the optimal concentration and treatment time to achieve the strongest synergistic effect. Furthermore, we aimed to assess the role of different CAP components in this process and to gain insights into the molecular mechanisms of the synergistic effect. A comparison of direct CAP treatment (contains all plasma components) with an indirect treatment using plasma treated medium (contains only plasma generated reactive species) revealed a reduced efficacy of the indirect treatment (IC50 of 24 s vs. 5 s). CAP treatment from the bottom of the well-plate (only the effect of electromagnetic fields) showed a reduced metabolic activity after 60 s treatment. These observations confirm that both electromagnetic fields and reactive species play an important role in the anti-cancer properties of CAP. In combination with the chromone derivative the strongest synergistic efficacy was observed after direct CAP treatment for 5 s in combination with 20 or 40 μM SM837 and after indirect CAP treatment for 10 s with 40 - 100 μM or 20 s with 20 - 40 μM SM837. These results indicate that a synergistic effect is only achieved in a specific range of treatment times and concentrations. Further ongoing experiments including transcriptome analyses will provide more detailed insights into the role of different CAP components for the synergistic efficacy as well as to the molecular mechanisms of action.

P212 | Identifying Novel Therapeutic Vulnerabilities in Malignant Melanoma by Proteomic Profiling of Melanoma Metastases

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Distant metastases is responsible for over 90% of deaths in cancer patients. 70% of melanoma are diagnosed in an early tumor stage (UICI). However over 55'000 patients die from melanoma annually. Metastasis is an exceedingly complex process that involves an interplay between cancer cells and the microenvironment as well as intrinsic pathways in cancer cells (PMID: 36478190). Cancer cells undergo important metabolic changes that enable them to survive dissemination through the blood to distant organs (PMID: 35909026). Yet, metastasis to specific organs is a non-random process and follows a "metastatic organotropism" ("seed and soil"-hypothesis). Multiple factors like the location of the primary tumor, its blood supply as well as the primary and secondary tumor microenvironment are being discussed to influence metabolic changes during the tumor progression. We hypothesize that these factors among others play an important role in metastatic organotropism. Proteins carry out important biological process within normal and cancer cells. The complete proteins set in a cell is called proteome. The aim of this project is to identify a proteome signature of melanoma metastases to better understand metastatic organotropism and to identify novel druggable targets.

We first performed top-down proteomic analysis using a LC-MS3 analysis on an Orbitrap Fusion Lumos in our spontaneous metastasizing human melanoma mouse models. For that reason, we used a comparative proteomic approach to identify and analyze proteins from primary human melanoma tumor cells and the matched melanoma metastases in distant organs including liver, lung, kidney and lymph node.

Our preliminary analyses showed significant proteomic differences between lung metastases and primary tumors. We identified differences in proteins for mitochondrial adaptations, which might be due to the change to an hyperoxic environment. In comparison, liver metastases only showed minimal differences in their protein expression profiles compared to the primary tumor. Among others we identified an overexpression in several keratins in the melanoma liver metastases most likely due to differences in tissue density.

Next, we will increase the samples size and the complexity of the proteomic analysis. To address this, we will combine novel proteomic data with matching RNAseq and global metabolic data from our lab to generate a comprehensive picture of melanoma organotropism. Finally, we want to strengthen our findings by analyzing primary patient samples.

In conclusion, we aim to characterize the proteome of melanoma metastases during cancer progression to better understand organotropism. The ultimate goal is to identify potentially targetable and druggable signal pathways to block cancer metastasis.

P213 | ctDNA Monitoring of metastatic Melanoma patients receiving Immunotherapy

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Objectives: Immunotherapy have revolutionized the treatment of patients with metastatic melanoma. Nevertheless, the subsequent treatment switch following the radiological assessments, can be too late to improve patient´s overall survival. Therefore, there is an unmet need for predictive biomarkers. Analysis circulating tumor DNA (ctDNA), shows promising potential to address this. ctDNA closely matches the genomic profile of the corresponding tumors and can be quantified to mirror response to treatment or minimal residual disease. The aim of this study is to determine wether ctDNA is suitable for therapy monitoring of immunotherapy in melanoma and which prognostic statements can be madet.

Methods: The mutational landscape is being examined in over 240 time points of 42 metastatic melanoma patients receiving immunotherapy, using Plasma SeqSensei(TM) Technology. We analyzed plasma-derived ctDNA within selected hotspots in 5 different gen es(BRAF,EGFR,KRAS,NRAS,PIC3CA) before and during the course of immunotherapy. This approach allows high sensitivity down to 0.07% mutant allele fractions (MAF) and an absolute detection limit of 7 mutant molecules (MM). Analyses of correlations with clinical data and volumetrically measured tumor load followed.

Results: ctDNA of the five genes has been determined with a mean of 45.2 Mutant Molecules per mL (MM/mL) in BRAF, 36.8 MM/mL in EGFR, 34.9 MM/mL in KRAS, 91.3 MM/mL in NRAS, 15.1 MM/mL in PIC3CA at any time. Biomarker status at baseline prior to treatment showed a mean ctDNA Level of 103.8 MM/mL. These ctDNA+ patients at Baseline showed a shorter Progression free survival (PFS) (HR:0.48; p-value 0.0231). There was a discordance in the BRAF mutational landscape between tissue and plasma in which treatment-relevant mutations could be detected (BRAFV600E mutations, WE476CE and G469A). Increasing ctDNA correlated to disease progression (PD) earlier than routine radiologic scans (7-13 weeks). Decreasing ctDNA was predictive for treatment response (stable disease, SD; partial response, PR; complete remission, CR). Lymphnode metastases were the most common metastatic site (53.6%), followed by lung metastases (30.7%), subcutaneous metastases (26.9%), brain metastases (26.9%). A strong and statistically significant correlation was observed between relative changes of ctDNA levels and total tumor burden ($r=0.87$; $p<0.05$). Discrepancies between MAF and MM/mL probably due to adverse autoimmune event releasing normal cfDNA could be observed.

P214 | Metabolite-driven regulation of T cells in human keratinocyte-derived skin cancer

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Keratinocyte-derived skin cancer (KDSC), such as basal cell carcinoma and squamous skin cell carcinoma, rank among the most common malignancies worldwide. Recent studies suggested that tumor-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 KDSC is limited. In this project, we are investigating metabolites in the tumor microenvironment of human KDSC. For our investigations, we have established a protocol to extract interstitial fluid metabolites from human KDSC samples. Therefore, we collect punch biopsies of human tumor skin and control skin. Metabolites are extracted by centrifugation and size exclusion and stored in methanol. Interstitial tissue fluids are subjected to metabolomic analyses using mass spectrometry. A pilot study of 14 plus 14 samples showed a significant increase of several metabolites in the tumor samples vs. the control skin. A follow-up analysis using interstitial tissue fluids from >100 tumor and control samples is in progress and should provide us a broader insight into the metabolic milieu of human KDSC. In a second part of the project, we are delineating the direct impact of tumor-enriched metabolites, as defined by our metabolomics approach on the function of human CD8+ T cells in vitro. First analyses reveal that single metabolites, enriched in KDSC, shape T cells functionally, shown by altered IFN-γ production. In the long-term, our study holds promise to identify immunometabolic targets for the treatment of human KDSC.

P215 | Apoptosis-independent functions of XIAP in melanoma.

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The X-linked inhibitor of apoptosis protein (XIAP) belongs to the family of human inhibitors of apoptosis proteins, whose primary function is to regulate programmed cell death. Expression of XIAP is observed in many cancers, including melanoma, and correlates with poor treatment outcomes. Besides its activity in regulating apoptosis, XIAP may modulate cellular programs other than apoptosis, as we previously showed for melanoma cell migration. That occurred by XIAP-mediated modulation of patterns and numbers of focal adhesions, possibly by a mechanism involving association with vimentin, an intermediate filament protein implicated in the cytoskeletal organization and formation of focal adhesions in cells adherent to fibronectin. In recent studies, we showed that XIAP-BIR2 mediated ubiquitination of RIPK2 leads to binding of TAK1/TAB1 complex that drives the production of IL8, leading to intra-tumor neutrophil infiltration promoting melanoma growth.

In further investigations, by deleting XIAP in melanoma cells, we have identified an alteration in the vascular mimicry (VM) process. VM is a process that describes the ability of tumor cells to generate vascular channels for tumor perfusion, known to occur in several tumors and suggested to underlie resistance to vascular therapies.

Without XIAP, cells seeded in matrigel failed to organize the actin and tubulin cytoskeleton, leading to a drastic decrease in formed tube-like structures compared to controls. This effect was not due to enhanced apoptosis without XIAP, as we detected similar numbers of apoptotic cells in controls. However, XIAP deletion reduced melanoma cell migration on matrigel-coated surfaces, which could partly explain the observed VM phenotype. To identify the XIAP-specific function implicated in the regulation of VM, we used murine melanoma cell clones carrying the deletion of XIAP domains in a tube-formation assay, thereby identifying the BIR2/3 domains as crucial for proper VM formation. In summary, these data suggest that XIAP is involved in various cellular functions unrelated to the regulation of apoptosis, including cell migration and vascular mimicry, mechanisms relevant to melanoma progression.

P217 (OP03/05) | Cutaneous squamous cell carcinoma patients harbour keratin 14 reactive T cells

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Background: Keratinocyte differentiation antigens (KDA) have recently been described as tumor-associated antigens and correlate with improved survival of non-small cell lung cancer patients under checkpoint blockade. KDA specific T cell responses eliminate tumor cells and at the same time cause skin-related adverse effects. Cutaneous squamous cell carcinoma is one of the most frequent tumors in Caucasians and can successfully be treated with PD-1 based

checkpoint blockade. However, T cell antigens of cSCC are poorly characterized.

Objective: This study aimed to investigate if cSCC patients harbor KDA-specific Tcells and if they can recognize and eliminate the tumor.

Methods: cSCC samples were immunohistochemically stained for CD3. Tumor samples were subjected to the HLA-I and HLA-II mass-spectrometry analysis. PBMCs were isolated from treatment-naïve cSCC patients. T cell stimulations were carried out using various keratin peptides. scRNA-seqencing was performed to identify the keratin-specific T cell receptors (TCRs) in the blood.

Results: The IHC staining showed high infiltrate of CD3+ T-cells in cSCC tumors. Immunopeptidomics data confirmed the presentation of KDA-specific peptides on the HLA-I and HLA-II in the tumor tissue. Autoreactive T-cell responses in PBMCs from cSCC patients were directed against keratins 1, 2, 5, and 14, with keratin 14 reactive CD8+ being the most frequent. After stimulation with keratin 14 peptides, activated cells (CD8+CD69+CD137+) were sorted and sequenced for single-cell transcriptome and V(D)J recombination analysis. The data revealed, that the TCRs of the keratin 14 reactive CD8+ are oligoclonal. Moreover, the patients, harboring keratin 14 reactive T-cells share the same HLA-B*07.

Conclusions: In this study, we have demonstrated, that cSCC patients develop autoreactive T cells, specific for KDAs. These KDAs are also presented on the HLA-I in the tumor, making them tumor-associated antigens in the cSCC.

P218 | PmCiC - The influence of a citrate transporter in different cancers of the skin

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Membrane transport proteins are involved in the movement of ions and small molecules across biological membranes. A recent study of our collaborators Mycielska et al. showed that extracellular citrate is supplied to cancer cells through a plasma membrane-specific variant of the mitochondrial citrate transporter pmCiC. These exciting findings prompted us to investigate whether pmCiC also plays a role in the pathogenesis of different skin tumors. Therefore, immunohistochemical analysis of pmCiC expression was performed on benign nevi, primary melanoma, metastatic melanoma, BCC, cSCC and Merkel cell carcinoma. Also in vitro an expression of pmCiC in melanoma as well as merkel cell carcinoma cell lines could be detected. In cells, that showed a high expression level of pmCiC, citrate has a positive effect on the proliferation of the cells. After 3- 5 days, cells treated with citrate, showed a significant higher proliferation compared to the same cells in the absence of citrate. This effect could only been seen in cells with a high expression level of pmCiC. After Mycielska et al. described with gluconate a potential inhibitor of pmCiC, cells were treated with citrate and gluconate as well. After adding the inhibitor gluconate, the proliferation rate of the cells was equal to the cells that had an absence of citrate. This effect could also be seen in vivo using the chorio-allantoic membrane assay. After a treatment of 5 days with gluconate once per day, tumor growing was significantly lower as well as less tumor surrounding vessels could be seen.

After we could show, cancer cells are consuming extracellular citrate, we next investigated if cancer associated cells are providing citrate to cancer cells. Here we could show a significant higher release of citrate in cancer associated fibroblasts that had a lack of extracellular citrate. The cytokines released from cancer cells differed a lot, after a lack of citrate. Also the cytokine release of fibroblasts, treated with conditioned media from cancer cells, differed a lot, depending of the presence of citrate.

P219 | Translational data from a multicenter phase II study on dimethyl fumarate treatment in relapsed and refractory cutaneous T cell lymphoma

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1. Introduction: We performed a multicenter phase II clinical study on dimethyl fumarate treatment in relapsed and refractory CTCL in Germany [1]. Dimethyl fumarate (DMF) inhibits NF-kappa B in the malignant T cells of CTCL patients and can specifically induce cell death [2].

2. Aims: The aim of this study is to investigate the mechanism of action of DMF as well as its influence on cellular signaling and microenvironment in CTCL patients in vivo.

3. Materials and Methods: Pre- and posttreatment, Formalin-fixed-paraffin-embedded (FFPE) samples were collected, and peripheral blood mononuclear cells (PBMCs) were isolated. Immunohistochemical (IHC) and CODEX highly multiplexed tissue imaging was performed as previously described [3]. PBMCs were isolated from patient blood samples as described before [4]. Whole exome sequencing is being performed at the Core Facility of the University Medical Center Mannheim.

4. Results: IHC stainings showed a significantly decreased expression of Ki67 in the responders upon end-of treatment compared to the non-responders and correspondingly a significantly increased

expression of Cleaved Caspase 3. Additionally, there was a tendency for a higher expression of NF-kappa B pp65 in the responders, although not reaching statistical significance. A tissue microarray was manufactured from FFPE-stainings from 6 responder and 3 non-responders pre- and post-treatment, whose quantitiative analysis is ongoing. The whole exome sequencing has not yet been completed.; **5. Summary:** DMF is a promising new therapeutic option in CTCL that already proved effective and well-tolerable in a clinical phase II study. The first results point towards a correlation between pre-treatment NF-kappa B activity and response to therapy. This would allow a pre-treatment therapy stratification to better identify patients suitable for DMF therapy.

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P220 (OP04/03) | Mechanisms of CD4+ T cell-mediated inflammatory cell death in melanoma

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Over the past few decades, immunotherapy has revolutionized the treatment of cancer. Unfortunately immunotherapy strategies focused on harnessing cytotoxic CD8+ T cells are often subjected to the emergence of treatment resistance due to tumour cell antigen loss or MHC-I downregulation. Recently, the ability of CD4+ T cells to contribute to anti-tumour immunity has been gaining more attention. Our laboratory established a combinational CD4+ T cell adoptive cell therapy protocol, which engages the adaptive and the innate immune responses to cooperate in order to eradicate MHC-deficient tumours that escape direct CD8+ T cell targeting. The effector CD4+ T cells combined with innate immune stimulation promote the modulation of the tumour-associated myeloid cell network towards interferonactivated antigen-presenting and iNOS-expressing tumouricidal effector phenotype. The concerted action between IFNy and TNFá as well as the effector monocytederived nitric oxide (NO) lead to inflammatory cell death of immune-evasive melanomas. To gain further mechanistic insights into the death of melanoma cells, we first examined the ability of the inflammatory mediators IFNy, TNFá and a NO donor (SNAP) alone or in combination to induce cell death in IFN-responsive CRISPR-ctrl and IFN-unresponsive JAK1-KO HCmel12 melanoma cells via live-cell imaging. Therefore, IFN-responsive and IFN-unresponsive melanoma cells were treated with combinations of IFNy, TNFá and SNAP and the viability as well as the cell number was recorded for 48 h. While IFNã and TNFá alone could not induce cell death of IFN-responsive HCmel12 melanoma cells, the combination of both lead to an increase of dead cells starting 10 h after treatment. Additionally, a single treatment with SNAP induced an increase of cell death faster in IFN-responsive melanoma cells (14 h) than in IFN-unresponsive melanoma cells (17 h). In these IFN-unresponsive melanoma cells, TNFa and SNAP synergised to further induce cell death. To further investigate the induced cell death pathways, we treated melanoma cells with the inflammatory mediators and used either the pan-caspaseinhibitor Z-VAD-FMK or RIP1-inhibitor Necrostatin-1. In IFN-responsive HCmel12 melanoma cells, the pan-caspase-inhibitor as well as the necroptosis-specific RIP1- inhibitor could reduce cell death induction caused by IFNã and TNFá. Strikingly, the effectiveness of cell death inhibition upon treatment with TNFá and SNAP was more effective when using the RIP1 inhibitor, rather than the pan-caspase inhibitor, in both IFN-responsive and IFN-unresponsive melanoma cells. This data suggests that in both cell lines, necroptosis is the main cell death pathway activated when melanoma cells are exposed to both TNFa and NO. Together, our results highlight that exploiting different cell death pathways in immune-evasive melanomas could provide possibilities to improve existing treatment protocols in order to enhance treatment efficacy and counteract treatment resistance.

P221 | Interaction between astrocytes and cancer cells mediate behavioural changes that contribute to the development of melanoma brain metastases

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Malignant melanoma is not only the deadliest skin cancer, but also the cancer entity with the third highest incidence of brain metastasis development. As brain metastases are associated with a very

poor prognosis for the patients, their clinical treatment represents an unmet clinical need. In the last decades it has been shown that the tumor microenvironment (TME) often plays an important role in tumor progression and therapy response, which suggests the hypothesis that the unique TME in the brain, including resident cells like astrocytes, also has an impact on the development of brain metastases. However, the exact interactions between cancer cells and resident cells in the brain are still poorly understood. Therefore, this research aims to unravel the interaction between astrocytes with cell lines derived from spontaneously developing brain metastases or primary skin melanoma, both established by using the MT/ret animal model.

Here we were able to show that our cell line established from the primary cutaneous melanoma expressed lower levels of the Epithelial-mesenchymal transition (EMT) regulating genes than cell lines generated from established brain metastases. Since EMT is an important axis of the metastatic process, these markers are also of interest for analyzing the effects of astrocytes on metastatic seeding of melanoma cells in the central nervous system. First we observed, that the cell lines of both tumor origins shift for EMT-gene expression levels after being brought in contact with astrocyteconditioned medium. Modulators of tumor heterogeneity connected to EMT like Twist, Zeb1, Snail, Sox9, Serpine1 were tested. The results differed between the cell lines in whether the markers were up- or down-regulated. However, the cutaneous cell line still expressed lower levels of the genes even after astrocyte conditioned medium contact, when compared with cell lines originated from metastases of the brain. Furthermore, we could observe changes in the secretome of melanoma cells after incubation with astrocyte-conditioned medium. For example, we detected an upregulation of SDF, which is associated with EMT as well as proliferation, migration, invasion and prognosis of malignancies. In addition, we detected down-regulation of markers such as IL-16 and IL-17, are associated with inflammation. Also direct contact between astrocytes and melanoma cells under co-culture conditions induces changes in the cells, such as the upregulation of Ki67 and Stat3 expression. The upregulation of Ki67, as an important proliferation marker, indicates an enhanced cell proliferation in all tested cell lines induced by direct contact with astrocytes. Furthermore, the upregulation of Stat3, known for its important multifunctional roles in oncogenesis and immune cell activation, but also described for its impact driving sensitivity of radioresistance, suggests an increased radioresistance in our cell lines after astrocyte contact. As radiotherapy is a common treatment option for brain metastases, we also performed a limited dilution assay (LDA) followed by radiation of our cell lines. Although the LDA results do not show a significant decline in radiosensitivity after astrocyte conditioned medium-treatment, our results demonstrate a slightly increased radiosensitivity in our brain cell lines compared to the cutaneous cell lines.

In conclusion, we anticipate our work to be a starting point for further investigation of the interaction between tumor cells and the TME in the brain, in order to identify possible influences of the TME on tumor cells that could further influence treatment, such as radiotherapy, and to use these findings to improve treatment responses.

P222 | MDA Specific T Cell Responses in Sentinel Lymph nodes and Periph-eral Blood Mononuclear Cells in Melanoma Patients

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Background: Much research has focused on elucidating T cell specificities in the peripheral blood and tumor infiltrating lymphocytes of melanoma patients. Draining lymph nodes (LNs) have been studied to a lesser extent, despite their significant role in regulating tumor-directed immunity. We therefore characterized T cell responses targeting the melanoma differentiation antigens (MDAs) gp100, MART1, tyrosinase and TYRP2 in sentinel LNs.

Methods: LN cells and patient-matched peripheral blood mononuclear cells (PBMCs) were acquired from patients undergoing sentinel LN extirpation. After stimulation with protein-spanning peptide pools, T cells were expanded with IL-2 for 9-13 days. Subsequently, they were restimulated with protein-spanning peptide pools and stained for flow cytometry using a viability stain and antibodies targeting CD3, CD45RA, CD4, CD8, TNF, and IFNγ. Medium only was used as negative control and a peptide pool containing immunogenic peptides of hCMV, EBV and influenza was used as positive control. Activation was defined as a frequency of TNF+ IFN-gamma + cells of >1% and >2x the medium control. Samples with less than 500 CD4+ or CD8+ cells were excluded.

Results: A total of 48 patients were included, 6 (12.5%) with detectable melanoma cell spread to the LN. In 41 cases single LNs were acquired, whereas 6 and 1 patients had 2 and 3 LNs available, respectively. In 34 patients matched PBMCs were stimulated.

MDA-directed reactivity was detected in LNs of 5 (10.6%, CD4+) and 5 (13.2%, CD8+) patients. CD4+ responses were directed against TYRP2 (3 LNs), gp100 (2 LNs) and tyrosinase (2 LNs), whereas CD8+ responses targeted gp100 (3 LNs), TYRP2 (2 LNs) and tyrosinase (1 LN).

The rate was higher in PBMCs with 27 (79.4%, CD4+) and 14 (41.2%, CD8+) patients responding. Here, responses were directed against TYRP2 (17 patients), tyrosinase (14 patients), gp100 (13 patients) and MART1 (7 patients) in CD4+ cells and gp100 (8 patients), TYRP2 (5 patients), MART1 (3 patients) and tyrosinase (2 patients) in CD8+ cells. If reactivity was recorded, it was shared against the same peptide pool between PBMCs and LNs in 3 (6.1%, CD4+) and 1 (5.3%, CD8+) cases. Reactivity was concordant between CD4+ and CD8+ cells in 0 (0%, LNs) and 7 (11.3%, PBMCs) of activated samples.

Concordant activation of either CD4+ or CD8+ cells in multiple lymph nodes of the same patient was not observed.

Conclusions: T cell responses directed against MDAs were observed in sentinel LNs of melanoma patients. The rate of activation was higher in PBMCs than in LNs. There was limited concordance in the reactivity patterns between LNs of the same patient, as well as between matched LNs and PBMCs. Additionally, CD4+ and CD8+ cells did not exhibit concordant reactivity. Therefore, immune responses

within lymphatic tissues appear highly compartmentalized and cannot be reliably assessed solely through sampling peripheral blood or individual T cell populations. Sentinel LNs serve as valuable resources for characterizing the locoregional immune modulation induced by melanoma cells and necessitate further in-depth analysis.

P223 | Elevation of the extracellular pH in the tumor microenvironment

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High tumor metabolism has been found to be responsible for a decrease in the extracellular pH (pHe) in the tumor microenvironment (TME) compared to healthy tissue. For malignant melanoma patients, strong acidic pHe compared to healthy tissue is linked to poor prognosis. Additionally, it has been shown, that low acidic pHe does have impacts on immune cells in the TME. Macrophages will be polarized towards the immunosuppressive M2 macrophage type, the predominant polarization type in tumor-associated macrophages (TAM) leading towards a low M1/M2 macrophage ratio in the TME, which is also associated with a poor prognosis. M2- TAM promote tumor growth through secretion of soluble factors and the suppression of anti-tumor effector cells present in the TME. Therefore our aim is to investigate changes caused by differences in the pHe.

To influence the pHe in cancer tissue, we designed specially adapted pH-regulating nanoparticles. Urea, produced and secreted by cancer cells in the TME, is used as target to modulate the pHe in the TME. By encapsulation of the enzyme urease in liposomes, urea is catalyzed to ammonia and carbon dioxide and results in the increase of the pHe value. Besides a repolarization from M2 to the beneficial M1 as the inflammatory macrophage type, we are also investigating changes by the elevation of the pHe in cancer cells and by the use of cytostatic drugs in combination.

First, we could confirm the production and secretion of urea in human malignant melanoma cells. The pH-regulating liposomes were able to elevate the pHe in the acidified cell culture medium depending on the urea concentration in the medium, confirming a still functional enzyme activity after its encapsulation. Doxorubicin and temozolomide (cytostatic drugs) have shown significant differences in drug uptake into tumor cells caused by pHe changes. Additionally, we investigated changes in proliferation of cancer cells that were also affected by the pHe. Energy metabolism in cancer cells and macrophages were shown to be decreased by low pHe. Incubation of the macrophages in different pHe indicate, that M1 macrophages seem to go through strength adjustments compared to M2 macrophages.

With these experiments and future studies, we are investigating the influence of pHmodulating nanoparticles on the composition of immune cells and their anti-tumor functions in the TME as well as on the efficacy of distinct drugs in more detail.

Our preliminary results show a successful modulation of the pHe in the TME with smart carrier systems, which can lead to an increase of anti-tumor responses and indeed does improve drug uptake.

P224 | Coagulation and antithrombotic medication influence the outcome of Immune Checkpoint Inhibition in Melanoma patients

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Introduction: Cancer immunotherapy has revolutionized melanoma treatment, but the high number of non-responders still emphasizes the need for therapy augmentation. One potential avenue for enhancing anti-tumor treatment is through the modulation of coagulation and platelet activity, which have been found to play an important role in the tumor microenvironment, with factors such as tissue factor and thrombin promoting tumor growth and metastasis. Although preclinical studies indicate a beneficial effect of antithrombotic therapy, clinical data has been inconsistent.

Methods: We conducted a study of 203 patients with stage III or IV Melanoma, who received treatment with ICI 2018 and 2022. Which we examined for various cell counts and blood markers. In addition, we studied a cohort of melanoma patients (n=2,419) derived from the german prospective multicenter skin cancer registry ADOREG, to investigate a potential preventive effect of concomitant antithrombotic medication. The patients were classified based on whether they received platelet aggregation inhibition (PAI) (n=137), anticoagulation (AC) (n=185), or no antithrombotic medication (n=2,097) at any point during ICI treatment.

Results: In the examination of blood levels, Patients with elevated D-Dimer levels at baseline had significantly decreased OS and PFS. The D-Dimer kinetics under therapy also seems to have prognostic ability. In the ADOREG cohort, where concomitant medication was analyzed, a significantly improved PFS was observed in patients receiving PAI with acetylsalicylic acid (ASA) (p=0.0023) as well as in patients receiving AC (p=0.01) compared to patients receiving no antithrombotic medication. The hazard ratio was 0.7 for patients who received AC (95% CI: 0.53 to 0.91) and 0.75 for those who received PAI (95% CI: 0.59 to 0.93) when compared to patients without concomitant antithrombotic treatment.

Discussion: Our study indicates a negative prognostic impact of elevated D-Dimer levels, a predictive ability for D-dimer kinetics and a potential protective effect of concomitant antithrombotic medication in melanoma patients receiving ICI therapy. However, more studies are needed to further investigate the use of D-Dimers as a

prognostic marker and the cancer-related benefit of adding anticoagulation or platelet inhibition to ICI treatment.

P225 | Downregulation of MHC-I molecules during melanoma evolution

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In recent years, immune checkpoint blockade (ICB) has revolutionized the treatment of patients with melanoma. The efficacy of ICB is predominantly attributed to the reactivation of CD8+ T cells, which recognize target antigens presented by MHCI molecules on tumor cells. Downregulation of MHC-I on melanoma cells has been described as an immune evasion mechanism. Here, we reassessed the frequency of MHC-I downregulation of melanoma cells during tumor evolution. For this, we analyzed the expression of MHC-I in a cohort of melanoma skin metastases, and observed a very low MHC-I expression in one third of samples. The downregulation of MHC-I was associated with a decreased number of infiltrating CD8+ T-cells. Due to the emerging evidence of CD4+ T-cells as contributor to antitumoral immune responses, we additionally assessed the expression of MHC-II molecules. We detected MHC-II expression on antigen presenting cells but only infrequently on melanoma cells. We also analyzed the expression of MHC molecules by singlecell RNA sequencing. In an independent cohort of 20 treatment-naïve melanoma metastases, we detected a marked transcriptional downregulation of MHC-I in four of these samples. Again, the expression of MHC-II was observed almost exclusively on antigen presenting immune cells. Importantly, transcriptional downregulation of MHCI was associated with poor response to immune checkpoint therapy. Taken together, the immunohistochemical and single-cell transcriptomic analyses indicate that downregulation of MHC-I expression is an early event during melanoma evolution. Currently, we are expanding our analyses of MHC-expression to include additional primary and metastatic melanoma samples. These studies will provide further insights into the evolutionary dynamics of MHC downregulation.

P226 | Tuning toxicity and immunogenicity of medical gas plasma-generated ROS in an SCC organoid model

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Medical gas plasma is a partially ionized gas operated at body temperature and generating a plethora of reactive oxygen species (ROS) simultaneously. The technology has been approved as a medical product in Europe since 2013 for the treatment of chronic and infected wounds and ulcers. We previously have found that exacerbated levels of gas plasma-derived ROS have tumor-toxic potential and potently combine with existing and novel anticancer drugs and immune checkpoint blockade (ICB) targeted at skin cancer in vivo. Now, we take this approach further, increasing the targetability of the approach using ROS-tuning that is enabled by modifying the type of gas and mixture fed into the medical gas plasma jet device. The hypothesis was that we would identify specific reactive species production signatures associated with increased toxicity in squamous cell carcinomas (SCCs) while maintaining the generally-well tolerability of gas plasma exposure. In the first step, we set 12 different gas plasma modes and characterized the ROS profiles by using optical emission spectroscopy and reactive species analysis assays. Second, these gas plasma conditions were separately applied to the hen's egg test of the chorioallantois membrane (HET-CAM), and algorithm-based image quantification was used to identify the irritation potential of gas plasma-derived ROS mixtures based on an in-house scoring system 5min, 24h, and 48h after treatment. Third, three SCC cell lines (A431, FaDu, SCC25) were used and separately seeded on the CAM of chicken embryos to grow realistic, three-dimensional, matrix-forming tumor organoids. Next, the tumors were exposed to the different gas plasma feed gas conditions and tumor weight was monitored. Finally, tumors were excised, digested, and subjected to multi-color flow cytometry and multiplex chemokine and cytokine analysis. From these data, we were able to extract a superiorly tuned gas plasma demonstrating increased tumor toxicity at SCC while maintaining a similar safety profile. The optimized gas plasma setting is currently being prepared for direct comparison in three in vivo xenograft models.

P227 | Bach-1 expression in melanoma cells treated with oncolytic viruses

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To date, only one replication-competent oncolytic virus, a modified herpes virus type 1 (HSV-1) termed talimogene laherparepve (T-VEC) (IMLYGIC®, Amgen), has been approved by the US Food and Drug Administration (FDA) for the treatment of advanced

melanoma. This makes T-VEC the first therapeutic agent in oncolytic immunotherapy.

However, the mechanisms underlying the regulation of HSV-1 replication in cancer cells and antitumour immunity are not well understood. In addition, it is not yet known which factors predict treatment response or failure.

Recently, we identified Nectin-1, an HSV-1 entry receptor, as a potential biomarker for T-VEC-induced melanoma regression (Schwertner et al., 2021). In addition, activation of intrinsic antiviral pathways may influence the antitumour activity of TVEC. Recently, BTB and CNC homology 1 (Bach-1), a protein involved in various cellular processes such as cell cycle, cancer metastasis and oxidative stress response, has also been shown to have antiviral activity. In this context, interferondependent stimulation of Bach-1 following HSV-1 infection leads to suppression of viral replication and antitumour activity. In contrast, Bach-1 deficiency promotes HSV-1-induced immunogenic cell death and antitumour immune responses (Pan et al. 2022, Zhang et al. 2018).

In our study, we aim to analyse the expression and regulation of Bach-1 in human melanoma cell lines and patient samples. We will determine the baseline expression of Bach-1 in several melanoma cell lines. We will then treat melanoma cells with T-VEC or d106S, a replication-deficient oncolytic virus, and interferon to see if this triggers Bach-1 expression. In addition, we will retrospectively analyse Bach-1 expression for the response of patient melanoma metastases to intralesional T-VEC injection.

P228 | Architecture of peripheral sensory and sympathetic neurons in Hgf-Cdk4 mouse melanomas transplanted into the skin

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Peripheral neurons densely innervate barrier organs like the skin and cooperate with immune cells to detect potentially noxious stimuli. Neuro-immune interactions have been studied in a number of different inflammatory disease models. Their role in the development and progression of skin cancer is only beginning to be explored. Based on published work so far, we hypothesize that peripheral sensory and sympathetic neurons in the tumor microenvironment participate in the regulation of anti-tumor immunity. To experimentally address this hypothesis we established genetically engineered mice expressing fluorescent reporter genes in sensory neurons (Nav1.8- CrexR26tdTomato) and sympathetic neurons (TH-CrexR26tdTomato). In initial experiments, we set out to characterize the architecture of peripheral sensory and sympathetic neurons of fluorescent tagBFP labeled Hgf-Cdk4 mouse melanomas transplanted into the skin. We could detect neuronal structures by standard fluorescence microscopy of cryosections. To get a more complete picture of the neuronal architecture of transplanted melanomas, we established a procedure of optical tissue clearing and 3D imaging using light sheet microscopy. Results so far show that sensory neurons are predominantly found at the tumor invasive margins, while sympathetic neurons predominantly extend along blood vessels. We are currently complementing these results using confocal and multi-photon microscopy. The establishment of this experimental system provides a basis to investigate mechanisms that govern the interactions between neurons, cancer cells and immune cells with the ultimate goal to improve cancer immunotherapy.

Miscellaneous

P229 | Human three-dimensional full-thickness skin models from donors of different ages show corresponding characteristics in the stage of skin aging

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Human skin serves as a model organ system to better understand aging processes and associated diseases as well as to develop preventive and therapeutic strategies. In order to investigate the molecular mechanisms of skin aging and associated diseases, human skin, animal models or cell culture systems are often used in vivo and ex vivo.

In accordance with the 3R rule of the animal protection law, we have cultivated threedimensional skin models as an organ system to examine the aspects of the aging process in the skin. Using primary dermal fibroblasts and epidermal keratinocytes isolated from donors of female juvenile skin (~20 years) and female mature skin (~80 years), we analyzed the morphological development over a period of 28 days. Within a few days of culture, the skin models developed from old donors showed a decrease in epidermal and dermal thickness compared to the models developed from young donors. This difference became even clearer after 21 days of cultivation period. Immunohistological studies showed that in the skin models of mature donors compared to juvenile donor models, the number of proliferating Ki67-positive cells was reduced, while the number of senescent p21-positive cells increased in both the epidermal and dermal layers. In addition, the content of hyaluronic acid was significantly reduced in aged skin models. Comparative transcriptome analyzes showed aging process-associated gene expression signatures in the models of mature skin compared to the models of juvenile skin. Biological processes such as the synthesis of matrix metalloproteases, degradation of the extracellular matrix and senescence in regard to skin aging were assigned by Gene Ontology (GO)

analysis. We were able to show that three-dimensional skin models are suitable to support skin aging research. The models, consisting of keratinocytes and fibroblasts from juvenile or mature donor skin, show differences in phenotypic signs of skin aging which correlate to previous in vivo studies.

P230 | Using in vitro human 3D skin models for the serological diagnosis of severe bullous autoimmune dermatoses

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Autoimmune bullous dermatoses (AIBD) are a heterogeneous group of diseases. In pemphigus, autoantibodies are directed against desmosomal proteins, which connect keratinocytes, and in pemphigoid diseases against proteins of the basement membrane zone. These abnormalities clinically result in intra- or subepidermal blister formation and secondary erosions on the skin and mucous membranes. If left untreated, these diseases are potentially life-threatening due to superinfection, fluid loss, and severely restricted food intake. AIBD cannot be diagnosed on the basis of the clinical picture alone, but by detecting tissue-bound and/or circulating autoantibodies using direct or indirect immunofluorescence (DIF/IIF), monospecific ELISA or immunoblot analyses. In particular, the IIF method commonly uses animal tissue substrates such as monkey esophagus to screen for suspected AIBD and to differentiate between pemphigus and pemphigoid diseases.

In the present study, we aimed to develop and test an in vitro human full-thickness 3D skin model as a replacement for animal tissue in the diagnosis of AIBD. In a first step, we generated 3D skin models comprising human dermal fibroblasts and epidermal keratinocytes from healthy commercial donors. These models consisted of a fully differentiated dermal and epidermal part separated by a basement membrane. In a next step, we prepared cryosections of these 3D skin models. In order to test whether these cryosections of 3D skin models are a suitable test method for the detection of AIBD, sera from patients with pemphigus vulgaris, bullous pemphigoid and pemphigus foliaceus were collected within a clinical study. Incubating the cryosections with sera of AIBD patients using the IIF technique we could differentiate between the different clinical features of pemphigus and pemphigoid diseases. Sera of pemphigus diseases showed intracellular fluorescence in the epithelium, while sera of pemphigoid diseases exhibited linear deposits on the basement membrane. These results correlated exactly with results of the IIF on monkey esophagus. Essential technical documentation for the test device was prepared following the In-Vitro-Diagnostic Regulation (IVDR) and applying quality standards for medical devices. Starting from user needs, the intended purpose for the new tool was determined, requirements defined and risks assessed, to assure safe and reproducible production and testing of the cryosections of the 3D skin models.

In summary, in accordance with the 3R principle, we present an alternative method for detecting AIBD in IIF using animal tissue. In a study using sera from the most common AIBD, we were able to demonstrate that cryosections from in vitro human 3D skin models are a suitable diagnostic tool. Since the final diagnosis is based on a combination of the clinical picture with the detection of autoantibodies against the individual target antigens, we present a new tool that can enrich the diagnostic methods for AIBD.

P231 | Comprehensive in vitro assessment regarding cytotoxicity, and sensitizing potential for food packaging composite materials

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Introduction: Several European Union legislations request the use of in vitro methods for toxicological evaluations, including sensitization, in order to increase consumer safety but also to reduce animal tests. The food packaging industry's primary concerns are to ensure the safety of food contents and to prolong their shelf life by protecting them from spoilage, oxidation, and external contaminants. However, in response to growing environmental concerns, there is a concerted effort to additionally reduce packaging waste through the increased use of biodegradable polymers, coatings, and films. Those natural polymers are more prone to induce sensitization potential in consumers, which can lead from skin irritation to more severe consequences, including tissue damage and health complications. There are multiple approaches to determine sensitization potential of single chemical compounds in vitro but no established protocol yet for finished products like composite materials. This study evaluates a sequence of in vitro assays to assess finished materials and there consumer safety.

Methods: Cytotoxicity was assessed using in vitro assays with human keratinocytes and fibroblasts exposed to various extraction media (saliva, sweat solution) that mimic environmental conditions. Additionally, the sensitization potential was evaluated using in-vitro testing methods regarding the second key event of the adverse outcome pathway for skin sensitization initiated by covalent binding to proteins.

Results: The data show good results in defining cytotoxicity for human keratinocytes and fibroblast for different kinds of extraction media and results from the skin sensitization assays demonstrate great potential to be transferred to 3D skin models next and be used in material evaluation.

Conclusion: This evaluation is a promising first attempt for an in vitro testing protocol to ensure material safety for consumers.

P232 | Enhancing the regenerative and pro-angiogenic properties of mesenchymal stem cells via RGD/integrin-induced mechanisms: application to the treatment of ischemic wounds

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Mesenchymal stem cells (MSCs) contribute to the wound healing process through angiogenesis and immunoregulation. Used as therapeutic cells, MSCs have demonstrated their potential to heal chronic wounds. This study reveals a novel regulation of MSCs induced by arginylglycylaspartic (RGD) motif/integrin interaction. After exposure to a gelatin-containing scaffold, adipose tissue-derived MSCs (or adipose tissue-derived stem cells, ASCs) altered their full gene expression profile and secreted proteome in favor of a regenerative profile, including increased production of angiogenic, remodeling and acute-phase inflammation factors. Using competitive inhibitors of RGD (cilengitide) and antibodies blocking integrins α5β1, αvβ3, and αvβ5, we demonstrate that these regulations depend on the interaction of ASCs with RGD motifs present in gelatin. At the functional level, RGD/integrininduced regulation resulted in a marked pro-angiogenic switch characterized by the secretion of factors promoting endothelial cell proliferation and migration, in vitro tubulogenesis and angiogenesis in the chick chorioallantoic membrane model. Furthermore, ASCs activated by the RGD/integrin pathway promoted rapid and enhanced re-vascularization of cutaneous ischemic wounds in rats, thereby leading to a more efficient healing as compared to polyurethan/silicon dressings. Together, we provide compelling preclinical evidence that RGD/integrin-activated ASCs undergo a pro-angiogenic switch that could prove essential in future therapies to restore effective healing of chronic wounds.

P233 | Regulating human scalp hair follicle pigmentation through vasoactive intestinal peptide

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Vasoactive intestinal peptide (VIP) is a neuropeptide found in the skin and associated with hair follicle (HF) immune privilege. Notably, intrafollicular VIP receptor expression is found to be defective in conditions like alopecia areata. In the current study, we aimed to explore the role of VIP in regulating melanogenesis in human scalp HF and determine the underlying mechanisms. We obtained frontotemporal human scalp skin specimens from healthy patients undergoing cosmetic facelift surgery or hair transplantation. HFs from male donors were treated "systemically" with two concentrations of VIP (100nM and 1μM) or vehicle for six days, every other day. Our quantitative immunohistomorphometry results showed that VIP at 100nM reduces melanin production (Warthin-Starry histochemistry), and significantly decreases the activity of tyrosinase, the key enzyme involved in melanin production, in anagen VI HFs. Instead, the intrafollicular protein expression levels of other melanogenesis-regulatory enzymes, such as tyrosinase hydroxylase and dopachrome tautomerase, remained unaffected. To confirm that the changes in melanin production were not due to a reduction in melanocyte cell number or an increase in melanocyte apoptosis, we performed MITF/gp100 and gp100/ TUNEL double-immunostaining. Our results showed no significant differences in melanocyte cell number or apoptosis in anagen VI HFs when treated with VIP compared to the control. Additionally, to our surprise, we discovered that none of the concentrations of VIP upregulate VPAC2 protein expression. Our initial research presents VIP as a new and physiologically significant regulator in the neuroendocrine and neuroimmunological aspects of human HFs pigmentation. This aspect of VIP has been ignored in conventional pigment cell studies and warrants careful examination, especially concerning hair graying.

P234 | Transient p53/p21-activation selectively protects healthy human hair follicles and their stem cells from chemotherapy

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Chemotherapy-induced alopecia (CIA) remains one of the most distressing adverse effects of cancer therapy. There are no pharmacological treatments available to selectively protect healthy hair follicles (HFs) and their epithelial stem cells (eHFSCs) from acute and long-term chemotherapy-induced damage without also awarding survival benefits to cancer cells.

To address this major unmet clinical need, we have explored in organ-cultured human scalp HFs whether ALRN-6924 can award such protection against two key CIA-associated chemotherapeutics, paclitaxel and the cyclophosphamide active metabolite 4-HC

(4-hydroperoxy cyclophosphamide) 4-HC. ALRN-6924 is a clinical-stage MDM2/MDMX dual inhibitor that activates p53 to upregulate p21, transiently arresting and selectively protecting healthy bone marrow cells from chemotherapy in patients with p53-mutant cancers without protecting cancer cells.

ALRN-6924 significantly increased the number of p21+ hair matrix keratinocytes and bulge eHFSCs, confirming cell cycle arrest ex vivo, yet without promoting premature transition of HFs from the growth (anagen) phase to regression (catagen) phase. Moreover, ALRN-6924 significantly inhibited paclitaxel- or 4-HC-induced premature catagen development and hair matrix damage, as shown by reductions in HF pigmentary abnormalities, apoptosis induction, and micronucleation. Importantly, ALRN-6924 pretreatment ex vivo also significantly reduced DNA damage, apoptosis, "mitotic catastrophe", and pathological epithelial-mesenchymal transition (EMT) in eHFSCs, mechanisms that potentially can cause permanent CIA. Finally, the CIA-protective effects observed in microdissected full-length HFs and organcultured full-thickness hairy skin could be reproduced when ALRN-6924 was administered topically to intact chemotherapy-treated human scalp skin ex vivo using an established vehicle for clinical HF treatment. These findings provide long-missing proof-of-principle that healthy human HFs and their eHFSCs can be selectively protected against multi-level toxicity induced by chemotherapy by temporary p53/ p21-dependent cell cycle arrest, and support the development of ALRN-6924 as a prophylactic treatment for CIA.

P235 | The common fragrance, linalool, promotes core pathology events associated with frontal fibrosing alopecia in human scalp hair follicles ex vivo

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It has been speculated that (as yet disputed) environmental factors may explain the greatly increased prevalence of frontal fibrosing alopecia (FFA) over the last decades. Since many FFA patients are sensitized to allergens found in leaveon skin products, we investigated if one of the most frequently FFA-associated allergens, linalool, which is ubiquitous in personal care products, promotes core FFA pathogenesis elements. To probe this, biopsies from healthy and non-lesional FFA scalp skin were treated with either vehicle or 1% linalool and organ-cultured for 6 days. Ex vivo, linalool significantly decreased the number of K15+ epithelial HF stem cells (eHFSCs), and upregulated bulge expression of MHC class Ia and the "danger" signal, MICA, both in healthy HFs and in non-lesional scalp skin from FFA patients with documented linalool sensitization. The latter also showed an increased number of eHFSCs undergoing EMT (K15+/vimentin+ cells). These pilot data strongly suggest that linalool can indeed promote core FFA pathobiology events, namely bulge immune privilege collapse and irreversible HF stem cell damage, not only in sensitized FFA patients, but also in healthy scalp HFs. We are currently probing by silencing experiments which role olfactory receptors that are specifically activated by linalool and expressed in human HFs may play in these bulge pathology-inducing effects of a very widely used fragrance.

P236 | Bitter taste receptor-mediated signaling regulates human organ remodeling: TAS2R4 stimulation inhibits hair growth ex vivo via cell cycle inhibition and TGFβ2 secretion

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Taste receptors (TR) exert many non-classical functions. Here, we have explored the as-yet-unknown functions of TRs in human skin biology by investigating whether and how human scalp hair follicles (HFs) utilize signaling via the bitter TR, TAS2R4.

We show that human HF epithelium prominently expresses functional TAS2R4. Its activation by the steviol glycoside, rebaudioside A (Reb-A), inhibits hair matrix keratinocyte proliferation, induces premature HF involution (catagen) ex vivo, and upregulates the intrafollicular production of hair growth-inhibitory TGF-β2. Instead, TAS2R4-knockdown in the presence of Reb-A promotes hair growth, demonstrating the TAS2R4-dependence of RebA effects, while TGF-β-neutralization antagonizes Reb-A-induced catagen. RNAseq analysis of Reb-A- or TAS2R4 siRNA-treated HFs reveals differential gene expression signatures consistent with TAS2R4-mediated changes in cell cycle and TGF-β pathway signaling.

By studying the role of TAS2R4 in human HF cycling as an instructive human model system for clinically relevant chemosensory research, this work introduces bitter TRs as potent regulators of tissue remodeling whose stimulation by a simple tastant impacts profoundly on growth factor production and keratinocyte proliferation within a human (mini-)organ. Besides novel mechanistic insights into TAS2R4-regulated TGF-β signaling and cell cycle control, this also identifies an innovative, drug-free strategy for the therapeutic inhibition of unwanted hair growth by TAS2R4 agonists.

P237 | Tacrolimus/FK-506 stimulates human scalp hair follicle growth ex vivo

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The immunoinhibitory calcineurin inhibitor, tacrolimus (FK-506), not only suppresses expression/secretion of several pro-inflammatory cytokines, but also restores IFNγ- induced human hair follicle (HF) immune privilege collapse ex vivo and is hair cyclemodulatory in mice in vivo. However, it is unknown how tacrolimus impacts on human HF growth. To explore this, organ-cultured human anagen scalp HFs were treated with tacrolimus (10, 100nM) for 6 days. Quantitative (immuno-)histomorphometry showed that tacrolimus (100nM) prolongs anagen/inhibits catagen, just as in mice, yet without significantly affecting hair matrix keratinocyte proliferation/apoptosis. However, expression of catagen-promoting TGFβ2 protein in outer root sheath keratinocytes was decreased, while that of anagen-promoting IGF1 was increased ex vivo. Furthermore, FGF/KGF protein expression was increased in hair matrix keratinocytes. Interstiungly, tacrolimus (100 nM) also increased protein expression of keratin 15 in the bulge and of keratin 85 in the precortical hair marix. Taken together, these preliminary data show that tacrolimus exerts similar hair growth-stimulatory effects on human scalp HFs as the other main calcineurin inhibitor, cyclosporine A, but likely via different pathways, and thus deserves systematic exploration as a candidate hair growth-stimulatory agent, namely in the management of alopecia areata, FFA and androgenetic alopecia.

P238 | Skin microbiome dynamics as predictor and pathogenesis mechanism for severe radiodermatitis in breast cancer patients

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Radiodermatits is commonly observed during radiotherapy in post-operative breast cancer patients, severly impacting the quality of life of the patients. So far, the interindividual differences regarding radiodermatitis severity and the pathomechanism are not well understood. To understand the role of the skin microbiome and skin physiology in the development of radiodermatitis, we conducted a longitudinal pilot study with 20 female breast cancer patients undergoing radiotherapy. From each patient, the skin pH and skin microbiome were assessed via nextgeneration sequencing of the V1-V3 region of the 16S rRNA and quantitative PCR on both the affected and non-affected body-sides before, during and after radiotherapy on a weekly basis (360 samples). Additionally, radiodermatitis severity was determined.
All patients developed radiodermatitis during the study. After five to seven weeks, four patients developed severe radiodermatitis. Strikingly, low (<5%) colonization with skin commensals (Staphylococcus epidermidis, Staphylococcus hominis, Cutibacterium acnes) at baseline was highly predictive for the development of severe radiodermatitis. Instead, different Corynebacteriaceae species were more abundant in severe cases. Corynebacteriaceae abundance was correlated positively, and commensal abundance negatively with skin pH. Strinkingly, only severe patients showed an increase in total bacterial cell numbers estimated via qPCR of the 16S rRNA copies in contrast to stable bacterial colonization in mild and moderate radiodermatitis cases prior to the onset of severe symptoms.
In summary, we have observed two logically linked phenomena exclusively in severe patients: a low baseline level of commensals, and an early increase in total bacterial load. Thus, our findings potentially show for the first time that microbes have a direct effect on the pathogenesis of radiodermatitis.

P239 | Identification of cell subsets across anatomical sites in human skin

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Research in mice has uncovered the significant heterogeneity of cell populations, both in terms of their subtypes and functions in the skin, which is closely linked to the developmental origin and body location. Recent studies on human skin suggest that similar cellular differences exist depending on anatomical location, but a full understanding is lacking. Interestingly, different areas of skin are predisposed to certain diseases or scarring. Deciphering the cellular identity in human skin depending on the anatomical location will allow a better understanding of the underlying mechanisms of this phenomenon.
In this study, we focus on analysing skin from different locations that are known to derive from different origins (mesodermal layers including cephalic mesoderm and neural rest) to identify differences in stromal cell, myeloid cell and lymphocyte cell subsets between skin location and unravel any correlation among those. As a result, published scRNASeq datasets were reanalyzed from healthy human skin (age range 25-60 yrs.) and skin collected from the face, head, chest, abdomen, and limbs of healthy donors. Moreover, skin was also collected from patients with scars or inflammatory diseases. To ensure data accuracy, the skin was also characterized with new scRNASeq data and multicolour FACS analysis.

Re-analyzing datasets from healthy human breast and forearm skin revealed that although fibroblasts in these two areas develop from the same embryonic origin (paraxial mesoderm), they have distinct gene expression patterns. This may suggest different developmental pathways of trunk and limb skin. Fibroblasts from the forearm displayed high expression of genes associated with the Wnt signalling pathway, immune-like functions, extracellular matrix (ECM)-related genes, and wound healing-related genes (e.g., Wisp2, DPP4, S100A4, CXCL12, COL1A1, COL3A1). Conversely, fibroblasts from the breast predominantly expressed genes linked to tissue homeostasis, and development, and exhibited myofibroblast-like characteristics (e.g., AKR1C1, DDIT4, PTX3, IL6).

Interestingly, when analyzing stromal cells (CD45-/CD326-/CD31-) in our skin samples via flow cytometry we also observed different frequencies of fibroblast subsets depending on the anatomical location. To define different fibroblast subsets, we used well-known markers such as CD90+ and PDGFRa+ in association with markers extrapolated from murine studies. Our analysis revealed that CD90+/ CD39+ fibroblasts in the upper dermis were notably elevated in lip tissue from infants and inflamed skin but were low in arm and limb scars. Meanwhile, CD90+/CD26+ fibroblasts from the upper dermis showed increased levels in the limbs and trunk, were missing in the buccal mucosa, lip, and capillitium and reduced in scar and inflamed skin.

When studying the immune cell compartment, we observed an increased presence of dendritic cells (CD3-/CD19-/CD56-/CD45+/HLA-DR+/CD11c+/CD14-) in facial skin, breast and scalp whereas macrophages (CD3-/CD19-/CD56-/CD45+/HLA-DR +/CD11c-/CD14+), specifically CD206+ macrophages, were more prevalent in upper leg and breast skin. Additionally, elevated levels of macrophages were also detected in fibrotic scars. In summary, our data indicate that the tissue-resident population in the skin differ between body locations. Further substantiating of these findings and integration of scRNASeq analyses are ongoing to shed light on the mechanisms predisposing to scarring and inflammatory diseases in different locations of the skin.

P240 | Measuring of calcium and ammonium ions on the palmar skin surface - comparison of two methods for ion assessment

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When measuring calcium ions or ammonium ions, partly linked biochemically, on the skin surface photometrically, a possible influence on the determination procedure of ammonium ions are amino acids or their derivatives. The potentiometric assessment of the ions along with the photometrical measurement might be an approach to evaluate possible interferences. Therefore, the aim of the present study is to assess calcium ions as well as ammonium ions in the palmar region using both approaches along with skin surface pH measurement and an in vitro evaluation of the influences of some amino acids characteristic for the skin on the photometrical test for ammonium ions.

Overall, 20 volunteers were included after informed written consent. The samples were taken by rinsing the skin surface with ion free water. The calcium ions were determined using a photometric test kit as well as a calcium ion selective combination electrode. The ammonium ions were assessed using a photometric test as well as an ammonia electrode based on a glass electrode with ammonia selective membrane. The pH of the skin surface was assessed using a standard glass electrode. After determining the absolute values per area of investigation the results from both methods were compared by statistical tests as well as by correlation and regression analysis. Moreover, it was tested whether some of the most common amino acids such as glycine, histidine or arginine as well as trans-urocanic acid contributed to a positive result of the photometric test.

The amounts of calcium ions showed almost identical results with regard to the average and the variation. The absolute values for ammonium ions, however, showed statistically significant differences, when comparing the results obtained by the different methods. The values were about twice as high using the photometric test in comparison to the potentiometric measurement, while there was little difference in the width of the values. The results from the correlation and regression analysis showed a statistically significant relation between calcium and ammonium ions when applying the photometric test. Moreover, a strong inverse relation between the potentiometrically assessed ammonium ions and skin surface could be found, while no significant relation was found between the calcium ions and pH of the skin surface. The amino acids and trans-urocanic acid showed a variety of positive reactions in the photometric test.

The almost identical results for the calcium ions suggest that they reflect a very similar information. In contrast, the results obtained for the ammonium ions showed very different results suggesting that the information contained is at least in part different. As the potentiometric assessments can be considered more selective when investigating samples with a large variety of molecules and as the values are lower, it can be concluded that the photometric measurements assessed additional information. This is also suggested by the correlation analysis in particular with regard to the correlation to the pH-values, where the results are more significant in the potentiometric measurement. For the relation between ammonium ions and calcium ions this would mean that other molecules such as those investigated in vitro are involved, too. More investigations are required to identify the role of amino acids and other nitrogen containing molecules for the measurements.

P241 | Patient-reported estimation of medical care for chronic inflammatory diseases: an enterprise-based survey

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Background: Chronic inflammatory skin diseases (CISD) are among the most common diseases in the Western world (1). Pain, itching, exudation, and visibility of the cutaneous alterations lead to physical and psychological burden, resulting in restriction of daily activity for those affected. Furthermore, due to annual loss of gross value added CISD have a profound negative socioeconomic impact (2). Current estimates of medical care for CISD are primarily based on surveys among patients in the medical care facilities and on health insurance data.
Objective: Survey-based examination to what extent CISD patients consider their skin disease to be controlled.
Methods: The survey of CISD patients was carried out in the best possible environment: among the employees of a pharmaceutical company located in Germany and Switzerland, two countries with a high standard of medical care and high level of income. Software-based, anonymous, self-reported questionnaires were used to collect data on the number, nature, and severity of CISDs the employees suffer from, the extent to which they consider their skin disease to be controlled, socio-economic and professional aspects, as well as the impact of the CISD on their private and professional life.
Results: The number of employees, who completed the questionnaire, was 868. Of these, 222 people (25.6%) reported having at least one CISD disease. 28.7% of employees with CISD described their disease as being hardly or not (i.e. poorly) controlled. In relation to the specific diseases, more than one third of the people suffering from hidradenitis suppurativa (HS) or psoriasis fell in this category. In contrast, the largest proportion of employees with chronic spontaneous urticaria or atopic dermatitis (>40%) considered their CISD to be completely or well controlled. Only 35.5% of employees with CISD stated that they were currently under medical care for their skin condition. Being under medical care had no influence on to what extent CISD patients consider their skin disease to be controlled. The number of active CISD phases but not the total number of symptomatic days per year was negatively associated with poor disease control (Kruskal-Wallis test, P=0.042 and P=0.856, respectively). Regarding the effects of disease control, there was a positive association of poor disease control with the extent of presenteism and daily activity restriction (Kruskal-Wallis test, P=0.005 and P=0.005, respectively). 41.4% and 20.7% of employees with poor disease control stated that their CISD had a moderate and severe or very severe impact on their overall lives, respectively (Chi2 test, P=<0.001 and P=<0.001, respectively). Employees with HS most often answered that the CISD had a severe to very severe impact on their lives.
Conclusion: Medical care for CISDs, even in an environment with a relative high socio-economic standard, still appears to be limited and has a negative impact on the personal and professional lives of those affected. There seems to be a particularly high need for care in HS and even in psoriasis.

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None

P242 | Effects of Persistent Organic Pollutants on EGFR Activity in Human Keratinocytes: Indications of a Direct Interaction with the EGFR Extracellular Domain

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The epidermal growth factor receptor (EGFR) regulates processes like cell proliferation, differentiation, and migration. Thus, the receptor is crucial for the development, maintenance, and repair of various tissues and organs. Consequently, dysregulation of EGFR signaling, for instance caused by EGFR-targeting drugs and antibodies used in cancer therapy, can induce skin toxicities. Recently, our group identified the EGFR extracellular domain (ECD) as a direct cell surface target of persistent organic pollutants (POPs), in particular dioxins and structurally related chemicals.
In the current study, our group investigated whether other POPs of very high concern are also capable of disturbing growth factor-driven EGFR activation in human keratinocytes. We focused on bioaccumulating compounds used in plastic production, namely UV-stabilizing phenolic benzotriazoles (BUV) and flame-retarding polybrominated diphenyl ethers (BDE). Via BrdU proliferation assay, we found that halogenated BUV and BDE significantly and dose-dependently reduced the EGFR ligand-induced DNA synthesis while a structurally similar non-halogenated BUV did not. Furthermore, western blot analysis showed that BUV and BDE decreased the level of ligand-induced EGFR phosphorylation in a significant manner. Subsequently, internalization assays could further confirm the interference with ligand-induced EGFR activation by the test compounds. At last, the presumably direct interaction of the tested compounds with EGFR could be confirmed by EGF/EGFR AlphaLISA binding assay.
Since the EGFR plays such a critical role in numerous cellular functions and tissue integrity, its newly discovered function as a target for a variety of POPs may help to better understand the pathomechanisms by which these chemicals trigger or exacerbate adverse health effects in humans. The data also suggest that the expression

level and activity of EGFR might contribute to potential cell- and tissuespecific toxicities induced by the respective chemicals.

P243 | Medical gas plasma-generated ROS depend on the characteristics of hydrogel models and human skin

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Cold physical plasma is a partially ionized gas operated at body temperature that generates abundant oxygen and nitrogen reactive species. The use of cold physical plasma in medical care has become increasingly widespread over the past years [1], especially in treating chronic wounds [2] and extending to other dermatology applications [3]. Clinical studies have often reported promising results with high efficiency and increased patient welfare while lowering therapy costs despite the high heterogeneity of certain conditions. Various types of plasma sources are now on the market and routinely used in clinics. Interaction between plasma and tissues is one of the keys to the healing process. However, many interrogations remain. For atmospheric pressure plasma jets such as the kINPen MED, it is unclear how the physico-chemical characteristics of the plasma change when it is in contact with its treatment target (e.g., skin), the so-called conductive mode [4]. To this end, we used the kINPen plasma jet and several hydrogel models to investigate the impact of the treatment target characteristics, such as organic and water content as well as polymer type and mechanical property, on the several plasma traits, which in turn may alter biological responses in plasma-treated tissues. In addition, we aimed to address the penetration depth of the reactive species into tissues by using agarosebased hydrogels to compare with human skin samples using several chemical probes for oxidation evidence. We could show that the generation of plasma jet ROS in the gas phase is affected by the characteristics of the treatment target and that plasmaderived reactive species penetrate hydrogels up to several millimeters deep. For the first time, we could also directly monitor ROS production in plasma-treated wounded skin. In future work, we will investigate how these findings relate to and potentially affect subsequent biological responses.

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P244 | T-cell receptor stimulation in human hair follicle organ culture leads to immune privilege collapse, which is prevented by farudodstat treatment

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The chronic immune-mediated hair follicle disorder, alopecia areata (AA) affects around 2% of the global population. It is characterized by hair loss in small circular patchy areas on the scalp which may develop to involve the complete scalp and/ or body. Increasing evidence points to AA as a stereotypic response pattern of the hair follicle to autoimmune-mediated (AAA) or autoantigen-independent (NAAIA) inflammatory responses. Regardless of the initiation mechanism, AA is recognized to primarily develop as consequence of excessive local expression of interferon-gamma (IFNg) and associated loss of immune privilege (IP) in the HF bulb, and intra-/peribulbar inflammation comprising particularly T-cells. The development of preclinical tools that recapitulate the different AA endotypes is of high importance to develop efficient therapies for AA. While we have previously shown that non-autoantigen driven AA-like response can be reproduced by co-culturing gdT-cells or ILC-like 1- cells with stressed hair follicles, ex vivo models mimicking AAA are currently lacking. Thus, in this project, we aimed to investigate whether the selective activation of the T-cell receptor (TCR) of resident intra-/peri-follicular T-cells by using anti-CD3/CD28 antibodies in human hair follicles ex vivo is sufficient to driven IP collapse. TCR stimulation resulted in proliferation and expansion of resident T-cells in HF organ culture, as reflected by an up-regulation in the number of T-cells in the HF epithelium and mesenchyme and by the significantly increase of proliferating CD3+ T-cells. While application of anti-CD3/CD28 antibodies did not affect hair matrix keratinocyte proliferation or catagen development, it promoted key features of IP collapse in the hair bulb. Specifically, MHC class I expression was significantly upregulated in the proximal outer root sheath (ORS) and dermal cup (DC), and significantly more MHC class II+ cells were

counted in the bulbar connective tissue sheath (CTS) in HFs challenged with anti-CD3/CD28 antibodies. Subsequently, we explored whether interfering with T-cell proliferation and Th1 responses prevents anti-CD3/CD28/ mediated bulb IP collapse. For this, we tested Farudodstat, a novel, potent, orally active dihydroorotate dehydrogenase (DHODH) inhibitor under clinical development for AA treatment. Farudodstat alone did not induce catagen entry nor HF cytotoxicity neither modulated keratinocyte proliferation nor IP marker expression in healthy HFs ex vivo. However, in a prophylactic assay, the drug efficiently prevented the increase in proliferating T-cells, up-regulation of MHC class I and II proteins, and the increase in MHC class II+ cells as observed upon anti-CD3/CD28 stimulation. Taken together, these preliminary data demonstrated that selective T-cell activation through anti-CD3/ CD28 treatment induces key features of AA ex vivo. Additionally our results underline the therapeutic potential of Farudodstat in patients suffering from AA.

AUTHOR INDEX

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KEYWORDS