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ALLERGY

P001 (OP01/04) | The gut-skin axis: bacterial stimulation in the gut shapes hapten-driven inflammation of the skin

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Methods/Results: We addressed the functional role of gut microbiota in experimental contact hypersensitivity (CHS), a CD8 + Tc1-mediated cutaneous inflammatory model, which largely resembles allergic contact dermatitis in men. We found that germ-free mice (GF) exhibited reduced ear swelling and cellular infiltration compared to conventionally housed (Conv) mice. Importantly, re-colonized GF mice showed an unaffected CHS reaction, excluding a general defect in the GF immune system. The hapten-specific Tc1 response (T cell proliferation, IFN-γ production) did not differ in Conv and GF animals. However, lymphocytes from skin-draining lymph nodes of CHS tolerant GF mice secreted higher amounts of IL-10. Unexpectedly, ablation of the intestinal microbiota in mice, by an antibiotics-cocktail (ABX mice), completely mimicked the GF phenotype with regard to clinical symptoms, T cell response and IL-10 secretion. This indicates that gut- but not skin-associated microbiota shape immunity towards contact allergens. Moreover, a distinct set of bacteria was able to restore CHS immunity in total. Indicating that extent and quality of bacterial stimulation in the gut alter hapten-driven inflammation. Increased numbers of IL-10 producing CD25 + CD4 + T cells and restoration of CHS responses in the absence of IL-10 producing FOXP3 + Tregs or CD4 + T cells in ABX treated mice demonstrated the functional relevance of T cell-related IL-10 in CHS inhibition. As haptens can activate TLR-2 mediated processes and as these molecules are involved in cutaneous inflammation and immune tolerance, we aimed to address the CHS response in mice lacking TLR-2 under ABX and GF conditions, respectively. In the absence of TLR2-mediated signaling, the CHS was largely unaffected in GF and ABX mice, revealing that TLR2 signaling is critically involved in the control of the CHS reaction by the gut microbiome. Interestingly, GF and ABX conditions restored IL-10 secretion in TLR-2 deficient mice, demonstrating TLR2 signaling upstream of IL-10 production.

Conclusions: Intestinal bacteria shape CHS inflammation via TLR2 and IL-10-mediated pathways.

P002 | Sodium is an ionic checkpoint for Th2 cell responses and shapes the atopic skin microenvironment

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There has been a strong increase in the incidence of allergic diseases over the last 50 years. Environmental factors most likely account for this phenomenon. However, the nature of these factors and the mode of action by which they induce the type 2 immune deviation, which is characteristic of atopic diseases, remain unclear. It has previously been reported that dietary sodium chloride promotes the polarization of Th17 cells with implications for autoimmune diseases such as multiple sclerosis. Here, we demonstrate that sodium chloride also potently promotes Th2 cell responses on multiple regulatory levels. Sodium chloride enhanced IL-4 and IL-13 production while suppressing IFN-g production in effector T cells. It diverted alternative T cell fates into the Th2 cell phenotype and also induced de novo Th2 cell polarization from naïve T cell precursors. Mechanistically, it exerted its effects via the osmosensitive transcription factor NFAT5 and the kinase SGK-1, which regulated Th2 signature cytokines and master transcription factors in hyperosmolar salt conditions. The skin of patients suffering from atopic dermatitis contained highly elevated amounts of sodium compared to non-lesional atopic and healthy skin. This demonstrates that sodium chloride represents a so far overlooked cutaneous microenvironmental factor in atopic dermatitis that can induce Th2 cell responses, the orchestrators of allergic diseases. Together, our data propose ionic signaling through sodium chloride as a novel checkpoint and potential therapeutic target for type 2 immunity and its associated allergic diseases.

P003 | Comparison of the safety profiles of three different Hymenoptera venom immunotherapy protocols - a retrospective two-center study of 143 patients

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Introduction: Venom immunotherapy (VIT) is highly effective and the treatment of choice for patients with a history of systemic anaphylactic reactions to a Hymenoptera sting. It has been assumed that VIT protocols with a rapid dose increase during induction phase are associated with a higher frequency of systemic reactions (SR); however, study data addressing this issue are conflicting.

Objective: The aim of this study was to compare the safety of three different Hymenoptera VIT protocols (half-day ultra-rush, three-day rush, three-week cluster).

Methods: This retrospective two-center study included 143 Hymenoptera venom allergic patients, who underwent 147 VIT procedures during the years 2015-2018. Twenty cluster, 75 rush and 52 ultra-rush VIT protocols were performed with honeybee (54 protocols) and wasp (93 protocols) venom. All documented side effects were classified into large local and SR (Ring and Messmer classification).

Results: SR were observed during 11 (7.5%) VIT procedures and did not exceed severity grade II. SR occurred more frequently in cluster compared to accelerated protocols. This result was observed for both honeybee (cluster: 25%, rush: 8.7%, ultra-rush: 15.8%) and wasp VIT (cluster: 12.5%, rush: 0%, ultra-rush: 6.1%), though the differences were statistically significant only in the wasp VIT subgroup. Honeybee venom elicited more SR compared to wasp venom (14.8% and 3.2%, respectively, $P = 0.01$). The risk for SR did not depend on age, sex, concomitant antihypertensive medication, hypertryptasemia or severity of the index sting reaction.

Conclusion: Accelerated VIT protocols, namely rush and ultra-rush protocols, are safe therapeutic options for Hymenoptera venom-allergic patients and displayed fewer SR compared to cluster VIT protocols in our study.

P004 | Supporting next-generation sequencing results via absolute quantification

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Background: Atopic dermatitis (AD) is associated with increased Staphylococcus aureus frequencies, especially during disease flares. Several S. aureus strains are capable of expressing toxins, which are exacerbating disease severity. Toxin expression is controlled by the quorum sensing system which is regulated by cell density. However, so far most microbiome studies are based on next-generation sequencing (NGS) which only describe the relative microbial abundance within the population. Varying copy numbers of 16S rRNA per species make this gene unsuitable for absolute quantification. Therefore, we aimed for absolute quantification of S. aureus based on a unique gene.

Methods: For quantification we used qPCR of a unique S. aureus gene and 16S copy number as proxy for bacterial cell number. Furthermore, the same samples were sequenced via a 16S rRNA amplicon NGS approach.

Results: qPCR could successfully be applied for bacterial quantification and correlated strongly with the relative abundance of S. aureus observed in an NGS approach. Interestingly, not only S. aureus abundance but also total 16S copies were higher in AD patients than in healthy controls. Whereas the bacterial abundance of heathy was patient-specific and remained stable over time, there was an association between S. aureus cell number and total bacterial load measured by 16S copy number in AD, hinting towards an S. aureus overgrowth and not only shift in distribution.

Conclusion: Absolute quantification of S. aureus confirms NGS data and could serve as a complementary method to NGS in the future.

P005 | Bidirectional activation of blood eosinophils and skin mast cells by secreted mediators and direct cell-cell contact

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Infiltrating eosinophils contribute to the late phase of allergic tissue inflammation either by physical contact with cells within their

environment or by the release of proinflammatory mediators. Together with mast cells (MCs), eosinophils can play a role in protection against foreign threats e.g. parasites, but, on the other hand, can contribute to chronic skin inflammation. For example, skin lesions of chronic spontaneous urticaria (CSU) patients present increased eosinophil numbers as well as eosinophil granule mediators such as major basic protein and eosinophil peroxidase, which are able to activate MCs. Based on these observations we hypothesized that tissue-infiltrating eosinophils contribute to the pathologic activation of MC in CSU.

To test this hypothesis we studied the interaction of primary human skin MC and human blood eosinophils in vitro and ex vivo. MCs or eosinophils were treated either with cell culture supernatants or by co-culture assays. Release of β-hexosaminidase or histamine was used as activation readouts for MC activation. Eosinophil activation was determined by measuring CD69 expression by flow cytometry. To mimic eosinophil infiltration into skin, we injected blood eosinophils into human skin explants and performed ex vivo skin microdialysis.

Supernatants from anti-IgE-activated skin MCs strongly increased the expression of CD69 on eosinophils (unstimulated MCs: 3.5% vs. stimulated MC: 28.6%). Interestingly, upregulation of CD69 expression remained stable even after lowering the MC stimulation strength. Vice versa, supernatants from platelet-activating factor (PAF)-stimulated eosinophils strongly induced MC degranulation (47%). However, MC degranulation levels dose dependently decreased with lower eosinophil activation strengths. Co-culture of unstimulated MCs and eosinophils induced a strong CD69 upregulation on eosinophils (46%), which was further increased (to 78%) in the presence of previously activated MC. Thus, eosinophils seem to be more reactive towards the physical presence of MCs than towards MC supernatants. Finally, injection of eosinophils into ex vivo skin induced histamine release by MCs (no eosinophils: 54 ng/ml vs. injected eosinophils: 109 ng/ml).

Here, we show that activation of primary human blood eosinophils can be induced by human skin MCs and vice versa. This crosstalk might result in a reciprocal activation loop that contributes to sustained disease activity in CSU. Therapeutic disruption of this activation loop may be a novel approach for the treatment of CSU.

P006 | In chronic spontaneous urticaria, basopenia/eosinopenia and basophil/ eosinophil numbers in lesional skin are linked to clinical and laboratory features of type IIb autoimmunity

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Background: In chronic spontaneous urticaria (CSU), peripheral basopenia and more recently also eosinopenia had been linked to features of type IIb autoimmune CSU. Furthermore, increased numbers of basophils and eosinophils had been described in CSU lesional skin, but their link to any subtype of CSU remains unclear.

Objectives: To investigate the association of blood and lesional skin basophil and eosinophil numbers with clinical and laboratory features of type IIb autoimmune in CSU. Ten healthy subjects served as control cohort.

Methods: In ten patients with CSU, the numbers of basophils and eosinophils in the blood were assessed by automated hematology analyzers (cutoffs for reduced counts: ≤0.02109 basophils/L and ≤ 0.08 eosinophils/L) and in the skin lesions (wheals) and non-lesional skin by quantitative histomorphometry. Patients were clinically assessed for physician global assessment (PGA), disease activity (UAS), body surface area (BSA), itch and quality of life impairment (DLQI, CU-QoI). Additional laboratory blood tests included eosinophil cationic protein (ECP), total serum IgE and basophil activation test (BAT) as a marker for type IIb autoimmunity.

Results: Of the 10 CSU patients analyzed, four and three had low blood levels of basophils and eosinophils, respectively. Low basophil and eosinophil count were significantly correlated in the blood of CSU patients ($r = 0.5731$, $P = 0.0112$). Basopenia in CSU patients significantly correlated with quality of life impairment (DLQI; $r = -0.693$, $P = 0.026$), but not with the other assessed clinical features.

Basophil and eosinophil numbers were significantly increased in the lesional skin of CSU patients as compared to control skin (BB1-staining of basophils: Lesional vs healthy control skin, 6.8 1.0 vs 2.0 0.4 cells/microscopic field [MF], $P = 0.0006$; HE staining of eosinophils: Lesional vs Non-lesional skin, 3.4202.79 vs. 0.11590.08 cells/ MF, $P = 0.0085$, n = 9). In the lesional skin of CSU patients, basophil and eosinophil numbers were moderately correlated ($r = 0.6$, $P < 0.01$). Higher basophils numbers in the lesional skin of CSU patients were linked to lower total IgE in the blood ($P = 0.04$), which is a feature of type IIb autoimmunity. Elevated eosinophil numbers in the lesional skin of CSU patients were associated with high PGA (rs = 0.755, $P = 0.01$), but not with other clinical features or eosinophil markers like ECP.

Of our analyzed CSU patient cohort, 2 patients exhibited a positive BAT, who were also baso- and eosinopenic. The remaining eosinopenic and/or basopenic CSU patients exhibited minimal donor dependent activation in the BAT, whereas the CSU patients with normal eosinophil and basophil numbers had clearly negative BAT results.

Conclusions: In CSU, the reduced numbers of basophils/eosinophils in the blood are linked to clinical and laboratory features of type IIb autoimmunity, which could also lead to the cellular influx of these cells in the lesions. Ongoing analysis are aiming on further characterization of the cellular infiltrate and their link to features of type IIb autoimmunity. Future studies should investigate the role and relevance of basophils and eosinophils in this subform of CSU.

Keywords: Basophil, eosinophil, autoimmune, Chronic spontaneous urticarial

P007 | Expression of histamine receptors H2R and H4R are predominantly regulated via the IL-4/IL-13 receptor type II on human M2 macrophages

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Introduction: Histamine is found in higher concentrations in atopic dermatitis (AD) patients' skin contributing to immunomodulation and pruritus. Specific antagonists of the histamine- H4-receptor (H4R), and dupilumab, currently approved for treatment of type II inflammatory diseases, have shown remarkable reduction of pruritic symptoms and clinical AD severity scores.

We investigated the H2R and H4R expression in M2 macrophages from AD patients versus healthy controls. Further H2R and H4R expression in response to IL-4 or IL-13 and the signaling pathways of these cytokines were analysed to find checkpoints for H2R and H4R regulation in human M2 macrophages.

Methods: Monocyte derived M2 macrophages were treated with IL-4 or IL-13 and antibodies blocking receptor complex subunits of type I (IL-4) receptor and type II (IL-4/IL-13) receptor. Also inhibitors of the down-stream Janus/Tyrosine kinases (JAK/TYK) and of the activator protein 1 (AP1) were added to the IL-4- or IL-13-activated cells. Histamine receptor mRNA expressions were detected by qRT-PCR.

Results/**Discussion:** Pre-incubation of M2 macrophages with antibodies targeting the IL-4Rα subunit (dupilumab) and inhibitors of TYK2, specific for type II (IL-4/IL-13) receptor signaling, significantly inhibited the IL-4-mediated H2R as well as the IL-4- and IL-13-mediated H4R mRNA up-regulation. Inhibiting of the transcription factor activator protein1, which can synergize with STAT6 to activate transcription reduced the IL-4-induced H4R up-regulation.

Our findings may help to better understand the regulation of histamine receptors with possible relevance for AD and the related mode of action of substances interfering with type II inflammatory responses in allergic skin diseases such as AD.

P008 | The H4R is upregulated via Th2-cytokines and has immunomodulatory effects on human eosinophils from atopic dermatitis patients

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In atopic dermatitis (AD) patients, eosinophils are part of the mixed inflammatory infiltrate in the dermis and severity correlates with elevated eosinophil numbers in the blood. It has been previously reported that eosinophils express a H4R. However, limited data regarding regulation and immune modulatory functions of the H4R are reported, especially in relation to AD. In our study, we isolated human eosinophils from peripheral blood of healthy donors and patients with AD or psoriasis. We showed that the H4R mRNA on human eosinophils can be upregulated by Th2 cytokines (IL-3, IL-4 and IL-5) and that the IL-4 induced upregulation is mediated by the JAK1/2 and STAT6 pathway. Moreover eosinophils from AD patients showed a significant higher H4R basal level and a higher upregulation after IL-4 stimulation compared to eosinophils from healthy subjects and psoriasis patients. The functionality of the H4R upregulation was verified via chemotaxis assay. Additionally, by means of RNA-seq we identified various genes, which are regulated by the H4R on eosinophils. Taken together our study indicates functional expression of the H4R on eosinophils, which is pronounced in cells from AD patients and under Th2-conditions, respectively. This provides further evidence for the H4R as a putative therapeutic target in AD.

P009 | Soluble IgE receptors and their potential use as biomarker in food allergy

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Introduction: Food allergy is triggered by the interaction of an antigen with its specific immunoglobulin E (sIgE) that is loaded on the high affinity receptor for IgE (FcεRI) on mast cells. However, till today it is unknown why sIgE is not synonymous with clinical manifestation.

Recently, it has been shown that atopic patients show significantly elevated serum levels of soluble IgE receptors (sIgER) like sFcεRI,

a truncated soluble version of the alpha chain; sCD23, the soluble form of the low affinity IgE receptor; and galectin-9, found uniquely as soluble receptor. Taking into account that sIgER can interfere with IgE detection, IgE synthesis and FcεRI binding, this project aims to assess the role of sIgERs in food allergy diagnosis.

Methods: Serum samples from well characterized adults diagnosed either 1) as food allergic (n = 24) or 2) sensitized but tolerant subjects (n = 26) were analyzed for sCD23 and galectin-9 levels by ELISA and correlated with clinical and diagnostic tests (SPT, OFC), total and sIgE levels. Food diagnosis followed the national and international guidelines including skin prick test (SPT), serum sIgE, and oral food challenge or convincing history.

Results: Preliminary experiments with sera from peanut and tree nut allergic and sensitized but tolerant subjects have shown similar values of sCD23 in tolerant (92.86 38.31) and food allergic subjects (92.86 38.31). No correlation between sCD23 and total IgE (r = −0.005) as well as peanut specific IgE (r = −0.005) was found. A trend of lower galectin-9 levels in tolerant (6.301 1.759 ng/ml) compared to allergic (10.03 2.275 ng/ ml; P = 0.2066) subjects is shown. Also, galectin-9 titers do not correlate with total (r = 0.086) and peanut specific IgE (r = 0.098). However, in peanut allergic patients there is a significant negative correlation between galectin-9 titers and the wheal size after SPT with peanut extract (r = −0.725, P = 0.027), indicating a possible inhibitory function in vivo.

Discussion: sIgERs should be further explored as biomarker candidates in food allergy in larger patient cohorts. Furthermore, sIgERs might provide new insights on the differences seen between sIgE levels, skin prick test reaction size and food provocation results.

P010 | Sub-pressure assisted application of sharp hollow microneedles for improved recovery of biomarkers from skin interstitial fluid (ISF) as compared to microdialysis

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The pathomechanisms of chronic inflammatory skin diseases such as urticaria, psoriasis, or atopic dermatitis are complex and challenging to investigate. Histomorphometric analyses of skin biopsies combined with the analysis of blood and serum as well as skin microdialysis (SMD) samples are gold-standard tests in skin research. However, these tests are either invasive, samples are difficult to obtain with the risk of infection or depend on subjective assessment of clinical parameters such as wheal and flare responses, itch, and blood flow. Moreover, locally produced biomarkers in the tissue are often diluted below detection thresholds of routinely used assays in the circulation.

To improve diagnostic options and facilitate biomarker discovery in dermatology, we have developed sharp-hollow microneedle chips for skin penetration and extraction of dermal interstitial fluid (ISF). Microneedle Chips, comprising of 37 microneedles (MN) with 420 µm length, were manufactured from monocrystalline silicon wafers and cleaned by plasma treatment. All studies were performed with ex vivo human abdominal skin samples obtained from plastic surgery. Optimal skin penetration of the MNs and ISF recovery required the application of a moderate sub-pressure of -70 kPa. To investigate the recovery efficacy of molecules from ISF, various concentrations of histamine (small molecule), recombinant GFP (medium molecule) or a fluorescently labeled antibody (large molecule) were applied by intradermal injection before ISF was i) collected by MN application for 20 mins or ii) sampled by SMD for 1 h at a flow rate of 0.8 µl/min. We found that MN ISF is superior to SMD with a recovery rate between 69-99% - independent of the molecule size, as compared to 5.22-12.48% for SMD. Moreover, injection of Codeine which is the strong IgE-independent mast cell (MC) activator led to a strong increase of histamine in the ISF extracted by sub-pressure assisted MN application indicating its usefulness to detect MC degranulation in the skin.

In summary, we have developed a novel, easy to handle and minimally invasive tool to sample ISF from the skin. The method also overcomes limitations regarding molecular size and recovery efficacy that apply to other sampling methods such as SMD making it a promising tool for research and diagnosis of various skin diseases.

P011 | Modulation of allergic contact dermatitis via unfolded protein response

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Background: Allergic contact dermatitis is a sterile T cell mediated inflammatory skin disease resulting in erythema and eczema formation. While the importance of the innate immune response for both sensitization and elicitation phase becomes more apparent, knowledge regarding mechanistic events initiating the skin inflammation remains sparse. Here, we hypothesized that both direct binding of sensitizers to proteins (the so called haptenization process) as well as the generation of reactive oxygen species (ROS) as a consequence of sensitizer interaction with skin cells would lead to changes in cellular protein structures and to generation and accumulation of misfolded proteins. This might then lead to the activation of the unfolded protein response, which has been linked to the generation of proinflammatory conditions.

Objective: We aimed to analyze the impact of different sensitizers and irritants on UPR activation/inhibition and the generation of a

pro-inflammatory micromilieu in vitro and the effect of UPR modulation on the strength of CHS responses in vivo.

Methods: Analysis of UPR activation in vitro and its effect on inflammatory responses in different cell types (human and murine) by (qRT-)PCR, Western blotting and immunofluorescence. In vivo studies using specific small molecule inhibitors for UPR signaling in the CHS model.

Results: Interestingly, all three UPR branches were activated by contact sensitizers, though sensitizer specific preferences were observed. Synergistic effects were observed after combination of different weak sensitizers/addition of irritants. Blocking UPR signaling resulted in decreased NF-κB activation, cytokine production and downregulation of activation marker expression in a HaCaT/THP-1 co-culture model. Modulation of the UPR in vivo before sensitization or elicitation by systemic application of UPR inhibitors modulates the strength of CHS responses. Ex vivo treatment of skin from ER-stress reporter (ERAI) mice with sensitizers proves that the inhibitors can be used topically to prevent sensitizer induced ER stress responses. This is reflected in abrogation of CHS responses after topical pre-treatment of mice with the inhibitors.

Conclusions: Our observations highlight an important role of the UPR in determination of sensitizing potency.

P012 | Autoallergic CSU patients exhibit a distinct sensitization profile to exoallergens

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Background: Mast cell activation is the key factor in the pathophysiology of chronic spontaneous urticaria (CSU). Autoreactive IgE is thought to be one of the most common triggers of this mast cell activation and has been shown to have distinct biochemical properties. Whether or not autoreactive IgE in patients with CSU shows cross-reactivity with exoallergens is currently not known.

Methods: Sera of 127 CSU patients and 18 healthy control subjects (HCs) were tested for their IgE reactivity using Immuno Solid-Phase Allergen Chip (ISAC) based screening for 112 exo-allergens. CSU patients were grouped in 2 subgroups, patients with IgE anti-IL-24 levels above (IgE anti-IL-24 +) or below (IgE anti-IL-24-) the cutoff value, 46 and 81 patients, respectively, as assessed by ELISA. ISAC signals were analyzed by Microarray Image Analyzer and normalized to ISAC standardized units, using ≥ 0.3 as threshold.

Results: Overall, IgE reactivity was similar in IgE anti-IL-24 + and IgE anti-IL-24- CSU patients and HCs. For example, IgE-anti-IL-24 + CSU patients had a similar rate of above threshold IgE reactivity to at least one exo-allergen (50%) as compared to IgE-anti-IL-24- patients (53%) and HCs (56%). However, IgE-anti-IL-24 + CSU patients had nominally higher rates of overall IgE reactivity and/or higher average levels of reactivity to a distinct set of exoallergens, including the London plane tree pollen allergen Pla a2. IgE reactivity to Pla a2, above and below threshold, was observed in 9 of 46 (20%) IgE-anti-IL-24 + CSU patients as compared to 6 of 81 (7%) and 2 of 18 (11%) IgE-anti-IL-24- CSU patients and HCs, respectively. Also, average IgE reactivity was significantly higher in sera of IgE-anti-IL-24 + as compared to IgE anti-IL-24- patients with CSU ($P = 0.025$).

Conclusion: Our results, albeit preliminary, suggest that autoreactive IgE, in CSU, could cross-react with exoallergens. This finding calls for further studies that establish exoallergen and autoallergen IgE cross-reactivity patterns in CSU and characterize their clinical relevance.

CELLULAR BIOLOGY

P013 | Molecular biological effects of laser-assisted tattoo removal and aftercare treatment studied in a novel 3D tattoo skin model

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Tattoo and pigment removal is an essential part of everyday dermatological practice.

Picosecond pulse duration lasers have recently entered the field of dermatology but their effects on cutaneous skin biology have not been completely understood since standardized in vitro test systems were not available. Therefore, we developed a human three-dimensional full thickness skin tattoo model system by injecting tattoo ink comparable to the in vivo application procedure. The tattooing process of skin models was carried out by piercing a one way sterile Dragon Hawk tattoo 0.3 mm needle with HPLC tested black tattoo ink into the dermal equivalent of a 3D skin model. Light microscopy examination revealed large deposits of dark ink particles in the dermal part of the model. Removal of tattoo pigments was achieved by application of a picosecond pulse laser with a non-thermal, photomechanical impulse that shattered the targeted pigment (1064 nm; 750 ps, 3 mm spot size, 2.8 J/cm^2). Morphological effects of laser treatment on tattoo models were examined directly and five days after irradiation. Biological effects were investigated after five days. Gene ontology of microarray data revealed an increased cytokine production, cell motility, cellular response on chemical stimuli and stress. Real time PCR data confirmed these findings by upregulated gene expression of IL18, CXCL14, RARRES 1 and cathepsin H. Daily treatment of topically applied dexpanthenol - containing aftercare

was done over a period of five days and compared to skin models without receiving any aftercare. Effects of an improved wound healing process were figured out by histological examination. In comparison to skin models without receiving aftercare treatment with dexpanthenol containing ointments, GO analysis revealed a positive regulation of development processes and cell migration, as well as regulation of angiogenesis and anatomical structure development. Furthermore qRT-PCR analysis revealed an enhanced expression of leptin, lipoxygenase, S100A4, MMP3 and fibroblast growth factor 2 in these samples receiving post-laser treatment, whereas a downregulation of CXCL14 could be detected. In conclusion, full thickness 3D skin models can be applied to investigate the morphological and biological effects of tattoo, laser-assisted tattoo removal and aftercare treatment.

P014 | Proliferating tumour cells mimic glucose metabolism of mature human erythrocytes

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Mature human erythrocytes are dependent on anerobic glycolysis, i.e. catabolism (oxidation) of one glucose molecule to produce two ATP and two lactate molecules. The phenomenon of glycolysis is not restricted to anucleated human and mouse erythrocytes. In nucleated cells, aerobic glycolysis (also named Warburg effect) is widespread. It mediates the metabolic basis for trained immunity. Activated immune cells (e.g. macrophages, dendritic cells, TH1 and TH17 cells) take advantage of this phenomenon to meet their enormous energy requirements (ATP), feeding biosynthetic pathways (DNA synthesis etc.) and facilitating proliferation and synthesis of inflammatory cytokines. Proliferating tumor cells also take advantage of the aerobic glycolysis phenomenon and pretend that they do not have any organelles, thus mimicking organelles-free human erythrocytes to glycolytically generate 2 ATP molecules. They deliberately avoid or switch off their respiration, i.e. tricarboxylic acid (TCA) cycle and oxidative phosphorylation (OXPHOS) machinery and consequently dispense with the production of additional 36 ATP molecules from one glucose molecule. The present work deals with the fate of a glucose molecule after entering a mature human erythrocyte or a proliferating tumor cell and describes why it is useful for a proliferating tumor cell to imitate a mature erythrocyte. Blood consisting of plasma and cellular components (99% of the cells are erythrocytes) may be regarded as a mobile organ, constantly exercising a direct interaction with other organs. Therefore, the use of drugs (e.g. anti-cancer drugs), which influences the biological activity of erythrocytes, has an immediate effect on the entire organism.

P015 | Induction of psoriasis- and atopic dermatitis-like phenotypes in 3D skin equivalents

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Introduction: Atopic dermatitis (AD) and psoriasis (Ps) are prominent cutaneous disorders characterized by skin barrier defects, abnormal differentiation and inflammatory events. Although both diseases share similarities in the phenotypic outcome, the immunological source of the pathologies differs; while psoriasis results from an imbalance leading to increased Th1 and Th17 subsets of T-helper cells, AD develops upon enhanced Th2 signalling. An increasing number of people worldwide suffers from these cutaneous disorders. Hence, further understanding of these pathologies and the opportunity to test new approaches and therapeutics under in vivo-like conditions is required urgently. This study provides model systems of AD and Ps that might provide such an opportunity for therapeutic research.
Methods: 3D skin equivalents consisting of dermal fibroblasts and primary epidermal keratinocytes were stimulated with cytokine cocktails (IL-4, IL-13, TNF, IL-31 for AD; IL-6, IL-17A, IL-22, TNF, IL-1alpha for Ps induction) for 12 days during airlift cultivation. Effects on morphology and production of structural proteins were histologically analysed using HE staining and immune histochemistry (IHC) approaches. In addition, RT qPCR was performed to characterize the skin models on gene transcript levels for specific cytokines and antimicrobial peptides (AMPs) as well as structural and further disease-associated proteins. Secretion of IL-6 and IL-8 cytokines was also determined by specific ELISA.
Results: Cytokine stimulation resulted in distinct changes in levels of structural proteins relevant for the barrier function of the skin. Filaggrin and keratin-10 deficiencies, a hallmark for both skin disorders, were induced by various cytokine stimuli. Interestingly, keratin-10 defects in psoriatic skin models were only observed when the cocktail included IL-1alpha. An increased number of Ki67 positive cells in the basal layer confirmed the hyperproliferative phenotype of psoriasis. Involucrin was decreased in models of AD but was not altered in psoriatic equivalents. Structural proteins were downregulated on mRNA levels and the psoriasis models showed an elevated expression of AMP and other disease-associated genes, e.g. LCN2. As for the AD models, a potent increase in CCL26, NELL2 and CA2 transcription was observed. ELISA analysis revealed an increased secretion of IL-6 upon psoriasis induction by Th1 and TH17 cytokines whereas IL-8 release was enhanced under both disease conditions.
Conclusions: Abnormal differentiation and other phenotypic characteristics of AD and psoriasis were successfully reproduced in skin equivalents upon Th1, Th17 and Th2 cytokine stimulation. Since the established models closely mimic the in vivo changes of these cutaneous diseases, they can be considered a suitable model to analyse and understand local pathogenic effects of AD and Ps. More importantly, they might also represent a reliable tool to apply new therapeutics and investigate their efficacy in a tissue-like system.

P016 | MSCs Escape energy crisis by rerouting metabolism towards β-oxidation implications for trauma and diabetic wound healing

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The role of Mesenchymal Stem Cells (MSCs) in wound healing of healthy skin is well explored for its unique ability to adaptively respond to sensing information of the microenvironment at the wound site. It has recently been observed that following severe trauma bone marrow derived mesenchymal stem cells (BM-MSC) are released from the bone marrow to the peripheral blood, and under the surge of chemo-attractants this release undergoes a series of rises and falls in BMMSC numbers. We observed both in vitro and in vivo that after a traumatic shock (thorax trauma), the microenvironment is rather hostile for MSCs themselves with elevated ROS concentrations, pro-inflammatory cytokines and hypoxia, which in consequence, leads to a profound energy crisis. This crisis in ATP generation is due to the inability to utilize glucose as a consequence of IGF-1 resistance, peroxidation and carbonylation of vital proteins on the MSCs, most likely impeding MSCs to unfold their full adaptive potential. We here set out to first dissect mechanisms how MSCs deal with energy depletion and the fundamental oxidative attack after trauma mimicking oxidative stress, and secondly, we searched for a possibility to metabolically support the enormous energy demand of MSCs to survive and better serve tissue regeneration after trauma. We here found that Adipose-Derived Mesenchymal Stem Cells (ADMSCs) up-regulate superoxide dismutase 1 (SOD1), a superoxide anion detoxifying enzyme in the cytoplasm in conjunction with glutathione-peroxidase-1 (Gpx1), which detoxifiers the resulting increase in H2O2 concentrations in the cytoplasm. By contrast, human dermal fibroblasts up-regulate the mitochondrial superoxide dismutase (SOD2) in conjunction with the cytoplasmic catalase. These data are previously unreported and imply that ADMSCs do not generate primarily ATP by oxidative respiration. Employing seahorse analysis and in vivo lipid trafficking to investigate the underlying metabolism, we here for the first time show that, under conditions of elevated ROS concentrations, when the IGF-1/ mTOR is shut down, ADMSCs can reroute fatty acids to generate energy using β-oxidation and, thus, raise the cellular ATP levels vitally demanded for tissue repair. Of note, when supplementing the amino acid L-cysteine under trauma simulating oxidative stress conditions to ADMSC cultures to boost the Glutathione redox system (e.g. Gpx-1), the resistance to insulin, the suppressed IGF-1/mTOR pathway and ADMSC survival was fully restored with persistence of β-oxidation as the major energy source till the levels of ROS was not restored. In aggregate, we uncovered a unique mechanism of energy rerouting. This is an important process and elaborate signaling mechanisms have to be unveiled in full minutiae. However, the implication of our finding is remarkable and resembles a diabetic wound situation where increased fat accumulation and curb IGF-1/mTOR or glucose utilization constitute the primary hindrance to successful wound healing and overall patient′s prognosis. Accumulated fats could be reutilized in these energy-starved cells to detoxify the ROS and potentially to heal diabetic wounds. Our findings are unprecedented and may substantially benefit patients with diabetic wounds in clinical routine.

P017 | MSCs rescue impaired wound healing in a murine LAD1 model by adaptive responses to low TGF-β1 levels

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Mutations in the CD18 gene encoding the common β-chain of β2 integrins result in impaired wound healing in humans and mice suffering from leukocyte adhesion deficiency-1 syndrome (LAD1). Transplantation of adipose tissue-derived mesenchymal stem cells (MSCs) restores normal healing of CD18-/- wounds by restoring the decreased TGF-β1 concentrations. TGF-β1 released from MSCs leads to enhanced myofibroblast differentiation, wound contraction and vessel formation. We uncover that MSCs are equipped with a sensing mechanism for TGF-β1 concentrations at wound sites. Low TGF-β1 concentrations as occurring in CD18-/- wounds induce TGF-β1 release from MSCs, whereas high TGF-β1 concentrations suppress TGF-β1 production. This regulation depends on TGFβ receptor sensing, and is relayed to microRNA-21 (miR-21), which subsequently suppresses the translation of Smad7, the negative regulator of TGF-β1 signaling. Inactivation of TGFβ receptor, or overexpression or silencing of miR-21 or Smad7, abrogates TGF-β1 sensing, and thus prevents the adaptive MSC responses required for tissue repair.

P018 | Fascia fibroblasts swarm to drive scar formation through N-cadherin

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Scars are more severe when the subcutaneous fascia beneath the dermis is injured upon surgical or traumatic wounding. Here, we reveal the mechanism of fascia mobilization in unprecedented detail by using deep tissue intravital live imaging of acute surgical wounds, fibroblast lineage-specific transgenic mice, and skin-fascia explants (scar in a dish - SCAD). We observe that injury triggers a collective swarming of fascia fibroblasts that progressively contracts the skin and form scars. Swarming is exclusive to fascia fibroblasts, and requires the upregulation of N-cadherin. Both swarming and N-cadherin expression are absent from fibroblasts in the upper skin layers and the oral mucosa, tissues that repair wounds with minimal scar. Impeding N-cadherin binding inhibited swarming and skin contraction, and led to reduced scarring in SCADs and in animals. Fibroblast swarming and N-Cadherin thus provide novel therapeutic avenues to curtail fascia mobilization and pathological fibrotic responses across a range of medical settings.

P019 | The role of scramblases for the shedding of CD137 in epithelial cells

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Psoriasis vulgaris is an inflammatory skin disease mediated by the cells and molecules of both the innate and adaptive immune systems. CD137 (TNFRS, 4-1BB) is a member of the tumor necrosis factor receptor family and a regulator of activation and proliferation of T lymphocytes as well as other immunoregulatory cells. While CD137 cannot be detected in the skin of healthy subjects, high expression levels are found in lesional and also in non-lesional skin of psoriasis patients. The cellular sources are activated T-cells, mast cells and neutrophils.

CD137 may represent a promising target for immunotherapy of different skin diseases. Soluble CD137 is released by activated lymphocytes and the loss of cell membrane-bound CD137 could be an outcome of proteolytic shedding of metalloproteases. Recently, our group presented evidence that Anoctamin-6 (ANO6), a Ca2 + –activated scramblase frequently expressed on epithelial cells, regulates sheddase function of disintegrin-like metalloproteases (ADAMs). Scramblases are proteins located in the cell membrane, which transport phospholipids along their concentration gradient in a non-specific, bidirectional and energy-independent manner across the membrane bilayers. Scramblases are therefore capable of disrupting the asymmetrical distribution of phospholipids in the plasma membrane. Surface exposure of phosphatidylserine (PS) upregulates the sheddase activity of ADAM10 and ADAM17, the two most prominent members of the ADAM family, which control diverse cellular functions with vital importance. The previous findings led to the question whether shedding of CD137 might be regulated by scramblase-dependent ADAM activation. Thus, we set out to analyze whether scramblases might be involved in the release of soluble CD137.

Ionomycin-induced shedding of CD137 in the epithelial cell line HEK293T transfected with CD137 was inhibited by the soluble PS-headgroup phosphorylserine as well as the PS-binding protein lactadherin, indicating a link between surface-exposed PS and shedding of CD137. Following that, we speculated that ANO6 might have a regulatory effect on CD137-shedding. Preliminary data from HT29 cells, a human colorectal adenocarcinoma cell line with epithelial morphology, co-transfected with CD137 and a hyperactive mutant of ANO6 showed increased shedding of CD137 in the absence of any stimulus compared to a control vector. This suggests that ANO6 could be a mediator of CD137-shedding via its activation potential of ADAM10/17.

Since the scramblase activity of ANO5 and XKR8 is characterized to a lesser extent than for ANO6 we sought to examine whether ANO5 and XKR8 may have a similar role as ANO6 in further experiments.

In preliminary experiments, we could demonstrate that hyperactive ANO5 was also able to induce PS-dependent shedding of CD137.

In summary, these results indicate that shedding of CD137 in epithelial cells is dependent on surface-exposed PS and scramblases are involved in the activation of CD137-shedding.

P020 | ADAM10/17 as regulator of CD137 function

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Transmembrane proteins are shed and released by “A disintegrin and metalloproteinases” (ADAMs). ADAM10 and ADAM17 are the most prominent members of the ADAMs family. As ectodomain sheddases they are able to cut off transmembrane proteins. ADAM10 and ADAM17 have many important substrates e.g. TNFα, EGF or adhesion molecules like cadherins or fractalkine, and so there are involved in several inflammatory diseases like Psoriasis. It is also well known, that ADAM17 is the major sheddase of the tumor necrosis factor receptor (TNFR) family. Several members of the TNFR family function after initial T cell activation to sustain T cell responses.

CD137 is an important member of the TNFR family. It is expressed on immune cells, such as activated CD4 + and CD8 + T cells and by other immune cells including neutrophils. Cell types linked with the pathogenetic mechanisms of psoriasis.

CD137 mRNA is elevated in psoriasis lesion. The receptor is discussed to have pro- as well as anti-inflammatory effects. Receptor activation is initiated by its trimeric CD137 ligand (CD137L). Activation of CD137 promotes cell proliferation and activation. It is known that the soluble CD137 is generated by differential splicing but it was reported once that it could also be the result of proteolytic shedding of a metalloprotease, which is unknown so far.

In this study, we wanted to investigate if CD137 is shed by ADAM10 or ADAM17 and to address the functional consequences in the context of clinical relevance.

Our studies in the epithelial cell line HEK293T have shown that the release of sCD137 in the supernatant of these cells is decreased by adding metalloprotease- or ADAM10/17-inhibitors.

We also stimulated HEK293T cells with ADAM activating stimuli such as PMA, Ionomycin and Melittin. It turned out that the sCD137 level significantly increased by treating the cells with calcium-ionophore Ionomycin. Retransfecting ADAM10/ ADAM17 double knockout HEK293T cells with ADAM10 or ADAM17 also increased the shedding of CD137.

Taken all these data together it turned out that ADAM10 might be mainly responsible for the release of sCD137.

Further studies are ongoing including the analyses of patient samples which will hopefully shed more light into the regulation and function of this important molecule in the context of Psoriasis.

P021 | mTORC1 activity in psoriatic lesions is mediated by aberrant regulation through the tuberous sclerosis complex (TSC)

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In psoriasis the tightly controlled balance between cell division (proliferation) in the basal layer and ordered maturation (differentiation) is severely disturbed. Despite increasing knowledge about the underlying pathomechanisms and the development of modern therapeutics, a large group of patients still cannot be treated adequately. Specifically, comprehensive knowledge about the deregulated signaling pathways that are potentially suitable as therapeutic targets is still scarce. We previously found that aberrant activation of the Akt/mTORC1 cascade contributes to the pathogenesis of psoriasis. In healthy skin mTORC1 signaling is only active in the basal layer and contributes to the control of proliferation while preventing differentiation. When cells leave the proliferative compartment, mTORC1 signaling is switched off which promotes differentiation. However, under inflammatory conditions this switch is hijacked by inflammatory cytokines, which prevents proper differentiation. Beyond this model, it is currently unknown how mTORC1 activity is regulated to promote these effects on keratinocyte differentiation. It is known from other tissues that mTORC1 is regulated through various pathways via the tuberous sclerosis complex (TSC). This complex, consisting of TSC1, TSC2 and TBC1D7 can be phosphorylated by different kinases such as Akt, Rsk1 or Erk, which cause its dissociation from the lysosome. Thus, TSC2, the catalytic subunit of TSC2, can no longer act as a GTPase activating protein for Rheb, which remains GTP-loaded and is able to activate mTORC1 on the lysosome. To elucidate the regulation of mTORC1 in inflammatory skin diseases, we investigated whether psoriatic cytokines that are known to activate these kinases are in turn mediating inactivation of TSC2. Treating keratinocytes with TNF-alpha of IL-1beta induced robust phosphorylation of TSC2 especially on Ser939 that is mediated via the PI3-K/Akt and the MAPK pathway. Interestingly we could not detect increased phosphorylation of this site in psoriasis patients. Instead, we found that TSC2 is strongly downregulated in lesional psoriatic skin compared to non-lesional skin of the same patients or healthy skin, which is in line with previously reported RNAseq data. Thus, we hypothesize that downregulation of TSC2 contributes to hyperactivation of mTORC signaling in psoriasis. To further study this phenomenon we generated a TSC2 knock-out keratinocyte cell line by CRISPR genome editing. This cell line shows constitutive activation of mTORC1, thus resembling a psoriasis-like phenotype. Using these cells, we studied the impact of mTORC1 regulation on proliferation, differentiation and tissue formation in 3D epidermal models. Our results further confirm the importance of the mTORC1 cascade in the pathogenesis of psoriasis and suggest to further explore topical mTORC1 inhibition as an antipsoriatic strategy.

P022 | Understanding impaired clearance of senescent fibroblasts from aging skin towards therapeutical implications

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Aging is defined as the progressive loss of physiological integrity, leading to impaired tissue and organ function and an increased susceptibility for age-related diseases. Upon DNA damage by a variety of endogenous and exogenous stressors, cells of distinct histogenetic origin either undergo apoptosis, differentiation or senescence, thereby preventing that damage-mediated DNA mutations enforce malignant tumor formation. Fibroblasts preferentially undergo senescence, and we previously showed that senescent fibroblasts profoundly accumulate in the skin. Apart from being locked in a p16INK4A-mediated senescence program with irreversible growth arrest, senescent fibroblasts adopt resistance to apoptosis and a Senescence Associated Secretory Phenotype (SASP). This SASP consists of the enhanced release of a variety of bioactive molecules, among them pro-inflammatory cytokines/chemokines, matrix-degrading metalloproteinases and other proteolytic enzymes as well as the suppressed release of growth factors, altogether leading to

a severe structural and functional decline in the connective tissue of the dermis and the overlying epidermis. By their soluble nature, SASP factors spread senescence to neighboring cells in the skin and likely to other organs. This is particularly the case as the SASP released by senescent fibroblasts is persisting in aged skin. This is in contrast to the transient release of SASP factors during embryogenesis and wound healing which is beneficial for transient tissue remodeling. Under these transient conditions and in some amphibians with high regenerative potential, senescent cells are successfully removed by cells of the innate immune system such as Natural Killer (NK) cells and macrophages. We here set out to understand whether senescent fibroblasts in aging skin are resistant to the removal by innate immune cell or, alternatively, cells of the innate immune system are themselves compromised to remove senescent fibroblasts from aging skin. In a first attempt to address these questions, we have established a reliable NK cell killing assay. Remarkably, we found that the NK-92 cell line and primary NK cells preferentially kill senescent fibroblasts. These data imply that in the employed system senescent fibroblasts are not resistant to the innate immune cell clearance. Of note, although there is no change in the absolute number of NK cells in peripheral blood and skin between young (~25 years) and old (~70 years) healthy human individuals, primary NK cell isolated from old individuals (~70 years) are profoundly less efficient in killing K562 myelogenous leukemia cell line as opposed to the excellent killing ability of primary NK cell isolated from young individuals (~25 years). Furthermore, release of granzyme B, the effector molecule which induces pores in cells to be killed by NK cells at the cell synapse, is significantly reduced in NK cells isolated from old individual (~70 years) when compared to enhanced perforin concentrations in NK cells isolated from young individuals. These data provide first unprecedented indication, that possibly aging of NK cells may contribute to reduced removal of senescent cells. These data contribute to advancing our understanding of mechanisms underlying senescent cell accumulation, and may even be exploited for future senolytic therapies.

*Contributed equally.

P023 | Insulin resistant fibroblasts impair angiogenesis in diabetic conditions - Implications for novel treatment strategies

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Diabetes mellitus is an urgent global health problem imposing a significant socioeconomic burden on societies. Around 600 million people are predicted to suffer from diabetes by 2045 and, in consequence, secondary complications like non-healing wounds will profoundly increase. Diabetic wounds initiated by acute or repetitive trauma is the outcome of a complex amalgam of factors. The most important among them are vascular pathologies including micro- and macroangiopathy, and on a cellular level impaired angiogenesis and regeneration. Limited advances in understanding the underlying molecular cause and currently unsatisfactory treatment options underscore the unmet need for novel, more effective preventive and/or curative strategies. We previously observed profoundly reduced wound healing and development of insulin resistance in fibroblasts from Leptin receptor-deficient hyperphagic mice (db/db), which develop type 2 diabetes and a variety of co-morbidities due to high glucose as well as saturated free fatty acids. In fact, db/db mice as opposed to wild-type mice, showed severe reduction of CD31 positive endothelial cells indicative of new vessel formation during wound healing. In this study, we set out to further explore the impact of insulin resistant fibroblasts on angiogenesis under diabetic conditions. For this purpose, we first established an in vitro model which mimics insulin resistance employing sodium palmitate, a free fatty acid that induces insulin resistance in the fibroblasts. We found that both wild-type fibroblasts in the presence of sodium palmitate and db/db fibroblasts when co-cultured with endothelial cells resulted in significantly reduced angiogenesis as indicated by reduced endothelial tube formation. Similar results were obtained for insulin resistant human dermal fibroblasts (HDF) co-cultured with human vascular endothelial cells. To uncover mechanisms underlying the fibroblast-dependent impairment of new vessel formation, we performed unbiased transcriptome analyses using Illumina platform based RNA-Seq. Several activated and inactivated pathways in HDF were observed following induction of insulin resistance. Interestingly, pathways for cellular senescence, Senescence-Associated Secretory Phenotype (SASP) among others are activated, while pathways responsible for Insulin/IGF-1 signaling, collagen formation, extracellular matrix organization are suppressed after sodium palmitate treatment, a finding nicely correlates with connective tissue aging. Of note, cyclin dependent kinase inhibitor p21, a senescence marker, was upregulated in sodium palmitate treated HDF and in the skin of db/db mice. Also, suppression of the insulin/IGF-1 signaling as indicated by reduced phosphorylation of AKT, and subsequent suppression of collagen type I and type III deposition was observed in vitro and in db/db skin in vivo. Antibody array demonstrated that among other interesting proteins, VEGF was significantly reduced in the supernatants of insulin resistant HDF and in fibroblasts from db/db mice. Preliminary data indicate that supplementation of VEGF in the supernatants of insulin resistant HDF attenuated impaired tube formation, while incubation of endothelial cells with supernatants from insulin sensitive fibroblasts in the presence of neutralizing antibodies against VEGF impaired tube formation. These data are unprecedented and imply that unsaturated fatty acids result in insulin resistance and senescence of dermal fibroblasts which - via enforcement of an anti-angiogenic SASP - profoundly impair vessel formation. These results hold substantial promise to be translated into preventive and therapeutic approaches for patients suffering from diabetes.

P024 | The adaptive response of ABCB5 + mesenchymal stem cells differs in old from young healthy individuals upon exposure to the pathogen signal LPS

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Previously, we uncovered that by contrast to fibroblasts, mesenchymal stem cells (MSCs) are endowed with the unique capacity to raise an adaptive response to environmental cues. This allows MSCs to control their direct neighborhood and endogenous stem cell niche. We also observed that in an uninfected microenvironment in vitro and in a murine model of non-infectious immune complex vasculitis in vivo MSCs suppress the release of reactive oxygen species (ROS), neutrophil expulsed DNA traps (NET) and proteolytic granules from activated neutrophils, and thereby suppress overall tissue damage. By contrast, upon exposure of MSCs with infection mimicking lipopolysaccharide (LPS), a wall component of gram negative bacteria, MSCs completely shift their transcriptome with the release of neutrophil activating chemokines. The LPS induced transcriptomic shift resulted in a significant increase in NETs and proteolytic enzymes. This adaptive response guarantees the defense from bacterial attack. Wound healing decreases with age and the propensity for infection significantly increases in elderly individuals. Therefore, we here set out to address the question whether MSCs from young healthy donors (< 30 years; n = 6) as opposed to MSCs from old healthy donors (>60 year; n = 6) may change their adaptive response upon LPS exposure towards a reduced microbicidal response. For this purpose, we employed ABCB5 + MSCs derived from human skin, a newly reported MSC population which by the expression of the ATP binding cassette subfamily B member 5 (ABCB5) can easily be isolated by MACS as a reproducible homogeneous MSC population. We explored whether upon LPS exposure sensing via LPS binding to Toll-like receptor-4 (TLR-4), the subsequent relay of the signal to the transcription factor NFkB, its activation and translocation to the nucleus and the transcriptomic shift to a microbicidal transcriptome differs in ABCB5 + MSCs from young when compared to old donors. Employing Western blot analysis, we did not observe quantitative changes in the expression of TLR-4, the sensing receptor for LPS from young as compared to ABCB5 + MSCs from old donors. This was confirmed on mRNA level with a similar time-dependent regulation of TLR-4 for young and old donors. Interestingly, data on NFkB translocation to the nucleus (activation) of LPS primed ABCB5 + MSCs as studied by immunostaining show significant differences between LPS primed ABCB5 + MSCs from young and old donors after three hours of activation. Old donors revealed a significantly delayed back-regulation of NFkB translocation. As to target genes IL-1â and IL-6, a significant up-regulation was observed for specific mRNA levels as assessed by RT-PCR and specific ELISAs in LPS primed ABCB5 + MSCS from old as opposed to LPS primed ABCB5 + MSCs from young donors. Both cytokines - if up-regulated - have earlier been reported to be associated with aging-related diseases as delayed wound healing, osteoporosis and frailty. This implies that upon LPS stimuli ABCB5 + MSCs from old donors contribute to unrestrained inflammation. In ABCB5 + MSCs from young individuals co-cultured with neutrophils, we monitored a markedly higher NE activity indicative for microbicidal NET formation as compared to ABCB5 + MSCS from old individuals. These data imply, that ABCB5 + MSCs from old individuals cannot at the same extent raise an adaptive response towards infectious cues of their microenvironment. This finding may preclude MSCs from old individuals to refine current MSC-based therapies employing LPS primed MSCs for the treatment of infected wounds in clinical

P025 | Angiogenin secreted from ABCB5 + MSCs improves healing of diabetic wounds by promoting angiogenesis

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Diabetes mellitus is an urgent global health problem imposing a significant socioeconomic burden on societies. Around 600 million people are predicted to suffer from diabetes by 2045 and, in consequence, secondary complications like non-healing wounds will profoundly increase. With high recurrence rates, nonhealing wounds in diabetic patients are highly susceptible for necrosis due to ischemia and often progress to lower limb amputation. These patients, unfortunately, have a high mortality after amputation. Though the underlying mechanisms are not fully resolved, severe angiopathy with reduced angiogenesis is a major driver for diabetes associated non-healing wounds and secondary complications. To dissect the underlying mechanisms, we here addressed the questions whether (i) the free fatty acid sodium palmitate - whose concentration is profoundly increased in type II diabetes - directly suppress human endothelial cell (HUVEC) functions, (ii) perivascular fibroblasts grown under diabetes mimicking conditions (sodium palmitate) or derived from diabetic db/db mice suppress angiogenesis and (iii) whether injection of skin derived ABCB5 + mesenchymal stem cells (MSCs), precursors of perivascular fibroblasts, can rescue suppressed angiogenesis and impaired wound healing in a full thickness wound model in diabetic mice. Employing next generation RNA sequencing and complementary bioinformatics analysis, we found marked alterations in the global transcriptome of HUVECs following palmitate exposure. GO analysis uncovered highly enriched genes

linked to vascular complications in palmitate exposed HUVECs (hypertensive disease, kidney failure, myocardial infarction). Strikingly, RUNX2 a member of the runt family of DNA-binding transcription factors involved in endothelial cell migration were significantly suppressed in HUVEC culture following palmitate incubation. Of note, fibroblasts treated either with sodium palmitate or derived from diabetic mice - when co-cultured with healthy HUVECs - also severely suppressed angiogenesis. Blood vessel numbers were significantly reduced in db/db mice and in diabetic foot ulcers as determined by immunostaining against endothelial CD31. Interestingly, injection of ABCB5 + MSCs around wounds rescued impaired angiogenesis and wound healing in db/db mice. Secretome analysis of ABCB5 + MSCs uncovered the ribonuclease Angiogenin to be mainly responsible for this. Silencing of Angiogenin in ABCB5 + MSCs significantly reduced angiogenesis and delayed wound closure in diabetic db/ db mice implying an unprecedented key role for Angiogenin in skin regeneration. Its proangiogenic action is partly due to phosphorylation of the Vascular Endothelial Growth Factor (VEGF) receptor. In addition, our data from Western blot analysis in conjunction with data from the literature suggest that Angiogenin via activation of the suppressed Insulin-AKT signaling reversed the suppression of RUNX2 in sodium palmitate treated HUVECs. Interestingly, Angiogenin is significantly reduced in human chronic diabetic ulcers. These data highlight that the specific delivery of proangiogenic favoring molecules holds significant promise for further refining MSC-based therapies for non-healing diabetic foot ulcers and other pathologies with impaired angiogenesis.

K. Singh and P. Maity contributed equally.

P026 (OP01/01) | A novel role of redox sensitive junb in fibroblasts disrupting stem cell niche homeostasis with severe skin aging

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Tissue atrophy and organ aging results from impaired stem cell functions which gradually fail to replenish senescent cells in distinct organ parenchyma. This failure is either due to an intrinsic dysfunction of stem/progenitor cells, the disruption of the corresponding stem cell niche, or a combination of both. Fibroblasts residing in the connective tissue of all organs mainly define the stem cell niche particularly in connective tissue rich organs like skin, skeletal muscle, bones and the cardiovascular system. Though the impact of the connective tissue resident fibroblasts on stem cell niches and organ aging is an emerging concept, the underlying mechanisms are largely unresolved. We here addressed the questions (i) whether the increase of superoxide anions (O¬2o-) - known to be increased in aged skin fibroblasts - may enhance redox specific transcription factors (TF), (ii) whether these TF may install senescence programs in fibroblasts in vitro and in the skin in vivo, (iii) whether this affects different stem cell pools in the skin, and (iv) whether it correlates with the atrophy of the corresponding tissue layers and functional properties of the skin. We studied the above questions in a fibroblast specific conditional Superoxide dismutase-2 (Sod2) deficient (Sod2 cKO) murine aging model which closely mirrors intrinsic aging of fibroblasts in a time lapse mode. In this model - due to the Sod2 deficiency - O¬2o- generated in mitochondria cannot be dismutated thus leading to profound redox imbalance. Employing an unbiased approach of combining RNA-sequencing with methods to study genome wide chromatin accessibility for transcription factor binding (ATAC-seq), in silico analyses and Chromatin Immunoprecipitation (ChIP) to confirm chromatin binding sites, we here uncovered a novel redox sensitive role of transcription factor JunB, a member of the Activating Protein-1 (AP-1) family, as the common denominator for the suppression of Insulin Growth Factor-1 (IGF-1) and the concomitant induction of the cell cycle inhibitor p16INK4A in fibroblasts. Thereby, fibroblasts impair the endogenous stem cell niches and entire skin homeostasis. The skin aging phenotypes with atrophy of all skin layers, reduced skin resilience and tensile strength were partly restored when p16INK4A was inactivated or IGF-1 was supplemented from osmotic pumps implanted in fibroblast specific Sod2 cKOs. However, these aging phenotypes and, the numbers of distinct stem cells detected by immunostaining (CD34, Sox9 for epidermal and hair follicle stem cells; Nestin for mesenchymal stem cells in the dermis, and Pax7 for muscle stem cells) were completely restored in the skin of fibroblast specific Sod2; JunB double cKOs. JunB expression was also enhanced in fibroblasts of intrinsically aged murine and human skin. We here show for the first time that JunB dependent IGF-1 suppression in p16INK4A+fibroblasts fundamentally enforce skin aging likely through the disruption of several stem cell niches. Our findings hold substantial promise for novel strategies to prevent and treat fibroblast aging-related pathologies.

P027 | TFIIEß mutation in trichothiodystrophy affects rRNA synthesis and performance

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The general transcription factor II E (TFIIE) consists of two subunits, TFIIEα and TFIIEβ, and plays a central role in transcription initiation by RNA polymerase II. Mutations in the gene GTF2E2 encoding for TFIIEβ lead to the rare autosomal recessive disorder trichothiodystrophy (TTD) characterized by a variety of symptoms including brittle hair, ichthyosis, and premature aging symptoms. However, our work reveals an unexpected involvement of TFIIEβ in RNA polymerase I transcription. By using immunofluorescent and confocal microscopy we show localization of TFIIEα and TFIIEβ in the nucleolus, at the site of active rRNA production. With specific inhibition of the RNA polymerase I transcription, TFIIEβ de-localizes from the nucleolus, implying RNA polymerase I transcription-dependent localization of TFIIEβ to the nucleolus. Additionally, chromatin immunoprecipitation (ChIP) analysis reveals interaction of TFIIE to the rDNA and RNA polymerase I. Furthermore, TTD patient-derived fibroblasts with a mutation in TFIIEβ show a decreased 18S ribosomal RNA (rRNA) abundance and increased translational infidelity, indicating that defective TFIIE disturbs the rRNA synthesis and performance. Our findings provide a better understanding of the pathophysiology for the wide range of clinical phenotypes observed in TTD patients.

P028 | A novel strategy to generate patient derived mast cells from human induced pluripotent stem cells for studying mastocytosis

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Mast cells (MCs) well for their contribution to the pathogenesis of a multitude of TH2-associated inflammatory diseases such as allergies, urticaria and mastocytosis, on the other hand, there is also increasing evidence that MC have important surveillance and protective functions orchestrating immune responses. However, research on human MC-driven disorder suffers from the scarcity of patient's primary cell resources and suitable cell lines. For certain genetic MC-driven disorders such as systemic mastocytosis, 95% patients burden the Kit mutation D816V. Unfortunately, the primary mutated MCs from patients cannot be generated so far, due to the limited availability of tissue for primary MC isolation and low mutation burden in the CD34 stem cell population. HMC1.2 is the only one mast cell line burdening this mutation as in mastocytosis; however, it lacks expression of a functional FcεRI and granules.

To address this, we developed a novel strategy for the rapid and efficient differentiation of MCs from human induced pluripotent stem cells (hiPSC). This technique is the first to generate mature functional mutated MCs from mastocytosis patient. Furthermore, the morphological phenotype and functional properties of the hiPSC-MCs were compared to the human skin MCs. hiPSC-MCs showed stable expression of MC-associated receptors such as CD117, FcεRIα and MRGPRX2, and exhibited histamine release in response to stimulation with IgE/anti-IgE. In addition, for better understanding the role of KitD816V mutation, we have also established the gene edited KitD816V mutated iPSC-MCs via CRISPR/Cas9 in a parallel experiment.

hiPSCs, reprogrammed from human somatic cells, provide a unlimited cell source for research due to their self-renewal property. This strategy of hiPSC-MCs can provide sustainable, homogeneous healthy and patient's cell source for drug discovery. It is advantageous in the MC-driven diseases modeling. It will open the door for applying novel approaches to the investigation of MC-driven diseases since it allows for the generation of disease- and patient-specific MC populations.

P029 | Characterization of different immortalized keratinocyte cell lines as models for epidermal differentiation studies

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In the past, human in vitro reconstructed skin models have been widely used to study epidermal stratification and maturation and further understand molecular (patho-)mechanisms. Most of these models, however, rely on primary human donor cells. These cells not only have a limited availability and life-span that limits genetic engineering but also show donor-specific variations, which hamper the understanding of general mechanisms. The spontaneously immortalized Keratinocyte cell line HaCaT displays chromosomal aberrations and is known to differentiate in an abnormal manner. To overcome these issues we validated differently engineered immortalized cell lines created from primary human keratinocytes as model systems to study the epidermis.

Cell lines either immortalized by the expression of SV40 large T antigen and hTERT (NHEK-SV/TERT) or by lentiviral transduction with HPV E6/E7 (NHEK-E6/E7) were analyzed for their growth and differentiation behavior using 2D and 3D culture systems and compared to primary keratinocytes. Both cell lines had a prolonged life-span for up to over 50 passages until now. Proliferation analysis using WST-1 and BrdU assays showed increased growth rates when compared to primary cells, nevertheless, cells were still sensitive to contact inhibition. Interestingly, the proliferation of NHEK-SV/TERT

immortalized cells was faster than NHEK-E6/E7 and primary cells, thus being more similar to HaCaT cells. This was in line with findings from 2D differentiation assays: While NHEK-E6/E7 showed a differentiation behavior comparable to primary cells, NHEK-SV/TERT displayed a delayed onset of differentiation. In 3D epidermal models, both cell lines were able to reconstitute a stratified epidermis; however, improved morphology could only be achieved when specifically adapted and optimized culture conditions were used. Analysis of proliferation and differentiation markers like Cytokeratins 14 and 10, Involucrin and Filaggrin showed a degree of differentiation, which was similar to primary human keratinocytes. Treatment of the epidermal equivalents with Lucifer Yellow showed, that both cell lines were able to form a functional barrier.

To address whether immortalized cells do reflect all features of primary cells, we tried to separate the cells into subpopulations. Primary keratinocytes present a mix of different epidermal cell populations such as keratinocyte stem and cells committed to differentiation, which can be separated based on their capability to adhere to Collagen IV. Immortalized cells did not show distinct populations, arguing that immortalized cells are a more uniform mainly proliferative cell population that needs an exogenous stimulus to commit to differentiation.

To evaluate whether these cell lines can be used for genome editing with CRISPR/ Cas9, we targeted different genes using ribonucleoproteins and plasmid-based methods. Genome editing was measured by ICE analysis after Sanger Sequencing. Both cell lines were suitable for the generation of monoclonal cell lines, with NHEKSV/TERT showing a higher transfection efficiency simplifying the isolation of edited single cell clones.

In summary, immortalized cell lines show a higher proliferative potential and partially similar differentiation pattern when compared to primary keratinocytes. Thus, immortalized keratinocyte cell lines are suitable substitutes for primary cells and have the potential to be transformed into inflammatory disease models through genetic engineering or treatment with pro-inflammatory cytokines in order to investigate molecular pathomechanisms and test novel therapeutics during preclinical evaluation.

P030 | A comprehensive picture of phosphoproteomic changes elicited by the SCF/ KIT axis in human skin mast cells: significance of PI3K versus ERK1/2 to different cellular programs

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Stem cell factor (SCF)-induced dimerization of the central tyrosine kinase KIT activates several signaling pathways in mast cells (MC) but the significance of individual cascades to distinct functional outputs is incompletely defined. Additionally, a comprehensive portrayal of phosphosignaling events after binding of SCF to its wild-type receptor KIT is lacking. Thus, we performed label-free global phosphoproteomic profiling using a state-of-the-art mass spectrometry approach followed by extensive validation.

Of over 11,000 class I phosphosites detectable in skin-derived MCs, more than 3,400 were identified to be regulated by SCF after 8 and over 4,700 after 30 min suggesting that events were not only stable over this period, but also that early operating kinases phosphorylated additional substrates by the later point. Of the regulated sites, around 2,900 and 4,100 were up-regulated (at 8/30 min). We found 94/113 kinases and 22/24 phosphatases affected by SCF, among which where KIT itself (multiple sites), MAPK1 (ERK2) and MAPK3 (ERK1). Enrichment pattern analyses based on the Reactome and KEGG databases revealed, that besides the expected term "signaling by SCF/KIT," the MAPK/ERK cascade was the most striking module. Therefore, our follow-up studies concentrated on the impact of ERK activity on MC functional programs in comparison to PI3K, as ERK and PI3K activities intersect at various points.

To elucidate a possible impact on survival, measurements of YoPro positivity were performed upon SCF withdrawal and re-addition. Individual inhibition of either ERK1/2 (SCH772984) or PI3K (Pictilisib) had no significant impact on MC apoptosis in the short term (24 h), while joint inhibition of both kinases strongly interfered with MC survival. Addressing proliferation, we found that either ERK1/2 or PI3K inhibition diminished BrdU-positivity, accompanied by an increase in G1/G0 cells and an elevated proportion of apoptotic cells over the 5-d-period. Again, effects on proliferation were stronger under concomitant suppression of both kinases. Combined, these data indicate that general programs like survival and proliferation are controlled by both kinases with a slightly higher impact from PI3K than ERK.

In contrast, however, SCF-triggered induction of immediate early genes (IEGs) like Fos, JunB and EGR1 was completely blocked by both ERK1/2 and ERK2 inhibitors, suggesting dominance of ERK2, while PI3K was not involved.

The SCF-mediated increase in cytokine transcripts (TNF, LIF and OSM) was likewise abrogated by ERK1/2 suppression. The pleiotropic LIF was of particular interest, as it has not been studied in human MCs before. We found that MCs spontaneously secreted LIF at substantial amounts and this secretion was further elevated by SCF in an obviously ERK2-dependent manner. Similarly, ERK inhibition nearly abrogated SCF-induced OSM production. Therefore, ERK is chiefly implicated in cytokine responses elicited by SCF in skin MCs. The uniform induction of IEGs and cytokines moreover indicates that these events may be inter-connected.

In summary, we present a broad picture of the human mast cell phosphoproteome after SCF-mediated KIT activation in a physiologically relevant MC subset, identifying the central role of ERK for specific functional programs driven by SCF.

P031 | Activation of STAT3 through the mTOR and MAPK pathway contributes to the 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 psoriasis and hyperactivation of STAT3 has been found in virtually all cell types that are involved in the initiation and maintenance of the psoriatic inflammation. Phosphorylation on Tyr705 has been regarded as the essential mechanism of STAT3 regulation. 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. However, for maximal transcriptional activity, additional phosphorylation of STAT3 on Ser727 is necessary. Therefore, we investigated the degree of STAT3 activation in inflammatory skin diseases by measuring the phosphorylation of STAT3 at Tyr705 and 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.

In contrast to phosphorylation on Tyr705 the second phosphorylation event on Ser727 is more complex and can be regulated by various kinases in different tissues. In order 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.

It was previously shown, that STAT3 hyperactivation interfered with keratinocyte differentiation. Therefore, we investigated the relationship between ERK1, mTORC1 and STAT3 regarding their effect on keratinocyte differentiation. First data show, that hyperactivation of STAT3 under inflammatory conditions blocked ordered differentiation in 2D keratinocyte cultures as well as in 3D epidermal models, which was dependent on ERK1 and mTORC1 activation.

This shows a novel mechanism how inflammatory cytokines can mediate their pathological effects beyond the known activation of Janus kinases. This is also interesting since the JAK/STAT signaling pathway has recently drawn some attention by being a novel therapeutic target for oral small molecule inhibitors. Our data suggest to not only inhibit activation of STAT3 via JAKs, but to also explore therapeutic intervention through the mTOR/MAPK pathway to fully block pathological STAT3 activity.

P032 (OP05/02) | Volume-regulated anion channel LRRC8 interferes with differentiation of human keratinocytes

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The human skin is constantly challenged by external cues such as mechanical or osmotic stress. Hypotonic stimuli lead to an influx of water into the cell, which keratinocytes counteract by the process of regulatory volume decrease (RVD). We showed that the volume-regulated anion channel LRRC8 heteromer is expressed in the epidermis and contributes essentially to RVD under hypotonic stress. As the proliferative potential of keratinocytes depends on their cell volume and recent advances in other stem cell areas suggest that the differentiation capacity depends on cell volume regulation, we hypothesized that the LRRC8 ion channel controls the switch from proliferation to differentiation in the epidermis.

First indications for this function of LRRC8 were given by analysing the expression of the essential subunit namely LRRC8A by Western blotting and RNAseq in keratinocyte subpopulations, separated by their ability to adhere to Collagen IV. High expression of LRRC8A was found in keratinocyte stem cells, while transient amplifying cells showed even stronger expression that fades when cells initiate differentiation. Immunostaining of healthy epidermis supported these findings, with LRRC8A being preferentially expressed in basal cells, while hardly any expression could be detected in suprabasal layers. Additionally, stimulation of primary keratinocytes by Ca^2+ switch in vitro, indicates a bell-shaped expression of LRRC8A over the time course of differentiation.

To further investigate the role of the LRRC8 ion channel during epidermal maturation, we established different approaches to manipulate LRRC8A. First, a siRNA-mediated knockdown of LRRC8A was optimized for 2D and 3D differentiation assays in primary human keratinocytes. In addition, we generated monoclonal LRRC8A-knockout cell lines from HaCaT cells and immortalized primary keratinocytes using CRISPR/ Cas9 and analyzed their proliferation and differentiation behaviour as well as the consequences on electrophysiological properties. Manual patch clamping showed that LRRC8A is essential for mediating swelling-induced anion secretion. Moreover, BrdU, WST-1 as well as clonogenic growth assays indicated a proliferation deficit in LRRC8A-/- cells. In the absence of LRRC8A ordered epidermal maturation was highly disturbed, as shown by aberrant expression of differentiation markers in 3D reconstituted skin.

Taken together, our findings suggest that LRRC8A contributes to the regulation of epidermal differentiation and maturation, by controlling the switch from proliferation to differentiation. Thus, controlling the expression and function of LRRC8 might be a possibility to restore epidermal homeostasis under conditions when keratinocyte differentiation is disturbed such as in inflammatory skin diseases.

Our wild-type and LRRC8A-knockout keratinocyte cell lines, not only allow us to analyse the role of LRRC8A in keratinocyte differentiation in detail but also offer a possibility to identify and evaluate small-molecule compounds that specifically target LRRC8A for cosmetic or medical skin care.

P033 | A psoriasis-like cytokine milieu modulates mechanotransduction and cell adhesion in human epidermis

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Aberrant mechanotransduction, a process of a biological system sensing and responding to mechanical signals, and disrupted epithelial barrier function are associated with numerous human pathologies including inflammatory skin disorders. However, the involved molecular mechanisms are not well understood. Here, we aimed to explore the changes in cell-cell adhesion, mechanosensitive effector molecules and mechanosignaling induced in epidermis during inflammation using psoriasis as a model disease.

Normal immortalized human keratinocytes N/TERT and primary human keratinocytes were stimulated with a specific cytokine cocktail (IL-17A, IL-22, Oncostatin M, TNFa, IL-1a) that recapitulates features of psoriatic epidermis in vitro. Upon the treatment, cultured cells exhibited disrupted adherens junctions and showed increased intercellular gaps and a moderate decrease in E-cadherin expression. However, other markers for epithelial-mesenchymal transition, such as N-cadherin, vimentin, Snail and Slug remained unchanged at either mRNA or protein level. Similar phenotype was observed in keratinocytes of lesional psoriatic skin by immunohistochemical analysis of patient biopsies.

Notably, we observed a consistent decrease in beta-catenin localization to cell junctions and in protein but not mRNA expression in both 2D cultures and 3D recapitulated human epidermis models after stimulation with M5 or individual inflammatory cytokines, and in lesional skin of psoriasis patients. No nuclear beta-catenin localization was found, pointing to inactive Wnt-signaling. Moreover, M5 cytokines enhanced T18/S19-phosphorylated myosin light chain (MLC) and induced nuclear translocation of a major mechanotransduction regulator YAP in cultured keratinocytes. To test if the YAP signaling could be at least partially induced by elevated myosin-dependent intracellular tension, we cultured cells on very soft 0.5 kPa gels, on which both MLC phosphorylation and YAP signalling are normally inhibited. However, M5 stimulation was able to increase nuclear YAP even in these conditions. Moreover, treatment of M5-stimulated keratinocytes with Y-27632, a small molecule inhibitor of Rho-kinase (both ROCK1 and 2) that phosphorylate MLC, further aggravated the integrity of cell adhesions and surprisingly induced even stronger nuclear translocation of YAP.

In conclusion, we demonstrated that a psoriasis-like cytokine milieu induces decrease in beta-catenin and cell-cell adhesion integrity but does not cause epithelial-mesenchymal transition in epidermis. Our data also indicate that YAP mechanosignaling in keratinocytes can be induced by inflammatory cytokines independently of the substrate stiffness or MLC phosphorylation.

P034 | Deciphering essential gene regulatory networks of cytokine-induced senescence

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Senescence establishes a tripartite cellular phenotype displaying permanent growth arrest, resistance to apoptosis and high secretory activity. The senescence-associated secretory phenotype (SASP) is characterized by the expression and secretion of several chemokines, interleukins and growth factors. Nevertheless, cellular senescence is considered an important antitumor mechanism which counteracts accelerated proliferation during tumor development. A breakthrough discovery demonstrated in benign human naevi that these pre-malignant cells are driven into senescence, and thus are permanently growth-arrested. Senescence can be induced by internal stressors, e.g. by oncogene overexpression, as in the case of melanocytes, DNA damage or anticancer drugs. In addition, external stimulation through cytokines may also lead to permanent growth arrest of different types of cancer cells, including melanoma cell lines. Although cytokine-induced senescence (CIS) has been repeatedly reported in the literature, the signaling networks leading to the senescent phenotype remained enigmatic. Here, we used two established models of CIS to decipher its fundamental signaling pathways and regulatory gene networks: (i) in vivo senescence induction of murine beta-cell tumors by adoptive transfer of T helper 1 cells into RIP-Tag2 mice and (ii) in vitro senescence induction of human A204 cancer cells by treatment with interferon (IFN)-gamma and tumor necrosis factor (TNF). Comparative transcriptomics demonstrated the persistent activation of IFN-gamma stimulated Janus kinase (JAK)/signal transducer and activator of transcription (STAT) and TNF-induced Nuclear Factor (NF) kappaB signaling pathways for up to 96 h leading to downregulation of cell cycle genes, upregulation of secretory factors, e.g. chemokines, and, surprisingly, activation of apoptosis-related genes. Yet, triggering of the senescence pathways

also led to concomitant induction of antiapoptotic factors at all levels of the apoptosis machinery thereby maintaining the survival of the cells. Western blot analyses confirmed the long-term STAT1 and NF-kappa-B activation for up to 96 h, and further showed that the surviving, growth-arrested cancer cells did not die by apoptosis. Thus, CIS is an external control mechanism of the immune system which continuously stimulates IFN-gamma and TNF signaling cascades to restrict the growth of tumor cells that are not destroyed by direct apoptosis induction.

P035 | Generation of the pTRE-fzd7 + K5tTA mouse model for studies on keratinocyte stem cell homeostasis

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Epidermal skin homeostasis relies on high cell turnover. The epidermis is composed of short-lived cells that require constant replenishment by keratinocyte stem cells (KSCs) and the corresponding signals from the dermal niche beneath. The inability of KSCs to maintain the homeostasis represents a major cause of skin aging with largely unknown underlying mechanisms. Previously, we have shown that members of the morphogenic Wnt family, amongst them Wnt receptor frizzled 7 (fzd7) is downregulated in aged KSCs, while high fzd7 expression regulates significantly the number and morphology of KSCs. Lentiviral transduction of old KSCs with fzd7, remarkably induces the expression of stem cell markers as detected by FACS, implying that induced fzd7 expression at least in part may rejuvenate old epidermal stem cells. In order to further elucidate the role of fzd7 expression in stem cell homeostasis, we established a pTRE-fzd7 + K5tTA mouse model for functional analysis. These mice are characterized by high expression of fzd7 in the basal stem cell layer of the epidermis. Interestingly, pTRE-fzd7 + K5tTA mice are characterized by a significant higher amount of hair follicles in the epidermis in comparison to the wild-type mice. These results support our previous findings that fzd7 expression has an impact on stem cell homeostasis. Downregulation of fzd7 as occurring with age most likely contributes to epidermal aging and hair loss due to reduced self-renewal of epidermal stem cells.

P036 | Distinctive responses of microvascular endothelial cells in skin fibrosis

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Microvascular damage is one of the earliest symptoms of fibrotic tissue remodeling in systemic sclerosis (SSc) primarily leading to reduced blood circulation and tissue hypoxia. Intriguingly, endothelial cells of SSc patients may acquire mesenchymal traits thus resembling effector cells of fibrosis, contractile myofibroblasts. However, how endothelial cells contribute to early and later stages of fibrotic skin remodeling and how they specifically interact with adjacent cell types such as dermal fibroblasts remains incompletely understood.
By analysis of central fibrotic and vascular markers as well as transcriptional pathways, we analyzed how endothelial cells are affected by dermal fibroblasts in homeostasis and early fibrosis. Therefore, we studied human microvascular endothelial cells in direct and indirect co-culture approaches together with native and short-term fibrotically activated dermal fibroblasts.
In both direct and indirect co-culturing approaches, we could show endothelial cell activation following interaction with fibrotically induced dermal fibroblasts. In more detail, gene expression analysis revealed induction of specific fibrotic/mesenchymal markers and minor changes in vascular marker expression. Furthermore, endothelial cell phenotype and Wnt/ß-catenin transcription factor signaling showed alterations after exposure to fibrotically activated fibroblasts. However, endothelial cell viability remained unchanged. Intriguingly, dermal microvascular endothelial cells directly triggered with fibrotic stimuli TGF-β1 or endothelin-1 showed partially resembling activation patterns. Now we are interested in later stages of endothelial cell interaction with fibrotic cells and tissue, in vitro and in vivo. Together, we revealed endothelial cell changes following cellular crosstalk with short-term fibrotically activated dermal fibroblasts with implications for improved understanding of fibrotic tissue remodeling.

P037 | A20 promotes ripoptosome formation and TNF-induced apoptosis via cIAPs regulation and NIK stabilization in keratinocytes

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The ubiquitin-editing protein A20 (TNFAIP3) is a known key player in the regulation of immune responses in many organs. Genome-wide associated studies (GWASs) have linked A20 with a number of inflammatory and autoimmune disorders, including psoriasis. Here, we identified a previously unrecognized role of A20 as a proapoptotic factor in TNF-induced cell death in keratinocytes. This function of A20 is mediated via the NF-κB-dependent alteration of cIAP1/2 expression. The changes in cIAP1/2 protein levels promote NIK stabilization and subsequent activation of noncanonical NF-κB signaling. Upregulation of TRAF1 expression triggered by the noncanonical NF-κB signaling further enhances the NIK stabilization in an autocrine manner. Finally, stabilized NIK promotes the formation of the ripoptosome and the execution of cell death. Thus, our data demonstrate that A20 controls the execution of TNF-induced cell death on multiple levels in keratinocytes. This signaling mechanism might have important implications for the development of new therapeutic strategies for the treatment of A20-associated skin diseases.

CHEMOKINES/CYTOKINES

P038 | Imiquimod-induced psoriasis-like dermatitis in mice depends on IL-17 signaling of keratinocytes

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Immunopathogenesis of psoriasis as well of its Imiquimod-induced animal model strongly depends on the Th17/IL-23 axis. Blocking of either the cytokine IL-17A or its receptor IL-17RA by monoclonal antibodies is highly beneficial in the treatment of psoriatic patients. Although the high efficacy of this treatment is generally acknowledged, it is not completely clear which cell type(s) need to respond to IL-17 signaling during disease development. We made use of full-body as well of cell type specific IL-17RA deficient mice to delineate which cell type exactly needs to sense IL-17 in order to develop IMQ-induced dermatitis. Using this approach, we found that the response to IL-17 by T cells, γδ T cells, neutrophils and macrophages is dispensable whereas the keratinocyte response is crucial for full blown disease development. Animals deficient for IL-17RA conditionally on keratinocytes developed strongly reduced disease parameters like erythema formation, scaling and skin thickening compared to controls and the residual disease scores were similar to the scores observed in the full-body deficient mice. The massively reduced clinical scores were concomitant with significantly reduced infiltration of neutrophils to the IMQ-treated psoriatic lesions in the skin.

P039 | Apremilast effectively inhibits TNFalpha-induced pro-atherogenic responses in human endothelial cells

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Epidemiologic studies revealed that patients with chronic inflammatory diseases (e.g. Psoriasis and rheumatoid arthritis) are at increased risk of atherosclerosis and cardio vascular diseases (CVD).
In this study, we found that the phosphodiesterase 4 (PDE4)-inhibitor apremilast, a well-established anti-psoriatic drug, effectively inhibited pro-atherogenic responses in TNFα-activated human umbilical vein endothelial cells (HUVEC).
We demonstrated, that apremilast suppressed the expression of important proatherogenic factors, including granulocyte-macrophage colony-stimulating factor (GM-CSF), interferon gamma-induced protein 10 (IP-10), monocyte chemoattractant protein-1 (MCP-1), vascular cell adhesion molecule 1 (VCAM-1) and E-Selectin.
Moreover, apremilast reduced the adhesion of human monocytes (THP-1 cells) to HUVECs and transendothelial migration (TEM) in response to TNFα. Mechanistically, we found that apremilast suppressed the activation of the nuclear factor κB (NF-κB) and mitogen activated protein kinases (MAPK) signalling pathways. Inhibition of MAPK and NF-κB differentially regulated the expression of GM-CSF, VCAM-1 and E-Selectin.
Finally, we found that apremilast inhibited the secretion of matrix metalloproteinase-9, a gelatinase that is involved in atherosclerotic plaque progression, in TNFα-activated THP-1 cells.
In conclusion, we demonstrated that apremilast has distinct anti-atherogenic effects in HUVECs, indicating that apremilast could have the therapeutic potential to prevent the higher risk for CVD in patients with psoriasis.

P040 | Differential involvement of IL-31/IL-31RA signaling in autoimmune skin diseases

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Interleukin-31 (IL-31) plays a prominent role in chronic pruritic skin disorders, such as atopic dermatitis (AD). Alongside its pruritogenic role, induction of proinflammatory cytokines, regulation of immune responses and cellular differentiation are recently elucidated effects of IL-31. Indeed, a novel biologic agent targeting IL-31 receptor showed promising results in the treatment of AD (Ruzicka et al., 2019 NEJM). There are, however, limited data available regarding the role of IL-31 in various autoimmune skin diseases. Despite the prominent pruritus in bullous pemphigoid (BP) and dermatomyositis (DM), it is still unknown how the IL-31 signaling is regulated in the pruritic skin lesions.

In situ expression of IL-31 and IL-31 receptor α (IL-31RA) was assessed by immunohistochemical staining for IL-31 and IL-31RA in skin samples of patients with autoimmune skin diseases including patients with BP (n = 10), pemphigus vulgaris (PV, n = 10), lupus erythematosus (LE, n = 10), DM (n = 8), systemic sclerosis (SSc, n = 4), lichen planus (LP, n = 10), psoriasis vulgaris (PsV, n = 6) patients and healthy skin sections (n = 14). The IL-31RA-expressing area in epidermis and peripheral nerves was analyzed using Image J software and the percentage of dermal infiltrating cells immunoreactive for IL-31 and IL-31RA was manually quantified. In addition, serum levels of IL-31 were measured by ELISA in patients with BP (n = 13), PV (n = 12), LE (n = 10), DM (n = 12), SSc (n = 6), LP (n = 5), PsV (n = 6) patients and healthy controls (n = 5).

Quantitative analyses revealed that the expression of IL-31RA in epidermis was significantly increased in BP lesion (median 34.9%) compared to PV (16.7%) (P = 0.0496) and healthy control (2.7%) (P = 0.0007). IL-31 + or IL-31RA+ cells in dermal inflammatory infiltrates were also significantly higher in BP (IL-31; 22.2%, IL-31RA; 31.5%) than in PV (IL-31; 6.3%, P = 0.0291, IL-31RA; 10.4%, P = 0.0215) and healthy control (IL-31; 2.9%, P = 0.0030, IL-31RA; 2.1%, P < 0.0001). Moreover, peripheral nerves in BP lesions presented significant higher IL-31RA expression (38.9%) compared to PV lesions (14.1%)(P = 0.0381). The difference was insignificant between BP and PV patients in serum IL-31 levels.

PsV lesion showed a significant augmented expression of IL-31RA in epidermis (mean 54.0%, P = 0.0242) and that of IL-31 (43.3%, P = 0.0422) and IL-31RA (56.5%, P < 0.0001) in dermal infiltrates compared with healthy control lesion. IL-31RA expression in the infiltrate was also enhanced in DM (38.4%, P = 0.0073), LP (37.0%, P = 0.0073) and LE (32.4%, P = 0.0337) than in healthy control. The serum levels of IL-31 was higher in PsV compared to SSc and LE patients.

Our results demonstrated IL-31 was differentially associated with autoimmune skin diseases. We found significantly increased levels of IL-31 and IL-31RA in BP skin lesion including peripheral nerves compared to PV lesion, suggesting the relevant contribution of IL-31 and its receptor to the difference between BP and PV in the induction of pruritus. Furthermore, the strong immunoreactivities in PsV, DM, LP and LE implied IL-31 could have a broader function as a proinflammatory and immunomodulatory cytokine, leading to the maintenance and amplification of ongoing inflammation. These data might suggest a new therapeutic potential targeting IL-31 pathway in autoimmune skin diseases.

CLINICAL RESEARCH

P041 | Tocilizumab treatment in patients with Schnitzler syndrome: An open-label proof-of-concept study

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Background: Schnitzler syndrome (SchS) is a rare acquired autoinflammatory disease characterized by urticarial rash and monoclonal gammopathy. Previously, IL-1-related cytokines such as IL-1ß and IL-6 were shown to be associated with skin and systemic inflammation in SchS. Also, IL-1 blockers demonstrated to be effective in SchS. Based on the lack of any approved treatment option, this study aimed at investigating the effects of tocilizumab, an anti-IL-6-receptor antagonist, on the signs and symptoms of SchS.

Methods: We performed a phase II, single-center, investigator-initiated, single-arm open-label study in patients with active SchS. The study comprised a 20-week open-label treatment phase followed by an optional study extension for patients with complete or partial treatment response for another 32 weeks. Patients received weekly tocilizumab 162 mg s.c. injections for a total of 20 or up to 52 weeks. Efficacy was assessed by changes in disease activity (total physician global assessment [PGA], 0-20), inflammatory markers (C-reactive protein [CRP], serum amyloid A [SAA], erythrocyte sedimentation rate [ESR], S100A8/9) and quality of life (DLQI, SF-36). Safety assessment comprised adverse event reporting and routine clinical and laboratory assessments. Statistical analyses include descriptive measures.

Results: Eight patients were included in the study. Patients experienced lower mean [SD] total PGA scores at week 20 (3.3 [3.6]) compared to baseline (12.1 [2.5], P < 0.001, change -8.7 [3.2]). Inflammatory marker levels were normalized at week 20 (CRP 0.6 mg/l [1.0]; ESR 12.6 mm/h [13.1]; SAA 4.1 mg/l [3.5]) compared

to baseline levels (CRP 31.7 mg/l [54.1]; ESR 37.6 mm/h [29.3]; SAA 176 mg/l [260.2]). DLQI and SF-36 scores did not change relevantly. Six patients with no or minimal overall autoinflammatory disease activity at week 20 participated in the optional study extension. Four patients discontinued the study (1 after 16 weeks, 1 after 20 weeks, 2 after 36 weeks) due to insufficient treatment response/loss of efficacy and/or occurrence of adverse events. In cases of decreased efficacy (n = 3), inflammation markers remained normal; however, total PGA scores increased as shown by elevated scores for urticarial rash, fatigue, arthralgia and myalgia. The most common adverse events were infections including skin, respiratory and urinary tract infections. No severe adverse events were observed.

Conclusion: In this pilot exploratory open-label study, tocilizumab treatment initially decreased clinical symptoms and inflammatory markers in patients with SchS. However, most of the patients showed loss of efficacy with an increase in clinical symptoms over long-term use.

P042 | Anatomical area-dependent wound healing duration in hidradenitis suppurativa after surgery

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Background: Estimated time until complete wound closure is a crucial factor for planning a surgical procedure. This holds especially true for hidradenitis suppurativa (HS), an inflammatory, debilitating disease for that wide local excision of the affected area with secondary wound healing is considered the treatment of choice for the inactive scarring form or after adequate anti-inflammatory medical treatment.

Objectives: In this study, we aimed to assess the duration of complete secondary wound closure after surgical intervention for HS.

Material and **Methods:** 23 surgical procedures in 17 consecutive patients (8 female, 9 male) were evaluated for duration of secondary wound healing at axillary or anogenital/inguinal sites. To investigate the contribution of hair follicle bulge progenitor cells to wound re-epithelialization, tissue samples of lesional and perilesional skin were analyzed for expression of the stem cell marker cytokeratin 15 (CK15).

Results: Initial wound size did not differ significantly between surgical wounds in the axillary (mean 30.0 cm2 5.4) and anogenital/inguinal (mean 35.3 cm2 5.7) region. However, healing time to complete wound closure was almost twice as fast in the anogenital/inguinal (mean 132 days 10.4) as compared to the axilla area (mean 254 days 39.1, $P < 0.01$). The accelerated wound healing in the anogenital/inguinal region was accompanied by a significantly enhanced expression of CK15 as compared to the axillary wounds ($P < 0.05$).

Conclusion: The anogenital/inguinal region showed a significantly faster secondary wound healing after surgical intervention for HS than axillary wounds. We suspect differences in pilosebaceous unit density and thus hair follicle progenitor cells (as mirrored by CK15 expression) to be the main driver behind this finding.

P043 | Raster-scan optoacoustic mesoscopy for objective and non-invasive evaluation of disease severity and therapy monitoring in atopic dermatitis

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Atopic Dermatitis (AD) is a widespread inflammatory skin disease, whose severity is usually assessed by subjective clinical scores such as Eczema Area Severity Index (EASI) and SCORAD (Scoring atopic dermatitis). Though highly effective treatments are available, objective assessment of disease severity and therapeutic response is still not established. Moreover, tools to diagnose AD without an invasive procedure such as a biopsy and to monitor the exactly same region of the skin several times are missing.

Raster-scan optoacoustic mesoscopy (RSOM) combines pulsed laser emission and detection of ultrasound waves generated by heat induced expansion of biomolecules and thereby achieves high optical contrast imaging and deep tissue examination. RSOM imaging is non-invasive, visualises the distribution of the optical absorbers within the epidermis and dermis and allows an objective characterisation of epidermal integrity and thickness, pigmented structures and vascular architecture. Previous studies proved that images obtained by RSOM correlate well with histology.

In this study, we aimed at quantifying AD severity using RSOM measurements and assessing response to different therapies consecutively. 14 patients were monitored during conventional in-patient treatment undergoing topical anti-inflammatory therapy (by means of topical steroids) and simultaneous phototherapy (311 nm NB-UVB or 340-400 nm UVA), while 6 patients were monitored during Dupilumab therapy (an anti-IL-4- and -IL-13 antibody). The first measurement was performed before the start of therapy (day 0), and measurements were taken every second day during inpatient stay. Moreover, follow-up measurements were performed at week4, 8, 12 and 16. The results of 145 longitudinal measurements of a total of 20 patients with atopic dermatitis were correlated to their clinical scores and we compared the images to 72 images of 10 healthy controls.

We found that in RSOM images, AD lesions exhibit a thicker epidermis, elongated and widened capillary loops and an increased mean vessel diameter in the dermis compared to healthy skin. During treatment, a notable decrease in epidermal thickness, hyper-vascularisation and mean vessel diameter could be detected. Of note,

RSOM detected subclinical changes in the skin and is thereby superior to conventional disease scoring tools such as SCORAD or EASI. Our study demonstrates that AD disease severity can be assessed objectively by means of RSOM. Repeated measurements of over time are useful to monitor therapeutic response. Hence non-invasive skin characterization by RSOM is a useful tool in the management of AD patients.

P044 | Investigation of cold atmospheric pressure plasma and small molecules as innovative therapies for the treatment of squamous cell carcinomas of the skin in vitro and in vivo

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The consortium ONKOTHER-H aims at establishing a translational development platform for novel cancer therapies. Innovative treatment with cold atmospheric pressure plasma (CAP) and new pharmacologically relevant small molecules (indole derivatives) as well as the combination of both are evaluated in vitro and in vivo using cutaneous melanoma and skin squamous cell carcinoma (SCC) as model tumors. As part of the joint project, this subproject focuses on the effect of novel therapies on genotoxicity and DNA repair in squamous cell carcinoma of the skin. Comparison of different cell culture media revealed significant differences in the detection of reactive oxygen species (ROS) and hence comparable culture conditions have been defined for all cell lines and experiments. Assessment of inhibitory doses of CAP and different small molecules at which 50 percent of cells die revealed an increased sensitivity of SCC cells compared to non-malignant keratinocytes. Quantification of ROS, using the oxidation of H2DCFDA as an indicator, confirmed an increased level of ROS after CAP treatment, while treatment with small molecules slightly reduced ROS-level. Combined treatment of CAP and small molecules also showed slightly reduced ROS-level compared to CAP alone. Besides reactive species, CAP also consist of small amounts of UV radiation. However, even after prolonged and toxic treatment with CAP, no UV-induced DNA lesions such as cyclobutane pyrimidine dimers (CPDs) or pyrimidine-pyrimidone (6-4) photoproducts (6-4PPs) have been observed by immunofluorescence staining. Oxidative DNA lesions (8-oxo-G), DNA crosslinks (anti-Cisplatin modified DNA CP9/19) as well as DNA double-strand breaks (gamma-H2AX detection) were quantified using specific antibodies and revealed a lack of genotoxic actions for the above mentioned therapies. Furthermore, the DNA repair capacity of the cells was assessed using a host cell reactivation assay and compared to the cellular toxicity of the respective treatment. A plasmid shuttle vector assay will be used to quantify the mutagenicity and toxicity of the treatments. It is expected that results from this subproject together with results from other subprojects will bring about novel cancer therapies and establish a development platform for other innovative anti-cancer therapies.

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P045 | Establishing CD154 as a novel activation marker for detection of autoreactive T cells in pemphigus vulgaris

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Analysis of T lymphocyte proliferative responses to antigenic or mitogenic stimuli is a vital parameter used in diagnosis of various immuno-deficiencies and monitoring a variety of immune responses. Most commonly applied techniques are based on the incorporation of tritiated thymidine (3H-TdR) or ELISPOT (enzyme-linked immunospot) analysis. Both rely on rather long ex-vivo expansion and stimulation protocols, and additionally contain inherent drawbacks such as the inability to distinguish specific cell populations (3H-TdR, ELISPOT) as well as the assumption of importance of certain cytokines (ELISPOT). Quick identification and quantification of very low antigen-specific T cell numbers, however, presents a problem in diseases such as pemphigus vulgaris (PV).

In this study, we aimed at investigating the rapid expression of intracellular CD154 as a marker for antigen-specific CD4 + T cells in PV patients. For establishing the CD154 proliferation assay we compared PBMCs (fresh and frozen) of PV patients and healthy controls (HC). Cells were left untreated for 24 hrs under cell culture conditions followed by an ex-vivo polyclonal or antigen-specific stimulation for 14 hrs including the addition of brefeldin A, a known blocker of inhibitor of Golgi-dependent protein secretion. Polyclonal T cell stimulation using phytohemagglutinin (PHA) resulted in a significantly higher CD154 expression in PBMCs of HC compared to PV ($P < 0.01$) pointing at a general T cell exhaustion caused by treatment or disease state. Upon stimulation with human desmoglein 3 (Dsg3), the major autoantigen in PV, the expression of CD154 has significantly increased on CD4 + T cells from PV patients compared to HC ($P < 0.001$). Using the noncollagenous (NC1) domain of collagen VII as stimulation control did not result in an increased expression in both groups. Furthermore, patients with active disease showed Th/Tfh cell subset-specific differences in expression of CD154 in cell subset with increased numbers of Dsg3-specific Tfh17 ($CXCR5^+ CXCR3^- CCR6^+$) and Th17 ($CXCR5^- CXCR3^- CCR6^+$) cells in comparison to remittent PV patients and HC. In contrast, expression of CD154 in the other CXCR5- (Th1, Th2, Th17.1) or

CXCR5 + (Tfh1, Tfh2, Tfh17.1) T cell subsets remained largely unaffected suggesting a predominant involvement of Th17 and Tfh17 cells in acute stages of PV.

Correlation of activated CD154 + T cells with a set of intracellular cytokines (i.e. IL-4, IL-17, Il-21 and IFNã) showed a significant increase of IL-21, a known inducer of immunoglobulin production and inhibitor of T regulatory cells. In contrast other cytokines were not found to be differently expressed in CD154 + T cells after antigen-specific ex-vivo stimulation.

In summary, we here show with a novel ex-vivo assay that expression of CD154 of PBMC from PV patients is linked to systemic activation which further support the concept that antigen-specific T cells are critical in the pathogenesis of PV.

P046 | Optical coherence tomography for monitoring therapeutic effects in plaque psoriasis and atopic dermatitis

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Optical coherence tomography (OCT) is a non-invasive imaging modality with a resolution of up to 100 times higher than that of ultrasound imaging. Threedimensional OCT images can be used to augment the conventional dermatological assessment of chronic inflammatory skin diseases, such as psoriasis and atopic dermatitis. We anticipate that OCT-based inflammation markers could allow the prediction of early treatment response or failure. The study is designed as a prospective monocentric clinical observational study. We aim to enroll 200 patients within five years. Patients are eligible if they are diagnosed with moderate to severe plaque psoriasis or atopic dermatitis and if they will undergo a systemic therapy. Patients with psoriasis are treated with a licensed drug, such as a biologic, small molecule, methotrexate or dimethyl fumarate. Patients with atopic dermatitis are either treated with dupilumab or ciclosporin. After the start of systemic treatment, patients will be scanned using OCT for evaluation of treatment efficacy at baseline and follow-ups after one, three, six and twelve months. A representative psoriasis or atopic dermatitis skin lesion and a perilesional control site will be selected and scanned at each visit. The measurements will be performed with the use of a clinically approved OCT system with angiography. OCT enables non-invasive, high-resolution imaging by detecting backscattered or backreflected infrared laser light from the tissue. Our OCT system can generate cross-sectional images of up to 2 mm in depth and en-face images of 6 mm x 6 mm.

By OCT, we detected typical histological features for psoriasis such as subcorneal Munro microabscesses, hyperkeratosis and parakeratosis. Psoriasis plaques larger than 1.5 mm in thickness caused a strong signal attenuation. Capillary loops were elongated perpendicular to the skin surface. Therefore, higher blood flow in the rete ridges was detected at about 200 micrometer tissue depth compared to the control site. Skin with atopic dermatitis exhibited an increased epidermal thickness due to spongiosis. In the dermis dilated vessels can be observed. The dermal-epidermal junction was less defined due to inflammation and edema. Horizontal scans showed typical vascular patterns such as “dotted” vessels in psoriatic skin and “comma-like” vessels in atopic dermatitis. In addition, vessel diameter and density were increased as a result of inflammatory processes. After beginning of systemic treatment, a reduction in epidermal thickness and an improvement or normalization of the microvasculature were observed.

Clinical scores such as psoriasis or eczema area and severity index (PASI or EASI) for grading the severity and extent of psoriasis and atopic dermatitis are observer-dependent. OCT as a diagnostic instrument could enable objective and quantitative scoring and monitoring of the severity of psoriasis and atopic dermatitis. Since OCT is a non-invasive imaging modality, lesions of interest could be monitored without the need for biopsies. Our results indicate that imaging markers could be epidermal thickness, skin structure and vessel features (diameter, density, location). In addition, the intensity curve as a function of tissue depth may also be used for further signal analysis (intensity peaks, dermal attenuation coefficient). We will aim to develop an OCT-based inflammation skin score that could contribute to individualized therapy.

P047 | Assessment of cold atmospheric pressure plasma as innovative therapy for treatment of radiation dermatitis using a mouse model

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A substantial number of cancer patients receiving radiotherapy develop radiation dermatitis caused by radiation damage of skin tissue leading to erythema, edema, moist desquamation, and ulceration. Radiation dermatitis usually is accompanied by pain and strong pruritus, which may lead to an interruption or, in severe cases, even to an abortion of the therapy. Cold atmospheric pressure plasma (CAP), a partially ionized gas, provides an innovative therapy option that exerts antiseptic and anti-inflammatory properties, reduces pain, and supports tissue regeneration as well as angiogenesis. As CAP

supports wound healing and regenerative processes without causing any relevant side effects, we hypothesized CAP treatment to reduce the severity of radiation dermatitis and the acute side effects of radiotherapy in cancer patients, which currently disallows an uninterrupted therapy. Hence, this study aimed to assess the clinical course and the molecular pathomechanisms of radiation dermatitis and their modulation by treatment with CAP. For this purpose, an acute radiation dermatitis was induced in nude but immunocompetent SKH-1 mice. The course of the disease was monitored using a scoring system and noninvasive imaging techniques such as hyperspectral imaging, laser scanning microscopy, and optical coherence tomography. To this end, an optimal radiation dose (65 Gy) was identified for inducing a moderate radiation dermatitis (score 2.5) using a gamma irradiator. Furthermore, we assessed the efficacy of CAP in treating such a moderate radiation dermatitis using an atmospheric pressure plasma jet in comparison to an untreated control group. In addition to the monitoring using imaging technologies, skin biopsies were collected for immunohistochemistry and transcriptome analyses. Molecular analyses of treated and untreated tissue will help unravel the pathomechanisms of radiation dermatitis and how this is modulated by CAP exposure.

P048 | Adjuvant melanoma treatment: real-world data from the DACH region

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Modern adjuvant melanoma treatment has revolutionized patient care. We report on efficacy and adverse events of adjuvant melanoma treatment in a real-life setting covering cancer centers in GER, AUT and CH.

Interim analysis of a retrospective study, assessing melanoma patients treated with adjuvant Pembrolizumab, Nivolumab or Dabrafenib/Trametinib between 1/2017 and 2/2020. Data of 1039 patients have been collected, of which 524 were ready for analysis in September 2020.

Demographic and tumor characteristics of patients treated with BRAF+MEK or anti-PD1 therapy were comparable. The majority of patients received adjuvant anti-PD1 therapy (n = 439; 83.8%; Nivo = 69.2%; Pembro = 30.8%). Average follow-up time was 13.1 months. Time from complete resection till start of adjuvant treatment was 2.5 months (STD = 1.55) for BRAF+MEK and 2.7 months (STD = 2.62) for anti-PD1 therapy. Average duration of treatment was 7.4 months (STD = 4.4) for both adjuvant regimens. 12-months progression-free survival (PFS) was calculated with 81.2% for anti-PD1, and 90.4% for BRAF+MEK treatment (OR 2.001; 95% CI: 1.045-3.830; $P = 0.036$). There were no differences in PFS between Nivolumab and Pembrolizumab treated patients.

Ulceration, greater tumor thickness, and higher number of metastatic lymph nodes were negative predictors for PFS in BRAF+MEK and anti-PD1 treated patients. Higher BMI (25-30) and male sex were positive predictive markers for favorable PFS in BRAF+MEK treated patients. There were no differences in PFS in patients receiving complete lymph node dissection or just sentinel lymph node biopsy. The safety profile of all drugs was similar to what is known from previous studies. The number of any drug-related adverse events (drAE) and any immune-related AE were similar in Nivolumab and Pembrolizumab treated patients (64.2-63.7% and 33.3-33.8%). DrAE were more frequent in BRAF+MEK treated patients (87%).

12-months PFS was better with adjuvant BRAF+MEK compared to anti-PD1 treatment. Additionally, there appears to be a group of patients with exceptional short-term outcome using BRAF+MEK inhibition. Further analyses will provide insights into patient and tumor characteristics of favorable response to adjuvant BRAF+MEK and anti-PD1 treatment.

P049 | Site- and autoantigen-specific associations in mucous membrane pemphigoid lessons learned from a retrospective bicentric observational study

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Background: Mucous membrane pemphigoid (MMP) is a rare autoimmune bullous disease with predominant involvement of various mucosal surfaces presenting with mucosal erosions and, when conjunctivae, larynx, or esophagus are affected, with scarring. The skin is involved in a minority of patients. Next to this clinical picture, diagnosis is based on the presence of tissue-bound and/or circulating autoantibodies against antigens of the basal membrane zone of the surface-close epithelia and the skin.

Objectives: To characterize clinical and immunopathological features as well as comorbidities of patients with MMP.

Methods: In this retrospective study, all patients with a diagnosis of MMP in 2001-2019 in two tertiary centers were included. Here, associations of involved mucosal surfaces and autoantibody profiles with disease characteristics were analyzed using univariate and multivariate logistic regression models.

Results: The study population included 154 patients with MMP. The mean age of patients at diagnosis was 66.2 (13.8, range 23-95) years and 62.3% of patients were females. The most frequently affected mucosal surface was the oral cavity (81.2%), followed by ocular (39.6%) and nasal (22.1%) involvement, whereas additional cutaneous involvement was observed in 22.7% of patients. While 54.5% of patients presented with an isolated mucosal surface involvement, 45.5% involved two or more mucosal surfaces. The soluble ectodomain of BP180 (LAD-1) was the most frequent target antigen, followed by the NC16A domain of BP180 and laminin 332 (27.3%, 26.0% and 8.5% of patients, respectively). In 60 (39.0%) patients, the target antigen could not be identified, of whom 10 showed blister roof binding by indirect immunofluorescence microscopy on salt-split skin. Any IgA reactivity was associated with involvement of multiple mucosal surfaces (OR 3.02; 95% CI, 1.56-5.84, $P = 0.001$) while any IgG reactivity was associated with concomitant skin involvement (OR 7.71; 95% CI, 1.01-59.41, $P = 0.023$). Ocular involvement was significantly associated with the involvement of multiple mucosal surfaces (adjusted OR 43.58, 95% CI 6.12-310.42, $P < 0.001$), with malignancy (adjusted OR 13.07, 95% CI 1.56-109.36, $P < 0.001$), and inversely with anti-BP180 NC16A reactivity (adjusted OR 0.09, 95% CI 0.01-1.00, $P = 0.05$).

Reactivity against laminin 322 was associated with male sex (adjusted OR 15.28, 95% CI 1.73-134.43, $P = 0.014$) and malignancy (30% of anti-laminin 332-MMP patients, adjusted OR 23.27, 95% CI 1.83-296.68, $P = 0.015$).

Conclusion: In addition to its well-established association with reactivity against laminin 332, malignancy was significantly associated with ocular involvement in MMP. In addition to a careful work-up to exclude malignancy in all anti-laminin 332-MMP patients, the high burden of malignancy imposed by ocular affection also necessitates a thorough tumor search in MMP patients with ocular disease.

P050 | Successful treatment of recalcitrant pemphigus vulgaris with the phosphodiesterase 4 inhibitor apremilast

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Pemphigus with its subtypes pemphigus vulgaris (PV) and pemphigus foliaceus (PF) is an autoimmune blistering disease that affects skin and mucosa. Autoantibodies directed against desmogleins (Dsg) are responsible for skin blistering. In PF, patients have autoantibodies against Dsg1, whereas in PV circulating autoantibodies against both Dsg3 and Dsg1 are present. Dsg are constitutive parts of desmosomes, protein complexes responsible for keratinocyte adhesion. Binding of Dsg by autoantibodies results in loss of cell adhesion and acantholysis. PV is clinically characterized by oral and cutaneous erosions, fragile cutaneous bullae up to strongly debilitating ulcerations of the nasopharyngeal region. In recent years, the development of a plethora of new treatments improved the prognosis, but adverse effects and complications especially from long-term immunosuppressive therapies still contribute to morbidity and mortality. Unraveling the pathogenic mechanisms helps to identify new therapeutic targets in pemphigus. Phosphodiesterases (PDE)

are a family of enzymes (PDE1 to 11), which are capable to degrade cAMP intracellularly. In the setting of inflammatory skin diseases, PDE4 seems to be the most relevant subtype since it is widely expressed in immune, endothelial and epithelial cells. Binding of IgG to Dsg3 is a cardinal point for disease onset in Pemphigus. This process causes steric hindrance but most importantly activation of different signaling pathways. IgG binding to Dsg3 activates p38MAPK, Src and cAMP signaling pathways, whereas, in the cutaneous restricted PF, binding of IgG to Dsg1 exerts its pathogenic effect in an Erk-, p38MAPK- and PKC-dependent fashion. As reported in experimental models, interference with these pathways can protect from development of disease. According to these experimental findings, we wanted to investigate whether the PDE4 inhibitor (PDE4i) apremilast ceases blistering in PV in human. Here, we report of a 62 years old patient with chronic debilitating and recalcitrant pemphigus not responding to several previous treatments. Treatment with apremilast was continued over a period of 32 weeks. Desmoglein levels were assessed by Enzyme-linked Immunosorbent Assay (ELISA) whereas disease severity and quality of life were assessed by Autoimmune Bullous Skin Disorder Intensity Score (ABSIS). In attempt to explain the effects of apremilast in pemphigus, peripheral blood mononuclear cells (PBMC) were analyzed for the whole period of treatment by flow cytometry for the distribution of specialized T cell subsets. T helper (Th) 1, Th2, Th17.1 Th17 and T follicular helper (Tfh) 1, Tfh2, Tfh17.1 and Tfh17 were discriminated by different expression of CXCR5, CCR6 and CXCR3. Further, based on the different expression of CXCR5, CD127 and CD25 we analyzed the T regulatory (Treg) and T follicular regulatory (Tfreg) compartment. In response to apremilast intake, Dsg-specific antibody titers decreased, blistering stopped and lesions healed, showing a longlasting effect. While most of the Th and Tfh cell subsets remained unchanged, we observed a continuous increase in Treg and Tfr cell levels. These findings on a single patient are encouraging and warrant extending this observation to a larger cohort of pemphigus patients.

P051 | Breaking down barriers: Oxygen as a potential factor in atopic eczema development

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Introduction: Atopic eczema (AE) is an inflammatory skin disorder that is already affecting 20% of children worldwide and is increasing in prevalence (Williams H., et al. 2008; Asher MI., et al. 2006). Most of the research done on this topic has focused on the disturbance of the aerobic microbial layer on top of the stratum corneum, but it is known that there are anaerobic microbes lying beneath that skin layer and AE inflammation may be caused by a death of those microbes (Nakatsuji, T. et al. 2013). The itchiness associated with AE leads to skin barrier damage that compromises the natural decreasing oxygen concentration in the deeper layers of intact, healthy skin (Stücker et al., 2002) and thereby disrupts the environment where the anaerobic bacteria live (Tabata N., et al. 1998; Nakatsuji, T. et al. 2013). It has been shown that the skin's microbiome diversity is significantly lower with the skin's barrier having been perforated than with it intact which implies the possibility that the size of the anaerobic microbiome may be drastically reduced, which indeed may be the cause of AE (Gong JQ et al 2006; Reiger M., et al. 2016). This study aims to explore the role of the anaerobic microbiome in atopic eczema and whether oxygen presence has an effect on the behavior and secretions of facultative anaerobe Staphylococcus aureus.
Methods: Since there is no known list of anaerobic bacteria within the skin, an anaerobic skin bacteria catalogue was made by performing a literature search and cross-referencing to existing data from skin microbiome samples obtained at our chair. The catalogue was then applied to determine if certain species are lost in AE patients as compared to healthy. In addition, for verification of the list and creation of a physical catalogue of anaerobic skin bacteria several methods of anaerobic skin bacteria cultivation were tested. Because of the relationship between the facultative anaerobe Staphylococcus aureus and AE, the "itchiness induced" shift from purely anaerobic conditions to aerobic conditions was investigated for effects on S. aureus' behavioral patterns and small molecule secretions by LC/MS-MS.
Results: Astonishingly, a large number of the skin strictly anaerobic bacteria are gram positive with species found in both healthy and atopic skin. This may be because DNA is not necessary equivalent to cultivable species, and can still be present after bacterial death. In both strains from healthy and AE participants, S. aureus has a dramatic increase in growth with the change in oxygen environment, from anaerobic to aerobic, as compared to a stable environment of either anaerobic or aerobic atmosphere. This suggests that overgrowth of S. aureus in AE may be due to the change in the oxygenated environment, where deeper layer strains of S. aureus benefit from a boost in oxygen. This environmental effect is also seen in the metabolome of the strains where Staphylococcus spp. cluster according to environment in contrast to species suggesting unique environmental metabolites. In summation, this research proposes an additional parameter, oxygen, must be taken into consideration for its potential role in development of atopic eczema.

P052 | Relevance of neutrophil extracellular traps during vessel damage in skin-limited IgA immune complex vasculitis

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Background: IgA vasculitis (IgAV), also referred to as Henoch-Schönlein purpura, is characterized by perivascular deposition of IgA1 and neutrophilic inflammation of postcapillary venules. It encompasses a systemic form involving kidneys, gut, skin or joints, and a skin-limited form.

We have previously shown that not only in systemic IgAV but also in skin-limited IgAV the deposited IgA is galactose-deficient IgA1 (GD-IgA1) as also seen in IgA nephropathy. So the pathomechanisms of vessel and renal damage are considered to be similar in systemic and skin-limited IgAV.

Aims: We want to dissect the pathomechanism of vessel damage which distinguishes vasculitis from all other inflammations. In this study, we analysed relevance and occurrence of NETosis in human IgAV, as well as its distinct regulation in vitro and in vivo (using psoriasis as comparator dermatosis with neutrophilic infiltrates).

Results: In biopsies we were able to visualize NETosis in vivo and detected it at the luminal vessel wall in lesions of IgAV and in association with neutrophils (PMN), while in psoriasis it was present in a much lesser extent and only in epidermis or dermis beyond vessels.

In vitro we revealed that neutrophils of IgAV-patients bound IgA and were prestimulated for increased NETosis with higher amounts of toxic proteins, and that they went into full NETosis only when adherent. In an in vitro flow-system IgA-binding neutrophils adhered readily and went into marked NETosis, which then lead to damage of the lining endothelial cells. The latter we could prevented when we applied degrading enzymes or inhibited release of NET in the flow-system.

Conclusion: IgA-IC prime PMN for increased adherence, NETosis and vessel damage in IgAV.

P053 | IMPROVE 1.0 - Individual monitoring of psoriasis activity via regular online-app surveys in combination with clinical expertise

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Psoriasis is one of the most prevalent chronic inflammatory skin diseases associated with a number of comorbidities such as inflammatory bowel disease and metabolic syndrome resulting more and more in the perception of psoriasis as a systemic disease rather than a pure skin condition. Furthermore, stress and psychological disorders play an essential role for disease dynamics. Although a number of specific therapeutics is now available, patients are treated inadequately and their individual risk profiles for associated comorbidities are not satisfactorily considered in therapy concepts. This is partly due to the limited doctor-patient contacts and partly to the lack of communication between physicians and patients.

We therefore developed the Smartphone-APP IMPROVE (Individual monitoring of psoriasis activity via regular online-app surveys in combination with clinical expertise) to get a more efficient doctor-patient-relationship by the regular use of an online app questionnaire, to enable the implementation of individual therapy concepts and to include patients actively in their disease management.

Via online app questionnaire patients document their lifestyle, stress level and skin condition every two weeks over a period of one year. The app contains questions based on scientifically recognized questionnaires such as Self-Administered Psoriasis Area and Severity Index (SPASI), Dermatology Life Quality Index (DLQI) and Perceived Stress Scale (PSS).

In addition, all patients are examined during medical visits every eight weeks to determine the severity of psoriasis and to identify possible comorbidities. Clinical parameters are assessed as well as serum parameters and markers of disease activity and inflammation.

The goal of this pilot study is to identify individual correlations of parameters of the course of the disease (e.g., stress and subsequent exacerbation of psoriasis) which may lead to personalized treatment concepts.

First results from this study show that patients benefit from a health manager app and are more actively involved in disease management. Above all, it became clear that individual clinical care and consideration of the respective life circumstances are essential for patients to positively contribute to the improvement of their disease.

P054 | Is tumor mutational burden a prognostic marker in AJCC stage II melanoma?

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Background: Tumor mutational burden (TMB) has been shown to be predictive of good response to immunotherapy in stage IV melanoma and also other tumors, and is starting to be used as an inclusion criterion in ongoing clinical trials. However, its prognostic value is jet to be validated, also in earlier stages. We analyzed data from primary melanoma of stage II patients from the TCGA database and found that TMB could be prognostic in a stage II collective. We intended to validate the prognostic value of TMB in a stage II cohort of melanoma patients from our department.
Material and **Methods:** We included patients with stage II melanoma diagnosed between 2000-2018 in the University Hospital of Tuebingen and for whom formalin-fixed, paraffin-embedded normal and tumor tissue were available. Tumor and normal DNA sequencing was performed using a next generation sequencing (NGS) panel that covers 693 genes, 7 promotor regions and the intronic
region of 26 genes with known fusion partners. TMB was expressed in mutations per megabase (mut/Mb) and the median TMB was used as cut-off to define high and low-TMB sub-groups. Descriptive analysis of patient characteristics and survival analysis were performed. The follow-up time was defined as the time between diagnosis and relapse or death.
Results: A total of 198 samples were included in the final analysis. The median TMB was slightly higher in the whole collective (median TMB = 14mut/ Mb) when compared to the subgroup of patients with BRAFV600E/K mutation (median TMB = 11mut/Mb). The highest TMB was observed in patients with other BRAF mutations (median TMB = 55mut/Mb). When analyzing the whole collective, we found no difference in terms of median relapse-free survival (mRFS; P = 0.4689) and median overall survival (mOS;P = 0.5534) for patients with high and low-TMB. In patients harboring a BRAFV600E/K mutation the same results were observed, when the median TMB for this cohort was used as cut-off (mRFS; P = 0.3235 and mOS; P = 0.7547). In the multivariate Cox Hazard analysis including gender, tumor localization, histological subtype, age, tumor thickness, ulceration and TMB as a continuous variable, only age and tumor thickness were significant (P < 0.0001 and P = 0.001, respectively).
Conclusions: Our analysis was unable to confirm the results from the TCGA database and the median TMB was not a prognostic marker in our cohort of stage II melanoma.

P055 | Neural networks for automated skin morphology segmentation

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Accurate segmentations of macroscopic and microscopic cutaneous structures like skin lesions, dermal nerve fibers, or single keratinocytes within the epidermis are crucial for automated analysis and quantification of skin features. Due to the vast amount of image data in many clinical and scientific fields, automated image segmentation methods are essential. Unfortunately, traditional algorithms often fail for many image segmentation tasks because of e.g. heterogeneities in image quality, the presence of image artifacts, or hardly comprehensible and understandable image feature parameters defining these dermal structures.
At that point, modern machine learning techniques allow for approaching many segmentation problems which were inaccessible up to now. Especially recent advances in deep neural networks - technically enabled by the rise in computing power with massively parallel execution utilizing current graphics processing units - pushed the possibilities in this field. An omnipresent problem regarding deep learning methods is the need for huge amounts of human-annotated image data for network training. Luckily, more and more pre-trained neural networks are publicly available which can be trained with significantly fewer images.
Using neural networks for skin morphology segmentation, we will present the many and varied advantages of these techniques compared to classical algorithms. Furthermore, we will examine the pitfalls of neural networks and show up ways to circumvent these.

DERMATO-ENDOCRINOLOGY

P056 | Autophagy and apoptosis in human sebocytes

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Apoptosis and autophagy are two crucial biological processes, which ensure the maintenance of cell homeostasis process. Production and

liberation of sebum on skin surface by sebocytes ensures suppleness of the skin and contributes to an intact barrier function. With increasing age this function is disturbed leading to dry skin and high susceptibility to microbial infections. Knowledge regarding the holocrine secretion of sebum by sebocytes still remains sparse, whereas a unique apoptotic process in sebocytes has previously been reported. In this study, we focused on the apoptotic and autophagy response of the human sebaceous gland cell line SZ95 to the four biologically relevant fatty acids, arachidonic acid, linoleic acid, palmitic acid and palmitoleic acid. We report for the first time that sebocytes exhibit a high basal apoptotic rate, which is strongly suppressed by the pan-caspase inhibitor QVD-OpH, thus underlining the dependency on proapoptotic caspase cascades. Furthermore, all four fatty acids induced intracellular lipid droplets with subsequent apoptosis and cell death, with arachidonic acid causing the most rapid effect. Fatty acid-induced apoptosis could be markedly inhibited by pre-incubation with QVD-OpH also resulting to further accumulation of intracellular lipid droplets. While cell viability after incubation with linoleic acid, palmitic acid or palmitoleic acid and QVD-OpH was comparable to controls, arachidonic acid alone and in combination with QVD-OpH caused a significant decrease in cell viability. Real-time cell analysis also showed a significant decrease of arachidonic acid-treated SZ95 sebocyte numbers, even in combination with QVD-OpH, compared with the three other fatty acids and untreated SZ95 sebocytes. Using electron microscopy we could detect typical autophagic structures such as autophagosomes as well as autolysosomes at the basal level, which became more pronounced after treatments with fatty acids. These findings contribute to a better understanding of the mechanisms underlying autophagy and cell death induction in human sebocytes.

P057 | Melatonin and its metabolites regulate mitochondrial function in human melanoma cells

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Melanoma is a leading cause of cancer deaths worldwide. Although immunotherapy has revolutionised the treatment for some patents, resistance to treatment and unwanted side effects remain a problem for a majority of patients. Herein, using melanotic human melanoma cells, we explore in vitro the new insights into the mechanisms of melatonin action and its selected metabolites which possess high safety profiles and are biologically meaningful. 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. Apart from the oncostatic responses and their negative impact on melanin content, melatonin, its precursor (serotonin), a kynuric (N1-acetyl-N2-formyl-5-methoxykynuramine, AFMK) and indolic pathway (6-hydroxymelatonin, 6(OH)MEL and 5-methoxytryptamine, 5-MT) metabolites distinctly affect mitochondrial functions. We observed significant mechanistic alterations in bioenergetics as follows: (i) uncoupling of oxidative phosphorylation (OXPHOS), (ii) attenuation of glycolysis (Seahorse assessment), (iii) dissipation of mitochondrial transmembrane potential (mt$\Delta\Psi$) (FACS analysis, IF live imaging), and (iv) differences in mitochondrial morphology (transmission electron microscopy). Collectively, these results together with previously published reports provide new perspectives and support the use of these indoleamines as either novel therapies or complementary future treatments of melanoma-affected patients.

P058 | The alpha7 nicotinic acetylcholine receptor mediates the modulatory effect of the pharmacological agonist PHA-543613 in experimentally induced skin fibrosis

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Previously, we reported that partial and full pharmacological agonists of the alpha7 nicotinic acetylcholine receptor (alpha7nAChR) have a promising potential in experimentally induced skin fibrosis. To address specifically the role of alpha7nAChR in vivo we utilized wild-type (WT) and alpha7nAChR-deficient mice and subjected them to bleomycin-induced skin fibrosis in absence or presence of PHA-543613, a full alpha7nAChR agonist. As expected, PHA-543613 led to the antifibrogenic effect in WT mice as shown by reduced collagen and alpha-smooth muscle actin (alpha-SMA) mRNA expression using real-time PCR analysis. At protein level, mice treated with both BLM and PHA-543613 also displayed reduced collagen and hydroxyproline content in their skin compared with BLM-alone treated animals. These findings were in accordance with histological analyses of skin sections demonstrating reduced dermal thickness and collagen expression in mouse skin treated with PHA-543613 versus BLM. In contrast, the antifibrogenic effect of PHA-543613 was completely abrogated in mice deficient for alpha7nAChR. Interestingly, alpha-7nAChR-deficient animals exhibited a significantly stronger expression of extracellular matrix genes upon NaCl-control injection compared with WT mice. Moreover, alpha-SMA but not collagen expression was significantly higher in BLM-treated alpha7nAChR-deficient animals than in WT mice. To test if murine dermal fibroblasts (MDFs) are direct targets for the antifibrogenic effect of

PHA-543613 we treated them with transforming growth factor-beta1 (TGF-beta1) and determined fibrosis read-outs. In MDFs from WT mice PHA-543613 led to in significantly reduced collagen expression in response to TGF-beta1. Gene silencing of alpha7nAChR by siRNA reversed effects of PHA-543613 on TGF-beta1-mediated collagen expression. In summary, we have identified here the alpha-7nAChR as an essential mediator of the molecular action of the anti-fibrogenic effect of pharmacological alpha7nAChR agonists such as PHA-543613. These findings further support therapeutic exploitation of alpha7nAChR receptor agonists in fibrotic skin diseases.

P059 | Hidradenitis suppurativa and comorbid disorders: An immunohistochemical real-world approach

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Hidradenitis suppurativa (HS) is a chronic, recurrent inflammatory skin disease that presents with painful nodules, abscesses, tunnel formation and subsequent scarring, most commonly localized in the axillar, inguinal and anogenital regions. Multiple genetic, immunological, endocrinological and environmental factors play a role in HS pathogenesis. Tobacco smoking is a widely discussed environmental predisposing factor, whereas the prevalence of smokers among HS patients ranges from 30% to 98%. The second important risk factor is obesity. Both factors have been associated with a more severe HS phenotype in several studies.

Our study comprises a group of 22 adult HS patients divided into two groups: 12 obese smokers (OS) and 10 normal weighted non-smokers (NN). Formalin-fixed, paraffin-embedded skin specimens were cut in 3.5 μm sections, were stained with hematoxylin and eosin, and were labeled with 8 different antibodies against proteins associated with smoking and obesity. Differential expression was assessed with the antibodies against the aryl hydrocarbon receptor, which is activated by dioxins and dioxin-like chemicals in cigarette smoke, irisin, with exhibits an antidiabetic effect, insulin-like growth factor-1 receptor and furthermore, epidermal growth factor receptor, S100A8, peroxisome proliferator-activated receptor-γ and IL-17 and its receptor. The project improves our understanding of the etiopathogenesis of HS disease and provides valuable knowledge on the differential expression of certain environmentally and metabolically triggered proteins in OS in comparison to NN.

P060 | The melanocortin tripeptide derivatives KdPT and WOL074-029 can modulate fibroblast activation in vitro

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The melanocortin peptide derivative Lys-d-Pro-Thr (KdPT) has previously been reported to possess promising anti-inflammatory effects. For example, KdPT suppressed IL-1beta-induced expression of proinflammatory cytokines in vitro and exhibited beneficial effects in animal models of inflammatory bowel disease. The latter observation was recently confirmed in a proof of concept study in patients with ulcerative colitis. A major advantage of KdPT over alpha-melanocyte-stimulating hormone appears to be the lack of pigment induction as this peptide does not bind to the melanocortin-1 receptor. However, whether KdPT has modulatory effects on fibroblasts, the key effector cells of fibrotic diseases, remains unknown. Here, we investigated if KdPT and another more stable derivative, WOL074-029, are capable of suppressing fibroblast activation following stimulation with the profibrotic cytokine transforming growth factor-beta1 (TGF-beta1). Both KdPT and WOL074-029 strongly suppressed mRNA expression of collagen type1, fibronectin I and alpha-smooth muscle actin in human dermal fibroblasts (n = 5 donors) compared with TGF-beta1 alone as shown by real-time RT-PCR analysis. Moreover, expression of connective tissue growth factor, a cAMP-dependent gene, was suppressed. Interestingly, these effects occurred not strictly in a dose-independent manner and were detectable in part at subnanomolar levels of the peptides. In contrast, KdPT and WOL074-029 alone did not have modulatory effects on these fibrosis-related gene read-outs. In accordance with these findings, secretion of collagen type I was significantly suppressed by KdPT and WOL074-029 using procollagen type I peptide ELISA. Currently, the mode of action of the two peptides is further being addressed including expression and functional studies on tripeptide transporters, e. g. PepT1, and signal transduction pathways induced by TGF-beta1. These findings may kick off therapeutic exploitation of KdPT and related peptides in fibrotic skin diseases such as scleroderma.

DERMATOPATHOLOGY

P061 | Novel cold atmospheric plasma wound dressing promotes healing of split skin graft donor sites

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Over the last decade, cold atmospheric plasma (CAP) has emerged as a new treatment option for the improved healing of acute and chronic wounds. We investigated in a prospective cohort pilot study the response and tolerability of a newly developed CAP wound dressing (PlasmaDerm Dress, CINOGY System GmbH, Duderstadt, Germany) for the acute healing of split skin graft donor sites compared to conventional therapy. We applied both treatments to each patient (n = 10) and measured different parameters of wound healing (deep tissue oxygen saturation, hemoglobin distribution, tissue water distribution and superficial hemoglobin oxygen saturation) over 7 days using a state-of-the-art hyperspectral imaging camera. Additionally, we evaluated clinical appearance and pain levels reported by the patients. CAP therapy significantly improved the deep tissue oxygen saturation ($P < 0.001$), hemoglobin distribution ($P < 0.001$) and the tissue water distribution ($P < 0.001$) compared to standard wound therapy. CAP was well tolerated, and pain levels were lower in CAP-treated wound areas. In summary, CAP as applied through an innovative wound dressing is a promising new tool for the improved healing of acute wounds.

P062 | Mutual interaction of S100A9 and saturated fatty acids is a central mechanism in the obesity-mediated exacerbation of skin inflammation

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Obesity exacerbates inflammation in multiple organs. Thus, obese people develop more severe skin inflammatory diseases such as psoriasis. In the present study, we identify S100A9 and its combined action with elevated levels of saturated fatty acids (SFA) as one central mechanism in the high fat diet- (HFD) and obesity-mediated exacerbation of skin inflammation. We show that both obese mice and non-obese/non-diabetic, short-term HFD-fed mice with elevated serum-SFA develop an amplified, prolonged skin inflammation and a delayed inflammatory resolution response. Additionally, these mice display increased and sustained S100A9 expression during skin inflammation at times when M2 macrophage activation and inflammatory resolution had already occurred in chow-fed control mice. Using in vitro and in vivo systems of macrophage polarization, we demonstrate that S100A9 impairs M2-like macrophage differentiation while promoting their pro-inflammatory activation. Mechanistic studies on increased S100A9 expression in conditions of HFD and obesity show that SFA together with S100A9 induce inflammasome activation and IL-1β release in macrophages. Macrophage-derived IL-1β in turn amplifies epidermal S100A9 expression, thus initiating a perpetuating S100A9 response that sustains inflammation and prevents its resolution. Consequently, inhibition of S100A9 by the quinoline-3-carboxamide derivative paquinimod or reduction of SFA by dietary intervention reverses the increase/prolongation of skin inflammation induced by HFD and obesity in mice and favors the activation of M2-like macrophages. Moreover, dietary intervention of fatty acid uptake reduces disease severity in patients with mild-moderate psoriasis. Reduction of disease severity correlates with the change of S100A8/A9 serum levels confirming the significance of SFA and S100A9 in the control of psoriatic inflammation.

P063 | Artificial neural networks and pathologists recognize basal cell carcinomas based on different histological patterns

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Recent advances in artificial intelligence, particularly in the field of deep learning, have enabled researchers to create compelling algorithms for medical image analysis. Histological slides of basal cell carcinomas (BCCs), the most frequent skin tumor, are accessed by pathologists on a daily basis and are therefore well suited for

automated pre-screening by neural networks for the identification of cancerous regions and swift tumor classification.

In this proof-of-concept study, we implemented an accurate and intuitively interpretable artificial neural network (ANN) for the detection of BCCs in histological whole slide images. Furthermore, we identified and compared differences in the diagnostic histological features and recognition patterns relevant for machine learning algorithms versus expert pathologists.

An attention-ANN was trained with whole slide images of BCCs to identify tumor regions (n = 820). The diagnosis-relevant regions used by the ANN were compared to regions of interest for pathologists, detected by eye-tracking techniques.

This ANN accurately identified BCC tumor regions on images of histologic slides (AUC of 0.993, 95% CI: 0.990-0.995; sensitivity: 0.965, 95% CI: 0.951-0.979; specificity: 0.910, 95% CI: 0.859-0.960). The ANN implicitly calculated a weight matrix, indicating the regions of a histological image that are important for the prediction of the network. Interestingly, compared to pathologists' eye tracking results, machine learning algorithms rely on significantly different recognition patterns for tumor identification ($P < 10\text{-}4$).

To conclude, we found on the example of BCC whole slide images, that histopathological images can be efficiently and interpretably analyzed by state-of-the art machine learning techniques. Neural networks and machine learning algorithms can potentially enhance diagnostic precision in digital pathology and uncover hitherto unused classification patterns.

P064 | Cellular expression of complement receptors C5aR1 and C5aR2 in pemphigoid diseases

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Pemphigoid diseases (PDs) are a group of chronic autoimmune blistering skin diseases defined by an autoantibody-driven immune response against different proteins of the hemidesmosomal complex. Complement activation at the dermal-epidermal junction (DEJ) is an immunopathological and diagnostic hallmark of PD patients. A key effector of the complement response is the activation fragment C5a, which exerts its effector functions through binding to the receptors, C5aR1 and C5aR2. The proinflammatory effector functions of C5aR1 in diverse autoimmune diseases are relatively well described. The role of the second C5a receptor, C5aR2, remains, however, to be determined. In this study, we aimed to further elucidate the role of C5aR2 in the regulation of skin inflammation in PD and the potential of C5aR2 as a target in the treatment of these diseases. To address this aim, perilesional skin biopsies, representative for the early phase of autoantibody-mediated skin inflammation, of patients with the by far most frequent PD, bullous pemphigoid (BP) were subjected to immunohistochemistry staining. Skin biopsies matched for biopsy sites, age and sex in patients with non-inflammatory dermatoses served as controls. Double immunofluorescence staining for C5aR1/2 and cell markers of neutrophils (myeloperoxidase), eosinophils (eosinophil peroxidase), macrophages (CD68), mast cells (tryptase), and T cells (CD3) revealed cellular sources of C5aRs in BP skin. Our data demonstrated that C5aR1 expression was predominantly detectable on T cells (34.7%) and macrophages (27.6%). In contrast, mast cells (45%) and eosinophils (22.5%) appeared to be the main cellular source of C5aR2 in BP skin, followed by macrophages (10%), T cells (7.2%), and neutrophils (3.5%). These results will allow us to further investigate the major C5aR1/2-expressing cell populations as potential targets for pharmacological interventions by C5aR1 antagonist or C5aR2 (ant)agonists and to explore their functional relevance in mouse models of PD.

P065 | New diagnostic criteria for ichthyoses: Delineation of seven histological patterns

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Ichthyoses are a rare and heterogenous group of genetic skin diseases that are caused by mutations affecting the epidermal barrier function.

Histological examination is a general and widely available diagnostic technique for ichthyoses, which may be complemented by genetic analyses. However, there are missing data on systematic histological diagnostic criteria for ichthyoses and palmoplantar keratoderma. Therefore, we analyzed histologies of 47 geno- and phenotyped patients with the most common and some extremely rare ichthyoses considering the epidermis, stratum granulosum (SG), stratum corneum (SC) and inflammation. We defined seven different histological patterns which are described as follows: 1) Orthohyperkeratosis with a reduced or well-developed SG, 2) hyperkeratosis with ortho- and parakeratosis with preserved or prominent SG, 3) orthohyperkeratosis and a well-developed SG and 4) epidermolytic ichthyosis. The fifth pattern features "perinuclear vacuoles and binucleated keratinocytes" which is associated with keratin mutations. The sixth pattern is defined by psoriasis-like features and the last pattern shows follicular hyperkeratosis. In our cohort > 80% of the histologies could be related to one of these patterns.

Many features could be correlated with ultrastructural analyses as well as clinical findings including criteria of psoriasis and atopic dermatitis. These observations may help with diagnosis and result in new therapeutic approaches making use of drug repurposing.

We conclude the seven histological patterns of ichthyosis reveal specific clues for some diseases and help to achieve a shortcut for targeted molecular analysis of ichthyosis subgroups.

P066 | Analysis of ribosomal composition in the premature aging disease Cockayne syndrome

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Cockayne syndrome (CS) is a rare "premature aging" disease characterized by childhood onset of degenerative symptoms reminiscent of the aging body, such as loss of subcutaneous fat, alopecia, cataracts, neurological degeneration and cachexia. These symptoms are accompanied by developmental delay, resulting in a severe phenotype that can lead to childhood death. CS can be caused by mutations in the genes ERCC6 and ERCC8 encoding for CSB and CSA respectively. Additionally a number of patients with mutations in XPB, XPD, XPG and XPF genes also show features of CS. CS proteins are involved in a branch of the Nucleotide-Excision Repair (NER) pathway, thus explaining the elevated UV-sensitivity of the patients. CS has been initially regarded as a DNA damage disease however, total loss of NER is not always followed by premature aging, suggesting that alternative functions of the CS proteins have a crucial role in the disease.

One alternative function of the CS proteins is the transcription of ribosomal RNA by RNA polymerase I. Our previous findings have shown that a disturbed RNA polymerase I transcription in CSA and CSB-deficient cells is followed by a decreased translational accuracy of the ribosomes. This results in a high level of misfolded proteins and increased carbonylation of these proteins. As a result, ER stress and unfolded protein response are activated in CS cells and results in further repression of RNA polymerase I transcription, especially by activation of the protein kinase RNA-like ER kinase (PERK) pathway. CS cells also suffer from a high level of reactive oxygen species (ROS). The unfortunate combination of misfolded proteins and high ROS leads therefore to unresolved ER stress and to increased oxidative hypersensitivity in CS cells. Moreover, TUDCA chemical chaperone can decrease ER stress and restore the deficient RNA polymerase I transcription and protein synthesis of CS cells.

To identify the molecular defect of ribosomes in CS cells, we used Northern Blot analysis to determine the levels of pre-rRNA processing during different steps of rRNA maturation. We can show that the pre-rRNA processing is delayed in CS deficient cells. Furthermore we isolated ribosomes under stringent conditions from patient derived fibroblasts and healthy controls and analyzed the composition by Mass Spectrometry and Western Blot. The western blot analysis revealed a disturbed ribosomal composition indicating an unstable assembly of the affected ribosomes. Moreover, we performed rescue experiments by using chemical chaperones (4PBA and TUDCA), and as a result, these drugs increased cell growth.

Our findings support a possible treatment for a devastating childhood disorder and may have an impact on our understanding of the molecular mechanisms underlying the aging process itself.

P067 | Epithelioid hemangiomas show somatic mutations in the MAPK pathway

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Epithelioid hemangioma (EH), also known as angiolymphoid hyperplasia with eosinophilia, is a benign vascular tumor with marked inflammatory cell infiltration, which exhibits a high tendency to persist and to frequently recur after excision. So far, the underlying pathogenesis is elusive. Using multiplex PCR-based panel sequencing of genomic DNA isolated from archival formalin-fixed paraffin-embedded (FFPE) tissue of 20 cutaneous EH patients, we could identify somatic mutations in genes of the MAPK pathway (MAP2K1, KRAS). By droplet digital PCR, we could confirm the recurrent presence of low-frequency mutations affecting MAP2K1 in EH biopsies. In total, 10 out of the 20 analyzed patients showed MAPK pathway mutations, which were mutually exclusive. Comparative analysis of tissue areas enriched for lymphatic infiltrate or aberrant endothelial cells, respectively, further revealed an association of these mutations with the presence of endothelial cells. Taken together, our data suggest that EH is caused by somatic mutations in cutaneous endothelial cells leading to the formation of a benign tumor.

P068 | Selective PI3K-delta inhibition normalizes aberrant kinase activity in the skin and has therapeutic effects in experimental models of pemphigoid diseases

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PI3K signaling is essential for immune cell activation. PI3Kδ, one of the predominant isoforms expressed in the hematopoietic lineage, is of greatest interest for treatment of autoimmune diseases. It has been demonstrated that selective inhibition of PI3Kδ can alleviate disease

manifestation in mouse models of autoimmune diseases such as rheumatoid arthritis and systemic lupus erythematosus through prohibition of B cell, T cell, and neutrophil activation. Pemphigoid diseases (PDs) such as bullous pemphigoid (BP) and epidermolysis bullosa acquisita (EBA) are a group of autoantibody-induced and neutrophil-associated autoimmune diseases. Here, we used a selective PI3Kδ inhibitor, INCB050465, in vitro and in vivo models of PDs. INCB050465 repressed generation of reactive oxygen species from immune complex stimulated neutrophil/ polymorphonuclear leukocyte (PMN) in a dose-dependent fashion. When cryosections of human skin were incubated with BP or EBA patient IgG, followed by addition of PMN from healthy blood donors, the extent of dermal-epidermal separation was blocked by INCB050465 in a dose response manner. Ultimately, we applied INCB050465 in therapeutic setting in a mouse model of immunization-induced model of EBA. In this pre-clinical trial, mice were randomized to treatments if 2% or more of the body surface area were affected by skin lesions. Mice were either treated with INCB050465 or vehicle. After 4 weeks of treatment, clinical disease manifestation in INCB050465-treated mice was significantly lower compared to vehicle-treated mice. Kinome activity profiling of the mice lesional skin corroborated that the PI3Kδ signaling pathway is affected by the INCB050465. In summary, we demonstrated that INCB050465 ameliorates tissue destruction in experimental PD by inhibition of neutrophil functions. Thus PI3Kδ inhibition may be a promising new treatment that warrants further clinical investigation for patients with PD.

P069 (OP03/02) | Profiling of Acne inversa reveals disease-specific but also psoriasis-related pathogenetic pathways, which may explain the increased prevalence of concomitant psoriasis among AI patients

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Acne inversa (AI) is a chronic inflammatory skin disease characterized by painful inflamed nodules, abscesses, and pus-discharging fistulas developing in axillary, inguinal, gluteal, and perianal sites. Its pathogenesis is largely unknown and very limited target-directed therapies exist so far.

In order to shed light on the molecular mechanisms underlying AI, we first analyzed the transcriptomes of lesional and peri-lesional skin from AI patients by RNA deep sequencing and compared them to location-matched healthy skin. 13% of all protein-coding genes were upregulated (fold change ≥ 2, $p \leq 0.05$) in AI lesions. A similar number of gene expressions was found to be downregulated. In order to understand the significance and specificity of the detected regulations, we compared them to transcriptomic changes in skin lesions of patients suffering from psoriasis (Ps), a pathogenetically well-understood disease. A large overlap of expressional regulations in both conditions, but also a range of AI-specific changes, were found. To validate these results, we individually quantified the expression of more than 100 key molecules by RT-qPCR in the skin of a second cohort of AI, Ps, and healthy participants. Molecules comparably involved in both diseases comprised the cytokines IL-17A, IL-17F, IL-26, IL-34, IL-36b, IFN-g and TNF-a, while molecules being rather specific for AI lesions included IL-1b, IL-6, IL-27 and G-CSF. Correlation analysis of the levels of these molecules with those of the other regulated genes as well as in-vitro stimulation studies revealed pathways these molecules are involved in.

The existence of pathogenetic pathways common in both AI and Ps suggested that each of these diseases might favor the manifestation of the other. Our prospective study with ~400 AI patients seen in a dermatological clinic indeed revealed an increased proportion (7%) of AI patients simultaneously suffering from Ps (AI/Ps). Similarly, German health insurance data showed a higher Ps prevalence among AI patients (4.4%) compared to age- and gender-matched controls (2.4%, $P = 0.000$). Further evaluation of our study data demonstrated that the proportion of patients with positive family history for Ps did not differ between AI/Ps patients and patients with only Ps, suggesting the association of both disorders being independent of patients' genetic background. Interestingly, AI/Ps patients were afflicted with more severe AI symptoms than AI patients without Ps. Moreover, these patients showed the highest prevalence of metabolic (central obesity, type 2 diabetes) and other comorbidities (e.g., back pain) among the three groups compared.

In summary, our data show that, apart from disease-specific pathogenetic pathways, a number of Ps-relevant pathways are active in AI lesions and therefore encourage the study of anti-psoriatic therapies in AI.

P070 | Soluble FcεRI is a potential biomarker for therapeutic response to omalizumab in patients with chronic spontaneous urticaria

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Introduction: Treatment with omalizumab, an anti-IgE biological, is effective in many patients with chronic spontaneous urticaria (CSU). Poor response to omalizumab is associated with low total IgE and failure to increase total IgE levels upon treatment, although reliable biomarkers of good response are still missing. Elevated serum levels of soluble FcεRI (sFcεRI; >2 ng/mL) are linked to IgE/FcεRI-mediated mast cell activation, a key feature of CSU pathogenesis. We, therefore, evaluated sFcεRI as a marker for omalizumab response in patients with CSU.

Materials and method: Serum samples from CSU patients (n = 67) were analyzed for sFcεRI levels by ELISA and correlated with clinical data. Healthy individuals were used as controls (HCs, n = 31). CSU patients received standard omalizumab treatment (300 mg s.c. every 4 weeks). Response to treatment was assessed by the Urticaria Control Test (UCT) 4 and 8 weeks after the 1st injection, and patients with a UCT ≥ 12 were defined as responders.

Results: Baseline serum levels of sFcεRI in omalizumab-treated CSU patients were significantly higher than in HCs (mean SEM: 5.01.1 vs 1.30.3 ng/mL) and correlated with total IgE levels ($r = 0.36$, $P < 0.01$). The rate of response to omalizumab treatment was 63% and 68% at the end of week 4 and8, respectively. Week 4 responders had higher baseline sFcεRI levels as compared to non-responders (6.51.8 vs 2.90.7 ng/mL; $P = 0.03$). Baseline sFcεRI levels of week 8 responders were also higher than those of non responders, but this difference was not statistically significant (6.31.7 vs 2.90.9; $P = 0.067$). Baseline sFcεRI levels > 2 ng/mL were linked to a 60% ($P = 0.049$) and 50% ($P = 0.037$) higher rate of response at week 4 and8, respectively, as compared to baseline levels < 2 ng/mL.

Discussion: In CSU, high baseline sFcεRI levels may predict good and early response to omalizumab treatment. Further studies are needed to confirm these findings in independent and larger patient population, to understand the role of sFcεRI in the pathogenesis of CSU, and to characterize how sFcεRI is linked to the mechanism of action of omalizumab.

P071 (OP04/03) | Myeloperoxidase plays an important role in epidermolysis bullosa acquisita

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Epidermolysis bullosa acquisita (EBA) is an acquired, autoimmune blistering disease clinically characterized by the emergence of erosions and blisters on the skin. The hallmark of EBA is characterized to the presence of IgG class autoantibodies to type VII collagen. EBA, in comparison to other autoimmune blistering diseases, has a decreased responsiveness to therapy, making it essential to investigate the role of various disease contributing factors. One such factor is myeloperoxidase (MPO), a peroxidase enzyme abundantly expressed by neutrophils, the main effector cells in EBA. MPO is known to contribute to tissue damage and the resulting inflammation in various inflammatory diseases.

In order to study the role of this enzyme with respect to EBA, we utilized the mouse model for bullous pemphigoid (BP)-like epidermolysis bullosa acquisita (EBA) to provide preclinical proof for the role of MPO during the disease. In the antibody transfer bullous pemphigoid (BP)-like EBA mouse model, C57BL/6J wild-type mice displayed clinical signs of the disease while the MPO-/- mice were completed protected and did not develop skin lesions. In order to determine the mechanism through which MPO plays an important role in our disease model we performed additional studies. In vitro experiments using age- and sex-matched C57BL/6J wild-type and MPO-/- mice indicated a complete reduction in radical oxygen species (ROS) and leukotriene B4 (LTB4) release from bone marrow-derived neutrophils in response to immune complexes (IC). Further experiments with neutrophils stimulated with IC indicated the absence of NET formation in MPO-/- mice. BM-derived neutrophils from WT and MPO-/- mice stimulated with phorbol-12-myristate-13-acetate (PMA) did not indicate a difference in activation marker CD62L. The specific role of MPO in the inhibition of ROS release and formation of NETs in MPO deficient neutrophils stimulated with IC could be confirmed with further studies by treating neutrophils from C57BL/6J wild-type mice with an irreversible and specific inhibitor of the peroxidation activity of MPO, at a non-toxic concentration, before stimulation with IC.

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

P072 | Interferon-gamma drives keratinocyte necroptosis in acute graft-versus-host disease

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Graft-versus-host disease (GVHD) is a major complication occurring upon hematopoietic stem cell transplantation (HCT) severely affecting epithelial layers. Within cutaneous manifestations, keratinocyte cell death, a pathological hallmark and diagnostic feature of allogeneic recognition is believed to result from apoptosis or unspecific necrosis. However, our present data demonstrate that enhanced programmed necrosis known as necroptosis, a novel pathomechanistical process is activated in cutaneous acute GVHD (aGVHD) lesions. Described key events in the cascade of necroptotic signaling are the activation of receptor interacting protein kinase 3 (RIP3) leading to mixed linage kinase domain-like protein (MLKL) oligomerization and lytic inflammatory necrotic cell death. Here, we found that initial interferon gamma (IFNγ) signaling induces the expression and upregulation of necroptotic mediators leading to MLKL dependent cell death execution upon persistent IFNγ exposure. We are able to show that IFNγ-induced keratinocyte necroptosis depends on STAT1 regulated signaling and the activation and interaction of de novo expressed Z-DNA-binding protein 1 (ZBP1) and RIP3. In contrast to the receptor interacting protein kinase 1 (RIP1) mutated murine system, the recently described ZBP1-dependent cell death appears to be physiological for human keratinocytes. In line with this, specific inhibition of STAT1 signaling using JAK/STAT inhibitor tofacitinib could halt necroptosis and confirm these observations in vitro and ex vivo. Therefore, our data highlight the indirect cytotoxic properties of IFNγ on epithelial keratinocytes by forcing the transcriptome towards a necroptotic profile and its consequence of subsequent execution. We hypothesize that this occurrence might resemble a common pathomechanism for type I, IFNγ-mediated inflammatory diseases.

EPIDEMIOLOGY

P073 | Divergence in the prevalence of self-reported and physician-reported diagnosis of atopic dermatitis in adults: Results from a population-based study

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Background: Atopic dermatitis (AD) is a chronic inflammatory skin disease characterized by itch, skin pain, sleep disturbances and multiple comorbidities. Until now, data from German studies regarding the epidemiology of AD in adults and the validity of self-reported AD in the general population are scarce. We aimed to analyze (i) the prevalence of AD based on self-report and physician-report and (ii) differences in AD prevalence by age and gender using data from a large cohort of adults.

Methods: Data from[3,0]54 participants from the population-based Study of Health in Pomerania (SHIP) aged 20 to 83 years were analyzed. All participants underwent a standardized dermatological examination encompassing a personal interview and a clinical examination. Population-weighted analyses were conducted to determine AD prevalence stratified by age and gender.

Results: The overall prevalence of self-reported and physician-reported AD was 2.5% and 4.2%, respectively. Prevalence was higher in women compared to men both in self-reported (2.8% vs. 2.2%) and physician-reported data (4.4% vs. 4.1%). Prevalence rates decreased across age.

A considerable proportion of participants stated not to know whether they were suffering from AD (overall: 2.4%, men: 3.2%, women: 1.7%).

Conclusions: Our study is the first to provide data on AD prevalence in the general adult population in Germany. A recent review reported a considerably higher AD prevalence based on AD diagnoses for other European countries such as Denmark (10%), France (8%) and Sweden (9%). We revealed a divergence between self-reported and physician-reported AD prevalence with lower rates in self-reported data, suggesting limited validity of these data. Interview data indicate that a significant proportion of the participants was presumably aware of having a skin disease, but did not know which one, indicating the need for improvements in patient information and promotion of health literacy.

P074 | NK cell transcriptomic signatures in skin of atopic dermatitis

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Background: Natural killer (NK) cells are cytotoxic effector cells of the innate immune system. In atopic dermatitis (AD), NK cells have been reported to be reduced in peripheral blood, whereas in lesional skin they appear to accumulate. Further, an altered composition of NK cell subsets both in blood and skin as well as a reversal of peripheral blood NK cell abnormalities during treatment with the anti-IL4R antibody dupilumab has been reported for AD.

Methods: We analysed NK cell signatures in skin transcriptome data from 57 treatment-naïve patients with moderate to severe AD of the TREATgermany AD registry and 31 healthy controls. In addition, changes after 12 weeks of systemic treatment (dupilumab n = 21, cyclosporine n = 8) were analysed. Deconvolution of leucocyte fractions including NK cells was conducted. Immunofluorescence staining of NK cells was performed on paraffin-embedded skin sections.

Results: Immunofluorescence staining revealed a relatively high abundance of both NK cells and NKT cells in lesional skin as compared to non-lesional and healthy skin. Lesional and to a lesser extent non-lesional skin showed a strong up-regulation of NK cell markers together with a dysbalanced expression of inhibitory and activating receptors, which were not reverted under treatment. Digital cytometry showed a decrease of activated and increase of resting NK cells in both lesional and nonlesional skin, which was reverted by treatment with both dupilumab and cyclosporine. The NK cell gene expression signature remained up-regulated after treatment; however, there was a shift on the qualitative level indicating a compositional change of NK cell subsets towards tissue-resident CD56 bright NK cells.

Conclusion: Our data indicate a disturbed NK cell composition and function as a potentially important disease mechanism in AD.

P075 | Associations of host traits, lifestyle and environment with the skin microbiota

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The skin harbors millions of microorganisms, but factors shaping the skin microbiota are still understudied. We tested the association of host traits, lifestyle, and environment exposition with human skin microbiota. We included 647 participants from two population-based German cohorts, PopGen (n = 294) and KORA FF4 (n = 353), totaling 1794 skin samples. The V1-V2 regions of the 16S rRNA gene amplicon were sequenced. Associations of host traits with beta diversity and amplicon sequence variants (ASVs) were tested. We validated known associations of the skin microbiota with skin microenvironment, age, body mass index (BMI) and sex. These factors were associated with beta diversity and ASV relative abundance. Most intriguingly, several ASVs were associated with the quantity of dietary macronutrients (fat, protein, carbohydrate, fiber) and total dietary energy. ASVs were also associated with smoking, alcohol consumption, skin pH, skin type, transepidermal water loss, education and several environmental exposures, including pets and dwelling. General patterns of association with skin microenvironment, sex, BMI and lifestyle found in PopGen were replicated in KORA FF4. Based on these observations, we hypothesize that the skin bacterial community is primarily shaped by the skin microenvironment and the host physiology, but fine-tuned by individual alterations in skin physiological conditions, lifestyle and environmental exposition.

P076 | Which antipsoriatic induction treatment sequence is the most time-effective? Economic modelling with time instead of monetary cost

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Background: Systemic antipsoriatic agents vary in regard to their cost and efficacy but also regarding their time until onset of action.

In case of non-response to a first induction treatment patients are commonly switched to another treatment. Some patients receive several treatments until they notice an improvement. We aimed to compare the time-effectiveness of different systemic induction treatments sequences. These sequences will consist of agents that are currently licensed in Germany for the treatment of moderate to severe plaque psoriasis. We used time instead of monetary cost in this decision model.

Methods: We first identified most commonly used induction treatment sequences in Germany. These were then compared to four theoretical treatment sequences each starting with a biologic. We defined two health states: responder (patients achieving a Psoriasis Area Severity Index (PASI) 75) and non-responder (< PASI 75). To identify input values for the model systematic reviews were performed. We defined PASI 75 response rates per agent as probability values. The mean change in Dermatology Life Quality Index (DLQI) was defined as the effectiveness measure. Time until onset was determined as number of weeks until a quarter of patients reach PASI 75. Sequence-specific time-effectiveness ratios were calculated. Ratios represented time until onset of action (TOA, in weeks) per minimally important difference (MID) in DLQI. Ratios were subsequently ranked.

Results: Sequences commencing with a biologic agent (IXE, INF, SEC: 1.4; INF, IXE, SEC: 2.05; SEC, IXE, ADA: 2.1; ADA, IXE, SEC: 2.8 weeks per DLQIMID) were more time-effective than the most commonly used induction treatment sequences (MTX, SEC, ADA: 6.8; MTX, ADA, IXE: 7; MTX, ADA, SEC: 7.2; MTX, FAE, ADA: 10.05; FAE, MTX, CSA: 11.5 weeks per DLQI-MID) in Germany. The findings were robust to the deterministic sensitivity analyses.

Conclusions: When monetary resources are allocated on the basis of cost-effectiveness considerations, regulators and policy makers may also want to consider weeks until patients experience a DLQI-MID as an outcome measure.

P077 | Incidence of different cancer types in dermatomyositis, polymyositis and dermatopolymyositis: results of a registry analysis

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Background: Dermatomyositis/polymyositis/dermatopolymyositis (DM/PM/DPM) are considered paraneoplastic diseases; however, data on the incidence of different cancer types in DM/PM/DPM patients are scarce.

Objectives: To investigate the linkage of DM/PM/DPM with cancer incidence and to identify the most frequently observed cancer types in these patients.

Methods: We conducted a retrospective registry analysis using data from the Danish National Patient Register (1 January 1977 C 31 December 2017). 2.576 individuals (18 years) with DM/PM/DPM were included. We calculated cancer incidence rates of these individuals and compared them to general population values (age and sex matched).

Results: In total, 710, 1530 and 336 cases of DM/PM/DPM were identified, respectively. During the year of initial diagnosis of DM/PM/DPM, patients had substantially higher cancer incidence rates compared to the general population (IR per 100 person-years: 152.9, 95% CI 116.8-200.2 (DM); 51.8, 95% CI 37.5-71.5 (PM); 24.9, 95% CI 21.9-28.4 (DPM)). However, five years after diagnosis incidence rates fell to values comparable to the general population.

Conclusion: Our results confirm high rates of cancer diagnosis in patients with DM/ PM/DPM; however, the difference over time as identified in other studies could not be confirmed. Detection bias needs to be considered. Furthermore, means to differentiate into paraneoplastic and non-paraneoplastic DM/PM/DPM could be of great value to distinguish between a detection bias and an actual association between cancer and DM/PM/DPM. Further analysis linking anti-p155/140 antibodies and routine myositis panel to cancer incidence rates, could strengthen the reliability of the results.

P078 | A randomized controlled feasibility trial assessing compliance with sun protection and quality of life in patients with melanoma stage I or II.

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Background: The incidence of melanoma has steadily increased over the past 50 years. The incidence of melanoma in Germany increased from 3 to 21 cases per 100.000 inhabitants between 1970 and 2008. Although it is under discussion, whether patients benefit significantly from sun protection, melanoma patients are advised to strictly follow the sun protection recommendations of the national German guidelines (S3-Guideline Prevention of Skin Cancer). Sun protection refers to behavioural, textile and chemical options that protect the skin from incoming UVA and UVB radiation. The aim is to minimize sunburn and thereby the cumulative damage of epidermal cells by UV-radiation. So far it is unknown to which degree patients not only profit from the physical or chemical effect of sunscreen but also have psychological benefits of its use, such as feeling safer or less anxious while performing daily outdoor activities.

Objectives

To investigate patient's compliance and application frequency with sunscreen, to compare active sun protection with standard sun protection and to analyse its effect on anxiety and quality of life and to prove the feasibility of the trial concept.

Methods: Age 18-75, stage I or II Melanoma patients without mucosal or ocular melanoma, after successful surgery and within the

first or second year of aftercare were eligible. All participants of the randomized controlled feasibility trial, 17 assigned to the intervention and 15 to the control group using block randomisation, completed the HADS-D, DLQI, EQ5D-5L and sun protection related questionnaires at baseline and after three months of follow-up. During the follow-up they filled in a patient diary to track the days and the total number of applications of sunscreen. The intervention group was provided with premeasured sunscreen tubes SPF50 + .

Results: 32/32 questionnaire sets, 30/32 patient diaries and 15/17 sunscreen sets were analysed. The participants were compliant following the study protocol along the 3-month follow-up. No significant differences neither in the number and days of application nor in the quality of life were detected between the intervention and control group. Based on the diary, the intervention group applied sunscreen more frequently than the control group. Patients tend to feel less inhibited in their leisure activities with the use of sun protection. The vast majority of patients in both groups generally find their used sun lotion to be easily applicable and have a pleasant feeling on the skin.

Conclusions: This feasibility trial did not show significant differences between both trial groups. Participant's scores in the QoL questionnaires were comparable to the normal population. Furthermore this trial adds quantifiable information of both frequency and quantity of sunscreen use in melanoma patients and proves feasibility of a patient diary-based, follow-up study concept. A larger, multi-centred follow-up trial is thereby feasible.

P079 | Sports-related skin complaints among physically active students in Germany: online survey

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Background & **Objective:** There is a lack of knowledge about sports-related skin complaints among physically active students and how those affected deal with it. These skin complaints are caused by e.g. allergens, sunlight or friction and can be a significant burden for the persons affected.

The aim of this study was to determine the frequency and characteristics of sports related skin complaints among physically active students and to what extent these complaints influence training or competitions.

Material & **Methods:** We developed an online questionnaire comprising items on frequency, duration and nature of sports activities, perceived stress on the skin, specific skin complaints (e.g. redness, dryness, pruritus), their location their impact on training and performance as well as sociodemographics.

The survey was administered among undergraduate sports students at two German universities (Munich, Magdeburg) and conducted through Unipark. Participation was incentivised by a raffle. We computed counts and percent for categorical variables, and means and 95%-confidence intervals (CIs) for continuous variables. To assess differences, the Pearson's Chi-square-test or student's t-test were used. Analyses were performed using SPSS.

Results: In our survey 259 persons took part (229 at Munich, 30 at Magdeburg). The majority (n = 90) was 20-21 years old (range 17-32). Most of the participants (n = 200) were enrolled in a bachelor's program and studied in the first (n = 66) or second (n = 48) semester (range 1st-13th), 45.7% were female. On average all students performed 6.8 ± 3.9 hours of physical activity per week (range 0.5-21 h). There were 123 (47.5%) participants who had the impression that their skin was specially stressed by sporting activities. 80 participants (30.9%) reported that their skin reacted more sensitively due to the sporting activities. Students mentioning that their skin was specially stressed by sporting activities were significantly more active (7.6 hrs/week, 95%-CI: 6.8-8.3 hrs) than those not affected by stressed skin (5.1 hrs/week, 95%-CI: 5.5-6.7 hrs, $P = 0.005$). No statistically significant differences were found for the statement that the skin reacted more sensitively in relation to the exercise duration per week: 7.5 hrs/week (6.5-8.5 hrs) vs. 6.5 hrs/week (6.0-7.0 hrs).

The most frequent skin complaints among the participants were: Blistering (57.3%), dryness (56.7%), redness (44.7%) and chafing (34%). The most frequent localizations for the affected skin areas were hands and feet (78% each) as well as arms and legs (48.7% each). 75/150 participants stated that an intact skin is important or very important for their physical performance. Whilst only 10% of the participants mentioned to be unsatisfied with their performance in training because of skin complaints, 60% mentioned to take a relieving posture because of the skin complaints. A negative impact on competition performance was only stated by 5%. Duration of exercise was not related to gender, neither was the perception of skin complaints on frequency of training, exercise intensity, perceived performance or relieving posture.

Discussion: To our knowledge this is the first study on skin complaints of physically active sports students in Germany. Almost half of the participants reported that their skin was stressed by their sporting activities, whereby an intact skin was considered to be essential for physical performance. Skin complaints among the participants varied widely but were mostly localised at the extremities. Duration or frequency of the physical activity was related to the skin complaints.

P080 | Incidence and mortality of melanoma proportional to the catchment area of dermatologists in Bavaria

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When melanoma is treated in an early stadium prognosis is very good, while it isn't in metastatic disease. In the last decades incidence of malignant melanoma is continually increasing. Interestingly the mortality stays stable. An explanation might be the better treatment of patients with metastatic disease. Also the frequently examination of the skin, which is available for all people in Germany older than 35 years since 2008, might be a reason for an increasing incidence of melanomas in an early stadium. In Bavaria the availability of dermatologists depends strongly to the region.

In this study, we analysed incidence and mortality of malignant melanoma according to the catchment area of dermatologists, looking at the administrative districts in Bavaria. We also compared the time before an examination of the skin was offered to everybody to the time after 2008. We used the data of the Bavarian cancer registry (Bayerisches Krebsregister) for incidence and mortality. The number of dermatologists per administrative district was provided by the Bavarian Chamber of Physicians (bayerische Landesärztekammer).

We saw substantial variation in melanoma incidence (20.95 to 6) between the administrative districts and also differences in mortality (2.6 to 0.8). An increase of the incidence after 2008 was clearly seen. All over Bavaria incidence changed from 11.9 (2002-2008) to 14.7 (2009-2014). By looking at the catchment area itself, we could distinguish some districts with a big density of dermatologists (16 dermatologists/ 100.000 inhabitants) and districts with a low density of dermatologists (2 dermatologists/ 100.000 inhabitants). A higher incidence of malignant melanoma was seen in regions with more dermatologists but no clear pattern was seen for mortality.

In conclusion, we saw variation in melanoma incidence as well as mortality between districts in Bavaria. The data suggest a relationship between the density of dermatologists and melanoma incidence, but not mortality.

P081 | Anxiety and depression predispose individuals to bullous pemphigoid - A large-scale population-based cohort study

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Background: While the association between bullous pemphigoid (BP) and neurological comorbidities is indisputable, the burden of psychiatric conditions among patients with BP has been poorly investigated. Recent research has denoted the implication of autoimmunity and neuroinflammation as pathoetiologic factors underlying the development of depressive symptoms. The co-expression of BP180 and BP230 both in the skin and the central nervous system suggests crossreactivity between the epithelial and neuronal isoforms of these autoantigens and the production of brain-reactive autoantibodies.

The association of bullous pemphigoid (BP) with anxiety and anxiety-depression comorbidity is yet to be established.

Objective: To evaluate the bidirectional association of BP with anxiety, depression, and anxiety-depression comorbidity, and to delineate the epidemiological features of patients with BP and the aforementioned psychiatric comorbidities.

Methods: A population-based cohort study was performed to assess the risk of anxiety, depression, and anxiety-depression comorbidity among patients with BP (n = 3,924) relative to age-, sex- and ethnicity-matched control subjects (n = 19,280). A case-control design was additionally adopted to estimate the odds of BP in individuals with a preexisting diagnosis of these three psychiatric conditions. Adjusted hazard ratios (HRs) and adjusted odds ratios (ORs) were estimated by Cox regression and logistic regression, respectively.

Results: A history of anxiety (OR, 1.17; 95% CI, 1.04-1.31), depression (OR, 1.26; 95% CI, 1.15-1.38), and anxiety-depression comorbidity (OR, 1.19; 95% CI, 1.04-1.35) was associated with subsequent development of BP. In the cohort study design, patients with BP were found to be at an increased overall risk of depression (HR, 1.17; 95% CI, 1.01-1.35), while female BP patients had an increased risk of depression (HR, 1.19; 95% CI, 1.00-1.42) and anxiety (HR, 1.29; 95% CI, 1.00-1.67). Patients with comorbid BP and depression exhibited a 19% increased all-cause mortality rate (HR, 1.19; 95% CI, 1.08-1.31), whereas patients with BP and anxiety depression comorbidity were less adherent to long-term topical corticosteroid treatment (89.0% vs. 93.8%; $P = 0.001$) and were less frequently managed by adjuvant agents (53.2% vs. 59.1%; $P = 0.045$).

Conclusions: A history of anxiety, depression, and anxiety-depression comorbidity predisposes individuals to BP, whereas patients with BP are at an increased risk of depression. Clinicians managing patients with anxiety and depression should take the increased risk of BP into consideration, and patients with BP should be monitored for depression. Further immunoserological characterization of patients with anxiety and depression may better elucidate the pathomechanism underlying the observed association.

P082 | The influence of complement on the clinical, immunological, and histological features of patients with bullous pemphigoid - Insights from a retrospective cohort study

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Background: Deposits of complement components, of which C3 deposition is of high diagnostic significance, are typically observed in patients with bullous pemphigoid (BP). Beyond the diagnostic importance of complement factors, it is though that complement activation plays an important role in the pathogenesis of BP. While early in the neonatal mouse model of BP activation of complement appeared as a prerequisite for blister induction by anti-BP180 IgG, subsequent in vitro experiments and studies in a humanized and an adult mouse model of BP challenged this conclusion by demonstrating complement-independent mechanisms of subepidermal blister formation. The practical implication of complement deposition in direct immunofluorescence (DIF) microscopy and its influence on the clinical and immunological features of BP is poorly understood.
Objectives: To investigate whether the presence of complement deposition in DIF microscopy gives rise to differences in the morphological, immunological, and histological characteristics of patients with BP.
Methods: A retrospective study encompassing patients diagnosed with BP throughout the years 2009-2019 in a specialized tertiary referral center. Logistic regression model was utilized to identify variables independently associated with complement deposition
Results: The study encompassed 233 patients with BP, of whom 196 (84.1%) demonstrated linear C3 deposition along the dermal-epidermal junction (DEJ) in DIF analysis. BP patients with C3 deposition had higher mean (SD) levels (645.2 [1,418.5] vs. 172.5 [243.9] U/ml; $P < 0.001$) and seropositivity rate (86.3% vs. 64.9%; $P = 0.002$) of anti-BP180 NC16A and less prevalent neutrophilic infiltrate in lesional skin specimens (29.8% vs. 52.4%; $P = 0.041$). C3 deposition was found positively associated with the detection of anti-BP180 NC16A autoantibodies (OR, 4.25; 95% CI, 1.38-13.05) and inversely associated with the presence of neutrophils in lesional skin (OR, 3.03; 95% CI, 1.09-8.33). Compared to patients with deposition of C3 alongside other immunoreactants, those with isolated C3 deposition had lower levels of anti-BP180 (273.6 [388.3] vs. 738.1 [1561.5]; $P = 0.001$) and anti-BP230 (16.7 [20.0] vs. 94.7 [175.4]; $P = 0.007$) IgG autoantibodies and lower frequency of eosinophil-dominant inflammatory infiltrate in lesional skin biopsies (63.2% vs. 83.7%; $P = 0.041$).
Conclusions: Complement deposition influences the immunological and histological features of BP. These findings are in line with experimental data describing the pathogenic role of complement in BP. However, BP also develops in complement negative skin, suggesting the involvement of additional complement-independent mechanisms.

P083 | The risk of Coronavirus disease 2019 (COVID-19) in patients with bullous pemphigoid and pemphigus: A population-based cohort study

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Background: The burden of Coronavirus disease 2019 (COVID-19) in patients with bullous pemphigoid (BP) and pemphigus is yet to be evaluated.
Objective: To assess the risk of COVID-19, and COVID-19-associated hospitalization and mortality in patients with BP and pemphigus, and to delineate determinants of severe COVID-19 illness among these patients.
Methods: A population-based cohort study was performed to compare patients with BP (n = 1,845) and pemphigus (n = 1,236) with their age-, sex- and ethnicity-matched control subjects regarding COVID-19 and its complications.
Results: The risk of COVID-19 (HR, 1.12; 95% CI, 0.72-1.73; $P = 0.691$) and COVID-19-associated hospitalization (HR, 1.58; 95% CI, 0.84-2.98; $P = 0.160$) was comparable between patients with BP and controls, whereas the risk of COVID-19-associated mortality was threefold higher among patients with BP (HR, 2.82; 95% CI, 1.15-6.92; $P = 0.023$). The risk of COVID-19 (HR, 0.81; 95% CI, 0.44-1.49; $P = 0.496$), COVID-19-associated hospitalization (HR, 1.41; 95% CI, 0.53-3.76; $P = 0.499$), and COVID-19-associated mortality (HR, 1.33; 95% CI, 0.15-11.92; $P = 0.789$) was similar in patients with pemphigus and their controls. Systemic corticosteroids and immunosuppressants did not predispose COVID-19-positive BP and pemphigus patients to a more severe illness.
Conclusions: BP patients experience increased COVID-19-associated mortality and should be monitored closely. Maintaining systemic corticosteroids and immunosuppressive adjuvant agents during the pandemic is not associated with worse COVID-19 outcomes.

GENETICS

P084 | Vitamin D status in distinct types of ichthyosis: Importance of genetic subtype and severity of scaling

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Inherited ichthyoses are disorders affecting the epidermal barrier function. The epidermis is the major source of vitamin D production. Data on vitamin D status in European patients with ichthyosis are scarce and mostly do not refer to distinct mutations.

In a prospective monocenter observational study, we determined levels of serum 25-hydroxyvitamin D3 (25-OH-D3) and parathyroid hormone (PTH) in a cohort of 87 deeply characterized ichthyosis patients. Moreover, we evaluated the importance of severity of cornification as a possible risk factor.

The group of epidermolytic ichthyosis showed in total the largest number of patients with deficiency (n = 16) and presented notably low vitamin D3 levels (n = 17; median: 10.5 ng/ml [minimum-maximum: 7.0-21.3]), similar to patients with Harlequin ichthyosis (n = 2; median: 7.0 ng/ml), rare syndromic subtypes and peeling skin disease (n = 3; median: 7.0 ng/ml [7.0-15.0]). Marked reductions in 25-OH-D3 levels were also observed in TG1-proficient lamellar ichthyosis (n = 15; median: 11.9 ng/ml [7.0-30.0]). Patients with TG1-deficient autosomal recessive congenital ichthyosis (ARCI) (n = 12; median: 11.7 ng/ml [7.0-32.6]) and Netherton syndrome (n = 7; median: 10.7 ng/ml [7.0-30.6]) revealed low vitamin D levels, too. We only detected feeble reductions in recessive X-linked ichthyosis (n = 8; median: 13.9 ng/ml [7.0-35.1]) and vitamin D status in ichthyosis vulgaris (n = 10; 19.7 ng/ml [13.3-32.1]) was borderline. Those types of ichthyosis usually show a mild phenotype. Moreover, we could show that the extent of scaling correlated with vitamin D levels (rs = −0.3; P = 0.01) implicating scaling as a strong risk factor. We measured PTH levels of 69 patients. In 12 cases we detected elevated levels associated with low vitamin D values (median: 7 ng/ ml). We conclude that frequent analysis of vitamin D levels should be performed in patients with inherited ichthyoses. We recommend vitamin D supplementation in patients with deficiency or insufficiency to prevent associated symptoms and secondary diseases.

P085 | Development of a non-invasive and non-viral in vivo RNA therapy approach for dystrophic epidermolysis bullosa

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Mutations in the COL7A1 gene lead to malfunction, reduction or complete absence of the type VII collagen protein in the skin's basement membrane zone (BMZ). For epidermolysis bullosa (EB), approximately 800 mutations in COL7A1 have been reported, which lead either to the autosomal dominant (DDEB) or autosomal recessive (RDEB) inherited dystrophic form of EB. DEB is a severe and rare skin blistering disease, associated with a high risk to develop an aggressive form of skin cancer (squamous cell carcinoma, SCC) associated with increased mortality.

In previous studies, an RNA trans-splicing approach has been utilized to correct mutations within COL7A1 via a 3' RNA trans-splicing molecule (3'-RTMS6 m) using a viral vector for cell delivery. Functional correction of the RDEB phenotype has been shown in keratinocytes and skin equivalents in vitro and in a mouse model in vivo. In another study, a murine Col7a1 targeting RTM, carrying a Flag tag, in a nonviral minicircle vector was delivered into mice via gene gun application. Flag staining of the gunned area confirmed accurate RNA trans-splicing and deposition of the reprogrammed protein at the BMZ.

We combine these two approaches, using the engineered 3'-RTMS6 m, to develop a safe, non-viral, non-invasive and efficient in vivo RNA therapy for DEB. Therefore, we cloned the RTM-S6 m into a non-viral Minicircle-GFP vector and analysed its in vitro trans-splicing efficiency in RDEB keratinocytes. As a result, we have detected accurate RNA trans-splicing at mRNA level via qPCR analysis and the restoration of full-length type VII collagen via immunofluorescence (IF) staining at cellular level and via Western blotting at protein level. Further, we complexed the 3'-RTMS6 m with liposomes and delivered the RTM onto generated RDEB skin equivalents. IF staining on cryosections showed a partial restoration of type VII collagen expression at the BMZ. Now, we want to analyse the in vivo efficacy of the RTM in a xenograft mouse model to investigate its clinical potential for a possible in vivo therapy for patients with dystrophic EB.

P086 | Double-nicking-based correction of junctional epidermolysis bullosa via COL17A1 reframing

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COL17A1 encodes the transmembrane protein type XVII collagen, which forms the anchoring filaments needed for the connection between the plasma membrane of basal keratinocytes and the lamina lucida of the basal membrane zone of the skin. Mutations within COL17A1 cause reduced or absent expression of type XVII collagen in junctional epidermolysis bullosa (JEB) patients.

A suitable approach for the correction of disease-associated frameshift mutations is gene reframing using the CRISPR-Cas system. The delivery of a Cas9/sgRNA ribonucleoprotein (RNP) complex to target cells induces DNA double strand breaks (DSBs) at the desired gene locus, which are subsequently repaired by cellular DSB repair mechanisms. Activated end joining pathways can thereby result in the formation of insertions and deletions (indels) at the target site. Approximately 33% of these indels are expected to result in a corrected reading frame. In this study, we extended this approach to Cas9 nickases. Paired Cas9 nickases were recently shown to have a reduced off-target activity without sacrificing the on-target editing efficiency.

Paired nickases were designed to specifically target a homozygous frameshift mutation within COL17A1 in primary JEB keratinocytes. After delivery of the Cas9 nickase pair as RNPs into the target cells, a high gene reframing efficiency was shown on RNA (>30% COL17A1 expression), protein (>40% collagen type XVII expression) and cellular (>45% collagen type XVII expression) levels, as analysed via sqRT-PCR, Western blotting and FACS analysis, respectively. Subsequently, correct membrane localization of restored type XVII collagen was observed in the majority of cells following immunofluorescence analysis of monolayers. Next generation sequencing confirmed a high on-target efficiency accompanied by no detectable off-target events in our paired-nicking COL17A1 reframing approach. Grafting of bioengineered skins, consisting of corrected cells, to immunodeficient mice is underway to assess the deposition of collagen type XVII at the basement membrane zone as well as the restoration of epidermal-dermal adhesion in vivo.

This study demonstrates the development of an ex vivo gene editing therapy for JEB, using CRISPR/Cas9 paired nicking to permanently treat the genetic basis of the disease without the need for single cell expansion or selection for corrected cells.

P087 | Cold urticaria associated with a novel Phospholipase-C-Gamma-2 (PLCG2) mutation

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Introduction: Phospholipase-C-Gamma-2 (PLCG2) regulates various important cellular functions such as apoptosis/cell survival, migration and immune responses. Mutations within PLCG2 are associated with rare dominantly inherited diseases with variable clinical phenotypes including cold urticaria, neutrophilic dermatitis, immunodeficiency and autoimmune features or autoinflammation (PLAID/APLAID). Known mutations in PLCG2 include in frame loss mutations of exon 19 and exon 20-22 and a missense mutation (p.Ser707Tyr).

Methods: We studied four members of a three-generation family, three of whom were affected by cold induced urticarial rash. Genetic analysis of the index patient comprised screening for autoinflammatory periodic fever syndrome genes by Sanger sequencing (NLRP3, NLRP12 and PLCG2) and was followed by segregation analysis of further family members. For detailed analyses of PLCG2, we prepared cDNA, amplified transcript versions and subjected them to Sanger sequencing. Moreover, we assessed the clinical history and routine laboratory markers, immune status, autoantibodies, immunoglobulins, inflammatory markers. In addition, cold contact provocation testing, immunoblotting, B cell proliferation assay and basophil activation test were performed. For further mutation analysis, we transiently transfected COS-7 cells with wild-type or altered PLCG2 (ΔExon 19 or ΔExon 18-19) constructs and determined inositol phosphate (IP) formation at different temperatures (ranging from 37 to 27°C).

Results: All three affected family members presented with cold-induced pruritic urticarial rash since birth. Segregation analyses indicated an autosomal-dominant inheritance. Antihistamine treatment partially reduced the skin symptoms. However, direct cold contact provocation testing was negative. Laboratory analyses revealed elevated levels of serum S100A8/9 and IgE, whereas serum levels of IgM were decreased. Genetic analyses of the index patient showed a heterozygous c.2054 + 5G>T variant in PLCG2. Transcriptional analysis revealed two additional splice variants indicating a single

(exon 19) and double (exons 18 and 19) exon skipping. Western Blot analysis of PLCG2 from PBMCs showed similar protein expression as compared to controls, suggesting a functional alteration. Basophil activation testing showed lower rates of activated basophils in an affected patient as compared to control subject. The examination of B cell proliferation was not different.

Transient transfection of altered PLCG2 constructs in COS-7 cells showed increased IP formation in comparison to wild-type, with a maximum at 31°C.

Conclusion: We identified a novel mutation variant in PLCG2 associated with a phenotype of cold-induced urticarial rash and immune dysregulation. Transcriptional analysis indicates the generation of two alternative splice variants due to exon skipping. These deletions are located within a region encoding autoinhibitory domains and results in PLCG2 signaling abnormality.

P088 | A novel NLRP1 mutation causes different phenotypes in siblings

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Objectives: The aim of the present study was to analyze the consequences of a novel NLRP1 mutation resulting in the amino acid substitution p.Leu813Pro in the LRR domain, in two siblings with different phenotypes: the younger sister (P1) had features of multiple self-healing palmoplantar carcinoma while the older (P2) had manifestations of familial keratosis lichenoides chronica.

Methods: RT-PCR was performed to analyze the expression of IL1A, IL1B, IL6 and IL18 in two siblings' affected skin. The keratinocytes isolated from a biopsy of P2 (P2K) were lost during culture. Fibroblasts isolated from the biopsy of P2 (P2F) and normal human fibroblasts (NHF) were cultured with DMEM. The expression of IL1A, IL1B, IL6, IL17A, IL18, TNFA, TGFB was analyzed by qPCR. Besides, P2F and NHF were cultured without FCS for 48 hours. The medium was collected and added to normal human keratinocytes (NHK) for6, 9 or 12 hours, respectively. Subsequently the expression of the above-mentioned genes was detected by RT-PCR. Finally, P2F and NHF were treated with IL-6 (50 ng/ml) for 30 min and 60 min and STAT3 phosphorylation was analyzed by Western blotting.

Results: We found increased expression of IL1B and IL6 in P1 as compared with P2 or the normal control, which is in agreement with the severe inflamed lesions in P1. Expression of IL1A, IL1B, IL18, and TGFB was increased in P2F compared with NHF while the expression of IL-6 decreased in P2F. The mRNA levels of IL1B, IL6, IL18, and TNFA in NHK cultured with P2F's medium were higher compared with the treatment with NHF's medium at6, 9, 12 h, respectively, with time-dependent effect. After the treatment with IL-6, pSTAT3 was higher in P2F compared with that in NHF at 30 min and at 60 min, respectively. pSTAT3 in P2F at 30 min was significant higher compared with that at 0 min or at 60 min.

Conclusions: We demonstrate that this novel NLRP1 mutation increased activation of the NLRP1 inflammasome, resulting in the increase of IL1B and IL18 mRNA levels, more prominent in the severely affected patient. Mutated keratinocytes with activated inflammasome underwent apoptosis in culture, as described before. Mutated fibroblasts responded to IL-6 treatment by enhanced STAT3 phosphorylation, and secreted soluble factors, which induced increased expression of cytokines in keratinocytes. Our data support the involvement of inflammation in the new syndromes associated with NLRP1 mutations. To the best of our knowledge, this is the first report of a mutation that leads to both multiple self-healing palmoplantar carcinoma and keratosis lichenoides chronica.

P089 | Development of a pathogenesis-based therapy for peeling skin syndrome (PSS type 1)

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Peeling skin syndrome (PSS type 1) is a rare and severe autosomal recessive form of congenital ichthyosis. It severely impairs the quality of life, and therapeutic approaches are totally unsatisfactory. The disease is due to nonsense mutations or complete deletion of the CDSN gene encoding for corneodesmosin (CDSN).

We wanted to develop the first steps toward a specific protein replacement therapy for CDSN deficiency. The aim is to restore the lack of CDSN and improve cell-cell cohesion in the transition area of the stratum granulosum (SG) to stratum corneum.

Human CDSN was recombinantly expressed in E. coli. A liposome-based carrier system, prepared with a cationic lipopeptide to mediate the transport to the outer membrane of keratinocytes, was developed. The liposomal carrier system was characterised with respect to size, stability and toxicity. Furthermore, the interaction with primary keratinocytes and human epidermal equivalents was investigated.

The liposomes showed an accumulation at the membranes of keratinocytes. CDSN deficient epidermal equivalents that were treated with liposomal encapsulated CDSN demonstrated presence of CDSN in the SG. Finally, the penetration assay and histological examinations revealed an improved epidermal integrity for CDSN deficient epidermal equivalents, if they were treated with liposomal encapsulated CDSN.

The study presents the first preclinical in vitro experiments for a future specific protein replacement therapy for patients suffering from

Peeling skin syndrome (PSS type 1), but topical CDSN replacement could become interesting for broader patient groups: Netherton syndrome, which is characterised by a secondary CDSN-deficiency, or atopic dermatitis, in which a reduced protein expression of CDSN has been reported. These disorders would likewise benefit from topical CDSN substitution.

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P090 | Next-generation sequencing reveals druggable targets in advanced melanoma besides BRAF/MEK inhibition

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Despite new therapy options such as immune checkpoint or BRAF/MEK inhibitors, numerous melanoma patients do not permanently benefit from these therapies due to primary or secondary resistance. For this reason, comprehensive genetic profiling of advanced melanoma has become part of routine care. We evaluated the results of 82 patients with advanced melanoma who received comprehensive panel sequencing. The cohort was composed of cutaneous (n = 42), acral-lentiginous (n = 14), mucosal (n = 9) and uveal melanoma (n = 8), as well as the melanoma of unknown primary (n = 9). Sequencing of tumor samples identified a total number of 1,650 somatic variants (SNVs and INDELs) and 2,137 somatic copy number alterations. It resulted in a median number of 10 SNVs per patient and a median TMB of 5.82.

The cohort included 23 BRAF-, 20 NRAS-, 5 NF1-mutated melanomas and 36 triple wild-type tumors. We observed simultaneously occurring hotspot mutations in the genes BRAF (V600E) and NRAS (Q61K and R, respectively) in two patients. Based on the Cancer Genome Interpreter annotation, we found a potential druggable target in 95% (78 out of 82) of the patients. In three-quarters of the patients (63 of 82, 77%), we found a potential drug target in the RTK/RAS signaling cascade, mainly in the genes BRAF/NRAS and NF1. Beside the inhibition of BRAF and NRAS, mutations in NF1 causing the loss of its inhibitory function could be partially compensated by an mTOR blockade, possibly in combination with a BRAF/MEK inhibitor.

In 45% of the patients (37 of 82) we found activating mutations in receptor tyrosine kinases (RTKs). And in 38% of the patients (32 of 82), this activation could be inhibited either by pan-tyrosine kinase inhibitors (against KIT, PDGFRA, ALK, MET), small molecule inhibitors (anti-EGFR, ERBB2, MET, FGFR1/3, AR), or monoclonal antibodies (anti-EGFR, ERBB2). Furthermore, the PI3K pathway contained targetable mutations (PTEN, PIK3CA, PIK3R1) in about a quarter of the patients that could be blocked by PI3K or AKT inhibitors. The activating/inactivating mutations in the genes TSC1, RICTOR, and FLCN, could probably be treated with mTOR inhibitors. In over 60% of the patients, the cell cycle control was deregulated. And in nearly half of the patients (49%), these mutations represent a potential target for CDK4/6 or aurora kinase inhibitors (CDK4/6, CNND1/2, and CDKN2A). The DNA damage repair pathway (DDR) was mutated in one-fifth of the patients. A possible treatment in these cases is therapy with PARP inhibitors or platinum-based chemotherapy. This therapy option would be particularly suitable for patients (12%) with inactivating mutations in the genes ATM, CHEK2, FANCA, FANCC and BRCA2. In patients with uveal melanoma, mutations in either the gene GNA11 (63%, 5 of 8) or GNAQ (37%, 3 of 8) were observed. Both belong to the family of guanine nucleotide-binding proteins (G proteins), functioning as transducers of transmembrane signaling. MEK inhibitors can theoretically inhibit their activation.

Here we present a workflow to integrate genetic tumor profiles into clinical routine. We showed that our approach enables identifying genetic driver alterations (SNVs and CNVs), which could also point to potential off-label label treatment options. This off-label uses have to be approved by molecular tumor boards and should be performed in controlled basked or umbrella trials.

Health Services Research

P091 | Scientific evidence management system: Proof of concept for COVID-19

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Rapid acceleration in new coronavirus and scarcely sourced data makes it difficult for researchers and decision makers to keep up with the flood of information. COVID-19 literature is doubling every 20 days and clinical trial data every 30 days. This poses the biggest challenge in terms of increase of scientific literature ever seen. At the same time, it is tremendously important for researchers and decision makers to be updated on pandemic data.

Several labor-intensive efforts such as Covid-19 TrialsTracker University of Oxford, dashboard of clinical trials from COVID-19

McMaster University programme (https:// covidtrials.org) are underway to bring together clinical trial registries on studies of COVID-19 and track them in real time. These trackers are helpful tools for an overview of COVID-19 clinical trials in real time. But they still do not address researchers' and decision makers' needs for tailored information on clinical trials and their related publications.

Manual categorization and annotation is tedious and hard to maintain and scale up. Based on these circumstances, using artificial intelligence (AI) to mine data became the ideal way to find the most relevant studies for researchers. The latest scientific findings, from clinical trials to publication and pre-publications, as well as verified knowledge on coronavirus disease are gathering, linking and compiling in an AI-driven database on a daily basis. The database is structured and annotated by utilizing biomedical concepts and natural language processing techniques.

As a result of this study, we developed a tool powered by natural language processing (NLP) technology and a COVID-specific terminology, which understands detailed concepts of research in the field of COVID-19 and provides researchers with the most relevant scientific evidence tailored data in real time. Since the system is fully automated and empowered by AI, the system can also be used for any kind of dermatology research field by customizing databases to collect relevant data. To validate the system performance of risklick.ch, several complex queries also ran on clinicaltrial.gov for clinical trials and PubMed advance search for publications. The results were compared with the fuzzy matching technique and were validated by independent experts. In all cases the Risklick system showed superiority in terms of precision and recalls.

P092 | Effects of the COVID-19 pandemic on care of melanoma patients in Berlin: the Mela-COVID survey

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Background: The COVID-19 pandemic imposes major challenges for care of cancer patients. On the one hand, cancer patients may be more prone to severe SARS-CoV-2 infections. On the other, delay of diagnosis or treatment of cancer due to concentration of resources on patients with SARS-CoV-2 infections or due to fear of COVID-19 may contribute to progression and unfavorable prognosis of cancer.

Objective: Our aim was to assess the effects of the pandemic on treatment and appointments of patients with malignant melanoma from the Vivantes Skin Cancer Center in Berlin, Germany and to identify reasons for and determinants of changes.

Methods: We conducted a postal survey with questions on impairment by the pandemic, fear of COVID-19 and melanoma, changes in therapy and/or appointments and reasons therefore immediately after the first COVID-19 wave in Germany. Determinants of postponed/missed appointments were examined with descriptive analyses and logistic regression.

Results: The response rate was 41.3% (n = 324, 57.4% males, mean age 67.9 years). Most participants suffered from melanoma AJCC 2017 stage I (48.5%), followed by stage II (20.1%), stage III (17.6%) and stage IV (13.9%). Treatment changes related to the pandemic occurred in 4 participants. Forty-eight (14.8%) postponed or missed appointments, most frequently, at their own request (81.3%) due to fear of SARS-CoV-2 infections (68.8%). Participants were most afraid of SARS-CoV-2 infections from other patients (54.2% of 72 answers), followed by infections during transport (25.0%) and infections transmitted by medical staff (20.9%). Current treatment was associated with a reduced chance of postponing/missing appointments (OR = 0.208, $P = 0.003$), whereas much or very much concern about COVID-19 (OR = 6.806, $P = 0.034$; OR = 10.097, $P = 0.038$), SARS-CoV-2 infections among close acquaintances (OR = 4.251, $P = 0.026$), anxiety disorder (OR = 5.465, $P = 0.016$) and AJCC stage IV (OR = 3.108, $P = 0.048$) were associated with a higher likelihood. By contrast, advanced age and other comorbidities did not significantly influence the chance of missing or keeping appointments.

Conclusion: Among our participants, treatment changes were rare and the proportion of missed/delayed appointments was rather small. Main reasons for delays/ cancellations of appointments were anxiety and concern about COVID-19.

Immunology

P093 | Salt generates anti-inflammatory Th17 cells but amplifies pathogenicity in proinflammatory cytokine microenvironments

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T helper cells integrate signals from their microenvironment to acquire distinct specialization programs for efficient clearance of diverse pathogens or for immunotolerance. Ionic signals have recently been demonstrated to affect T cell polarization and function. Sodium chloride (NaCl) was proposed to accumulate in peripheral tissues upon dietary intake and to promote autoimmunity via the Th17 cell axis. Here, we demonstrate that high NaCl conditions induced a stable, pathogen-specific, anti-inflammatory Th17 cell fate in human T cells in vitro. The p38/MAPK pathway, involving NFAT5 and SGK1,

regulated FoxP3 and interleukin (IL)-17A-expression in high-NaCl conditions. The NaCl-induced acquisition of an antiinflammatory Th17 cell fate was confirmed in vivo in an experimental autoimmune encephalomyelitis (EAE) mouse model, which demonstrated strongly reduced disease symptoms upon transfer of T cells polarized in high NaCl conditions. However, NaCl was coopted to promote murine and human Th17 cell pathogenicity, if T cell stimulation occurred in a pro-inflammatory and TGF-b-low cytokine microenvironment. Taken together, our findings reveal a context-dependent, dichotomous role for NaCl in shaping Th17 cell pathogenicity. NaCl might therefore prove beneficial for the treatment of chronic inflammatory diseases in combination with cytokine-blocking drugs.

P094 (OP02/01) | Immune complexes induce a patrolling response in non-classical monocytes

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Immunoglobulins are of central importance in immune defense. Bivalent Fab domains confer recognition of autoantigens, tumor antigens or microbial pathogens forming immune complexes (ICs), whereas the Fc domains mediate a wide range of effector functions by engaging Fc receptors. Previously, we defined human slan-monocytes (slanMo), a population of non-classical monocytes (CD16 + CD14-), as having a unique capacity to bind and handle immune complexes (ICs) and demonstrated their role in Lupus erythematosus. Studying the cellular response of slanMo engaging immobilized ICs we now observed the induction of a hitherto unrecognized migratory response limited to slanMo and non-classical monocytes. Consequently, we also defined the molecular requirements and characteristics of this migratory response including biophysical aspects of ligand engagement and ligand disengagement. Live imaging combined with automated cell tracking identified non-classical monocytes and slanMo in contrast to classical monocytes (CD14 + CD16-) and intermediate monocytes (CD14 + CD16 +) to engage in a distinct migratory function. This migration was characterized by paths of pronounced directional persistence and decreased migration speed compared to migration on other substrates promoting adhesion, e.g. RGD peptides. To identify minimal and optimal IgG ligand densities inducing migration of slanMo, we used precisely defined nano-patterns of immune complexes of defined size with densities tunable by more than one order of magnitude from 145 to 7000/μm2. Therefore, matrices with different spacings (4-75 nm) of gold nanoparticles were designed and decorated with human IgG. Cellular adhesion and migration occurred only at high densities of ICs on matrices with spacings < 50 nm. The initial spreading and migration was inhibited when slanMo were treated with an FcγRIII (CD16) blocking antibody. In general, forward migration of cells requires rear detachment with defined mechanisms of disassembling ligand engagements. IC interaction at the matrix surface may be terminated by cellular uptake of ICs. Another possibility would be the extracellular cleavage of FcγRIII engaging with the immobilized ICs. We found slanMo to perform both mechanisms. First, we identified retraction fibers with large FcγRIII-containing aggregates at the trailing edge of the cells, a process also called migracytosis which describes a cell migration-dependent mechanism for releasing cellular contents. Moreover, we found that also shedding of FcγRIII takes place. FcγRIII is shed by the activation of ADAM17 as previously reported by us and others. The highly ADAM17-specific inhibition by the monoclonal antibody (clone D1(A12)) allowed for slanMo to normally engage ICs; however, the forward migratory response was inhibited.

The capacity of ICs recruiting slanMo from the blood flow and inducing a specific migratory behavior of non-classical monocytes as demonstrated here, indicates a potentially specific role of this distinct monocyte subset in mediating early IC dependent immune responses. Understanding molecular mechanisms of migration at sites of IC deposition will help to get a deeper insight into IgG mediated immune responses in health and disease as well as into the role of non-classical monocytes in homeostatic conditions and immune surveillance in the vasculature. As the described migratory response required intact FcγRIII and ADAM17, these molecules may serve as therapeutic targets.

P095 | C-type lectin receptors sense a Malassezia allergen and its human homologue, a damage-associated molecular pattern molecule

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Skin microbiota-derived antigens have been proposed to act as potent trigger factors in atopic dermatitis. In this study, we demonstrate that a major allergen from the skin-colonizing yeast Malassezia sympodialis, Mala s 13, is recognized by the pattern-recognition receptors Dectin-1 and Dectin-2 on myeloid cells. Mala s 13 is evolutionary highly conserved and its human paralogue, thioredoxin, is a well-described damage-associated molecular pattern (DAMP) and crossreactive autoallergen.

Stimulation of human monocyte-derived dendritic cells or macrophages with Mala s 13 or hTrx was followed by rapid internalization. In vitro assays revealed remarkable secretion of IL-1β and IL-23. Inhibition of receptor-binding confirmed that these proinflammatory cytokine responses were mediated to substantial extent via Dectin-1 and/or Dectin-2. Direct interaction of Mala s 13 and hTrx with Dectin-1 as well as Dectin-2 could also be observed on a fusion-protein screening platform. Further on, we demonstrate that Syk kinase is involved in intracellular downstream signaling.
We hypothesize that the resulting cytokine response may promote a Th2/Th17-polarizing milieu. Our findings strengthen the hypothesis that microbial antigens as well as DAMPs can influence antigen-presenting cells via C-type lectin receptors and thereby mediate skin inflammation and the process of allergic sensitization.

P096 | A deficiency in receptors for short chain fatty acids impairs the activity of regulatory T cells

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There is evidence for an association between psoriasis and a dysregulation of the microbiome both of the gut and the skin. The altered skin microbiota in psoriasis which includes an abundance of Firmicutes, Actinobacteria, Proteobacteria and Bacteroidetes exerts immunomodulatory effects, in particular reduction of the activity of regulatory T cells (Treg). Gut and skin microbiota mediate part of these effects via production of short chain fatty acids (SCFA). SCFA exert their effects via binding to G-protein coupled receptors (GPR). We recently observed that expression of GPR43 and GPR109a (HCA2) are significantly reduced in the skin of psoriatic patients. Furthermore, there is increasing evidence that Treg are impaired in their capacity to control the inflammatory responses in psoriasis. By using 16s sequencing we detected higher abundance of Firmicutes and Actinobacteria and other alterations of the microbiota in the skin of HCA2 KO mice. Thus we asked whether the deficiency of HCA2 influences the suppressive activity of Treg. To address this issue, Treg were isolated from lymph nodes and spleens of wild-type (WT) and HCA2 KO mice. Using FACS analysis we found a significantly reduced expression of Foxp3 on Treg from HCA2 KO mice. Furthermore, the expression of IL-10 which plays a crucial role in the development and the suppressive activity of Treg was remarkably reduced in HCA2 KO Treg. Accordingly, Treg isolated from TNCB sensitized HCA2 KO mice exerted weaker suppressive activity upon adoptive transfer experiments in comparison to Treg obtained from WT mice. Together this suggests that expression of HCA2 is required for Treg to develop their full suppressive activity. Since HCA2 is a major receptor for SCFA, this may indirectly indicate that SCFA are involved in the development and function of Treg.

P097 | Injection of immunocytes into mice with psoriatic skin grafts induces lesion flare up, but no systemic disease

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Psoriasis is one of the most common, chronic T cell-mediated inflammatory skin diseases; comorbidity includes affection of joints and/or the cardiovascular system. It is known that keratinocytes and lymphocytes play an important role in the pathogenesis of psoriasis. While a variety of different treatments exist, the pathogenesis is not yet fully understood. Mouse models have proven essential to fill this gap. In the present study, we aimed to establish and further develop a humanized psoriasis model consisting of human skin grafts and PBMC transfer into NOD-Scid (no T/B cells) and NOD-Scid gammac-/- mice (lacking T/B & NK cells). Non-lesional and lesional skin from psoriatic patients were transplanted onto murine back skin, and 12 weeks after xenotransplantation, 1-3x10E6 PBMC from psoriatic skin donors were activated by stimulation with IL-2 and Staphylococcal enterotoxin B (SEB) and transferred intradermally into skin grafts of immunodeficient mice. By studying Ki67-expression by immunostaining, we found a strong proliferative activity of epidermal cells not only in lesional skin, but also in non-lesional grafted skin; in addition, upon PBMC transfer, a large number of Ki67 + cells were also found in the dermis indicating infiltrating human leukocytes/lymphocytes. At the end of the experiment (week 15), an increased epidermal expression of cytokeratin (CK) 16, an important marker of psoriasis, in lesional and non-lesional skin was found, independent of the mouse strain. CK16 expression was stronger in lesional skin and upon PBMC transfer in all samples. In contrast, filaggrin expression (downmodulated in psoriasis compared to healthy skin) was barely detectable in PBMC-transferred skin, whereas the majority of samples harbored filaggrin+ cells both in lesional and non-lesional skin without PBMC. These findings indicate that characteristic psoriatic changes were induced in the human skin transplants upon PBMC transfer into lesional, but also - to a lesser degree - into non-lesional skin. However, clinical symptoms, such as hyperkeratosis or erythema, were barely visible. In addition, systemic signs of disease (e.g. alteration of survival curves, joint affection) were not detected in any of the mice. All in all, the present psoriatic skin transplant model may represent a valuable tool to investigate the contribution of various immunocytes to the psoriasis phenotype; an assessment of disease modification by e.g. treatments may require additional optimization of the model.

P098 | Immunological patterns and therapeutic consequences of chronic inflammatory skin diseases

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Chronic non-communicable inflammatory skin diseases (ncISD) are a heterogeneous group of skin conditions with hundreds of diagnoses currently known. Aside from the two most common entities, atopic dermatitis and psoriasis, there are several other diseases with little knowledge on pathogenesis or potential treatments. Clinical trials to develop medication for these rare conditions are scarce. Patients have to rely on off-label treatments, often based on weak evidence for efficacy.

According to our preliminary data and published work on effects of T cells on keratinocytes, our group established the hypothesis that non-communicable inflammatory and autoimmune skin disorders can be subdivided into five main immune response patterns: Pattern 1/lichenoid, Pattern 2a/eczematous, Pattern 2b/ bullous, Pattern 3/ psoriatic, Pattern 4a/fibrogenic, Pattern 4b/granulomatous, Pattern 5/autoinflammation.

To investigate these patterns in more detail, bulk RNASeq data from 287 patients (punch biopsies from lesional and non-lesional skin) affected by 13 different chronic inflammatory skin diseases (psoriasis (n = 90), atopic eczema (n = 48), nummular eczema (n = 52), lichen ruber (n = 30), cutaneous lymphoma (n = 20), lupus (n = 11), Pityriasis rubra pilaris (n = 8), guttate psoriasis (n = 7), psoriasis pustulosa (n = 5), dishidrotic eczema (n = 5), hyperkeratotic-rhagadiform eczema (n = 4), cutaneous side effect of biologics (n = 4), parapsoriasis (n = 3)) have been analyzed. Certain genes showed to be suitable for classifying different dermatoses into the mentioned patterns. We identified genes associated with cytotoxicity for pattern1, allergic inflammation/parasite defense for pattern2, antimicrobial peptides for pattern 3 and anti-inflammatory/pro-fibrotic activity for pattern 4. Specific primers for these genes were designed and PCRs were conducted in extracted mRNA from punch biopsy samples of patients suffering from different ncISD. Previously, deep histological and clinical characterization of patients and samples have been conducted.

We showed that several ncISD can be classified into specific immune response patterns according to their gene signature. All diseases within one pattern potentially respond to the specific treatment of the pattern. As there are drugs for treating some of these patterns, this opens a therapeutic door for patients suffering from rare inflammatory skin diseases.

The aim for the future will be to establish a classifier to distinguish between the immune response patterns. Furthermore, therapy response data will be included in future analysis to define therapy-related patterns and predict therapy response in patients with ncISD.

P099 | PAD4-mediated pathways contributes to dermal fibrosis via fibroblast activation

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Background: Inflammatory cells of myeloid and lymphoid origin play an important role in tissue fibrosis. A variety of diseases with fibrotic outcome are preceded or accompanied by inflammatory infiltrates. Neutrophiles via the release of extracellular traps or NETs are known to drive inflammation through recruitment of monocytes. One important protein is PAD4 which catalyses histone deamination and is therefore essential in NET formation.

Methods: By daily intradermal injection of hypochlorine acid (HOCl) dermal fibrosis was induced, resulting in cellular infiltration (flow analysis) and collagen accumulation (Goldner trichrome histology) after 4 weeks. We analysed inflammation and fibrosis in mice lacking PAD4 after 1 and 4 weeks of treatment with HOCl.

Results: Treatment of WT mice with HOCl induced an inflammatory response after 1 week of treatment with prominent monocytic infiltration and a subsequent march towards massive fibroblast activation after 4 weeks of treatment. We also observed a pronounced accumulation of mature Ly6G+ MHCII+ neutrophils compared to WT mice. Also monocytic infiltration and differentiation of macrophages did not differ between WT and PAD4 KO mice in week 1 or 4 of the treatment, we observed a significant reduction of dermal fibrosis after 4 weeks in mice lacking PAD4. When we analysed the fibroblasts isolated from PAD4 KO mice we found CD31-CD90-CD24- CD29 + CD140a+ fibroblast expressed equal amounts of vimentin and less MPO (Myeloperoxidase). PAD4 KO fibroblast were hypoproliferative (Ki67) compared to WT fibroblasts.

Conclusion: We conclude that pathways mediated by PAD4 contribute to fibrosis development by direct activation of dermal fibroblasts.

P100 (OP04/02) | High-dose immunoglobulins induce T regulatory (Treg) cells which downregulate desmoglein 3-specific IgG antibodies in a human leukocyte antigen-transgenic mouse model of pemphigus vulgaris

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Our group has established a human leukocyte antigen (HLA)-transgenic mouse model of pemphigus vulgaris (PV) using the HLA-haplotype (HLA-DRB1*04:02-DQ8) that is highly prevalent in PV patients. Immunization with recombinant human desmoglein 3 (Dsg3) protein leads to the HLA-DRB1*04:02-restricted activation of Dsg3-reactive CD4 + T lymphocytes and subsequently to the production of pathogenic Dsg3-specific IgG antibodies (ab). High-dose immunoglobulins (IVIg) are successfully used in the treatment of severe or recalcitrant PV leading to significant reduction of circulating autoab. However, the mode of action of IVIg in PV is still not completely understood. The aim of this study was therefore to investigate the immunological effects of IVIg treatment in a prevention preclinical model of PV. HLA transgenic mice (n = 8 per group) were immunized with recombinant human Dsg3 (day 0 and day 14) for the induction of a Dsg3-specific CD4 + T-cell and B-cell response and were treated with IVIg (2 g/kg body weight) intraperitoneally once a week over a 4-week treatment period. Control mice received phosphate buffered saline (PBS) instead of IVIg. Blood was taken weekly for the analysis of circulating anti-Dsg3-IgG by human Dsg3 enzyme-linked immune assay (ELISA) and mice were sacrificed at different time points during treatment. T- and B-cell subsets, including T regulatory cells (CD4 + CD25 + FoxP3 + Treg) and Dsg3-reactive interferon-gamma (IFN-ã) producing Type I T cells were identified among spleen and lymph node-resident cells by flow cytometry and enzyme linked immunospot (ELISpot) assay, respectively.

Dsg3 immunized mice showed a robust anti-Dsg3-IgG response which is detectable after the second Dsg3-immunization (day 14) and up to at least day 70 post-primary immunization. Dsg3-specific antigen stimulation ex-vivo resulted in the identification of proliferating (3H-Tdr) and IFNã-producing T cells. Treatment with IVIg resulted in a significant reduction of Dsg3-specific T cells at days 21, 35 and 70 post-immunization. Dsg3-specific splenic Treg cells were significantly induced by concomitant IVIg treatment compared to control at days 21 and 35, while cell proliferation and IFNã secretion from splenocytes were reduced. Furthermore, compared to control, IVIG treated mice initially showed a significant reduction of marginal B cells, known for their pathogenic potential in autoimmune diseases as they play a unique role in response to antigens delivered to the marginal zone area.

This effect was apparent in the lymph nodes as well as in the spleen and disappeared by d70.

Our study strongly suggests that IVIg treatment induced a Treg cell-dependent immunomodulatory effect on the humoral immune response to Dsg3 in the preclinical model of PV. This prevention model provides a unique in vivo system to further investigate the IVIg-induced anti-inflammatory effects in PV and will be further characterized to test its full potential in a therapeutic setting.

P101 | PPAR-gamma promotes proliferation of pathogenic Th2 cells through regulation of IL-2 signaling

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Recently, a subset of allergen-specific Th2 cells has been identified and termed “pathogenic” Th2 (pTh2) cells, based on their crucial role in mediating type-2-mediated immunopathology. pTh2 cells secrete high levels of IL-13, IL-5, and IL-9, and express high levels of the ligand-activated transcription factor peroxisome proliferator activated receptor gamma (PPAR-g). The functional role of PPAR-g for pTh2 cells, however, remains incompletely understood.

Here, we analyzed the effect of PPAR-g inhibition on basic T cell functions such as IL-2- or T cell receptor (TCR)-induced proliferation in pTh2 cells. Strikingly, PPAR-g inhibition strongly reduced IL-2-induced proliferation, but not TCR-induced proliferation, suggesting specific control of cytokine signaling events by PPAR-g.

To investigate the underlying mechanisms, we performed transcriptomic analysis of T cell clones treated with GW9662, a chemical inhibitor of PPAR-g. Pathway analysis revealed that the IL-2 signaling pathway is strongly affected by PPARg inhibition, in line with our observation from the proliferation assay. To assess the impact of PPAR-g inhibition on IL-2 signaling, we systematically measured its impact on phosphorylation of signal transducer and activator of transcription (pSTAT) molecules. Cells treated with GW9662 showed a significantly reduced phosphorylation of STAT3 and STAT5, while phosphorylation of STAT6 remained unaffected.

Together, our findings suggest that PPAR-g is a positive regulator of the IL-2 signaling pathway in pTh2 cells. Since IL-2 is crucial for T cell proliferation and survival, PPAR-g might provide a selective advantage for pTh2 over conventional Th cells under conditions of limited IL-2 availability in the tissue.

These findings further highlight the potential of PPAR-g as therapeutic target in type 2 immunopathology.

P102 | Profiling of tumor-infiltrating T cells for guidance of melanoma salvage therapy in immune-checkpoint-blockade resistant patients

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Successful anti-CTLA-4 and anti-PD1/PD-L1 immune-checkpoint-blocking (ICB) therapy depends on the reactivation of cytotoxic CD8 + T cells, killing tumor cells upon recognition of cognate tumor antigens. However, additional inhibitory receptors (TIGIT, LAG3, TIM3) on CD8 + T cells can dampen their anti-tumor activity and contribute to therapy resistance. Since antibodies targeting TIGIT, LAG3, or TIM3 immune checkpoints have recently been translated or are on the way into clinic, we assume that profiling of functional TIL phenotypes from anti-CTLA-4/anti-PD1/PD-L1 resistant patients could guide rational clinical decision in salvage therapy.

In the current project, we investigated the phenotype of patient-derived CD8 + tumor infiltrating lymphocytes (TILs) using multi-color flow cytometry. Within the 24 patient samples analyzed, PD1 (44.3%) and TIGIT (42.6%) were most frequently expressed on CD8 + TILs followed by TIM3 (18.7%) and LAG3 (18.0%). CD8 + TILs from ICB therapy-resistant lesions showed a heterogeneous co-expression of PD1 with other inhibitory receptors. To determine the functional impact of different immune checkpoints on the anti-tumor activity of CD8 + TILs we set up autologous TIL/tumor models. CD8 + TILs were cultured in the absence or presence of either an antibody cocktail targeting all immune checkpoints (PD1, TIGIT, LAG3, TIM3) or antibodies directed towards single immune checkpoint. Notably, the proliferation of CD8 + TILs with severely exhausted phenotype (PD1 + TIGIT+LAG3 + TIM3 +) could be significantly enhanced upon repeated stimulation with autologous tumor cells in the presence of ICB antibody cocktail (anti-PD1/TIGIT/TIM3/LAG3). Even low numbers of tumor reactive CD8 + TILs from immune-cell poor tumors could be efficiently expanded by autologous tumor cells in the presence of the ICB antibody cocktail. Furthermore, we demonstrated that single anti-TIGIT treatment was superior and achieved effects similar to the ICB antibody cocktail in different patient models.

Taken together, our study on functional TIL phenotypes in tumor lesions provides a strategy for identifying salvage therapy targets to achieve long-lasting patient benefit.

P103 | Inhibitory effects of baricitinib on a human skin model of atopic dermatitis

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Introduction: Atopic dermatitis (AD) is a common inflammatory and pruritic skin disease characterized by complex cytokine signaling involving cross-talk between keratinocytes (KCs), neurons, immune cells, and inflammatory mediators. Baricitinib is a Janus kinase (JAK)1/JAK2 inhibitor in Phase 3 clinical development for AD.

Objective: We sought to decipher the role of JAK1/2 signaling with baricitinib using a 3-dimensional (3D) model possessing AD-like pathology.

Materials and **Methods:** Human skin equivalents were processed by MatTek (Ashland, MA) using neonatal human foreskin KCs overlaid on a collagen matrix embedded with fibroblasts. Skin cultures raised to an air-liquid interface were maintained for 3 days either in medium alone or in medium containing a cytokine cocktail with interleukins (ILs) known to be elevated in lesional AD skin (IL-4, -13, -31). Cultures were treated with or without baricitinib (150 nM). Skin samples were processed for immunohistology and filaggrin expression, with RNA isolated and analyzed using microarrays.

Results: Treatment with this cytokine cocktail induced histopathological alterations classically observed in eczematous lesions such as diminished granular cell layer and increased spongiosis accompanied by filaggrin immunostaining reduction. Gene expression analyses focusing on the epithelial differentiation complex revealed similarities between the AD 3D model and lesional tissue. Adding baricitinib to the cocktail reduced the AD-like pathology induced in the skin model.

Conclusions: These data indicate that it is possible to create a physiologically relevant 3D model resembling AD pathology using a combination of different cytokines. This 3D model provides an opportunity to further investigate the potential role of different cytokines and inflammatory pathways in AD. These data support that JAK1/2 signaling contributes to the cytokine-induced pathology of AD, with baricitinib treatment reducing pathological alterations, providing a path forward for further defining the mechanism of action of baricitinib in AD.

P104 | Absence of functional Tregs in scurfy mice leads to blister inducing autoantibody directed against Type VII Collagen

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Dysfunction of regulatory T cells (Tregs) contributes to the development of different autoimmune diseases. Scurfy mice have a missense mutation in the transcription factor foxp3 which leads to the absence of functional Tregs. This results in the uncontrolled development of autoreactive CD4 + T cells. We have previously shown that sera of scurfy mice contain high titers of autoantibodies with reactivity against both desmosomal and hemidesmosomal structural proteins of the skin. Furthermore subepidermal blister formation in scurfy mice indicates the development of autoimmune blistering diseases (AIBDs) in the absence of functional Tregs.

Previously, we generated hybridomas of spontaneously activated B cells from scurfy mice with subepidermal blistering. One of these autoantibodies (H510) showed linear staining at the dermal side of the basal membrane zone by indirect immunofluorescence (IIF) on murine salt-split skin.

Western blot analysis (WB) identified the murine von-Willebrand-factor A like domain 2 (mvWFA2) of type VII collagen (Col7) as the target antigen. By using skin from Col7 knock-out mice in comparison to wild-type skin, Col7 was confirmed as the antigen of H510 by WB and IIF. To investigate the pathogenicity of H510, this autoantibody was s.c. injected into neonatal wild-type mice followed by histological examination of the skin. H510 caused subepidermal blisters in 75% of mice indicating the pathogenicity of the antibody as well as its' relevance for the development of AIBD.

In summary, we show that in the absence of functional Tregs a pathogenic autoantibody with reactivity against Col7 develops. Moreover, the injection of this IgG1 anti-Col7-antibody causes blister formation and thus represents a novel mouse model for epidermolysis bullosa acquisita that is based on the transfer of antibodies rather than xenogeneic (i.e., rabbit) autoantibodies.

P105 | Low levels of IgA in chronic spontaneous urticaria are associated with low levels of IgE and autoimmunity

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Introduction: Type IIb autoimmune chronic spontaneous urticaria (CSU) is an immunologically distinct subtype of CSU associated with severe disease and poor response to treatment with antihistamines and omalizumab. Low total IgE levels have been linked to type IIb autoimmune CSU, whereas the role of IgA in CSU is still unknown.

Methods: We analyzed data of 606 patients with CSU by dividing them into four groups based on their IgA and IgE levels: i) low IgA (≤0.7 g/l) and low IgE levels (≤40kU/l), ii) low IgA and normal or elevated IgE levels, iii) low IgE and normal or elevated IgA levels and iv) normal or elevated IgA and IgE levels. The patient groups were compared for their spectrum of symptoms, disease activity, autoimmunity, specifically concomitant autoimmune diseases and autoantibodies, and routine laboratory markers. Several features of type IIb autoimmune CSU were assessed including basophil activation test (BAT) and autologous serum skin test (ASST). Moreover, anti-thyroid peroxidase (TPO)-IgE was measured.

Results: Of 606 patients with CSU 4.8% (n = 29) had low IgA and 37.5% (n = 227) had low IgE levels. Of the patients with low IgA 82.8% (n = 24) also had low IgE levels and in the total cohort IgA and IgE levels correlated significantly ($r = 0.316$, $P < 0.001$). Patients with low IgA and/or IgE showed a higher prevalence of concomitant autoimmunity ($P < 0.001$). A positive BAT ($P < 0.001$), a positive ASST ($P = 0.008$), as well as double positive results in both tests ($P < 0.001$) were also more present in these patients. IgA and IgE levels correlated with basophil (IgA: $r = 0.161$, $P < 0.001$; IgE: $r = 0.245$, $P < 0.001$) and eosinophil counts (IgA: $r = 0.134$, $P = 0.001$; IgE: $r = 0.300$, $P < 0.001$). Patients with elevated anti-TPO-IgE levels had lower IgA ($P = 0.007$) and IgE levels ($P = 0.001$).

Discussion: Our results show that reduced levels of IgA in CSU are linked to low IgE levels and features of type IIb autoimmune CSU. The pathomechanisms underlying the decrease in immunoglobulin levels and their interaction in CSU are still not well understood. However, our findings encourage to screen CSU patients for serum IgA and IgE levels and to further assess their role as diagnostic or therapeutic biomarker.

P106 | How do neutrophil extracellular traps contribute to enhanced Staphylococcus aureus skin colonization?

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Introduction: Staphylococcus aureus causes life-threatening infections and about 20-30% of the human population is colonized mostly in the nose. It is already known, that a disrupted skin barrier leads to rapid infiltration of neutrophils which results in enhanced S.aureus colonization. Previous experiments of our group showed, that the enhanced S.aureus skin colonization is mediated by the interaction of neutrophil extracellular traps (NETs) with keratinocytes. However, the exact mechanism how NETs contribute to enhanced S. aureus skin colonization is not yet understood.
Objectives: In this work we investigated the timing of NET formation after coincubation with keratinocytes and S. aureus infection as well as the signaling pathways induced in keratinocytes by NETs that might play a role in the colonizing enhancing effect.
Material and **Methods:** Using an in vitro co-culture model with human primary keratinocytes and neutrophils, we analyzed how long neutrophils and keratinocytes must be pre-incubated prior to S. aureus infection to see a colonizing enhancing effect using colony-formation units (CFU) assays. We analysed the formation of neutrophil extracellular traps by SYTOX Green staining and live cell imaging. Furthermore, the induction of pro-inflammatory cytokines was analysed via ELISA and activated signaling pathways were elucidated by western blot and blockade studies.
Results: We show that with extended co-culture incubation neutrophil were able to form more NETs. Additionally, the enhanced NET formation correlates with increased S. aureus colonization. A significant colonizing-enhancing effect was observed after 4 h of co-incubation; however, the effect increased further with longer incubation time. During the co-incubation, keratinocytes released increasing amounts of CXCL8. Moreover, we saw an increased induction of pro-inflammatory cytokines (IL-33, CXCL8 and IL-1β) in keratinocytes upon S.aureus infection co-cultivated with neutrophils or neutrophil extracellular traps which is dependent on NFKB and TLR2/ TLR4 signaling. Stimulation of keratinocytes with the induced pro-inflammatory cytokines also resulted in enhanced S.aureus colonization suggesting that NETs induce an inflammatory state in keratinocytes.
Conclusion: Our data suggest that during the co-incubation of neutrophils with keratinocytes neutrophils are alarmed and ready to form neutrophil extracellular traps after S. aureus infection. Neutrophil extracellular traps are able to induce proinflammatory cytokines in keratinocytes, which contribute to enhanced S.aureus skin colonization.

P107 | Staphylococcus aureus membrane vesicles participate in S. aureus skin colonization by activating innate immune responses and neutrophil recruitment

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Introduction: The human skin is constantly exposed to pathogens but is only rarely colonized by them. *Staphylococcus aureus* is a pathogenic bacterium that causes human infections like mild skin lesions up to invasive, life-threatening infections. *S. aureus* produces membrane vesicles (MVs), which can contribute to skin inflammation. The membrane vesicles consist of a bilayered membrane in which proteins, polysaccharides, nucleic acids, and lipids are encapsulated. Objectives: The aim of this work was to investigate the role of MVs of *S. aureus* and skin commensals in *S. aureus* skin colonization and the induction of pro-inflammatory cytokines. Materials and **Methods:** We used a protocol for the efficient isolation of MVs from Gram-positive bacteria using size-fractionation and enrichment by a MV-precipitation reagent (ExoQuickTC). To quantify the amount of released lipids we used the specific fluorescent membrane dye FM4-64, a dye that fluoresces upon incorporation into a membrane lipid environment. Cytokine induction in primary human keratinocytes was analyzed using ELISA or LEGENDPlex analysis. In addition, an established co-culture system of keratinocytes and neutrophils was taken for the analysis of neutrophil migration and neutrophil extracellular traps formation.
Results: We demonstrate that skin commensals can secrete MVs in equal amounts and with nearly the same lipid content as *S. aureus*. Furthermore, we show that pretreatment of primary human keratinocytes or human skin explants with *S. aureus* MVs results in an induction of pro-inflammatory cytokines comparable to treatment with living *S. aureus*, which is dependent on NFκb signaling and partially on TLR-2 signaling. Interestingly, the MVs of skin commensals show a protective effect on *S. aureus* skin colonization. Furthermore, we show that *S. aureus* MVs are itself able to recruit neutrophils and can lead to the induction of neutrophil extracellular traps in neutrophils. Interestingly, *S. aureus* strains isolated from the lesional or non-lesional skin of atopic dermatitis patients showed a differential induction of pro-inflammatory cytokines in keratinocytes and a lower ability to recruit neutrophils, both correlated with a lower lipid content of the MVs.
Conclusion: These data suggest that released MVs of commensal and pathogenic staphylococci play itself an important role in the inflammatory skin response and the modulation of *S. aureus* skin colonization.

P108 | ARG1 + IL10 + polymorphonuclear myeloid-derived suppressor cells are elevated in patients with active pemphigus and correlate with an increased Th2 response

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Myeloid-derived suppressor cells (MDSCs) are a heterogeneous population of immature myeloid cells, which are characterized by their capability to suppress T cell responses. While MDSCs have been traditionally associated with cancer diseases, their role as regulators in autoimmune diseases is rapidly emerging. Pemphigus is a chronic autoimmune blistering skin disease characterized by dysregulated T cell responses and autoantibody production. The role of MDSCs in pemphigus disease has not been defined yet. The aim of this study was to characterize MDSCs in pemphigus patients and to dissect their relationship with CD4 + T cell subsets and clinical disease assessments. For this purpose, we performed a cross-sectional analysis of 20 patients with pemphigus. Our results indicate that a population of CD66b+CD11b+ polymorphonuclear-like MDSCs (PMN-MDSCs) is expanded in the peripheral blood mononuclear cell fraction of pemphigus patients compared to age-matched healthy donors. These PMN-MDSCs have the capability of suppressing allogeneic T cell proliferation in vitro and show increased expression of characteristic effector molecules such as arginase I and Interleukin-10. We further demonstrate that PMN-MDSCs are especially expanded in patients with chronic active pemphigus, but not in patients in remission. Moreover, MDSC frequencies correlate with an increased Th2/Th1 cell ratio. In conclusion, the identification of a functional MDSC population in pemphigus suggests a possible role of these cells as regulators of Th cell responses in pemphigus.

P109 | Characterization of T cell - Melanoma Interactions for an in vitro Melanoma Skin Model

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Immunotherapy has greatly improved the outcome of advanced melanoma but a substantial number patients still has limited benefit from this approach. The development of new immunotherapeutics is highly needed but comes at the cost of excessive animal testing. To date, in vitro systems for the testing of new immunotherapeutics are lacking but hold promise to reduce animal testing. Our aim is the creation of an allogeneic and autologous in vitro melanoma skin model with infiltrating T cells to test new immunotherapeutics and reduce animal models for preclinical testing. Therefore, we evaluated T cells for their interaction with melanoma cells to introduce them into a melanoma skin model.

We first investigated the recognition of melanoma cell lines by allogeneic T cells using human CD3 + T cells isolated from healthy donors and coculturing them with different melanoma cell lines. We found that naïve CD3 + T cells were only scarcely capable of inducing melanoma cell death, independently of melanoma mutational status. Pre-activation of CD3 + T cells, however, profoundly increased melanoma cell killing. The presence of PD-1 inhibitor nivolumab in the cocultures had no effect on T cell-mediated killing, proving that PD-1 inhibition has no effect in in vitro 2D T cell - melanoma cocultures.

In Immunotherapy, recognition of tumor antigens is a crucial step in the development of anti-tumor immunity. In this perspective, we created CD8 + T cells from healthy donors transduced with antigen-specific T cell receptors targeting the common melanoma antigens gp100 and MART-1. These receptors are functional since the IFN gamma is produced by the transduced T cells upon exposure to melanoma cells presenting the cognate antigens. In a next step, the T cell receptor transduced T cells will be introduced in the melanoma skin model alongside several melanoma cell lines to investigate antigen-specific T cell activation and to evaluate established and new immunotherapeutics. In future studies, we want to establish autologous melanoma skin models with expanded tumor infiltrating lymphocytes and autologous melanoma cell lines to test immunotherapeutics without animal experiments.

P110 (OP02/02) | Delineating the pathogenetic relevance of autoreactive T cells against desmoglein 3 in pemphigus vulgaris

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Pemphigus vulgaris (PV) is a potentially lethal autoimmune disorder of mucous membranes and skin and is associated with pathogenic

IgG autoantibodies against desmoglein 3 (Dsg3), a desmosomal adhesion molecule. As T cells are critical for B cell help leading to anti-Dsg3 IgG production, we employed suitable in vitro assays to further characterize T cell responses against Dsg3. A total of 72 PV patients and 43 age- and sex-matched healthy controls (HC) were included in this study. By ELISpot assay, frequencies of type 1 (IFN-gamma), type 2 (IL 5), and regulatory (Treg; IL-10) cells reactive with Dsg3 were studied. Moreover, utilizing HLA class II-Dsg3 peptide dextramers in 20 PV patients, we sought to identify autoreactive T cells specific for four immunodominant Dsg3 peptides (p1, p2, p3, p4) residing in distinct extracellular (EC) domains of Dsg3 in context of HLA-DRB1*04:02. By ELISpot assay, autoreactive Dsg3-specific type 2 T cells, but not type 1 or Treg cells were significantly elevated in PV patients (n = 47) compared to HC (n = 30; $P = 0.019$). By dextramer staining, a majority of Dsg3-specific T cells from PV patients were specific for at least one of the four immunodominant Dsg3 epitopes. Of note, HLA class II-matched HC also showed T cells specific for the same set of Dsg3 peptides. In PV patients, frequencies of T cells reactive with Dsg3-p4 residing in the EC3 domain of Dsg3 were increased in comparison to HC ($P = 0.0263$) and were preserved in patients off therapy ($P = 0.0294$). Of note, Dsg3-p4-reactive T cell frequencies correlated with serum anti-Dsg3 IgG levels ($r = 0.693$; $P = 0.023$) and immunization of HLA-DRB1*04:02-transgenic mice with Dsg3-p4 induced pathogenic IgG antibodies as determined by keratinocyte dissociation assay. Of note, using the same PV mouse model induction of T cell responses against Dsg3-p1 residing in the EC2 domain of Dsg3 induced IgG antibodies with the highest pathogenicity. As T cell reactivity against Dsg3-p1 in PV patients was directly correlated with T cell reactivity against peptides Dsg3-p2 ($r = 0.647$; $P = 0.038$) and Dsg3-p3 ($r = 0.968$; $P = 0.001$) all residing in the EC2 domain, autoreactive T cell responses against the Dsg3 ectodomain are rather polyclonal and may act synergistically in inducing pathogenic IgG autoantibodies. Our findings strongly support that type 2 T cell responses against distinct HLA class II-restricted epitopes of the Dsg3 ectodomain promote pathogenic IgG autoantibody production and further underline the critical role of autoreactive T cells in the pathogenesis of PV.

P111 | Targeted inhibition of complement at the basement-membrane zone in bullous pemphigoid

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Bullous pemphigoid (BP) is a blistering skin disease in which autoantibodies against basal keratinocyte antigens cause loss of cell adhesion to the basement membrane zone (BMZ). Biopsies usually show IgG and C3 at the BMZ in direct immunofluorescence (IF) microscopy, indicating complement activation that results in attraction and continuous activation of inflammatory cells and, ultimately, dermal-epidermal separation. We cloned more than 20 anti-BP180-NC16A single chain variable fragment (scFv) monoclonal antibodies (mAbs) from the IgG and IgE repertoires from two active BP patients using antibody phage display. All mAb clones obtained were recombinantly expressed and validated by BP180-NC16A ELISA and indirect IF. Unexpectedly, our scFvs displaced bound patient IgG from the immobilized antigen in vitro. Confirming previous data, monovalent anti-BP180-scFvs tested were non-pathogenic when injected into human skin organ culture, because for pathogenicity bivalent IgG abs with cross-linking and complement-binding properties are required. As a proof-of-principle study we then exploited this non-pathogenicity observed by designing proteins that allow targeted complement inhibition at the BMZ: Fusing an anti-BP180-NC16A scFv with a C1s-inhibiting compound domain we were able to test for targeted inhibition of the classical complement activation pathway at the BMZ, in an BP ex vivo model. This recombinant molecule bound to normal human skin sections pre-incubated with BP sera and efficiently inhibited C3 fixation as shown by IF microscopy. This finding was then independently confirmed by decreased C5a anaphylatoxin levels in corresponding supernatants from the same experiments, as detected by ELISA. Another fusion protein designed for targeted inhibition of the alternative pathway at the BMZ was not effective in our model, illustrating the dependence on the classical complement activation pathway in human BP. Because BP180-NC16A is expressed at the BMZ of skin, mucous membranes and the retina, this innovative approach may be translated to other complement-dependent diseases affecting these tissues, e.g., mucous membrane pemphigoid, epidermolysis bullosa acquisita, or age-related macular degeneration. Alternatively, the compound domain may be exchanged for other pharmaceutically active drugs, allowing for targeted delivery to the BMZ in a broad range of other diseases of the skin.

P112 | Autocrine IL-9/IL-9Rα signaling induces a pathogenic phenotype in Th2 cells

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IL-9 is a common gamma-chain cytokine, for which a range of pleiotropic functions have been proposed. However, an overarching role in humans remains elusive. IL-9 and its receptor, IL-9Rα, are specifically expressed by pathogenic Th2 cells (pTh2) residing in the skin, suggesting an important function of autocrine IL-9 signals in cutaneous immunity and allergy. Yet, the regulation of IL-9Rα expression on pTh2 cells and the auto- and paracrine functions of IL-9 remain incompletely understood.

Here, we confirmed that IL-9Rα is strongly enriched in CRTh2 + memory Th2 cells isolated from blood and skin. Since previous data showed that these cells are associated with the expression of the transcription factor PPAR-γ, we hypothesized that PPAR-γ controls IL-9Rα expression. Indeed, we found that PPAR-γ inhibition downregulates the expression of IL-9Rα at the RNA as well as the protein level in Th2 clones.

To decipher the autocrine function of IL-9 on Th cells, we isolated human Th cells from blister fluid of acute atopic contact dermatitis (aACD), expressing high levels of IL-9Rα. Transcriptional profiling of these cells in presence and absence of recombinant IL-9 showed that approx. 800 genes are differentially expressed in response to IL-9. Pathway analysis indicated that the upregulated genes are associated with conventional Th2 immune response. Strikingly, we observed a strong induction of genes specifically associated with the pathogenic Th2 phenotype, such as IL9, IL17RB and HPGDS.

In summary, we discovered that PPAR-γ - a transcription factor closely linked to the pathogenic Th2 phenotype - regulates IL-9Rα expression and that autocrine IL-9 signals promote pathogenic features of Th2 cells. Together, our data provide a functional explanation for the consistently observed coexpression of PPARG, IL9, and IL9R in single cell transcriptomic data and suggest that Th2 cells might induce their pathogenic phenotype through autocrine IL-9 signaling.

P113 | Immune signatures of treatment response and immune-related adverse events in melanoma patients under checkpoint inhibitor therapy

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A substantial number of stage IV melanoma patients does not benefit from currently available immune-checkpoint inhibitor (ICI) treatment. Moreover, patients often experience adverse events. Therefore, it is of high clinical relevance to identify predictive markers of clinical response, treatment failure and immune-related adverse events (irAEs). To identify potential biomarkers, we initiated a pilot immune monitoring study in stage IV melanoma patients using flow cytometric analysis of peripheral blood mononuclear cells (PBMC). Here, we report about the first 10 patients. Patients were treated with either nivolumab (every two weeks) or pembrolizumab (every three weeks) alone or with a combination of nivolumab and ipilimumab every three weeks. Treatment responders (n = 6) as compared to nonresponders (n = 4) were characterized by enhanced PD-1 expression on CD3 + T cells immediately before treatment (median 40.0 + /- 7.2% vs 19.8 + /-13.8%). Interestingly, responders showed a higher T cell responsiveness after T cell receptor stimulation with anti-CD3/anti-CD28 antibodies as determined by re-induced PD-1 expression on CD3 + T cells. The percentage of CD8 + effector memory (CD8 + CD45RACD45RO+ CCR7-) T cells was higher in responders as compared to non-responders immediately after the first and before the second cycle of treatment (median 37.8 + /- 5.7% vs 26.1 + /- 8.3%; median 45.0 + /- 8.8% vs 30.3 + /- 12.9%). In contrast, the percentage of activated (HLA-DR+CD38 +) NK cells was higher in non-responders compared to responders immediately after the first cycle of treatment (median 7.0 + /-1.7% vs 2.2 + /- 0.9%). Immune-related adverse events (irAE) were accompanied by a lower percentage of CD4 + effector (CD4 + CD45RA+CD45RO-CCR7-) T cells after three months of treatment (median 1.9 + /- 0.6% vs 4.8 + /- 1.5%) and by a higher percentage of activated CD4 + (CD4 + CD38 + HLADR+) T cells before the second treatment cycle (median 15.6 + /- 6.3% vs 5.3 + /- 1.6%). In summary, immune monitoring of ICI treatment in melanoma using flow cytometric measurement of PBMC appears to be a promising approach to identify early markers of treatment response and resistance and irAEs.

P114 | Investigating pemphigus vulgaris pathogenesis using a human skin organ culture model

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Pemphigus vulgaris (PV) is an autoimmune skin blistering disease. Autoantibodies (IgG) form against desmoglein (Dsg) 1 and 3 and induce acantholysis leading to intraepidermal split formation of the skin and / or mucous membranes. The current treatment options are not considered satisfactory as they consist mostly of systemic corticosteroid application. To improve treatment options, the underlying pathogenesis causing the loss of cell adhesion needs to be better understood. Therefore, comprehensive insights into anti-Dsg 1 / 3-induced signalling in the course of split formation are needed.

To address this, a human skin organ culture model was employed and intraepidermal split formation, induced by injecting an anti-Dsg 1 / 3 single-chain variable fragment (scFv), was observed over the course of 24 hours (h). Immunofluorescence stainings showed a honeycomb-like binding pattern of the scFv, as well as co-localisation with the main target structures of PV autoantibodies, Dsg 1 and 3. Additionally, skin sections were analysed by semi-quantitative histomorphometry of Haematoxylin and Eosin-stainings. First blisters could be observed already 5 h after scFv injection while the first significant increase in split formation was reached after 7 h.

Further, we performed whole proteome analyses of the scFv treated skin from the time-course experiments, aiming to identify novel candidate proteins that might be involved in PV pathogenesis. The overlap of proteins and peptides between different skin donors suggested comparability between different samples. Clustering of unique, quantifiable proteins showed a cluster of upregulated proteins at 5 - 7 h after injection of the scFv and confirmed the role of previously described proteins that are suspected to be involved in the pathomechanisms of PV such as kallikrein and epidermal growth factor. Since increased proteolytic substrate processing is associated with inflammatory processes in the skin, whole proteome analysis of scFv treated skin was complemented by terminal amine isotopic labelling of substrates (TAILS). TAILS allows for the identification and quantification of proteolytic substrates by enriching for protease-generated N-terminal peptides. N-terminal enrichment by TAILS confirmed increased proteolytic substrate processing of several PV associated proteins, as well as novel candidates, at the on-set of split formation at 5 h.

The identified proteins and proteolytic peptides, identified after incubation of the anti-Dsg 1 / 3 scFv, could potentially act as target structures for future treatment options.

P115 | Inhibition of phosphodiesterase-4 reverses anti-laminin 332 IgG mediated signal transducing events and functional alterations in vitro

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Autoantibodies (aab) against BP180 (type XVII collagen) or laminin 332 and a predominant mucosal involvement are major characteristics of the autoimmune blistering disease mucous membrane pemphigoid (MMP). Until now, only little data are available about the pathophysiological mechanisms of MMP. To explore the very early events of aab-mediated tissue destruction, i.e. the phase immediately after the aab bind to their respective target protein, cultured murine keratinocytes and fibroblasts were treated with anti-murine laminin alpha 3 (anti-mLAMα3) IgG. Both keratinocytes and fibroblasts released significantly more IL-6 ($P < 0.05$) and CXCL2 ($P < 0.01$) upon incubation with anti-mLAMα3 IgG compared to normal rabbit (NR) IgG in a dose-dependent manner. The release of IL-6 and CXCL2 from keratinocytes, but not from fibroblasts, could be dose-dependently and significantly reduced by the phosphodiesterase-4 inhibitor roflumilast. Roflumilast has previously been shown to prevent the development of oral, but not skin, lesions in experimental murine anti-laminin 332 MMP. Furthermore, anti-mLAMα3 IgG treatment of keratinocytes significantly increased spreading of cells compared to normal rabbit (NR) IgG, as determined by in vitro scratch assay. Roflumilast decreased the migration speed of keratinocytes induced by anti-mLAMα3 IgG to levels of NR IgG-treated cells, while no effect of roflumilast was observed on keratinocyte migration or proliferation without IgG treatment. Our data indicate a specific signaling cascade and altered keratinocyte migration induced by the binding of anti-mLAMα3 IgG. Future transcriptome analysis and proteome profiling in this model will help to uncover the anti-mLAMα3 IgG-mediated mechanisms and effect of phosphodiesterase-4 inhibition. This information may be helpful to specifically inhibit the skin and mucosal inflammation in MMP.

P116 (OP02/04) | Identifying hidden drivers of heterogeneous inflammatory skin diseases

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Chronic inflammatory diseases of the cardiovascular system, brain, gut, joints, skin and lung are characterized by complex interactions between genetic predisposition and tissue-specific immune responses. This heterogeneity complicates diagnoses and the ability to exploit omics approaches to improve disease management, develop more effective therapeutics, and apply precision medicine.

Using skin inflammation as a model, we developed an analysis pipeline (AuGER) that assigns deep clinical phenotyping information including clinical picture, histological architecture, laboratory parameters and information on the patients' history to transcriptome data of lesional and non-lesional skin (564 samples) to identify biologically relevant gene signatures. AuGER revealed multiple previously unknown hub genes for biologically relevant processes such as neutrophil biology in tissue, disturbed epithelial architecture, or symptoms such as pruritus. In particular, CCAAT Enhancer-Binding Protein Beta (CEBPB) was found as a master regulator of neutrophil biology in the skin, and Pituitary Tumor-Transforming 2 (PTTG2) was identified as a hub gene for acanthosis. Both CEBPB and PTTG2 were validated using genetically modified human skin equivalents, migration assays, and in situ imaging. Thus, by combining deep clinical phenotyping and omics data with sophisticated biocomputational

algorithms we identify hidden drivers of clinically relevant biological processes within omics datasets.

P117 | Processing of Leishmania parasites by human dendritic cell

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Leishmania are intracellular parasites that cause various human diseases ranging from self-healing cutaneous to fatal visceral leishmaniasis. The host cells are phagocytes, primarily macrophages, where the parasites neutralize innate immune defenses, proliferate and finally lyse the cells. Despite that, Leishmania induce vigorous T cell responses, which require antigen presentation and stimulation by phagocytes, importantly dendritic cells. So far it is not clear how to align the blockade of phagocyte functions with the efficient immune stimulation. We found that, in contrast to other phagocytes, dendritic cells destroy the parasites through what appears to be an apoptotic process in acidified compartments that express components of the MHC class I and II antigen processing pathways. The infection leads to enhanced activation of dendritic cells triggered by inflammatory cytokines, and efficient induction of CD4 + and CD8 + T cell responses including IFNf production. Our findings suggest the control of Leishmania by dendritic cells with implications for a preventive vaccine.

P118 | Diagnosis of epidermolysis bullosa acquisita: Comparison of different assays for serum anti-type VII collagen reactivity

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Epidermolysis bullosa acquisita (EBA) is a rare autoimmune bullous skin disease characterized by autoantibodies against type VII collagen (Col7). EBA is diagnosed by direct immunofluorescence microscopy (IF) of a perilesional biopsy and/or by serology. This study aimed at comparing the sensitivity and specificity of various diagnostic assays for the detection of serum anti-Col7 IgG. In total, sera from patients with EBA from Europe and Japan (n = 101), pemphigus vulgaris (n = 40), bullous pemphigoid (n = 99), anti-p200 pemphigoid (n = 65) and anti-laminin 332 mucous membrane pemphigoid (n = 6) were analyzed by six assays: 1) Col7 NC1/2 ELISA (MBL, Nagoya, Japan), 2) Col7 NC1 ELISA (Euroimmun, Lübeck, Germany), 3) indirect IF test based on the expression of the recombinant NC1 domain in a human cell line (NC1 BIOCHIP mosaic; Euroimmun), 4) full-length-Col7 ELISA, 5) immunoblotting (IB) with the full-length Col7 from human dermis extracts, 6) and IB with recombinant Col7 NC1. EBA patients with positive direct IF or/and IgG reactivity in at least one of the Col7-specific assays were included. IB with recombinant NC1 revealed a sensitivity of 93.1% and a specificity of 100% followed by the NC1 BIOCHIP mosaic (sensitivity, 89.1%; specificity, 100%), IB with human dermis (sensitivity, 87.1%; specificity, 100%), NC1-ELISA (sensitivity, of 82.2%; specificity, 98.6%), NC1/NC2 ELISA (sensitivity, 88.1%; specificity, 93.3%), and fulllength Col7 ELISA (sensitivity, 80.2%; specificity, 93.8%). Our data demonstrate that the detection of serum anti-Col7 IgG in EBA patients shows considerable variation among recently reported test systems and usually requires confirmation by visualization of tissue-bound antibodies.

P119 | Aldehyde dehydrogenase inhibition does not resolve skin and mucosal lesions in experimental laminin-332 mucous membrane pemphigoid

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Mucous membrane pemphigoid (MMP) is a rare autoimmune blistering disorder primarily affecting mucous membranes lining the oral cavity and conjunctivae. The skin may also be affected in about 25% of patients. MMP lesions in the larynx, trachea, esophagus, skin, and particularly, in the conjunctivae tend to heal with scarring, which can lead to strictures and irreversible blindness. Effective treatment options for MMP are currently unavailable and therapies mainly rely on the use of high-dose corticosteroids in combination with potentially corticosteroid-sparing agents such as azathioprine, mycophenolate mofetil, cyclophosphamide, or intravenous immunoglobulin. Aldehyde dehydrogenase family 1 (ALDH1) has been implicated in immune-mediated mucosal scarring and ALDH1 inhibition proposed as an antifibrotic therapy option. Previously, ALDH1 inhibition led to reduced fibrosis in a mouse model of scarring allergic eye disease. Here, we evaluated the ALDH1 inhibitor disulfiram in a recently established antibody transfer mouse model of anti-laminin 332 MMP using IgG directed against the laminin alpha 3 chain. C57BL6/J mice (n = 7/group) were treated prophylactically with p.o. 200 mg/kg disulfiram once daily or the respective solvent over a period of 12 days. Disulfiram- vs vehicle-treated mice did not show any improvement in the severity of oral mucosal lesions. In addition, no significant changes in affected body surface area were observed

in both treatment groups, though disulfiram-treated mice showed a trend towards improvements in clinical skin scores (P = 0.0521). Furthermore, the local use of disulfiram to improve conjunctival lesions was evaluated. Disulfiram was dissolved in a concentration of 300 μM in 2% (w/v) methocel and administered to the diseased mice (n = 7/group) daily from Day 8 to Day 20 in the form of eye drops. On Day8, the mice had an average affected surface area of the palpebral conjunctiva of approximately 20%. Over the treatment period of 12 days, no improvement in the conjunctival lesions was observed as a result of the topical treatment in both treatment and control groups. No reduction in fibrosis was seen, as assessed by immunohistochemistry. Taken together, ALDH inhibition failed to improve clinical and histological signs in a mouse model of antilaminin-332 MMP suggesting that immunopathological pathways in diseases with a propensity for scarring are different.

P120 | The impact of high fat diet on contact hypersensitivity

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Allergic contact dermatitis (ACD) is an inflammatory skin disease caused by low molecular weight chemicals or metal ions like cobalt and nickel. ACD affects approximately 20% of the Western population and is abundant as an occupational disease. The mouse model for ACD is contact hypersensitivity (CHS). We used 2,4,6-trinitro-1-chlorobenzene (TNCB) as the contact allergen. Previously, we have shown that mice lacking Toll-like receptor 2 (TLR2) and TLR4 (TLR2/4 mice) are resistant to CHS. Interestingly, these mice become susceptible to CHS at an age of about 16 weeks (our unpublished data). Obesity affects an increasing part of the Western population and is known to have a pro-inflammatory impact on the organism. As older mice gain weight and show increased body fat compared to young mice, we hypothesized that the increase in adipose tissue contributes to the loss of resistance to CHS due to its association with inflammation.

The aim of our work was to investigate the effect of high fat diet on the sensitization and the elicitation of CHS. To address this, wild-type (wt) or TLR2/4-deficient mice were fed normal chow, control diet or high fat diet for four weeks prior to sensitization. Only a small effect of high fat diet on the bodyweight, the leptin levels, the size of the fat pads and the amount of fat in the liver was found. Despite this, feeding the high fat diet increased the reaction of wt mice to TNCB and broke the tolerance of TLR2/4 mice to TNCB while the normal chow-fed mice were resistant to CHS induction. Unexpectedly, the control diet-fed mice showed a similar response to TNCB like high fat diet-fed mice. Using flow cytometry, antigen-specific in vitro T cell re-stimulation and ELISA, we showed an increased ear swelling, a pro-inflammatory infiltrate into the ears and an antigen-specific T cell response in the local lymph nodes (LNs) in high fat diet and control diet fed mice upon TNCB treatment. These results suggest that the increased fat content or the fatty acid composition of the diet is a risk factor for the development of CHS.

In further studies we will address the question whether a certain fatty acid or a combination of fatty acids are responsible for the break of the resistance of the TLR2/4 mice to CHS. Furthermore, we want to investigate whether the effect of high fat diet and control diet are reversible.

P121 | Low invasive microbiopsies are sufficient to perform immune-based molecular classification of inflammatory skin diseases

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Eczema and psoriasis are two of the most common chronic inflammatory skin diseases. A correct diagnosis is essential for a successful therapy. Clinical and histological evaluations are still the gold standards for diagnostic decisions, but in some cases, it is difficult to differentiate between both diseases as they often reveal similar characteristics. Therefore, several molecular classifier (MC) were developed to predict the right diagnosis with high sensitivity and specificity based on the gene expression of NOS2, CCL27 or IL36G. So far, MC were only tested on 4 - 6 mm skin biopsies ("macrobiopsies") or paraffin-embedded tissue samples. Microbiopsies of 1 mm size are a new possibility to collect tissue samples less invasive and painful than conventional biopsies. Additionally, suture or local anaesthesia are no longer required, so that valuable time in the clinical routine can be saved. However, it is unclear if tissue samples collected by microbiopsies can be used to perform MC based on NOS2 and CCL27 gene expression. Therefore, the comparison of the molecular signature of macro- and microbiopsies from the same patients diagnosed with eczema or psoriasis was the aim of the study. A total of 51 Patients were included: 27 clinically and histologically confirmed eczema, 19 psoriasis and 5 mixed phenotypes. Gene expression of NOS2, CCL27 and IL36G was detected by qRT-PCR. Finally, NOS2 and CCL27 values were utilized to construct the MC. We found that relative gene expression of NOS2, CCL27 and IL36G were almost equal between macro- and microbiopsies (mean values: NOS2macro = 14.1; NOS2micro = 14.4; CCL27macro = 0.7; CCL27micro = 0.8; IL36Gmacro = 39.5; IL36Gmicro = 46.0) (P > 0.05). We observed an overexpression of NOS2 and IL36G in psoriasis to eczema for macro- as for microbiopsies (log2FC: NOS2macro = 5.7; NOS2micro = 4.6, IL36Gmacro = 2; IL36Gmicro = 2.2), while CCL27 was decreased in psoriasis (log2FC: CCL27macro = -2; CCL27micro = -2.6). 6 micro- and 2 macrobiopsies were classified as eczema, while the clinical picture was consistent with psoriasis. In the eczema cohort, 1 microbiopsy was classified as

psoriasis and all macrobiopsies were classified as eczema. Overall the MC showed high correlation between macro- and microbiopsies with a mean difference of 5% in the eczema cohort and a mean difference of 13% in the psoriasis cohort. Thus, microbiopsies appear to be as useful as conventional biopsies to perform molecular diagnostic tests, such as the MC.

P122 | Genetic C5AR1 variant and C5A levels associated with pemphigus

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Introduction: Pemphigus is a group of blistering skin autoimmune diseases. It is characterized by acantholysis and by the production of autoantibodies against, mainly desmoglein 1 (DSG1) in pemphigus foliaceus (PF) and DSG3 in pemphigus vulgaris (PV). Both occur sporadically around the world, but PF is endemic in Brazil, being commonly known as "fogo selvagem" (Portuguese for "wild fire"). Skin blistering of both PF and PV involves several components of the complement system (CS) and polymorphisms of CS genes may alter the efficiency of cascade activation and regulation. Recently, we identified an association of increased endemic PF susceptibility with the rs10404456 C>T single nucleotide polymorphism (SNP) in the C5AR1 gene, which encodes the receptor for the C5a anaphylatoxin.
Aim: To replicate the association of the rs10404456 C>T in a sporadic pemphigus foliaceus cohort, as well as to measure C5a levels in endemic PF and PV.
Methods: We genotyped the rs10404456 C>T SNP in 59 sporadic PF patients and 140 controls with the iPLEX platform of the MassARRAY system (Agena Bioscience, San Diego, USA). We also measured C5a levels in the sera of 35 endemic PF and 18 PV patients, and 35 controls using the MicroVue C5a EIA (Quidel) kit.
Results: The distribution of the rs10404456 genotypes was in Hardy-Weinberg equilibrium. The same allele previously associated with endemic PF (rs10404456*C) was also associated with sporadic PF (OR = 2.41 [95% CI = 1.45 - 3.99] $P < 0.001$). Serum C5a levels were higher in both PF (mean = 130.7 [69 - 187] ng/mL) and PV (mean = 138.9 [89 - 198] ng/mL) patient groups compared with controls (mean = 87.2 [30 - 135] ng/mL, unpaired Student T test $P < 0.001$ for both comparisons).
Conclusion: These results highlight the proinflammatory role of the C5aR1-C5a axis and ultimately of the complement cascade in PF pathogenesis, regardless of its sporadic or endemic etiology, as well as in PV. They also lead us to suggest a shared role of the complement system in different pemphigus forms and a beneficial effect for its therapeutic inhibition.

P123 | Up-regulation of SIRPA expression and high LDH levels in human skin organ culture mimicking pemphigus foliaceus

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Introduction: Pemphigus foliaceus (PF) and pemphigus vulgaris (PV) are autoimmune blistering diseases, characterized by the production of autoantibodies against, mainly, desmoglein 1 (DSG1) and 3 (DSG3). The role of cell death in this group of diseases is still poorly understood. Current evidence disqualifies apoptosis as the major cell death mechanism for PV and PF. We recently identified an association of polymorphisms of genes involved in apoptosis resistance and induction of pyroptosis, necroptosis, necrosis, parthanatos and immunogenic cell death (ICD) with the susceptibility to PF.
Aim: To investigate the gene expression of formerly identified cell death-associated genes in human skin organ culture (HSOC) in PF and PV-like skin blisters, as well as to investigate the lactate dehydrogenase (LDH) levels in the supernatant of the HSOC cultures.
Methods: We injected exfoliative toxin of Staphylococcus aureus in seven HSOCs and anti-Dsg1/Dsg3 in eight HSOCs, to mimic PF and PV phenotypes, respectively, and injected eight HSOCs with IgG from control individuals. To confirm the formation of blisters in the epidermis, we used hematoxylin-eosin staining and electron microscopy. We also extracted the RNA from frozen biopsies to measure gene expression by RT-qPCR for: CD47, SIRPA, EIF2AK3 (ICD); TNF, TRAF2 (apoptosis and necroptosis); IL1B, IL18 and IL1A (pyroptosis and inflammatory response). Furthermore, we measured LDH levels in the supernatants from 5 HSOCs from each group.
Results: As expected, the PF and PV models presented blisters in the granular and deep layers of the epidermis, respectively. For SIRPA (signal-regulatory protein alpha), we found higher gene expression in the HSOC PF model ($P = 0.016$), and a trend for higher gene expression in the HSOC PV model ($P = 0.08$). No significant differences for the expression levels of other genes were found. Still, high levels of LDH were detected in supernatant from PF model ($P < 0.001$), for the PV model, a trend was found ($P = 0.09$).
Conclusion: The increased levels of LDH in PF model, a marker that leaks out of the cytoplasm of dead cells into culture medium, suggests a higher cell death rate in this model. The expression of the

SIRPA gene, which has an important role as a phagocytic regulator in the ICD pathway, was up-regulated in the HSOC model mimicking PF. We suggest that the immunogenic cell death pathway may have a causal role in PF acantholysis. This result contributes to the understanding of PF etiology and to the development of new therapies for the disease.

P124 (OP02/03) | Innate lymphoid cells contribute to the control of cutaneous leishmaniasis

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Innate lymphoid cells (ILCs) are relatively newly discovered cells of the innate immune system found in various tissues, including the skin. ILCs share similar transcriptional and functional features with T helper (Th) cell subsets, however lack T cell receptors. In analogy to the Th1, Th2 and Th17 subsets, ILCs are commonly subdivided into three major groups ILC type 1-3. Under healthy, non-inflammatory conditions, the skin harbors mainly group 2 ILCs (ILC2s), with fewer numbers of group 1 ILCs (NK cells and ILC1s) and low numbers of group 3 ILCs (ILC3s). Under inflammatory conditions however, ILCs have been reported to participate in different skin diseases, including psoriasis, atopic dermatitis and wound healing. Cutaneous leishmaniasis, induced by infection with the intracellular parasite Leishmania major (L. major), manifests in skin lesion formation. L. major evokes a Th1/Tc1-dominated immunity in both immune-competent humans and C57BL/6 mice, while in immune-compromised patients and BALB/c mice, a Th2/Treg/Th17-immune response dominates supporting progressive disease. Only few prior publications studied the role of ILCs during L. major infection and mainly focused on type 1 ILCs. Yet, these publications reported rather conflicting results and did not adequately discriminate NK cells from the ILC1 subset. Furthermore, the role of ILC2s in L. major infection was not addressed so far. We therefore set to investigate the role of ILCs in cutaneous leishmaniasis in more detail by using a physiologic low dose L. major infection model. Following L. major infection of C57BL/6 mice, we observed that both NK cell and ILC1 numbers strongly increased in the lesion site. Furthermore, by gating on lesional CD3 negative cells, we identified an early innate source for IFN-gamma that was important for controlling the infection, as selective IFN-gamma neutralization at early time points post-infection (week 2-3) led to aggravated disease. We found that in response to L. major infection, NK cells and ILC1s serve as an early source of IFN-gamma. Similarly, antibody mediated NK cell and ILC1 depletion (aNK1.1) at early time points post-infection, resulted in aggravated disease. In line with that, constitutive genetic ablation of group 1 ILCs (NCR1Cre x DTA LSL mice) led to increased disease pathology and reduced levels of IFN-gamma, which was associated with increased parasite burdens and delayed recovery. In contrast to group 1 ILCs, ILC2 numbers increased primarily at later stages post-infection, suggesting these cells may play an important role in tissue regeneration. Supporting this notion, infected ILC2-deficient mice (Tie2Cre x Ror-alpha fl/fl mice) exhibited prolonged disease pathology, highlighting the importance of ILC2s contribution to disease recovery. In summary, our data indicate a well-coordinated ILC division of labour, with NK cells and ILC1s contributing to L. major parasite clearance, at least partly by providing an early source of IFN-gamma, whereas ILC2s appear to be important for disease recovery and tissue regeneration.

P125 | Cleavage of C1-Inhibitor by Mast Cell chymase results in abrogated control of FXIIa activity - a connection between histaminergic and bradykinin-mediated angioedema

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Chronic inflammatory conditions have been described to increase disease activity in patients with C1-inhibitor-dependent hereditary angioedema (HAE). One of the features in mast cell (MC) -associated chronic inflammation is the release of preformed mediators including proteases such as tryptase and chymase from activated MCs. These proteases are known to cleave a large array of proteins and are suggested to contribute to the development of recurrent AE. In some patients, occurrence of AE has been proposed to be due to the cleavage of C1-Inhibitor. Therefore, we assessed the effects of the MC proteases tryptase and chymase on C1-Inhibitor. We found that supernatant obtained from degranulated, cultured human MCs cleaves purified as well as recombinant C1-Inhibitor, resulting in a protein with reduced size as shown by SDS-PAGE and coomassie staining. Using purified tryptase and chymase, we could confirm that chymase cleaves C1-Inhibitor, potentially at the N-terminus of the molecule. Importantly, chymase-cleaved C1-Inhibitor showed reduced inhibitory capacity in a chromogenic FXIIa/kallikrein activity assay. These findings demonstrate that C1-Inhibitor is a potential target of MC chymase. Cleavage by chymase results in decreased efficacy of C1-Inhibitor to control the contact system. This may contribute to FXII/kallikrein-activation, bradykinin release, and angioedema formation in patients with MC activation.

P126 | Development of combination therapy to delay resistance in BRAF-inhibitor treated melanoma

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Introduction

Dendritic cells (DC) have the ability to capture and cross-present antigens for activation of T cells. However, the tumor microenvironment actively suppresses antitumor immunity partly by suppressing DC activation and antigen accessibility. The aim of this project is to develop combination therapies, allowing prolonged control of melanoma growth by boosting DC function during BRAF inhibitor (BRAFi) treatment of melanoma

Methods: A preclinical melanoma mouse model carrying the BRAFV600E mutation was tested for tumor growth control by BRAFi and mRNA expression of melanoma-associated antigens. The immunological alterations in the tumor microenvironment were investigated by multi-color flow cytometry. We combined BRAFi with various adjuvants to delay resistance development in the transplantable tumor model. Moreover, we designed DC vaccines by cloning melanoma antigens into the anti-DEC-205 antibody and tested them in vitro and in vivo for their potential to boost DC function.

Results: We observed that, BRAFi boosted T and NK cell function in transplanted tumors. Interestingly, DC infiltrated tumors shortly after start of BRAFi treatment accompanied by an enhanced expression of the melanoma-associated antigens gp100 and trp2. After prolonged BRAFi exposure tumors developed resistance, and tumor milieu shifted from inflammatory to immunosuppressive. This resistance development can be delayed by combining BRAFi with DC-stimulating adjuvants.

Conclusion: We learned from our project that DC are essential players in tumor-targeted therapy by modulating the immunological responses. How this knowledge can be used to improve therapeutic strategies by combination with DC vaccines is currently under investigation.

P127 | Differential role of reactive oxygen and nitrogen species in contact hypersensitivity reaction

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Introduction: Reactive oxygen and nitrogen species (ROS/RNS) are important modulators of inflammatory immune responses which can promote pro- or antiinflammatory effects. However, the impact of ROS/RNS mainly provoked by neutrophils and macrophages during acute and chronic contact hypersensitivity reactions (CHR) is controversially discussed. Aim of our study was to dissect the dominant ROS/RNS sources during acute and chronic trinitrochlorobenzene (TNCB)-induced CHR by using mice with differently impaired ROS/RNS production; NADPH oxidase-deficient (gp91phox-/-), myeloperoxidase-deficient (MPO-/-), and inducible nitric oxide synthase-deficient (iNOS-/-) and wild-type mice.

Methods: Mice were sensitized with 5% TNCB at the abdomen and challenged with 1% TNCB at the right ear 7 days later to elicit acute CHR. To induce chronic CHR we challenged mice every 48 h up to 5 times. We determined ear-swelling responses and conducted non-invasive in vivo optical imaging (OI) of ROS/RNS production using the chemiluminescence probe L-012 (n = 11-12 per group). Additionally, we conducted extensive ex vivo analyses of inflamed ears focusing on ROS/RNS production and the biochemical and morphological consequences.

Results: During acute and chronic cutaneous CHR non-invasive in vivo L-012 OI measurements revealed a completely abrogated ROS/RNS production in ears of gp91phox-/- mice, an up to 90% decrease in ears of MPO-/- mice and an unaffected ROS/RNS production in the ears of iNOS-/- mice when compared to wild-type mice. Ex vivo DHR flow cytometry analysis of inflamed ears with acute CHR exhibited a reduced ROS/RNS production in gp91phox-/- and MPO-/- mice and almost similar values in iNOS-/- mice when compared to the wild-type mice, confirming our in vivo L-012 optical imaging results. We determined no significant differences in ear swelling response between all experimental groups. Nevertheless, histopathological analysis of inflamed ears from gp91phox-/- mice exhibited a slightly enhanced inflammatory immune response during acute CHR but a slightly reduced inflammation during chronic CHR. In contrast, inflamed ears from MPO-/- mice with chronic CHR yielded the most severe inflammatory immune response when compared to inflamed ears of wild-type mice. Analyses of lipid peroxidation,

8-hydroxy-2'deoxyguanosine levels, redox related metabolites and genomic expression of antioxidant proteins revealed similar oxidative stress in all experimental groups. Furthermore, inflamed ears of wild-type and gp91phox-/- mice displayed neutrophil extracellular trap (NET) formation exclusively in acute but not chronic CHR.

Conclusions: The analysis of mice with differently impaired ROS production revealed that MPO and NADPH oxidase 2 are the dominant ROS sources in acute and chronic CHR. Nevertheless, reduction of ROS/RNS production by depletion of predominant ROS/RNS sources exhibited only moderate but conflicting clinical consequences.

P128 | Characterization of the skin myeloid cell landscape upon L. major infection

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During the course of L. major infection, different types of myeloid cells can be found at the skin lesion. These cells differ in terms of their ontogeny, cellular subset identity and functional contribution in combating the parasite. Once entered the skin, parasites invade skin-resident macrophages (Mac) and use them to shield themselves from detection and elimination by other immune cells. This so-called "silent phase" normally lasts for three weeks, during which the parasite converts to its intracellular life form and replicates. Once a critical mass is reached, the parasites are released and taken up by neighboring cells. Different skin APCs then incorporate the parasite, process it and migrate to the skin-draining LN, where they present L. major antigens and initiate acquired immunity. This in turn leads to an accumulation of several myeloid cell types in lesions, where they play important roles both for fighting the parasite and restoring skin homeostasis. In an attempt to better understand the division of labor among these different cell subsets during cutaneous leishmaniasis, we set to characterize the different myeloid cell populations following physiologically-relevant low dose L. major inoculation, by performing a time-kinetic analysis. This was carried out using a combination of surface and intracellular protein staining as well as the use of reporter mouse lines. Furthermore, a comparison of myeloid cell composition found in Leishmania-resistant/self-resolving C57BL/6 mice and those of Leishmania-susceptible/non-resolving BALB/c strain was performed. This approach permitted us to generate a comprehensive skin myeloid cell landscape following L. major-infection. Our data demonstrate the contribution of selected myeloid subsets i.e. Langerhans cells (LC), dermal (d)DC (both cDC1 and cDC2), monocytes (Mo), Mo-derived Mac (Mo-Mac) and Mo-DCs to different disease stages. Accordingly, at early stage post-infection (first days), we found tissue-resident Mac/LC to be the main myeloid subset that responds and proliferates. 10 days post-infection, a short influx of Mo was seen at the lesion vicinity, though these were short-lived cells, as they disappeared shortly after (either die, convert or migrate - not yet clear). This is followed by dDC activation and migration away from the skin. By week 3 post-infection, dDCs (both dDC1 and dDC2) expand and reach their numeric peak. This is followed by a rapid decline in numbers, with a second DC wave reoccurring at week 6 onwards (likely being Mo-DCs, which downregulated Ly6c expression). Unexpectedly, however, when comparing C57BL/6 and BALB/ c mice, we did not find significant differences in terms of the timing of skin myeloid landscape appearance or major differences in cellular distribution. This, however, needs to be further investigated using new genetic tools, which are now available at our laboratory. By linking the skin myeloid cellular composition, their origin and protein expression to the different disease pathological stages, we plan to pinpoint potential cellular targets relevant for new interventions for combating leishmaniasis.

P129 | Preclinical evaluation of a combination of checkpoint blockade and dendritic cell vaccination against Merkel cell carcinoma

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Merkel cell carcinoma (MCC) is a rare and highly aggressive skin tumor that may be caused by the Merkel cell polyomavirus. Under occasional circumstances, this virus integrates into the host cell's genome and expresses a truncated form of one of its proteins, the large T antigen (truncLT). Apart from surgical excision, radio- and chemotherapy, treatment with checkpoint inhibitors has shown promising results in metastatic MCC. These antagonistic antibodies target inhibitory receptors on T cells and their ligands to enhance anti-tumor immune responses. Nevertheless, approximately one third of the patients does not respond to this checkpoint blockade, so that additional treatment options need to be investigated. To make the tumor visible for the immune system, dendritic cell-based therapeutic vaccination, which is currently evaluated in several clinical trials, can be used. Dendritic cells (DCs) process and present antigens (Ags) to T cells and thereby control Ag-specific T-cell proliferation and differentiation. Therefore, ex vivo generated DCs loaded with the viral target antigen could be used to treat virally induced cancers.

The aim of this project is to evaluate a possible combination of checkpoint inhibitors and therapeutic DC vaccination against MCC. We hypothesize that this combination induces more and better immune responses against Merkel cell carcinomas than each treatment alone. Therefore, we analyze whether the immunogenicity of truncLT-expressing DCs towards autologous T cells is influenced by

different checkpoint blockade antibodies alone and in combination in human ex vivo cell culture systems. The fraction of T cells, specific for the truncLT Ag, is quantified by IFNγ ELISpot assays. Initial results indicate that checkpoint inhibition seemed to have an effect on quantity and quality of antigen-specific T cells in healthy donors. The findings of this project can contribute to improved clinical treatment of MCC and other virally induced malignancies.

P130 | Topical treatment with1,8-dihydroxy-9-anthrone induces IL-6 and ROS and recruits myeloid cells with immunosuppressive features

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Psoriasis is a systemic inflammatory disease causing epidermal hyperproliferation, dermal endothelial cell proliferation and immune cell recruitment to the skin. Cytokines associated to the IL-23/IL-17 axis of immune responses are critically implicated in disease pathogenesis and highly expressed in psoriatic lesions. The most effective and most rapid topical treatment modality of psoriasis is the application of the oxidative compound1,8-dihydroxy-9-anthrone (Anthralin). Anthralin is a skin irritant and its exact mode of action is not fully understood. In this experimental study, we compared the immune response of skin treated topically with either1,8-dihydroxy-9-anthrone or with imiquimod, a TLR agonist that typically induces psoriasis-like inflammation in mice. We followed the degree of ear swelling, pathology and molecular changes in these two models. Interestingly, 1,8-dihydroxy-9-anthrone led to a more rapid and stronger ear swelling than imiquimod. Analysis of skin pathology revealed some similarities but also differences in epidermal aspects and immune cell composition. Myeloid cells were more prominent in the skin of mice treated with1,8-dihydroxy-9-anthrone compared to imiquimod. As expected, imiquimod treatment resulted in a dominant IL-17/IL-23-immune response as determined by quantitative PCR analysis of cytokines. In contrast, 1,8-dihydroxy-9-anthrone did not induce IL-17 expression, although IL-6 expression was very prominent. To better understand the underlying effects of 1,8-dihydroxy-9-anthrone we determined the generation of oxidative stress by this chemical compound using in vivo imaging. Our data indicated that a milieu rich of IL-6 and ROS promotes myeloid cells with possibly suppressive features on Th17 responses. Therefore we isolated myeloid cells from skin of mice treated with 1,8-dihydroxy-9-anthrone and could confirm their suppressive function on T cell proliferation and Th17 immune responses in vitro and in vivo. The suppression of Th17 responses by 1,8-dihydroxy-9-anthrone was also true in human psoriasis as determined by studying human skin samples before and during treatment. In summary, we could unravel a novel suppressive action of 1,8-dihydroxy-9-anthrone on the immune system that explains its beneficial effects on Th17-mediated psoriasis.

P131 (OP03/04) | Type 17 T follicular helper cells promote desmoglein 1/3-specific autoantibody production in pemphigus

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Pemphigus is characterized by autoantibodies (auto-ab) directed against desmoglein (Dsg) 1 and 3. Binding of pathogenic auto-ab to their desmosomal targets results in blisters and erosions of skin and mucosa. Autoreactive T cells are thought to stimulate B cells for Dsg-specific auto-ab production. T helper (Th) 2 cells were thought to be the disease-promoting subset. However, recent findings delineate a more complex immune mechanism. We aimed to identify the pathogenic T cell subset relevant for auto-ab production.
By performing transcriptome analysis of lesional skin from patients with pemphigus we revealed an IL-17A-dominated immune signature compared to healthy skin. KEGG pathway analysis revealed an upregulation of the IL-17A pathway. Differently expressed genes included MMP1, MMP13, S100A8/9, CXCL5/8 and IL6. By quantitative qPCR we found high levels of IL17A, IL21, IL23, IL1B, IL19 and IL24 in lesional skin compared to healthy skin. Analysis of circulating T cell subsets in patients with pemphigus (n = 74) by flow cytometry demonstrated an increase of T follicular helper (Tfh)17 and Tfh17.1 cell subsets in pemphigus patients, especially during active disease. Interestingly, numbers of Th17 and Tfh17 cell subsets correlated with levels of Dsg3-specific CD19 + CD27 + memory B cells and levels of Dsg3 autoreactive Tfh17 cells were strongly increased in acute pemphigus patients, thus indicating that the IL-17-producing T cell subsets could be relevant for auto-ab production. Therefore, we performed T/B cell co-culture experiments. As expected, all Tfh cell subsets induced IgG production by memory B cells. Importantly, the Tfh17 cell subset was predominant in inducing Dsg3/1-specific auto-ab production by memory B cells, especially in acute pemphigus patients. Taken together, we show for the first time that Tfh17 cells are associated with active pemphigus disease, correlate with the number of Dsg3-specific CD19 + CD27 + memory B cells and are capable to promote Dsg3-auto-ab production in B cells.

P132 | The inflammatory milieu in cutaneous lichen planus is predominantly dominated by IFN-γ and IL-21

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Cutaneous lichen planus (CLP) and psoriasis (PSO) are both common chronic inflammatory skin diseases for which development of new treatments requires the identification of key targets. While PSO is a typical Th17/IL-17-disorder, there is some evidence that Th1/IFN-γ dominate the inflammatory process in CLP. Nonetheless, the immunopathogenesis of CLP is not fully explained and key immunological factors still have to be recognized. In this study, we compared the immune signature of CLP lesions with the well characterized inflammation present in PSO skin. First, we analyzed the histological and immunohistological characteristics of CLP and PSO. Second, we assessed the cytokine expression (IL1A, IL1B, IL4, IL6, IL8, IL10, IL17A, IL19, IL21, IL22, IL23A, IL13, IFNG, TNF, IL12A, IL12B, IL36G) of lesional skin of CLP with PSO by qPCR. Histology revealed a similar epidermal thickness in CLP and PSO. Immunohistochemically, both diseases presented with an inflammatory infiltrate mainly composed by CD3 + CD4 + T cells rather than CD3 + CD8 + . Importantly, mRNA analysis showed a distinct cytokine signature: while levels of IL12B, IL1A, IL6 and IL23 were similar between the two groups, the characteristic PSO-associated cytokines IL8, IL17A, IL22, IL19 and IL36G were expressed at very low levels in CLP. In contrast, CLP lesional skin was dominated by the expression of IFNG, IL21, IL4, IL12A, and TNF. Immunohistochemistry confirmed the dominance of IL-21, IFN-γ and pSTAT1 in the dermal infiltrate of CLP, while IL-17A was more present in PSO. Collectively, this study improves our understanding of the immunological factors dominating CLP. The dominating cytokines and signaling proteins identified suggest that future anti-cytokine therapeutics like JAK inhibitors may be beneficial in CLP.

P133 | Cutaneous lichen planus induced under anti-IL-17A treatment is immunologically different from spontaneous occurring lichen planus

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Inflammatory skin diseases like psoriasis or lichen planus that develop under treatment with TNF antibodies are called paradoxical skin reactions. One possible explanation for the appearance of such paradoxical skin reactions is a misbalance of cytokines, triggering pathologic reactions. Some preliminary studies suggest that these skin lesions phenotypically and histologically resemble the original skin disorders with some distinct immunological characteristics. In this case study, we present a patient with plaque type psoriasis (psoriasis vulgaris, PV) under treatment with an anti-IL-17A antibody, which developed lichen planus (LP) like lesions of the skin as confirmed by clinical and histological investigation. We evaluated mRNA expressions level of several cytokines (IL1A, IL1B, IL4, IL10, IL17A, IL19, IL21, IL22, IL23A, IFNG, IL12A, IL12B) by RT-qPCR and compared these parameters to spontaneously developed LP (n = 7). Additionally, we sought to understand the effect of anti-IL-17A treatment on psoriatic skin by comparing residual PV skin to untreated PV skin (n = 7). Our approach revealed relevant immunologic differences between standard LP and paradoxical LP. Although LP is thought to be IFN-ɣ; dominated, we could find only minimal levels of IFNG in paradoxical LP skin compared to spontaneous LP. In addition, IL12B, IL21 and IL24 expression were also minimal in paradoxical LP, while strong expression of these cytokines was detected in spontaneous LP skin. Of note, we also found substantial differences between untreated PV and persistent PV skin lesions under anti-IL-17A blockade. We observed decreased levels of IL19, IL22, IL1A and IL1B under anti-IL-17A treatment compared to untreated PV skin. The level of IL17A was strongly overexpressed in the patient's PV skin when compared to untreated PV patients. Although our findings are based on a single case study, they demonstrate distinct immune phenotypes of paradoxical LP and persistent PV skin under anti-IL17A blockade compared to spontaneous LP and untreated PV skin, although clinical and histological features were similar.

P134 | UV-irradiation potentiates type I interferon signaling in fibroblasts of patients with cutaneous lupus and TREX1-deficiency

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Background: Lupus erythematosus is a multifactorial autoimmune disease that can manifest in patients carrying predisposing heterozygous mutations in the three prime DNA exonuclease TREX1. Mutations impairing the function of TREX1 lead to a cellular stress response and self DNA accumulation in the cytoplasm that induces a type I interferon response. Lupus patients with TREX1 mutation are sensitive to sun light which can induce disease flares.

Objective: In order to understand how this external trigger factor leads to disease exacerbation, we analyzed patient fibroblasts for reactive oxygen production, DNA damage response and type I interferon production after exposure to cold or UV-irradiation.

Results: We found that UV-irradiation induced enhanced formation of reactive oxygen species, oxidized DNA and DNA cyclobutane pyrimidine dimers in TREX1 deficient fibroblasts compared with wild-type cells. This was associated with a strong DNA damage response resulting in upregulation of type I interferon stimulated genes. This type I interferon response was dependent on the cytosolic DNA sensor cGAS as assessed by siRNA mediated sensor inhibition.

Conclusion: The data demonstrate that UV irradiation can potentiate a DNA damage response induced type I interferon signaling pathway. This cytokine is of utmost importance in the pathogenesis of cutaneous lupus. Demonstration of its sensitivity to cGAS inhibition in patient fibroblasts opens a new opportunity for treatment in patients with cutaneous lupus who have currently only access to limited therapeutic options.

P135 | Cellular stress due to RNA repeat expansions in myotonic dystrophy type II

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Background: Myotonic dystrophy (MD) type II is characterized by autosomal dominant progressive myopathy and multiorgan involvement including the skin and an increased risk for developing autoimmune disorders. The disease is caused by (CCTG)n expansion in CNBP (Cellular Nucleic Acid-Binding) leading to stable CCUG RNA repeat expansions. Their impact on cellular function and role for disease manifestation is incompletely understood.

Objective: To investigate the impact of CCUG RNA repeat expansions on the cellular stress response in human fibroblasts.

Methods: Fibroblasts isolated from skin of MDII patients were analysed by RNA FISH for detection of repeat expansions and reactive oxygen species (ROS). Using immunohistochemistry repeat associated non-AUG (RAN) proteins were stained. Basal and thapsigargin induced endoplasmic reticulum (ER) stress was analysed by RT PCR and western blot. MitoTracker were used to determine mitochondrial stress.

Results: Using RNA FISH technique, we demonstrated that fibroblasts of MDII patients accumulate CCUG RNA repeat expansions in the nuclear and cytoplasmic compartment. These cytoplasmic repeats can be translated by a mechanism called repeat associated non-AUG (RAN) translation, which led to RAN protein accumulation in the skin of MDII patients. Patient fibroblasts responded with chronically elevated mRNA levels of binding immunoglobulin protein (BiP) to this uncontrolled protein accumulation. BiP centrally regulates the unfolded protein response of the ER. Challenge of the pathway by thapsigargin treatment resulted in enhanced protein kinase RNA-like endoplasmic reticulum kinase (PERK) phosphorylation in patient fibroblasts compared to wild type indicating a chronically active ER stress response. This chronic PERK activation can lead to a higher calcium transfer between the ER and mitochondria, which results in mitochondrial stress. We showed that fibroblasts of MDII patients suffer from mitochondrial stress by using MitoTracker green and red. This was associated with elevated levels of ROS that correlated with the intensity of repeat expansions in the cell. We propose that this intracellular stress response impairs with regular cell function leading to muscle impairment and predisposition to autoimmune diseases in patients.

Conclusion: The chronic ER stress response and mitochondrial stress in fibroblasts demonstrate an ubiquitous reaction to RNA repeat expansions in patients with MDII and may provide an explanation for

myopathic disease manifestation as well as the predisposition to autoimmune disorders.

P136 | BRAF and MEK inhibitors affect dendritic cell maturation and T cell stimulation

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Small-molecule-kinase inhibitors and advanced immunotherapeutics have revolutionized melanoma therapy. Different combinations of BRAF and MEK inhibitors (BRAFi/MEKi) are currently standard treatment for patients with BRAFV600-mutated melanoma. Future treatment strategies will involve combining those with various types of immunotherapy, notably checkpoint inhibitors, but also experimental therapies such as adoptive transfer of receptor-transfected T cells or dendritic cell (DC) vaccination. Since the RAS/RAF/MEK/ERK-pathway is crucial for the function of different immune cells, it is expected that the inhibitors influence these cells and may thus interfere with anti-tumor immunity.

Therefore, we examined the influence of two approved BRAFi/MEKi combinations on the maturation of monocyte-derived DCs and their subsequent capacity to stimulate autologous CD4 + and CD8 + T cells. We matured the DCs with IL-1β, IL-6, TNF and PGE2 in the presence of the BRAFi vemurafenib (V) or dabrafenib (D) or the MEKi cobimetinib (C), or trametinib (T), as well as the combinations VC and DT. DCs matured in presence of V or VC secreted large quantities of IL-8 and some IL-12p70. The expression of various maturation markers was also influenced. V, alone or in combination with C had the strongest negative effect on most of them. However, all other inhibitors, except for D as single treatment, also had a negative impact on most of the maturation markers. Only CCR7 expression showed some opposite effects. While being reduced upon treatment with V, it was increased by both MEKi and by DT.

Subsequently, we used the DCs, which had been matured in presence of the inhibitor(s), in a T-cell stimulation assay with autologous TCR-transfected T cells, again in the presence or absence of the respective inhibitor(s). Activation of CD4 + and CD8 + T cells, analyzed by measuring CD25- and CD69-expression on the T cells, was negatively influenced by all inhibitors, except for D. The strongest effect was seen with VC, the most moderate with DT. Similar effects were observed for the secretion of pro-inflammatory cytokines like IL-2, TNF, and IFNγ. The antigen-specific interaction between CD4 + T cells and DCs is a bi-directional process, in which, next to the T-helper cell, also the DC responds with cytokine release and up-regulation of surface molecules. We observed that V and VC completely abolished the helper T cell-mediated up-regulation of CD70, CD80, and CD86, but not of CD25 on the DCs.

In conclusion, this work shows that the combination of VC affects DC maturation as well as T-cell activation stronger than combined DT. Hence, for a potential combination with immunotherapy, our data indicate that DT treatment may be superior.

NS and JD share senior authorship.

P137 | Autoantigen generation of the HLA-C*06:02-mediated autoimmune response against melanocytes in psoriasis is ERAP1-dependent

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Genome-wide association studies revealed that variants of the gene encoding endoplasmic reticulum aminopeptidase1, ERAP1, are important risk factors for psoriasis in patients carrying the strongest psoriasis risk gene, HLA-C*06:02. The pathogenic mechanisms of this gene-gene interaction in psoriasis risk are unknown. By the unbiased analysis of a Vα3S1/Vβ13S1 T-cell receptor (TCR) from a lesional CD8 + T-cell clone from the pathogenic epidermal psoriatic T-cell infiltrate, we have previously shown that in psoriasis vulgaris, HLA-C*06:02 mediates an autoimmune response against melanocytes through presentation of an autoantigenic peptide from ADAMTS-like protein 5 (ADAMTSL5) to CD8 + T cells as the underlying pathogenetic mechanism.

To examine the functional interaction between ERAP1 and HLA-C*06:02, we utilized a TCR activation assay. It integrated the five key components of the psoriatic autoimmune response: HLA-C*06:02, melanocytes as autoimmune target cells, the psoriatic ADAMTSL5 autoantigen, different ERAP1 variants, and the pathogenic HLA-C*06:02-restricted melanocyte-directed ADAMTSL5-specific Vα3S1/Vβ13S1 TCR expressed in a reporter hybridoma cell line. ERAP1-/- clones (MCCs) were generated by CRISPR-targeted genome editing from two HLA-C*06:02-positive melanoma cell lines, WM278 and WM793, which can replace normal human melanocytes for analyzing the melanocyte-specific autoreactivity of the Vα3S1/ Vβ13S1 TCR.

We show that ERAP1 generates the causative ADAMTSL5 peptide autoantigen of the HLA-C*06:02-restricted autoimmune response against melanocytes in psoriasis from precursor peptides. ERAP1 knockout significantly reduced the expression of HLA-C and the antigenicity of MCCs for the psoriatic Vα3S1/Vβ13S1 TCR. Reconstitution with an ERAP1 psoriasis risk haplotype restored HLA-C expression and the antigenicity of ERAP1-/- MCCs to a significantly greater extent than an ERAP1 haplotype protective for psoriasis. In in vitro-digestion experiments, the psoriasis risk haplotype of ERAP1 generated the autoantigenic HLA-C*06:02- presented ADAMTSL5 epitope from NH2-extended precursors more effectively than the protective ERAP1 variant. Coordinated upregulation

of ERAP1 and HLA-C in psoriatic inflammation enhances melanocyte autoimmunogenicity.

Thus, the generation of the causative autoantigen of the psoriatic autoimmune response is ERAP1-dependent. ERAP1 variants regulate the autoimmune potential of melanocytes and strength of the psoriatic autoimmune response by different yields of the ERAP1-dependent self-epitope for presentation by HLA-C*06:02 and recognition by CD8 + T cells. These results identify a functional principle by which epistasis between ERAP1 and HLA-class I alleles control the development of CD8 + T-cell mediated autoimmunity, and they define ERAP1 as a therapeutic target for psoriasis.

P138 (OP05/03) | Complex interactions between Gut Microbiota and the Mitochondria contribute to the pathogenesis of Autoimmune Skin Diseases

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Autoimmune diseases are thought to be caused by complex interactions of genetic and environmental factors. Shifts in gut microbial communities and the reduction in their diversity are found in a variety of diseases, including autoimmune diseases. Bullous pemphigoid is the most common autoimmune blistering skin disease (AIBD), which is mediated by autoantibodies targeting type XVII collagen. To the best of our knowledge, no experimental studies have been conducted to elucidate the impact of gut microbiota in AIBD. Therefore, we first induced a well-established experimental epidermolysis bullosa acquisita (EBA) in germ-free mice. Germfree C57BL/6Tac mice, which received pathogenic IgG against type VII collagen exhibited significantly milder disease severity compared to the conventionally housed C57BL/6Tac mice (padj. < 0.0001), suggesting that microbiota modulate the disease severity in experimental EBA. In parallel, using 16S rRNA sequencing we recently observed a distinct pattern of gut microbiota in C57BL/6J-mtFVB/NJ (B6-mtFVB) mice, which carry a single variation in the mitochondrially encoded mt-Atp8 gene (m.7778G>T), compared to wild-type C57BL/6J (B6) mice. In the human equivalent gene, MT-ATP8, polymorphisms are associated with bullous pemphigoid in a German cohort. In line with this human study, B6-mtFVB mice demonstrated significantly less skin inflammation than B6 mice when experimental EBA was induced ($P < 0.05$). To further confirm a potential interaction of the gut microbiota and AIBD, shotgun metagenomics of cecum content, RNA-seq of cecum epithelium samples, and untargeted metabolomics of liver samples of B6-mtFVB and B6 mice were performed. Integrated analysis of shotgun metagenomics and untargeted metabolomics analysis is currently being conducted to identify the gut microbiome derived metabolites that may modulate host immune cell response. Additionally, we evaluated the immune cell metabolism in CD4 + T cells, a pivotal player in the pathogenesis of skin inflammation in the experimental EBA. CD4 + T cells from B6-mtFVB mice demonstrated a higher ratio of glycolysis to oxidative phosphorylation than those from B6 mice ($P = 0.0008$). This change may further modulate CD4 + T cell function, as we observed less cellular proliferation capacity upon immunological stress induced by anti-CD3 and anti-CD28 antibodies ($P < 0.05$) and less proinflammatory cytokine IL-17 production in CD4 + T cells from B6-mtFVB mice than B6 mice ($P < 0.01$). We also evaluated gut permeability via gene expression of tight junction proteins (Claudin-2, Claudin-15, Occludin and ZO-1). Expression levels of these genes were reduced in small intestine, cecum and colon samples of B6-mtFVB compared to those of B6 mice. Our findings show 1) experimental evidence demonstrating the importance of the gut microbiota in the pathogenesis of AIBD, and that 2) a single polymorphism in the mitochondrial genome impacts the composition of gut microbiota, host metabolites and immune cell metabolism, which further modulate disease severity in autoimmune skin inflammation in mice. To explore the link between gut microbiota and immune cell interaction, we plan to identify gut microbiota-derived metabolites and evaluate their modulatory effect on immune cells contributing to autoimmune skin inflammation in vitro and in germ-free B6-mtFVB and B6 mice which are currently being generated.

P139 | Skin immune cell infiltration: multidimensional single cell mass cytometry and flow cytometry studies of acne inversa lesions

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Chronic immune-mediated skin disorders represent an important health problem due to their high prevalence and the tremendous impact on patients' quality of life. Understanding of the underlying immune mechanisms requires accurate characterization of the nature and function of immune cell subsets populating the diseased skin of these patients. Due to the complexity of the skin organ; however, skin immune cell characterization remains challenging.

Our study aims at the establishment and application of a methodology to characterize the skin immune cell infiltrate of patients suffering from acne inversa, a poorly characterized disease with high

medical need. Lesions comprise inflamed nodules, abscesses, and pus-discharging fistulas, developing in axillary, inguinal, gluteal, and perianal sites, altogether lead to high burden for the patients. For these analyses, we chose single cell mass cytometry (CyTOF) in combination with multi-color flow cytometry, using a variety of markers that allows immune phenotyping of resident and non-resident immune cells. In contrast to multi-color flow cytometry, CyTOF has been scarcely used so far within the field of investigative dermatology. CyTOF allows the simultaneous assessment of up to 45 cellular markers thus allowing deciphering the immunological heterogeneity in the human skin.

As the first critical point in this approach, we developed an experimental method comprising of mechanical and enzymatic digestion of human skin suited for downstream cytometric applications that enable accurate data acquisition by optimizing signal detection and epitope recovery while minimizing background noise and low cell yields. We developed a 40 markers CyTOF panel for human leukocyte subsets identification comprised of a variety of markers for tissue homing, cytotoxicity and effector functions. Notably, chemokine receptors such as CXCR3, CCR6 and CCR4 vital for phenotyping of immune subsets of our interest are highly sensitive to isolation and preservation methods. Our results show that digestion with Collagenase I or IV with Benzonase for 6 hours at 37 C maintain epitope stability but also does not alter cell viability while obtaining adequate cell yields. In contrast, Dispase II, which is often used in skin digestion protocols, digests even vital immune markers such as CD4 and thus is not preferable for our applications. First analysis of AI skin-derived cells revealed that memory CD3 + TCR/ab+ T cells represent the dominant 60% population among single live CD45 + mononuclear cells. Notably, B cells, NK cells and monocytes are also infiltrating immune subsets in AI. Further characterization of the T cell subsets revealed both CD4 + and CD8 + T memory subsets as well as distinct subsets of CD103 + CD69 + , CD103- CD69 + and CD103- CD69- among CD4 + and CD8 + T cells are present in the AI tissue.

We have developed an optimized protocol for downstream multidimensional single cell cytometric applications which is applicable for various human cutaneous diseases and healthy tissue thus allowing comparisons between different tissues, blood but also donors as well as distinct time points of biopsy collection. The accumulating knowledge of the skin immune cell infiltration in acne inversa is of vital importance regarding the pathogenetic understanding of the disease thus the clinical management highly needed for these patients.

P140 | Phenotypic characterization of T cell subpopulations of patients with chronic spontaneous urticaria

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Chronic spontaneous urticaria (CSU) is characterized by the reoccurrence of intense pruritic wheals on the skin, without any identifiable exogenous trigger. While the key mediator responsible for CSU-related clinical symptoms, histamine, is wellknown, the underlying mechanisms leading to exaggerated histamine release by mast cells and basophil granulocytes still remain unclear. It has been suggested that pathophysiological processes of CSU involve either an IgE-mediated autoallergy, e.g. against the autoantigen thyroid peroxidase (TPO), autoantibodies against IgE or its high-affinity receptor FcεRI, or abnormal signaling pathways in mast cells or basophils. In addition, we recently could show that patients with CSU can be divided into three immunologically and clinically distinct subgroups based on the frequency and reactivity of their basophils. Furthermore, alterations of distinct T cell populations were induced by treatment of CSU patients with the anti-IgE antibody omalizumab accompanied with clinical improvement. Therefore, we wanted to shed more light on the involvement of T cells in the pathophysiology of CSU by analyzing whether CSU patients with different basophil phenotypes vary in their predominant T cell subsets.

We examined a cohort of 59 CSU patients, which was divided into three subgroups, i.e. reactive (CU-R; n = 39), non-reactive (CU-NR; n = 12) and non-reactive patients with basopenia (CU-NR-B0; n = 8), according to their respective basophil frequency and reactivity after anti-FcεRI stimulation. Peripheral blood mononuclear cells (PBMC) of the patients were used to determine the frequencies of IL-5-, IL-10-, IL-17-, IL-31- and IFN-γ-secreting T cells upon stimulation with anti-CD3/CD28 dynabeads by ELISPOT analysis. Disease-specific alterations in CSU patients were compared to a control group consisting of nine healthy individuals. Furthermore, serum autoreactivity was determined using the basophil activation test (BAT), i.e. the activation of basophils from a healthy donor was evaluated after stimulation with (potentially) autoreactive sera from CSU patients.

Looking at the T cell frequencies a substantial difference between CSU patients and healthy controls was found. Patients with CSU irrespective of their basophil phenotype had significantly less IL-10-, IL-17-, IL-31- and IFN-γ-secreting T cells than healthy controls, which could be due to migration of T cells into the skin of CSU patients. Since a prevalent role of Th2 and Th17 was suggested in CSU, we took a closer look at the relationship between these two cell T subsets. By calculating the ratio of IL-5- to IL-17-secreting T cells, we observed an inverse correlation with the serum autoreactivity of CU-R patients indicating a Th2-dominated cell response in this cohort. In contrast, the CU-NR-B0 group showed a trend towards increased Th17 cell levels associated with higher autoreactive potential.

Moreover, an inverse correlation was found between the concentration of anti-TPO-IgG antibodies in the serum of CSU patients and the number of IL-31-secreting T cells.
Thus, these findings led us to conclude that different T cell subsets may play a predominant role in CSU dependent on the respective pathophysiological process.

P141 | Artificial 3D human skin for translational and diagnostic utilization

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Pemphigus vulgaris (PV), epidermolysis bullosa acquisita (EBA), pemphigus foliaceus and bullous pemphigoid (BP) are autoantibody-mediated blistering skin diseases with increasing incidence and prevalence, both due to an aging population and improvements in diagnosis and awareness. Disease models are helping to understand disease pathophysiology, facilitating optimization of diagnosis and treatment.
We here established a 3D full thickness human skin model which consists of an artificial dermis, the dermal-epidermal junction and the epidermis, with cells derived from healthy human donors. The structure of our 3D skin fully resembles normal human skin, as shown by hematoxylin/eosin staining. It is fully stratified, as shown by immunofluorescence (IF) against the basal marker cytokeratin (CK) 15 and the suprabasal markers CK 10 and CK 14. Differentiation is shown by IF against filaggrin and loricrin, markers for terminal differentiation of the skin. Double stainings for desmoglein (Dsg) 1 and3, as well as single stainings for Dsg1, Dsg 3 and desmocollin 1 show a similar expression of the cell-cell adhesion proteins as compared to normal human skin cryosections. The keratinocytes of the stratum basale have proliferative activity, shown by Ki-67 staining. The qPCR results show that collagen VII, the autoantigen of EBA, is expressed on 3D skin and the IF shows a deposition around the basal keratinocytes. This indicates that a basement membrane is formed. The dermal-epidermal linker proteins BP 180 and BP 230, autoantigens in BP, show a deposition like a string of pearls on 3D skin, in contrast to the linear deposition on normal human skin. The dermal marker protein fibrillin 1 is missing on the 3D skin. Only a few fibroblasts started to produce fibrillin 1 and express this protein. In contrast to healthy human skin, the 3D skin is CK 6-positive which indicates wound healing characteristics. Expression of the following desmosomal and hemidesmosomal proteins was confirmed by qPCR: type VII collagen, Dsg1, Dsg3, BP180.
For diagnosis, sera form patients with pemphigus and pemphigoid are routinely incubated with monkey esophagus (ME) substrate. However, this routine immunofluorescence-based test is known to exhibit a rather low sensitivity and is based on use of animals, conflicting with the 3R principles. In a pilot study, we here used the above 3D skin equivalents for diagnosis of PV, where routine ME stainings often show false positive intercellular fluorescence (ICF): All PV patient sera (n = 6) showed ICF, whereas false positives from ME testing (n = 4) were all negative on sections of artificial 3D skin.
In summary, our innovative protocol allows for many translational applications, including diagnosis. Our preliminary results suggest a high sensitivity for the serological diagnosis of PV, potentially replacing ME-based IF.

P142 | Dysregulation of mTOR and JAK/STAT signaling pathways in monocyte-derived macrophages from sarcoidosis patients

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Background: Sarcoidosis is an inflammatory granulomatous disease characterized by aggregates of mature macrophages in one or multiple organs. Key molecular pathways leading to sarcoidosis still remain largely unknown.
Objectives: To acquire new insights into the pathomechanisms of sarcoidosis and granuloma formation we established a functional in vitro assay with monocyte-derived macrophages from patients with sarcoidosis and healthy controls.
Methods: In our study we included patients with progressive sarcoidosis and age-matched controls. Macrophages were differentiated from peripheral blood monocytes using GM-CSF supplemented media for 6 days to determine spontaneous cluster formation and transcriptomic regulation.
Results: We observed that monocyte-derived macrophages from non-familial sarcoidosis patients formed more clusters that were significantly larger in their surface area compared to age-matched controls. On transcriptomic level, sarcoidosis-derived macrophages showed an upregulation for genes involved in metabolic pathways, such as glycolysis and fatty acid synthesis as well as mTOR and JAK/STAT signaling pathways.
Conclusion: We established a functional readout for macrophages that provides new information on intrinsic deregulation of macrophages in sarcoidosis. Activation of metabolic and signaling pathways gives novel insights into pathomechanisms of granulomatous diseases in humans. The newly established in vitro assay might present a platform to test compounds specifically targeting granuloma formation.

P143 | Resiquimod reveals great efficacy in inflammatory cytokine induction and T cell recruitment

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Immune response modifiers such as imiquimod, a toll-like receptor (TLR) agonist, have demonstrated high efficacy in the local treatment of pre-cancerous lesions such as actinic keratosis (AK). It has been recently reported that the TLR8 agonist resiquimod could induce the clinical clearance of AK, and this, at much lower concentrations than the therapeutic form of imiquimod.

In our studies, we investigated the effect of resiquimod in vitro, ex vivo and in patients' skin. To measure cytokine expression, we exposed PBMCs from healthy donors to increasing concentrations of imiquimod or resiquimod. qPCR analysis showed a significant upregulation of IL-1B, IL-6, IL-8, IL-12, IL-36γ and TNF after a 4 hour incubation with resiquimod. In contrast, their expression was barely detectable in imiquimod-treated cells. Furthermore, we could show high secretion of IL-1B upon resiquimod treatment by ELISA.

To assess increased cytokine expression by resiquimod in a physiological model, we prepared ex vivo skin explant cultures that were treated with increasing concentrations of imiquimod or resiquimod for 72 hours at 37oC. After incubation, skin explants were enzymatically digested and analyzed flow-cytometry. As expected, treatment with imiquimod or resiquimod had no significant impact on the percentages of dendritic cells. In contrast, resiquimod treatment at both 0.01% and 0.03% resulted in a significant increase of T lymphocytes in the explant whereas imiquimod had no impact on their number. Moreover, cytokine expression levels in skin explants were measured by qPCR after RNA isolation. While imiquimod and resiquimod upregulated the expression of IL-12, IL-17 and IL-6 to similar levels, a treatment with 0.01% resiquimod resulted in a further increased expression of IL-1β, IL-36γ, IL-23 and IL-8.

To further study the effects of resiquimod on the cell infiltrate in AK, biopsies were taken from AK patients' lesions before, during and after resiquimod treatment in the course of the multicentre, partly placebo-controlled, double-blind clinical trial NCT01583816. Interestingly, we could identify massive dermal T cell infiltrates in close proximity to the dermo-epidermal junction zone during treatment which was followed by regression of the lesions.

Taken together, our data suggest that resiquimod present as an immune response modifier of great efficacy than imiquimod with possibly reduced side effects associated with the use of very low therapeutic doses.

P144 | Acute generalized exanthematous pustulosis - Not only a T cell driven disease? Impact of innate immunity in adverse drug reactions of the skin

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Acute Generalized Exanthematous Pustulosis (AGEP) is a severe cutaneous adverse drug reaction characterized by an acute onset of sterile pustules on an erythematous background, fever and peripheral blood neutrophilia. Even though it is hypothesized that the massive neutrophilic infiltration seen in skin lesions is mediated by memory T cells via the production of IL-8, little is known about the early pathophysiology of AGEP. Our studies could demonstrate that innate cytokines, in particular IL-36γ, IL-1β, IL-6 and IL-8 are highly expressed in lesional skin of AGEP patients. Such an IL-36γ, IL-1β, IL-6 and IL-8 overexpression was not observed in patients with drug-induced maculopapular rash (MPR), the classical delayed-type drug hypersensitivity reaction. Furthermore, we could detect high levels of IL-36γ, IL-6, IL-8 and serum amyloid A (SAA), a protein being highly induced by IL-1β, in the serum of AGEP patients.

In vitro, the causative drug specifically induced IL-36γ and IL-1β expression in peripheral blood mononuclear cells. Such culprit drug induction of innate cytokine secretion in vitro was specific for AGEP. Interestingly enough, when separating the cell types, the pure monocyte population showed high cytokine secretion upon drug treatment in the absence of any T cell. These results suggest that innate cytokine secretion by monocytes/macrophages in response to culprit drug exposure likely plays a key role in the pathogenesis of AGEP and that the immune activation is not T cell dependent.

IL-1β secretion from AGEP PBMC could be reduced significantly by the pancaspase inhibitor Z-VAD, indicating an active inflammasome in AGEP cells upon drug treatment. Notably, treatment of AGEP PBMC with the culprit drug induced autophagy, as demonstrated by positive LC3 immunofluorescence staining. Amoxicillin, a drug frequently causing AGEP, induced LC3 cleavage in THP-1 cells. Moreover, knock-down of Beclin-1, an autophagy regulator, led to higher IL-1β and IL-36γ mRNA expression in response to Amoxicillin. These findings indicate a possible role of a defective autophagy in the pathophysiology of AGEP.

Taken together, this is the first report of a significant impact of the innate immune system in the pathogenesis of AGEP. The study of the early mechanisms in the pathogenesis of this severe cutaneous drug eruption should help us to improve on an early diagnosis and treatment of the affected patients.

P145 | Effects of Cold Atmospheric Plasma (CAP) on the metabolic activity and survival of human T cells and cancer cell lines

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The fourth state of matter, called plasma, is generated by subjecting gas to an electro-magnetic field. The discovery of cold atmospheric plasma (CAP) enabled completely new applications within mild physiological temperatures (less than 40°C at the point of application). Because of this, medical interest arose quickly, as the use of CAP allowed in vitro and in vivo studies and new therapeutic approaches. CAP consists of excited atoms, electromagnetic fields, UV-light and free radicals, amongst them reactive oxygen and nitrogen species (ROS/RNS). CAP is already in clinical use in wound therapy and skin regeneration due to its disinfection effects. Besides that, it has been object of research because of its pluripotent effects such as cell detachment and cancer cell death. Yet the specific underlying functional mechanisms of CAP in cancer are still elusive. Therefore, we analyzed the effects of CAP on the metabolic activity and survival of human T cells and human cancer cell lines.

Cancer cell lines and human T cells were treated with CAP for different time periods. CAP effects on the metabolic activity were analyzed via the Seahorse mito-cell stress test assay, Seahorse T cell activation assay and Resazurin-Assay, while the effects on the cell survival as well as on phenotypic changes were analyzed via flow cytometry. Increasing CAP treatment lead to lower viability of the cancer cells. Already low doses of CAP had significant effects on the metabolic activity of the cells. Interestingly, different cancer cell lines from the same tumor entity differ in their susceptibility towards CAP treatment. Using natural radical scavengers abrogated the CAP effects.

In comparison, human T cells responded like cancer cells upon CAP treatment. CAP reduced viability and proliferation of CFSE labeled T cells after stimulation. Metabolic activity and T cell activation were also negatively affected by CAP treatment, even at low doses. In contrast to cancer cells, natural substances were only able to restore cell viability to a certain extent.

Taken together, CAP exerts toxic effects on cancer and immune cells. Since those effects can be compensated by natural substances which are known to be radical scavengers, it can be concluded, that CAP mainly acts on cells through radical species of oxygen and nitrogen.

P146 (OP04/01) | Mast cells instruct keratinocytes to produce TSLP - relevance of the tryptase/ PAR-2 axis

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Background: Numerous studies have established a role for thymic stromal lymphopoietin (TSLP) as a cytokine promoting Th2 inflammation. Notwithstanding, the endogenous factors inducing TSLP and their mechanisms of action are not well defined. We previously reported that skin irritation triggers TSLP in the skin, and that PAR-2 activation is involved in this response. We hypothesized that mast cells (MCs) may likewise elicit TSLP responses owing to their abundant expression of MC tryptase (an endogenous PAR-2 activator).

Methods: MC degranulation in mouse belly skin was induced by compound 48/80 (c48/80) in vivo and ex vivo in the presence/absence of neutralizing antibodies or antagonists. PAR-2 knockout mice were treated with c48/80 in vivo and skin explants were stimulated with mMCP6 (murine tryptase) ex vivo. MC-deficient C57Bl/6J-Mcpt5-CrexB6;129S7Dicer1tm1smr/J mice and the Cre-negative B6;129S7Dicer1tm1smr/J littermate control mice were also investigated. Murine skin explants were stimulated with c48/80 alone or in combination with mMCP6. In complementary experiments, murine skin explants and primary human keratinocytes (KCs) were stimulated with lysate or supernatant of human skin MCs. Additionally, murine skin explants and human KCs were stimulated with tryptase alone or together with IL-1. TSLP levels were measured in explant or cell supernatants and skin lysates by ELISA. Immunofluorescence (IF) staining served to visualize TSLP in intact skin. TSLP mRNA was quantified by RT-qPCR.

Results: Epidermal TSLP was induced by intradermal c48/80 injections into murine skin as shown by IF staining. PAR-2 deficiency was associated with strongly reduced c48/80-mediated induction of TSLP. TSLP responses could also be elicited in skin explants by mMCP-6, whereas skin from PAR-2 knockout mice was refractory to mMCP-6 stimulation. Skin TSLP responses elicited by c48/80 were MC dependent, as evidenced using skin explants from MC-deficient compared to MC sufficient littermate controls. Stimulation of MC-deficient skin with exogenous mMCP6 rescued the TSLP response, indicating that tryptase (and no other mediator) was indeed accountable for this activity. As with murine skin, tryptase-dependent TSLP production was observed in human KCs stimulated with MC lysate or purified human tryptase. Tryptase and IL-1 acted in concert to enhance TSLP production in murine explants and human KCs. Following elimination of tryptase from the MC lysate (by immunoprecipitation) or in the presence of a tryptase inhibitor (nafamostat),

MC elicited TSLP responses in human KCs were abolished, suggesting that pathways are conserved between human and mouse.

Conclusion: Mast cell degranulation results in activation of the PAR-2 pathway in adjacent keratinocytes, evoking TSLP production. MC tryptase (or its murine counterpart mMCP-6) is the causative mediator in this scenario. The crosstalk between dermal MCs and epidermal keratinocytes seems more important than currently recognized, and may have implications for the clinical management of skin disorders, e.g. by the selective targeting of PAR-2 or mast cell biomolecules, especially tryptase.

P147 | IL-36γ drives skin toxicity induced by EGFR/MEK inhibition and Cutibacterium acnes

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Epidermal growth factor receptor (EGFR) and MEK inhibitors are beneficial for the treatment of solid cancers but frequently induce cutaneous adverse reactions, such as acneiform eruptions, which affect a patient's quality of life with their therapeutic regimen. The pathophysiology mechanisms underlying these side effects remain elusive. EGFR/MEK-inhibitor-induced acneiform eruptions manifest as neutrophilic folliculitis and develop in pilosebaceous units of sebum-rich regions of the skin. Gene expression profiling in skin biopsy samples from patients suffering from acneiform eruption by EGFR/MEK inhibitors revealed elevated IL-36γ, which could be the initiating cytokine of this neutrophilic cutaneous inflammation. In line with gene expression data, histological analysis of patients' lesions showed elevated IL-36γ expression in follicular keratinocytes. Using primary human keratinocytes, we found synergistic IL-36γ elevation induced by EGFR/MEK inhibitors, acting in concert with the skin commensal bacterium Cutibacterium acnes. Analysis of human IL-36γ promoter region combined with database search for transcription factor binding profiles identified Kruppel-like factor 4 (KLF4) as the critical factor for elevated IL-36γ, in addition to C. acnes-induced NF-B activity. EMSA and DNA pull down assays demonstrated the presence of a KLF4-binding site in the human IL-36γ promoter, and overexpression and knockdown experiments supported the role of KLF4 in IL-36γ production in primary keratinocytes. We found elevated KLF4 levels by blockade of the EGFR-MEK-ERK pathway, due to attenuated proteasomal degradation and subsequent accumulation of KLF4. Therefore, our data suggest that acneiform eruptions may result from the combination of EGFR/MEK inhibition and commensal C. acnes on keratinocytes and identify IL-36γ and KLF4 as possible targets to limit cutaneous toxicities of EGFR/MEK inhibitors.

P148 | Initial analysis of T cell receptor (TCR)β repertoires in perilesional skin biopsies of bullous pemphigoid patients

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Objective: Bullous pemphigoid (BP) is an autoimmune disorder characterized by blister formation induced by binding of IgG autoantibodies to target antigens of the basal membrane. The mechanism of blister formation includes complement activation by autoantibodies, inflammatory infiltrates and liberation of proteolytic enzymes that directly interferes with the adhesion function of the autoantigens. The presence of CD4 + T cells in lesional skin has been reported previously; however, their clonality is unknown.

Methods: We performed high-throughput sequencing of perilesional skin biopsies of 5 BP patients and 5 age- and sex-matched control patients with other cutaneous diseases (mainly basal cell carcinoma) and identified their T cell receptor (TCR)β-repertoires.

Results: The frequency distribution of TCRβ clonotypes and the absolute number of unique TCRβ sequences was similar in skin tissues of BP patients and of controls, respectively, except that the most hyperexpanded TCRβ clonotypes were found preferentially in BP patients. Focusing on the number of shared TCRβ clonotypes we identified 12 TCRβ sequences that were present and oligoclonal expanded in at least 2 BP patients but completely absent in controls. In contrast, only one TCRβ clonotype fulfilled these criteria in control patients. Further, four from these 12 TCRβ clonotypes expressed the TCRβ V20-1 segment.

Conclusion: These data demonstrate that BP lesions harbour a highly polyclonal TCRβ repertoire. However, the presence of oligoclonal expanded and shared TCRβ clonotypes suggests a potential accumulation of autoreactive autoantigen-specific T cells in BP skin lesions. In line, immunohistological staining revealed lymphoid tissue-like structures containing CD4 + and CD20 + cells in MPO+ lesions. Further longitudinal studies with a higher number of patients are required.

P149 | Introgression of HLA-class I alleles from Neanderthals and Denisovans causes autoimmunity in psoriasis and other HLA-class I associated immune-mediated diseases

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The association of HLA-class I risk alleles with common immune-mediated diseases has been known for up to 50 years. In view of their unresolved autoimmune pathogenesis, these inflammatory diseases were summarized under the term MHC-I-opathies. Several genome-wide association studies (GWAS) have shown that statistical epistasis, i.e. non-additive gene-gene interaction between HLAC* 06:02, HLA-B*27 or HLA-B*51, and gene variants of the endoplasmic reticulum aminopeptidase 1 (ERAP1) controls the risk of psoriasis, ankylosing spondylitis, and Behcet's disease. Gene-gene interactions between HLA-class I alleles and ERAP1 were also identified in type I diabetes, multiple sclerosis, Crohn's disease, and other immune-mediated diseases. This suggests common autoimmune pathways, the elucidation of which should be a key to better understand the pathogenesis of these MHC-I-opathies.

Psoriasis is a T-cell mediated autoimmune disease with complex genetic traits. By unbiased screening, we have previously shown that in psoriasis, HLA-C*06:02 mediates an autoimmune response against melanocytes through presentation of an autoantigenic peptide from ADAMTS-like protein 5 (ADAMTSL5) to CD8 + T cells. We furthermore observed that ERAP1 generates the autoantigenic ADAMTSL5 peptide from precursor peptides. Accordingly, different aminopeptidase activities of different ERAP1 haplotypes control the autoimmune response against melanocytes and the risk of psoriasis by different autoantigen supplies for presentation by HLA-C*06:02. By analyzing archaic genomes, we now find an evolutionary cause of the pathogenic interaction between ERAP1 and HLA-class I alleles. We establish that HLA-C*06:02, HLA-B*27 or HLA-B*51 originate from now extinct archaic hominins, Denisovans and Neanderthals. Thus, heightened immunity from introgression of these archaic HLA-alleles causes a risk of autoimmune responses against ERAP1-dependent self-peptides in modern humans. The risk-associated ERAP1 haplotypes instead originate from either Denisovans, Neanderthals or modern humans. Different evolutionary origins of the archaic HLA-class I alleles and ERAP1 haplotypes cause functional mismatches that further increase the risk of autoimmune diseases by disproportionately high autoantigen supplies. This mechanism controls the risk of psoriasis and is a likely common pathogenic principle in the other HLA-class I mediated autoimmune diseases.

P150 | Inhibiting acantholysis in pemphigus vulgaris by novel systemic and topical therapeutics

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Pemphigus vulgaris (PV) is an autoimmune bullous skin disease which is defined by intraepidermal split formation. Clinically, blister formation of the skin and mucous membranes can be found. Acantholysis is caused by autoantibodies (IgGs) directed against desmoglein (Dsg) 1 and Dsg 3. So far, PV is mainly treated with high doses of corticosteroids which may involve severe side effects. Therefore, new treatment strategies are of interest.

Known functional inhibitors (A66, BIRB796, GW441756, Selumetinib and Vandetanib) from the SelleckChem Target Selective Inhibitor Library and the standard therapy prednisolone were tested in a human skin organ culture model. Here, we induced intraepidermal split formation by injecting a well-defined human anti-Dsg 1/3 single-chain variable fragment (scFv) antibody intradermally. Five inhibitors and prednisolone were injected intradermally, two inhibitors were applied topically. Immunohistochemical stainings for the scFv confirmed binding to the intended targets Dsg 1 and 3 and the scFv within the epidermis. Furthermore, skin sections were hematoxylin and eosin-stained and analyzed using semi-quantitative histomorphometry.

As expected, injecting prednisolone resulted in significant reduction of split formation for six patients in two different experimental settings. The two topically applied inhibitors reduced split formation significantly in three patients. One inhibitor (GW441756) performed in three applied concentrations (1, 2 and 5%), while the other inhibitor (Vandetanib) showed significant reduction only in the highest concentrations (5%).

The two inhibitors Vandetanib and GW441756 will be analyzed in more detail as possible future treatment options for PV.

P151 | Diverse apoptotic effects of TNFα on malignant and normal human keratinocytes

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Tumour Necrosis Factor alpha (TNFα) is a pro-inflammatory cytokine that may paradoxically induce either apoptosis or cell survival. The diversity of its signaling makes TNFA also important for resistance to infections and cancer. Besides, TNFα is involved in several immunological processes such as potentiating innate immunity or co-stimulating T-cell activation and differentiation. The targeting of TNFα is a well-established and efficacious therapy for several autoimmune diseases like psoriasis, rheumatoid arthritis, or hidradenitis suppurativa. It mediates its activity through binding of TNF-receptor (TNFR) 1 or 2. TNFR1 is mainly responsible for transmitting apoptotic signals. The activation of apoptotic mechanisms can either be intrinsic (mitochondrial) or extrinsic (death receptors). Death ligands such as TNF-Related Apoptosis-Inducing Ligand (TRAIL) specifically induce extrinsic apoptosis, while cytostatic drugs as 5-fluorouracil (5FU) induce intrinsic apoptosis. To investigate the effects of TNFα on apoptosis in malignant and normal human keratinocytes, human cutaneous squamous cell carcinoma (SCC) cell line SCC-13 and immortalized human keratinocytes HaCaT as well as primary normal human keratinocytes (PNHK) were stimulated with TNFα and then treated either with TRAIL or 5FU. Cell viability and proliferation, DNA fragmentation, apoptosis and cytotoxicity were determined by WST-1 proliferation assay, ELISA, flow cytometry, and colorimetric analysis of lactate dehydrogenase (LDH), respectively. Western blotting was performed for analysis of caspase-3. TNFα affected viability of SCC-13 and HaCaT cells in combination with 5FU or TRAIL. On the other hand, TNFα did not influence cell viability of PNHK. Mechanistically, TNFα enhanced the apoptotic effects of both extrinsic and intrinsic stimuli in SCC-13 and HaCaT. However, TNFα protected PNHK against TRAIL and 5FU-induced apoptosis. The effects were dose-dependent and TNFα-specific; and the apoptosis pathway was caspase-dependent. In conclusion, opposing effects of TNFα on malignant versus normal human keratinocytes were observed with possibly relevant clinical interpretation.

P152 | Alopecia areata is governed by a reversible, senescence-like growth arrest

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Alopecia areata (AA) is hypothesized to be an organ specific autoimmune disease mediated by T lymphocytes directed to the hair follicles and the most common immunological cause of hair loss. Although genetic predispositions and environmental factors may trigger the initiation of the disease, the exact cause is still unknown. The hair follicle is generally known for maintaining a privileged immune status. In AA autoreactive CD8 + T cells and NK T cells lead to an attack on the hair follicle and therefore to hair loss. Interestingly, this hair loss is not associated with scarring, as we know from other autoimmune diseases such as lichen planopilaris and lupus erythematosus. The molecular profile of cytokine pathways in AA tissues that lead to an arrest of hair growth is still unclear. Although data from several studies indicate a Th1 / IFNf mediated disease course, recent studies, showed a much broader cytokine signature as CD8 + NKG2D+ T cells play a major role in the pathogenesis of AA. In our previous studies we have shown that the treatment of proinflammatory autoimmune diseases with dimethyl fumarate (DMF) reduces the severity of psoriasis and the relapse rates of MS in humans. DMF promotes an IL-23lowIL-12lowIL-10 + DC phenotype, suppresses the differentiation of pathogenic Th17/Th1 cells and promotes the induction of IL-4 + Th2 cells. With rising evidence that Th2 cells play a major role in disease activity we conducted a study with DMF in 40 patients with therapy resistant alopecia areata in whom multiple therapies had failed. All the patients had a diagnosis of moderate to severe scalp AA, defined as > 20% AA, for at least 6 month duration. After an initial screening all patients received DMF daily according to the titration known from the treatment of Psoriasis. 60% of the included patients showed an improvement in AA, with a decrease in the Severity of Alopecia Tool Score (SALT) and an increase in the patients self-assessment. qRTPCR and FACS analysis from patients after DMF treatment showed a significant decrease in the expression of the proinflammatory cytokines IFN-f, IL-17A, IL-15, CxCL10 and GZMB.

In further analyses, we quantified the mRNA expression of different cell regulating genes. Interestingly, biopsies of AA patients showed a significantly higher expression of CDKN2A compared to healthy donors. At the same time, we were able to show that patients under DMF treatment showed a lower CDKN2A expression. Our findings

highlight that the therapeutic effects of DMF in the treatment of AA are not only based on the reduction of proinflammatory cytokines but in addition, an altered expression of cell cycle regulatory genes is manifested in AA patients before and after DMF treatment. We believe that AA is a reversible, senescence-like growth that needs to be further characterized.

P153 | Prevalence of Candida species in Psoriasis

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Psoriasis patients are more frequently colonized with Candida species. Candida species may trigger and exacerbate psoriasis, but the correlation between fungal colonization and clinical severity of psoriasis remains unclear. Interleukin (IL)-17 inhibitors are associated with an increased risk of candidiasis, but the influence of IL-17 based therapies on the prevalence of Candida species has not been studied.

The aim of this study was to examine the prevalence of Candida species in psoriasis patients and control subjects, to investigate the influence of IL-17 inhibitors and clinical severity, and to examine whether a colonization with Candida species leads to an altered immune response in psoriasis patients.

The prevalence of Candida species was examined in 265 psoriasis patients and 200 control subjects. Swabs (from the oral cavity, the armpit, and psoriatic lesions) and stool samples were analyzed for fungal growth. Peripheral mononuclear blood cells (PBMCs) from 20 psoriasis patients colonized with Candida species and 24 uncolonized patients were isolated and stimulated with Candida albicans and lipopolysaccharides (LPS). The expression of interferon (IFN)-γ, IL-17A, IL-22 and tumor necrosis factor (TNF)-α was measured by quantitative real-time polymerase chain reaction (qPCR).

A significantly higher prevalence of Candida species was found in psoriasis patients ($\chi 2$; $P = < 0.001$). The highest prevalence was found in stool samples. Candida albicans was the most common fungus. Older participants (≥ 51 years) were more frequently colonized. No correlation was found within the sexes. A more severely clinical affection or treatment with IL-17 inhibitors did not lead to an increased colonization. Colonized and uncolonized patients did not differ in their immune response.

The prevalence of Candida species is higher in psoriasis patients but is not associated with clinical severity and colonization does not lead to an altered immune response. IL-17A antagonists do not increase the prevalence of Candida species.

P154 (OP04/04) | Topical application of Adenosine A2 receptor agonists to skin prevents contact hypersensitivity reactions in mice by affecting skin residing Dendritic cells

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Introduction: Adenosine (Ado) has well documented immunosuppressive capacities, affecting tumor immunity and inflammatory reactions. For skin, we have recently shown that the production of Ado by CD73 + dendritic cells (DCs) is critical for tolerance development in a contact hypersensitivity (CHS) model.

Objectives: To exploit the immunosuppressive effects of Ado for treatment of CHS reactions by topical application of A2-type Ado receptor (AdoR) agonists to the skin.

Materials & **Methods:** A2A (CGS1798) and A2B (BAY0120) AdoR agonists were epicutaneously applied to skin prior to sensitization, or prior to challenge with the hapten DNFB.

The ear swelling and the immunologic outcome was analyzed.

Results: A reduction of the ear swelling was apparent as compared to solvent treated groups, even when AdoR agonists were given 30 min before sensitization. Thus, indicating a defective sensitization. In the ear tissue, AdoR agonists reduced the production of proinflammatory cytokines by sensitization and reduced numbers of skin migrating DCs (DCs) (CD207 + , CD207-CD11b+ and CD207-CD11b-), which produced less IL-12 and had a lower expression of CD86 and CD80, were recorded in draining LN. When CD3 + T cells were isolated from LNs and were re-stimulated ex vivo with haptenized DCs, less proliferation of CD4 + and CD8 + T cells derived from A2A and A2B AdoR agonists-treated mice versus controls was recorded. In line with these data, we also observed reduced expression of CD25, diminished production of granzyme B in CD8 + T cells, as well as reduced production of pro-inflammatory cytokines such as IFNγ, TNFα, IL-6, IL-17A and IL-1b in A2A and A2B AdoR agonist treated groups.

Conclusion: These data indicate that topical application of AdoR agonists to skin can prevent sensitization of T cells against haptens by reducing migration and activation of skin derived DCs.

P155 | Regulatory T cells prevent neutrophilic infiltration of skin during contact hypersensitivity reactions by strengthening the endothelial barrier

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The healing phase of contact hypersensitivity (CHS) reactions is critically dependent on regulatory T cells (Tregs). But even the early inflammatory phase, i.e. 6 to 24 hours after induction of a CHS reaction, is susceptible to Treg mediated suppression. To investigate

the underlying mechanisms, we injected Tregs prior to challenge in a DNFB CHS model and analyzed the skin infiltrating cells as early as 6 hours later. We found mainly neutrophils in challenged skin, but no T cells. This influx of neutrophils was blocked when Tregs were injected before challenge, indicating that they are able to prevent the first wave of leukocytes that enter the skin. These innate cells are responsible to induce a full blown immune reaction. As underlying mechanism, we identified that Tregs can tighten endothelial junctions by inducing intracellular cAMP, leading to PKA-RhoA dependent signaling. This eventually reorganizes endothelial junction proteins, such as Notch3, Nectin2, FilaminB and VE-cadherin, which all contribute to tightening of the endothelial barrier. As consequence, Tregs prevent the leakage of proinflammatory cells from and into the tissue. This mechanism establishes a novel function of Tregs in the downregulation of immune reactions.

P156 (OP03/01) | Interferon-independent enhancement of melanoma cell immunogenicity by RIG-I activation

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Clinical efficacy of checkpoint blocking (ICB) melanoma therapy is critically dependent on the anti-tumor activity of IFN-gamma, which augments the processing and presentation of antigens presented on HLA class I (HLA-I) molecules to cytotoxic CD8 + T lymphocytes. Upon recognition of cognate HLA-I surface antigens, CD8 + T cells become activated and kill melanoma cells. Thus, HLA-I-low melanoma cells escape CD8 + T cell recognition, but can be sensitized to T cells by HLA-I upregulation in the presence of IFN-gamma. Recently, we and others demonstrated that melanoma cells can acquire resistance to IFN-gamma by inactivating genetic alterations in different components of the JAK1/2-STAT1 signaling pathway, enabling them to maintain a HLA-I-low phenotype in the presence of the cytokine. Here, we pursued an approach to enhance HLA-I antigen processing and presentation of melanoma cells in an IFN-independent manner. Patient-derived melanoma cells harboring a JAK1/2 mutation and being insensitive to T cell effector functions were transfected with a short dsRNA (3pRNA), an activating ligand of the pattern recognition receptor RIG-I. The thereby initiated innate immune response induced HLA-I expression independent of JAK1/2-STAT1 signaling and enhanced melanoma recognition by autologous CD8 + T cells. To address these findings in an anti-PD 1 non-responder patient model, we asked whether combination of 3pRNA and immune checkpoint blocking antibodies could improve anti-tumor T cell responses. In fact, 3pRNA-transfected melanoma cells were able to enhance T cell activation in presence of anti-PD-1 and anti-TIGIT blocking antibodies.

In summary, this study demonstrates a beneficial effect of RIG-I activation on HLAI antigen presentation and CD8 + T cell recognition of IFN-deficient melanoma cells. Moreover, combination of 3pRNA and ICB treatment improves T cell responses in ICB-non-responding patient models suggesting that combinational therapy could be a strategy to overcome T cell resistance in melanoma.

P157 | [18F]FDG PET/CT-based imaging method to characterize the therapeutic effects

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Data from clinical and preclinical studies have shown that fumarates like dimethyl fumarate (DMF) - by suppressing the Th17 response - improve psoriasis and multiple sclerosis in human and experimental autoimmune encephalomyelitis (EAE) in mice. In our previous studies, we analyzed the anti-inflammatory effects of DMF on the immune response by methodologies like intracellular cytokine staining and flow cytometry or by performing quantitative mRNA expression from isolated cells. Here, we aimed to establish an in vivo method to follow T cell activation and the anti-inflammatory properties of DMF in mice immunized for developing EAE. We decided to investigate whether it is possible to characterize the therapeutic effects of DMF treatment in vivo using a non-invasive imaging technique. As proliferating T cells have been shown to uptake the clinically-used radioactive glucose analog on [18F]FDG (18F-fluorodeoxyglucose), we applied this tracer to mice immunized for EAE. Our aim was to follow T cell activation in the lymphatic system at different time points. In addition, we established a PET/CT-based imaging method to characterize the therapeutic effects of DMF treatment after active EAE induction. The PET/CT imaging data were analyzed by region of interest (ROI)-based methodology and validated by biodistribution studies and ex vivo mRNA expression analysis. Our findings show that the [18F]FDG and PET/CT-based imaging methodology can be used to characterize the effects of therapeutic compounds in the disease course of actively induced EAE in mice.

P158 | Dimethyl fumarate-induced IL-17 low IFN-γ low IL-4 + Th cells protect mice from severe encephalomyelitis

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Dimethyl fumarate (DMF) is the first modern small molecule approved for the treatment of psoriasis and multiple sclerosis (MS), two organ-specific inflammatory autoimmune diseases dominated by IL-17-producing T helper (Th) cells. As reported, DMF modulates the immune response by inducing IL-10 and suppressing IL-23 in antigen-presenting cells (APC). As a consequence, DMF promotes antigen-specific Th2 responses in vitro and in vivo. Based on previous observations we confirmed that oral application of DMF ameliorates Th17-dependent encephalomyelitis and induces a Th2 phenotype in a mouse model of experimental MS (EAE) induced by active immunization. Here, we asked, whether DMF-treated autoreactive Th cells can be used to control the severity of EAE. Myelin-specific Th2-like cells activated in the presence of DMF induced no disease in recipient mice after adoptive transfer. Moreover, mice that received DMF-induced Th2-like cells (IL-17lowIFN-γlow IL-4 +) showed delayed onset and reduced severity of EAE even after active immunization. An early in vivo recall of transferred Th2-like cells was required for longterm protection in mice that were immunized a second time after adoptive transfer. Our findings indicate that DMF's potency in inducing autoreactive Th2-like cells with protective features is one conducive mechanism in the treatment of patients with relapsing MS.

P159 | Pityriasis rubra pilaris - a primarily IL-23 mediated inflammatory skin disease

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Pityriasis rubra pilaris (PRP) is a rare chronic inflammatory skin disease, which presents with follicular hyperkeratotic papules that merge into salmon-colored scaling plaques with islands of unaffected skin. A rapid onset leading to erythroderma appears quite frequently in adults and often depicts a diagnostic and therapeutic challenge. Owing to shared clinically features between PRP and psoriasis, treatment strategies often follow anti-psoriatic therapeutic choices, but unfortunately are not always effective. Therefore, we aimed at understanding pathogenic mechanisms in PRP in order to target the disease more precisely.

Clinical and histological characteristics of PRP patients (n = 32), were compared to patients suffering from psoriasis (n = 30) and eczema (n = 30). We furthermore performed RNA sequencing of lesional and non-lesional skin biopsies and analyzed patients' antigen presenting cells and T cells by flow cytometry as well as immunohistochemistry. We thereby found an increased Interleukin (IL)-23 production by dendritic cells that was comparable to IL-23 levels in psoriasis, whereas hardly any T cells produced IL-17. Neutrophils were virtually absent. No intrinsic deficiency in cytokine production of keratinocytes was observed.

In summary, our results suggest that PRP is caused by a truncated Th17 immune response, with presence of IL-23, but low numbers of Th17 cells and neutrophil granulocytes in lesional skin. A good clinical response of PRP patients to anti-IL-23 treatment further supports this concept of the PRP pathogenesis.

P160 | From bench to beside: An experimental approach to identify skin derived Th2-determining tissue signals

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Atopic dermatitis (AD) is a wide spread chronic disease characterized by Th2 immune cell driven inflammation of the skin. AD is caused by complex interactions of genetic, immune and environmental factors that are not yet fully understood. The aim of this study was to identify tissue factors expressed in the skin that are involved in the initiation of Th2 immune response and thereby likely to play a role in predispositioning patients for AD.

A common model for investigating the properties of Th2 immunity is experimental pathogen infection of BALB/c mice. An infection of BALB/c mice with pathogens such as Leishmania major, or Staphylococcus aureus both induces a Th2-response characterized by IL4 and IL13 secretion.

We infected BALB/c mice with either Leishmania major or Staphylococcus aureus and analyzed changes in gene expression, 6 hours after infection, by a genome wide microarray. To identify potential general regulators of Th2 immunity we identified genes which were similarly regulated in both cutaneous infection models We then compared those candidate genes with previously published expression data of AD patients (lesional and non-lesional skin) and healthy controls (Illumina sequences, GSE121212) in order to find genes with similar expression profiles in both murine and human Th2 dependent contexts. Our approach yielded genes known to be involved in AD such as PTGES, IL-34 and OSMR but also a number of genes that have so far not been linked to Th2 immunity in general

or atopic dermatitis specifically. We are now in the process of validating and analyzing the 25 most differentially expressed candidate genes in more detail.

Using this approach we are confident to identify new factors involved in generation of Th2 immunity and in the pathophysiology of atopic dermatitis.

P161 | B-cells in psoriasis?

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Psoriasis is a chronic-inflammatory disease with an immunogenetic background. T-cells and cells of innate immunity are currently regarded as dominant in the pathogenesis. In this context, the role of B-cells beyond antibody production has been hardly studied sofar. Therefore, B- and T-cells were immunohistochemically examined in psoriasis and compared to cutaneous lupus erythematosus (CLE), atopic dermatitis (AD) and acrodermatitis chronica atrophicans (ACA) (n = 10 each). As expected B-cells are prominent in ACA located in the dermis whereas in both psoriasis and CLE B-cell infiltrates could be shown in the majority of samples in upper dermis. In AD samples only few and scattered B-cells were seen. No relation of B-cell number and distribution to clinical manifestation or inflammatory activity could be seen sofar in psoriasis. Prospective sampling of sequential biopsies from psoriasis lesions of different stages as well as under therapy will be done to further examine this relation. B-cells may play a regulatory role in the pathogenesis of psoriasis and cooperate with T-cells and cells of innate immunity.

Infectious Diseases

P162 | First systematic analysis of Darier Disease patients' cutaneous microbiome and transcriptome reveals S. aureus dominance and a strong anti-Staphylococcus immune response

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Darier disease (DD), also known as keratosis follicularis is a rare autosomal dominant genodermatosis with a prevalence range between 1/30,000 and 1/100,000. It is caused by a mutation in the ATPA2 gene disrupting intracellular Ca2 + signalling, leading to disadhesion of suprabasalar cells (acantholysis) and apoptosis (dyskeratosis). Cutaneous infections, either bacterial or viral, are known to be associated with exacerbation of DD. However, the structure of the microbiota and the role it may play in this disease are still poorly understood. Therefore, we investigated the skin microbiome of 14 patients with moderate to severe DD. Skin swabs were collected from three predilection sites (sub-mammary, inguinal and axillar) then analyzed by 16S ribotyping. The obtained data revealed a distinct microbiome profile characterized by a reduced α-diversity. Darier lesions were strongly colonized by Staphylococci at the expense of other genera, including Propionibacterium or Moraxella. Similar to atopic eczema (AE) Staphylococcus aureus was dominant both on lesional and non-lesional DD skin. However - different to AE - relative abundance of Corynebacteria was not reduced on DD lesions. Furthermore, S. epidermidis did not expand on DD lesions - as observed in AE - whereas higher relative abundance of C. simulans was characteristic in DD. The altered microbiome is reflected by the upregulation of genes involved in anti-infectious immune mechanisms, particularly in response to S. aureus. Expression of genes involved in IL-17 signalling, T-cell activation, leucocyte migration and keratinocyte differentiation was significantly altered in DD lesions as well. This is the first sequencing based report on skin microbiome and transcriptome in DD. It allows a deeper understanding of the microbial dynamics and identification of bacterial key players and facilitates our understanding how they may be involved in DD pathogenesis

and especially exacerbations. Further analyses will need to focus on preventive measures based on microbiota modulation.

P163 | Analysis of epidermal defense molecules in tinea pedis

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Tinea pedis is a superficial fungal skin infection typically caused by dermatophytes. Tinea pedis is very common and often chronic or recurrent, but not all individuals are equally susceptible to this fungal infection. It is known that dermatophytes are able to induce the expression of antimicrobial peptides (AMPs) in human keratinocytes. Furthermore, recent surveys have demonstrated that certain AMPs can inhibit the growth of dermatophytes in vitro. The focus of this study was to analyze the secretion of relevant AMPs, especially of RNase7, human beta-defensin-2 (hBD2) and the S100 protein psoriasin (S100A7), in a standardized manner in patients (n = 13) with confirmed tinea pedis. To verify the diagnosis, skin scales were obtained from all patients and the fungi were identified by KOH mount, fungal culture and PCR. To determine the AMP concentrations, the affected skin area of the foot was rinsed with a buffer that was subsequently analyzed. Anatomically identical skin areas of the contralateral unaffected foot as well as defined healthy skin areas of the forearm and forehead served as controls. Samples from age and gender matched healthy volunteers (n = 13) were used as additional controls. After centrifugation, the supernatants of the rinsing fluid were analyzed by standardized ELISAs to determine the concentration of AMPs. As a result, we were able to show that the AMP concentrations in tinea pedis were significantly higher than in the healthy controls. In particular, concentrations of hBD2 and psoriasin were markedly elevated. The induction of AMPs in tinea pedis might be triggered directly by the dermatophytes; furthermore, attendant inflammation or differentiation processes may play a role. Our results indicate that there is no defect in the constitutive expression and induction of the analyzed AMPs by dermatophytes in the epidermis of affected patients. However, we cannot exclude that the induced AMPs fail to efficiently combat dermatophyte growth (e.g. due to structural changes of the AMPs or development of resistance towards the AMPs), leading to long-lasting and recurrent infection. In addition, other AMPs not investigated in this study may be involved in tinea pedis. Additional studies are necessary to gain more insight into the pathogenesis and predisposing factors (e.g., the microbiome, further AMPs) of tinea pedis.

P164 | Induction of toll-like receptor expression in primary human keratinocytes by cytokines

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The innate immune system supports the physical skin barrier in antimicrobial defense. Human keratinocytes constitutively express various members of the Toll-like receptor (TLR) family important for innate immunity including TLR1, TLR2, TLR3, TLR4, TLR5, TLR6, TLR9 and TLR10. TLR1 and TLR6 are the receptors for triacyl lipopeptides and diacyl lipopeptides, respectively. TLR2 recognizes pathogen associated molecular pattern of gram-positive bacteria such as lipoteichoic acid. TLR4 recognizes lipopolysaccharide, a cell wall component of gram-negative bacteria. The ligand of TLR5 is flagellin, a protein expressed in flagellated bacteria. Double-stranded RNA and CpG-DNA motives are recognized by TLR3 and TLR9, respectively. In contrast, TLR10 is believed to have an inhibitory function in innate immune defense.

We investigated the influence of cytokines on the expression of different TLR in primary human keratinocyte culture in vitro. The expression of human various TLR was assessed by realtime PCR analysis.

For stimulation experiments we used cytokines which have been described to be involved either in Th1 or Th2 dominated immune responses: interleukin (IL)-1β, IL-4, IL-5, IL-6, IL-13, IL-15, IL-17, IL-18, IL-20, IL-31, interferon (IFN) γ, tumor necrosis factor (TNF) α and tissue derived growth factor (TGF)α. We revealed IFNγ as the main inducing cytokine for TLR expression in primary keratinocyte cell culture. As IFNγ is a typical Th1 cytokine it may be responsible for the induction of TRL2 expression seen in psoriatic skin, thus resulting in upregulation of innate antimicrobial defense known to be typical for psoriasis.

P165 | High frequency of point mutations within the squalene epoxidase gene of Indian Trichophyton mentagrophytes and rubrum strains is responsible for terbinafine resistance

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Aim: T. mentagrophytes strains of ITS genotype VIII collected from India (1, 2) show high frequency of single point mutations in the squalene epoxidase gene leading to terbinafine resistance (3, 4). Important subsequent amino acid alterations in the squalene epoxidase of clinical Trichophyton isolates were identified by screening of

clinical isolate or analysis of transformants (5). Alterations at position Phe393, Leu397, Phe415 and His440 of the squalene epoxidase were associated with terbinafine resistance (5).

Results: Of 90 T. mentagrophytes strains examined, 61% showed Leu397 mutation causing terbinafine resistance followed by 23% with Thr448 alteration while 7% were double mutants. Terbinafine resistance caused by mutations like Phe393 was found in 2% of isolates and the wild-type sequences were detected in 6% of the strains. All strains feature identical ITS regions; however, 11 different genetic squalene epoxidase variations were detected. Single point mutants of Leu397 are based on different codon types (CTC, TTA and TTG) demonstrating this mutant genotype evolved several times independently. T. rubrum strains from India showed similar tendency for squalene epoxidase alterations. Of 7 analyzed T. rubrum strains, 2 carried Phe393 mutations, 2 harbored Leu397, 1 is a double mutant (Phe393, Thr445), and 2 showed the wild-type sequence.

Conclusions: The high frequency of missense mutations within the squalene epoxidase gene demonstrates the selection pressure for amino acid alterations of the squalene epoxidase in India. Misuse of terbinafine in India apparently leads to an increase of resistant Trichophyton isolates expressing the importance of monitoring for resistance genes.

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P166 | Vitamin D and IL-10 reciprocally regulate antimicrobial peptide expression and intracellular iron accumulation in human

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Human macrophages play central roles in host defense against invading pathogens, as well as in iron homeostasis. On the contrary, pathogens have developed strategies to escape host defense mechanisms and exploit host cell iron metabolism for their own needs. Meanwhile, vitamin D critically supports macrophage host responses and was also found to regulate iron metabolism. This prompted us to investigate the parallel effects of vitamin D on the induction of a vitamin-D-dependent antimicrobial pathway and on iron metabolism in human macrophages. We studied primary human monocyte-derived macrophages and used Neisseria gonorrhoeae, a gram-negative bacterium, well known to interfere with host iron metabolism, as a model pathogen. We find that N. gonorrhoeae, as well as the TLR4 agonist LPS induce HAMP, a master regulator of iron metabolism, in an IL-10-dependent manner. Moreover, N. gonorrhoeae and LPS downregulate the expression of the only known iron exporter ferroportin and increase intracellular iron levels. Meanwhile, both 25-hydroxyvitamin D and the highly bio-active form 1,25-dihydroxyvitamin D counteract the upregulation of HAMP expression. Furthermore, 25D promotes TLR4 induction of cathelicidin expression, a central antimicrobial peptide containing three vitamin D response elements in its human promoter. However, TLR4 activation of macrophages also leads to the induction of IL-10, which in turn dampens cathelicidin expression. In sum, we show that vitamin D and IL-10 opposingly regulate cathelicidin and HAMP expression, thereby defining a regulatory network, which potentially could be targeted for treatment of bacterial infections in humans.

P167 | HSV-1 upregulates MRGPRX2 expression in human mast cells

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Mast cells are pleiotropic immune cells most abundantly found at host-environment interfaces, such as the skin, respiratory and gastrointestinal mucosa. Mast cells act as sentinel cells to sense and fight pathogens. In order to do this, they are armed with a plethora of bioactive mediators that initiate immune cell recruitment, promote the development of adaptive responses and contribute to defense mechanisms of the host against infections. Activation and subsequent mast cell degranulation are mediated through several receptors including the novel human G protein-coupled receptor (GPCR), known as Mas-Related G Protein-Coupled Receptor-X2 (MRGPRX2). Ligands of MRGPRX2 include the neuropeptide substance P, major basic protein, eosinophil peroxidase, opioids, cationic drugs and antimicrobial host defense peptides such as human â-defensins and the cathelicidin LL-37. MRGPRX2 might therefore be a central switch point linking infections, itch, pain and drug reactions. Herpes simplex virus type 1 (HSV-1) can cause infections in humans ranging from orolabial lesions to life-threatening conditions such as herpes simplex encephalitis. In the present study, we found that freshly isolated human skin mast cells are susceptible to HSV-1 infection in a dose- and time-dependent manner. Interestingly, human skin mast cells upregulated the expression of MRGPRX2 after 12 hours post-infection in vitro and maintained a higher expression of this receptor until 72 hours post-infection compared to uninfected control cells. In contrast, the expression of c-Kit and FcRI was downregulated on human skin mast cells after HSV-1 infection. Furthermore, we assessed the spontaneous mast cell degranulation by the surface expression of CD63 after HSV infection. Importantly, all CD63 + mast cells were also MRGPRX2 + but c-Kit- and FcRI-. Our results suggest that HSV infection modulates mast cell surface receptor expression which in turn could influence the development of anaphylactoid reactions, neurogenic inflammation, pain and itch via MRGPRX2

upregulation in the infected host. A better understanding of the mechanisms underlying mast cell activation after HSV-1 infection will allow the development of novel antiviral strategies as well as therapies to treat concomitant symptoms during viral infections

Pharmacology

P168 | Anti-osteopontin treatment ameliorates fibroblast dysfunction in radiation-induced morphea and prevents skin fibrosis in mice

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Background. Radiation-induced morphea (RIM) is a poorly understood entity of disfiguring inflammatory skin fibrosis affecting 1 in 500 breast cancer patients after radiotherapy. Currently, there is no satisfying treatment available since the knowledge about the underlying mechanisms is limited. In systemic sclerosis, the inflammatory cytokine osteopontin (OPN) has emerged as a stimulator of myofibroblast differentiation and subsequent tissue fibrosis. Here, we shed light on the role of OPN in RIM pathogenesis and provide proof-of-concept for repurposing mesalazine as a novel treatment for RIM.

Methods and Results. Primary human mammary skin fibroblasts of control and RIM patients were isolated via outgrowth from biopsies and subsequently studied functionally, by qPCR and by immunofluorescence (IF). Compared to control fibroblasts, we identified a strong myofibroblast phenotype in RIM reflected by increased expression of the myofibroblast marker alpha-smooth-muscle actin (+16.17%), reduced proliferation rates (-48.11%), and reduced cell migration (-31.77%). Western blot analysis revealed a striking upregulation of OPN in RIM fibroblasts. To test if radiation induces OPN, we exposed fibroblasts to 6 Gy γ-radiation. OPN expression was induced in both groups, but was significantly higher in RIM fibroblasts compared to control. In line, an ELISA of peripheral blood samples from healthy individuals and RIM patients revealed significantly higher plasma osteopontin concentrations in RIM (≈40 ng/mL) patients, compared to healthy individuals (≈20 ng/mL).

Mechanistically we identified higher OPN-inducing reactive oxygen species (ROS) in RIM fibroblasts under basal cell culture conditions. Therefore, antioxidative treatment with mesalazine abolished osteopontin expression and successfully reversed the myofibroblast phenotype in vitro. To determine the feasibility of an anti-fibrotic mesalazine treatment in vivo, we characterized the dermal phenotype of polo-like kinase 2 (PLK2) wild-type (WT) and knockout (KO) mice, which develop OPN-driven cardiac fibrosis and might therefore resemble the cutaneous RIM phenotype. In histology and IF we found spontaneous dermal fibrosis and skin thickening in PLK2 KO mice compared to their WT littermates. Moreover, primary PLK2 KO skin fibroblasts expressed significantly more OPN which was abolished by mesalazine. Orally administered mesalazine (150 mg/kg BW for 6 months) prevented spontaneous fibrosis development by inhibiting dermal myofibroblast differentiation in PLK2 KO mice. Based on these findings, one RIM patient participated in an individual healing attempt with 2 g mesalazine daily for 6 weeks. The treatment attenuated inflammation, led to tissue softening, and reduced VAS pain score from 8 to 1.

Conclusion and outlook. Here, we provide the first characterization of RIM fibroblasts. We identified increased ROS-production and excess OPN release as disease-relevant mechanisms leading to myofibroblast differentiation and subsequent fibrosis. Furthermore, we introduce a novel mouse model to study RIM pathophysiology, demonstrate the potential of mesalazine in vivo, and provide proof-of-concept for its repurposed clinical use in inflammatory skin fibrosis.

P169 | Rifampicin in Hidradenitis suppurativa

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Hidradenitis suppurativa (HS), also known as Acne Inversa, is a chronic inflammatory skin disease of the sebaceous glands and hair follicles. The lesions are characterized by painful nodules, scars, fistulas, and abscesses, which can lead to the formation of sinus tracts after rupture. Increased levels of pro-inflammatory cytokines were observed in lesional and perilesional HS skin.

Today, apart from the TNF inhibitor adalimumab, there are still no approved systemic therapies for the treatment of moderate-to-severe HS. However, the guideline gives therapeutic recommendations for the treatment of HS. One of these recommended medications is rifampicin, mostly combined with clindamycin. Based on that, HS is mainly an immune-mediated disease and not bacterial triggered; it can be assumed that the effect of antibiotics is more anti-inflammatory than caused by the inhibition of the DNA dependent RNA polymerase in this condition. In the literature, such an antiphlogistic effect is already described for other inflammatory diseases. In these studies, the downregulation of different pro-inflammatory cytokines was shown after rifampicin treatment.

The aim of our study is, therefore, to investigate whether rifampicin is also antiinflammatory in HS. To test this hypothesis, we treated different HS cell cultures with rifampicin in vitro. Cell culture supernatants were collected for ELISA and CBA analysis of different inflammatory cytokines, e.g., TNF. Our first results indicate an anti-inflammatory effect in rifampicin treated cell culture systems of HS patients.

P170 | Validation of a novel immunomodulatory compound for restoring tolerance in allergy

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Introduction: Present treatments of allergies are mainly aimed at the prevention or amelioration of signs and symptoms rather than curing patients. Specific immunotherapy can achieve this, but comes with many limitations including low compliance and local or systemic side effects such as asthma attacks and life-threatening anaphylactic shock. Small molecule compounds that restore tolerance against allergens by inducing functional regulatory T cells (Tregs) may overcome these limitations, but are not available yet.
Methods: From a library of 40,000 small molecules, we identified 32 compounds that significantly upregulated the expression of Foxp3, the master transcription factor of Tregs, in mouse CD4 + T cells. One candidate molecule (hereafter referred to as C5) was selected and further validated using primary human CD4 + T cells. We performed dose response studies, analyzed long term toxicity, and cell proliferation. In addition, we assessed the production of IL-4, IFN-y, and IL-2 by C5-treated total and Foxp3 + naïve and memory CD4 + T cells on a single cell level by flow cytometry.
Results: C5 significantly increased Foxp3 expression in a dose-dependent manner in primary human naïve and memory CD4 + T cells, by up to 28% and 39%, respectively, without impairing cell proliferation and viability. In addition, C5 dose-dependently decreased the frequencies of IL-2 and IL-4-producing total naïve and memory as well as Foxp3hiCD4 + T cells. In contrast, we observed an overall increase in frequencies of IFN-y producing CD4 + T cells after C5 treatment.
Conclusion: Our novel molecule, C5, potently induces Foxp3 expression and modulates cytokine production in human naive and memory CD4 + T cells. Thus, C5 is a promising candidate for treating allergies via restoring immune tolerance by converting effector T cells into Tregs.

Photobiology

P171 | Enhancement of antibody-dependent cellular cytotoxicity is associated with treatment response to extracorporeal photopheresis in Sézary syndrome

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Importance: Sézary syndrome (SS) is a rare, leukemic type of cutaneous T-cell lymphoma (CTCL), which can be successfully controlled with extracorporeal photopheresis (ECP). Reliable biomarkers to objectively monitor the response to ECP in patients with SS are missing.
Objective: We examined the quantitative and qualitative impact of ECP on natural killer (NK) cell activity in SS patients, and especially their functional ability for antibody-dependent cell-mediated cytotoxicity (ADCC). Further, we addressed the question whether the magnitude of the effect on ADCC can be associated with the anticancer efficacy of ECP in SS patients.
Design: We collected blood samples before starting therapy and after an average of 9 months of uninterrupted ECP treatment from a case-series of 13 SS patients (8 women, 5 men).
Setting: This is a single-centre study.
Participants: All patients were diagnosed according to EORTC-WHO criteria and gave written informed consent to the use of their material and data for research purposes. Blood from healthy volunteers was obtained anonymously from a blood bank.
Exposure: ECP.
Main Outcomes and Measures: Number of NK cells by flow cytometry, ADCC activity by LDH release assay, treatment response based on blood tumour staging.
Results: NK cell numbers were reduced in SS patients compared to healthy individuals and showed a tendency of recovery after long-term ECP treatment, independent of the clinical response to treatment. Patients with a marginal increase (≤1.5 AU-fold) or lack of increase in ADCC activity failed to respond clinically to treatment, while patients with an increased ADCC activity showed a reduction in blood tumour burden.
Conclusions and Relevance: NK-mediated ADCC is selectively enhanced and might be a mechanism underlying the effect of ECP while in addition, it can serve as a reliable biomarker to objectively monitor response to ECP in patients with SS.

P172 | UVA induced changes in glucose metabolism in dermal cells could be part of a cellular antioxidant strategy

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The ultraviolet (UV) radiation with wavelengths above 320 nm fall into the UVA-range, It has been known to induce reactive oxygen species (ROS) and DNA mutations, such as 8-oxoguanosine. The solar UVA exposure of normal people, considering the presence and quantity of UVA radiation in everyday life, is high and the damaging effects of UVA to human skin can reach from accelerated skin aging (photoaging) to skin cancer like melanoma. Although there is an increasing knowledge about enzymatic defense strategies of cells against ROS, not much is known about metabolic defense strategies of cells.

We have recently shown that UVA irradiation and UVA-induced ROS increase glucose metabolism of melanoma cells. In the following work we investigate the UVA-induced changes in glucose metabolism and the functional relevance of these changes in normal non-malignant cells of the skin. We treated primary human and murine fibroblasts and keratinocytes, as well as primary human melanocytes, with repetitive physiological doses of UVA radiation for 4 days. These cells showed an UVA induced increase of glucose consumption and lactate production. Interestingly we could show changes in pyruvate production upon UVA irradiation in fibroblasts and it has been hypothesized that pyruvate could have antioxidant properties. Therefore, we tested the functional relevance of pyruvate as protector against UVA induced ROS.

We show that pyruvate treated with H2O2 or UVA is non-enzymatically transformed to acetate. Interestingly, we can show that in fibroblasts pyruvate has antioxidant properties as enhanced levels of pyruvate protect from UVA induced ROS as well as, to some extent, from the UVA-induced DNA mutation 8-oxoguanosine.

Furthermore, UVA induced expression of matrix metalloproteinases (MMP) especially matrix metalloproteinase 1 (MMP 1) and matrix metalloproteinase 3 (MMP 3) are important features in the process of UV-induced skin aging (photoaging). In addition to this MMP1 is an important protease during carcinogenesis of melanoma. The UVA induced expression of MMP1 and MMP3 are decreased when 1 mM pyruvate is present in the culture medium.

These findings indicate that UVA induced enhanced glucose consumption and lactate production is a general phenomenon in normal skin. Furthermore we show that pyruvate, a product of glycolysis, has antioxidant effects and is involved in the protection against UVA induced ROS and UVA induced DNA mutations and ameliorates the effects of UVA induced expression of aging associated matrix metalloproteinases.

P173 | Influence of indocyanine green (ICG) in combination with water-filtered nearinfrared irradiation (wIRA) and hyperthermia on human keratinocytes - a new photodynamic therapy?

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Photodynamic therapy (PDT) is characterized by application of photosensitive agents, their activation by particular types of light and is mostly used in cancer therapy. The influence of water-filtered near-infrared (wIRA) under hyperthermal conditions on wound healing and extracellular matrix generation was previously shown. Clinically it is described that wIRA has a positive impact on radiotherapy. Aim of this study was to investigate whether indocyangreen can be used as photosensitizer during wIRA irradiation of neoblastic cells under physiological as well as hyperthermal conditions. After investigating cell morphology and cell viability we focussed on its influence on proliferation and apoptosis.

Human keratinocytes (HaCaT) and the epidermoid carcinoma cell line (A431) were pre-treated for 1 h with different indocyangreen concentrations. Thereafter, the cultures were kept for 56 min at temperatures between 37°C and 44°C in a water-bath connected to a peristaltic pump. During this time, the cultures were either kept light protected or exposed to 360J/cm2 generated by a wIRA irradiator (780 nm-1400 nm). Proliferation and apoptosis induction were monitored.

Our results show that the combinatory treatment (wIRA/ICG) reduced proliferation at all investigated temperatures. Monitoring DNA-fragmentation as well as the concentration of various proteins involved in the apoptotic signalling cascades showed that the extent of DNA-fragmentation of cultures treated with ICG and wIRA was higher than in cultures that had only been treated with ICG under light protected conditions. Furthermore we observed that the concentration of anti-apoptotic proteins e.g. bcl-xL was clearly reduced in ICG/wIRA treated cultures.

The herein presented data suggest wIRA in combination with indocyangreen and/or heat as a promising therapy for neoblastic carcinoma due to the observed reduced proliferation and increased apoptosis induction.

P174 | Pre-clinical trial platform for assessing the effect of cosmeceutical agents on epidermal melanogenesis in human skin organ culture

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Brown or black hyperpigmentation of the skin results from increased focal melanin production, causing a variety of skin pigmentation abnormalities, including lentigines (age spots) and melasma (larger dark patches). Moreover, in many cultures, multiple methods to reduce the predominant ethnic epidermal pigmentation ("skin lightening") are popular, but vary greatly in their effectiveness and associated potential adverse effects. Unfortunately, novel skin lightening agents with strong in vitro potency often fail clinical trials due to insufficient in vivo efficacy. A similar problem exists for melanogenesis-promoting cosmetic preparations ("UV-free tanning"). Even the currently employed preclinical 3D skin "equivalent" models for studying hypo- or hyperpigmentation-inducing agents only inadequately capture the complex neuroectodermal-mesodermal signaling interactions that control human epidermal melanogenesis, characterized by the concerted interplay between multiple different cell types. Therefore, we have explored whether the serum-free organ culture of full-thickness human over 6-7 days is a suitable ex vivo assay for testing the effects of candidate cosmeceutical agents that up- or downregulate epidermal melanogenesis, despite the short culture window. Here, we report that melanogenesis in healthy human can be significantly promoted within 3 days by treatment with [Nle4, DPhe7]-alpha-melanocyte stimulating hormone (NDP-MSH, 1 μM) which promotes human skin hyperpigmentation in vivo, or forskolin (25 μM), which stimulates melanogenesis in vitro, as demonstrated not only by quantitative immunohistomorphometry (NDP-MSH, 1.5-fold and forskolin 1.6-fold, $P < 0.05$, assessed by Warthin-Starry staining for melanin), but also by increased intraepidermal activity of tyrosinase (NDP-MSH, 2.0-fold and forskolin 1.7-fold, $P < 0.05$, assessed by in situ enzyme assay), the rate-limiting enzyme of melanin production, and increased (pre-) melanosome formation (NDP-MSH, 1.6-fold and forskolin, 2.6-fold, $P < 0.05$, assessed by gp100 immunofluorescence microscopy). Conversely, hydroquinone, an established anti-melanogenetic agent, significantly suppressed melanogenesis using the above read-outs. This confirms the robustness, sensitivity, and instructiveness of our human skin organ culture assay and its suitability for testing both hyper- and hypopigmentation-inducing agents under clinically relevant ex vivo conditions and on multiple levels of epidermal melanogenesis (i.e., melanocyte number and activity, pre-melanosome formation, tyrosinase activity, melanin synthesis). This assay can be customized and combined to test the candidate skin lightening/tanning agent in the presence of hyperpigmentation-inducing stressors (e.g., UV irradiation, ROS, histamine).

PRURITUS

P175 | Cortistatin, a MRGPRX2 agonist, is expressed by skin mast cells of patients with pruritic skin diseases, can be released upon activation with anti-IgE, and degranulates human skin mast cells in vitro and in vivo

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Cortistatin (CST), a neuropeptide expressed by neurons, endothelial, and immune cells, has been shown to bind Mas-related G protein-coupled receptor X2 (MRGPRX2) and to induce mast cell (MC) degranulation in a cell line that overexpresses MRGPRX2. In addition, CST was shown to be expressed in the skin of healthy humans. Here, we asked whether cortistatin is present in the skin of patients with chronic inflammatory skin diseases, which cells can produce it, and whether cortistatin can activate human skin MCs in an autocrine manner in vitro and in vivo. Quantitative immunohistochemistry of lesional skin of patients with pruritic skin diseases, e.g. chronic prurigo, showed markedly increased numbers of CST-positive cells as compared to nonlesional skin. Double staining using anti-tryptase and anti-cortistatin antibodies revealed that CST-positive (CST+) MCs are present in the lesional and nonlesional skin of patients with chronic prurigo as well as in the skin of healthy controls. Skin lesions of patients with chronic prurigo exhibited significantly higher numbers of CST+MCs than nonlesional skin. Importantly, supernatants of anti-IgE-stimulated MCs contain CST indicating that it is stored as pre-formed mediator in the secretory granules and released during degranulation thereby potentially enhancing MC activation in an autocrine manner. Accordingly, CST dose dependently induced degranulation of human skin MCs (EC50 4 μM) in vitro and in vivo. Skin prick testing of CST in healthy individuals showed a concentration dependent induction of itchy wheal and flare responses within minutes. As assessed by skin microdialysis, intradermal injections of CST resulted in rapid and substantial histamine release. Our findings suggest that CST is involved in the pathogenesis of mast cell-mediated inflammatory skin diseases possibly via autoactivation of MCs via MRGPRX2 and should be considered as a potential target of novel treatment options for patients with these conditions.

P176 | Exploratory biomedical basic research study to investigate the potential influence of amino acids on the skin of haemodialysis subjects with uremic pruritus (UP)

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Due to inadequate dialysis settings, end-stage renal disease (ESRD), hemodialysis patients can develop the characteristic itching of uremic pruritus (UP). Currently, little is known about the pathophysiology of UP. Several hypotheses suggest, for example, various substances, microinflammation or Xerosis cutis as a trigger of UP.
This work's hypothesis implies that uremic pruritus is caused by an impairment of the natural protective barrier of the skin due to unphysiological urea deposits. This accumulation of urea could lead to chemical modifications of specific amino acids, such as lysine or arginine, in essential skin proteins and thereby disrupting the natural barrier function. The stratum corneum includes the so-called natural moisturizing factor (NMF), which contains protein components that are particularly susceptible to chemical modification, so called carbamylation, caused by urea.
This study aims to examine the NMF and the carbamylation status of proteins in the skin of hemodialysis patients with uremic pruritus treated with an amino acid cream. The subjects receive either a cream with specific amino acids or a comparable topical application without amino acids for six weeks. Both the attending physician and the patient are blinded. Skin biopsies are taken for molecular studies at the beginning and after six weeks of treatment.
Cell culture experiments in vitro will support the results of the clinical research study. Therefore, epidermis models will be treated with urea as well as the corresponding amino acids. Additionally, we will investigate skin samples from in vivo experiments with rats treated with an ESRD simulation and amino acids. The epidermis models, the skin biopsies from the study and the skin samples from rats will be processed for immunohistochemistry and fluorescence staining. We will then investigate changes in carbamylation as well as alterations of the epidermis structure with different proliferation and differentiation markers, for example Filaggrin, and Involucrin.

P177 | Chronic nodular prurigo: Revealing the clinical profile and associated burdens of a neglected disease. A multi-center cross-sectional european study

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Introduction: Chronic nodular prurigo (CNPG) is a disease, which results from a prolonged scratching behavior due to chronic pruritus and is characterized by the presence of pruriginous nodular lesions. There is scientific lack of knowledge regarding the pathophysiology, clinical profile, associated burdens and treatment targets of this condition. Although no epidemiological data are available, CNPG is considered relatively rare and thus multicenter studies are needed to study this disease in depth. Aim of this study was to gain insight on clinical characteristics and impact of quality of life of CNPG.
Methods: Patients were enrolled at 15 centers across 12 European countries (Germany; Norway, Sweden [Northern Europe]; Austria, France, Switzerland [Central Europe]; Poland, Russia [Eastern Europe]; Italy, Portugal, Spain, Turkey [Southern Europe]). Patients with CNPG were invited to complete a questionnaire either on paper or electronic format.
Results: A total of 509 patients (210 male, median age: 64 years) were enrolled in the study. Of these, 406 reported having experienced pruritus and CNPG lesions in the previous 7 days and were thus eligible to complete the whole questionnaire. We recorded moderate to severe itch intensity scores in the previous 24 h assessed by the numerical rating scale (median [interquartile range]: 7 [4; 8]; NRS 0-10, n = 391). Scores were higher in Eastern and Southern Europe compared to Germany and Northern Europe ($P < 0.05$); however, these differences were of small magnitude. Pruritus was considered the most burdensome aspect of the disease (49%, n = 150/304), followed by the visibility of skin lesions (21%, n = 65/304) and the bleeding of lesions (14%, n = 43/304). The majority of patients experienced symptoms often or always (71%, n = 286/402) and their everyday life was rather or very affected by the disease (53%, n = 215/405). Most patients were unaware of an underlying condition leading to CNPG (64%, n = 141/395), while in a substantial number of cases psychiatric/psychosomatic conditions were mentioned in association with CNPG (19%, n = 21/109).
Discussion: This large European study raised awareness on CNPG and broadened the understanding of the clinical profile and humanistic burden of this disease. Itch is the most burdensome aspect of CNPG and should be targeted when planning a therapy.

P178 | Disease-specific responses to experimental itch in chronic inflammatory skin diseases

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Pruritus is a frequent and usually distressing symptom in chronic inflammatory skin diseases. Depending on the respective disease, there are different factors involved in mediating pruritus in the skin. While histamine is known to be the main factor responsible for itch in chronic spontaneous urticaria (CSU), the relevant itch inducers in atopic dermatitis (AD) and psoriasis (Pso) are less clear. Furthermore, it is unknown whether disease-specific peripheral sensitization processes occur that can contribute to chronic pruritus in patients suffering from chronic inflammatory skin diseases. Here, we aimed to characterize these sensitization processes to histaminergic or

non-histaminergic stimuli. To this end, we performed skin provocation with histamine, cowhage spicules (containing mucunain, a protease known to induce itch via activation of PAR-2 receptors) and saline as control on the volar forearm of patients with CSU, AD and Pso (20 each) and 20 healthy controls. In AD and Pso, itch provocation was performed both in lesional and non-lesional skin. Itch intensity was monitored on a visual analogue scale (VAS, 0-100) every minute until the subjects no longer experienced pruritus.

As compared to healthy control skin, lesional skin of AD patients showed considerably stronger itch intensity (expressed in Area Under the Curve) and higher itch peak values after provocation with cowhage and histamine, while the intensity of itch in non-lesional skin of AD, Pso and CSU patients was comparable to control subjects. Duration of itch after provocation with cowhage was longer in both lesional and non-lesional skin compared to all other groups while in response to histamine provocation, only lesional AD skin showed longer itch duration. Neither Pso nor CSU patients exhibited differences in itch duration as compared to HC. Comparing lesional and non-lesional skin, provocation with both cowhage and histamine resulted in stronger itch intensity in lesional skin of AD patients while the itch intensity was lower in lesional as compared to non-lesional skin of Pso patients.

Taken together, experimental itch provocation shows striking disease-specific pattern, not only between non-inflamed and inflamed skin, but also within diseases with inflammatory skin, which can give insights into peripheral sensitization mechanisms in pruritic chronic inflammatory skin diseases.

P179 | Development and validation of the Prurigo Control Test (PCT) - A patient reported outcome measure to assess disease control in chronic prurigo

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Chronic prurigo (CPG) is a common and difficult-to-treat disease with a significant impact on quality of life. Important characteristics of CPG, i.e. itch, sleep impairment, scratching behavior, and impairment in quality of life cannot be assessed objectively. Accordingly, patient reported outcome measures (PROM) are important tools to assess the status of CPG in patients. While itch intensity is determined by standardized assessments (e.g. numeric rating scale), there is no simple and validated PROM that has been specifically developed to determine disease control and to guide treatment decisions in CPG patients. Therefore, we developed and validated the Prurigo Control Test (PCT), a novel, easy to administer and fast to evaluate PROM for the retrospective assessment of disease control in CPG patients. In a first item generation phase, potential items were developed by literature research, patient interviews, and expert group input. Subsequently, item selection was performed in a combined approach based on impact analysis and additional criteria for item selection, such as item-item correlations, floor and ceiling effects and face validity. The resulting PCT instrument was then tested for its validity and reliability.

As a result, a 5-item PCT that addresses the severity of skin lesions, scratching, sleep, quality of life, and treatment efficacy with a recall period of 4 weeks was developed based on 69 potential PCT items in the item generation and selection phase. In the subsequent validation study, 95 patients from the CPG centers of Berlin (n = 44) and Münster (n = 51) were recruited. Finalization of analyses regarding the PCT's internal consistency reliability, test-retest reliability, convergent validity and known-groups validity, as well as its cut-off value to distinguish poorly and well-controlled CPG are currently ongoing.

P180 | Going skin deep - pH changes within the epidermis of chronic inflammatory skin diseases

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The pH of the skin is considered importantly involved in various aspects of skin homeostasis, including regulation of skin barrier function, epidermal differentiation and the composition of the microbial flora. Typically, the term "skin pH" refers to the pH on the skin surface, where the pH is slightly acidic and therefore often called the "acid-mantle" of the skin. In patients with chronic inflammatory skin conditions such as atopic dermatitis, contact dermatitis, acne or psoriasis, the pH on the skin surface is higher as compared to healthy skin and it is thought that this increase in pH contributes to pathophysiology. Differences in pH in deeper layers of the epidermis, i.e. beneath the stratum corneum, may however be more important for the modulation of disease-relevant factors. We, therefore, assessed the pH gradient throughout the epidermis in lesional and non-lesional skin of patients with atopic dermatitis (AD, lesional and non-lesional), psoriasis (Pso, lesional and non-lesional) and chronic spontaneous urticaria (CSU, non-lesional) and of healthy subjects (in 20 subjects each) on the volar forearm using a pH-meter with an electrode. After the superficial skin pH was assessed, epidermal layers were removed using up to 80 subsequent tape strips with D-squames and pH was assessed after every 10th D-squame.

The overall pattern of the pH gradient in the epidermis was comparable in all conditions with mean surface pH values ranging between 5.1 and 5.4, a subsequent reduction in pH in the upper epidermis (after 10th-20th D-squame) and a following increase in the middle and lower epidermis, reaching mean pH values ranging from 6.0 to 6.3 after the 80th D-squame. In contrast to both healthy and CSU skin, the reduction of pH in lesional AD and Pso skin is far less pronounced and shows a more rapid increase, with significantly higher pH values between the 10th and 60th D-squame for both lesional AD and Pso, as compared to healthy skin. After 40 tape strips (i.e.

below the stratum corneum), the mean (interquartile range; IQR) pH of healthy skin was 5.1 (4.9-5.2) while the mean pH of lesional AD and Pso skin was 5.6 (5.3-5.9; $P < 0.005$) and 5.9 (5.6-6.3; $P < 0.001$), respectively.

Taken together, we show that the so-called acid mantle of the skin lies beneath the outermost layer of the skin and that active chronic inflammatory skin diseases with epidermal changes (AD and Pso), but not those without (CSU), show a robust elevation of the pH in middle layers of the epidermis.

P181 | Cutaneous nerve fiber architecture is altered in lesional psoriatic skin and recovers upon treatment with Secukinumab

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Psoriasis is a chronic inflammatory skin condition with involvement of cutaneous TRPV1/Nav 1.8 positive nerves in the pathogenesis. However, there are conflicting reports about the morphological cutaneous nerve fiber characteristics. We therefore investigated the cutaneous nerve fiber anatomy in detail within a multicenter, randomized, double-blind placebo-controlled trial (NCT02362789) with monthly injections of Secukinumab (SEC). Skin biopsies were collected at baseline from lesional (LS) and non-lesional (NLS) skin, at week 16 (end of run-in phase) and week 32 (end of randomized withdrawal phase; half of patients with placebo or SEC). Intraepidermal nerve fiber (IENF) density and semiquantitative branching pattern were assessed (PGP9.5 staining); IENF length and epidermal thickening were calculated in a computer assisted approach. Clinical response was evaluated by PASI-scores and pruritus intensity-rating.

Extensive skin clearance (PASI ≥ 98) and pruritus control was achieved by SEC for 61.5% of the initially 130 included patients up to week 16. During withdrawal phase full, symptomatic control was sustained with SEC whereas partial recurrence of psoriasis was observed in the placebo group. The absolute length of IENFs (spatial determination basement to horny layer) was higher in LS at baseline as compared to NLS. As expected, epidermal thickening was much more pronounced in LS compared to NLS ($P < 0.01$). Corrected for epidermal thickening by calculating a fiber-length / epidermal-height ratio, relative length of IENFs was lower in LS compared to NLS (0.29 vs. 0.49; $P < 0.01$). IENF density (11.33 vs. 8.55; $P < 0.01$) and branching (2.56 vs. 1.44; $P < 0.001$) were reduced in LS at baseline. All nerve fiber characteristics recovered after 16 weeks of treatment and remained stable irrespective of the following treatment arm until week 32.

In sum, we demonstrated significant alterations in nerve fiber anatomy with lower density and branching of epidermal nerves in lesional psoriatic skin which recovered upon treatment with SEC. Furthermore, they remained stable after therapy termination despite worsening of pruritus and psoriasis indicating that nerve fiber alterations are not only responsible for induction of itch in psoriasis.

P182 | The opioid receptor (KOR) is part of a specific co-expression pattern in various pruritus entities

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In recent years, the -opioid receptor (KOR) has come into focus as a potential target in the treatment of chronic pruritus. KOR is expressed within the skin mainly on keratinocytes and neurons of pruritus perception. Several independent studies have shown that treatment with KOR antagonists, such as nalfurafine hydrochloride, showed a significant improvement of pruritus. In our study, we analyzed different pruritus-associated diagnoses for a potential co-localization of KOR and immunoreactive cells, as well as co-expression with structure proteins of the cytoskeleton. Studies were performed on biopsies of patients affected by prurigo nodularis (n = 30), lichen planus (n = 20), atopic dermatitis (n = 50) and psoriasis (n = 30). Biopsies of healthy individuals were used as controls (n = 30). The analysis of coexpression was performed by direct immunohistochemical double staining using the sandwich method. Co-localization between KOR and CD4/ CD8 positive cells as well as cytokeratin 5 (CK5) and Vimentin was investigated. Our analyses showed that not only the expression and presence of KOR, CD4/ CD8 positive cells, CK5 and vimentin itself is different in the individual diagnoses, but that there is a clear pattern of co-expression and absence of co-expression between the pruritus-associated diagnoses. With this study, we show for the first time that there is a distinct pattern of co-expression of the -opioid receptor and immunoreactive cells and structural proteins in the epidermis between different diagnoses with pruritus as symptom.

P183 | Cutaneous dysesthesias differ in chronic pruritus patients

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Chronic pruritus (CP) is often accompanied by cutaneous dysesthesias presumable caused by sensitization mechanisms. Many studies have already been conducted on the topic of central sensitization in chronic pain conditions but the assessment of somatosensory disorders in CP patients remains rare. With functional assays we aimed to the perception of alloknesis and hyperknesis in the state of CP.

Alloknesis is a dysesthetic symptom in which an innocuous non-itchy stimulus results in the perception of pruritus. The state of increased pruritus in response to a mild itchy stimulus, which can be induced either chemically (e.g. cowhage) or mechanically (e.g. von Frey filaments) is defined as hyperknesis.

In our study, we tested CP patients affected by atopic dermatitis (AD; n = 30) and brachioradial pruritus (BRP; n = 30) which are representatives of inflammatory and neuropathic origin. All subjects were analyzed in skin of the upper and lower arm characterized by pruritic lesional (P-L) and non-pruritic non-lesional (NP-NL) skin. Results were compared to matched healthy controls (HC; each n = 30).

Functional assays included cowhage stimulation applied in NP-NL skin to assess the induced pruritus intensities. Cotton swabs were used to examine perception of alloknesis in all described skin areas. Using von Frey filaments we analyzed the pruritus/pain threshold in NP-NL skin and hyperknesis in all skin areas respectively.

Our findings show increased intensities of pruritus induced by cowhage in patients affected by AD or BRP compared to healthy controls. Moreover, the area under the curve (AUC) in cowhage stimulation showed a significant inverse correlation with the pruritus/pain threshold in HCs ($P < 0.01$; n = 28) and patients affected by AD ($P < 0.05$; n = 18) leading to a comparable perception of chemical and mechanical stimuli. However, investigations of hyperknesis showed no differences between the localizations or between the groups. In contrast, alloknesis was more distinctive in P-L in comparison to NP-NL skin of BRP patients, indicating a present neuropathy with central sensitization. Additionally, patients affected by BRP showed pronounced alloknesis in comparison to AD patients in pruritic skin. This could be explained by increased central sensitization in patients with neuropathic versus inflammatory pruritus. Taken together, our data provide new insights about different perception of cutaneous dysesthesias in chronic pruritus patients emphasizing the important analysis of central sensitization in the state of chronic pruritus.

P184 | Gene expression and intraepidermal neuronal branching differs between pruritic entities

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Chronic pruritus (CP) is a major symptom of many different diseases (e.g. inflammatory or neuropathic). One common finding among CP is the reduced number of nerve fibers crossing the basement membrane to innervate the epidermis. For a deeper understanding we focused within this study on the nerve fiber anatomy within the epidermis and on gene expression of relevant markers (e.g. NGF, SEMA3A, IL-4, -6,-8, NFKB, TNF, ARTN). For that, we included patients (each n = 40) with an inflammatory, pruritic skin disease (atopic dermatitis, AD), neuropathic itch (radiculopathy-induced brachioradial pruritus, BRP) and with chronic scratch lesions (prurigo nodularis, PN) as well as 40 sex- and age-matched healthy controls (HC). Biopsies were obtained from all participants for assessment of epidermal neuroanatomy by PGP9.5 staining and for analysis of gene expression by means of SYBR green based qPCR. Epidermal nerve fiber branching was assessed semiquantitatively and revealed surprisingly distinct branching patterns. Whereas mainly linear epidermal nerve fibers were present in HC and AD the fibers in BRP and PN showed abundant branching (AD vs. BRP and PN: $P < 0.05$; HC vs. BRP and PN: $P < 0.05$). Furthermore, branching was most prominent in PN (BRP vs PN: $P < 0.01$) with abundant nerve fiber branching in the lower epidermis while nerve fibers in BRP had linear appearance in the lower epidermis and showed branching in the upper epidermis levels (stratum granulosum). Gene expression analyses confirmed different pattern for the CP entities. Especially the nerve attracting factor NGF and the distracting factor SEMA3A showed interesting profiles. The latter one was expressed higher in all CP entities compared to HC (HC vs PN $P = 0.018$; vs BRB $P = 0.009$; vs AD $P < 0.001$). But, AD patients (which had a normal branching pattern) showed the highest expression of this marker. Furthermore, the highest expression of NGF was present in PN patients (PN vs HC $P = 0.002$; vs BRP $P = 0.006$; vs AD $P = 0.05$) which had the most abundant branching pattern. Thus, a disbalance of these neural factors as well as of others may be responsible for cutaneous neuroanatomic changes in CP. Taken together, we were able to show for the first time distinct branching pattern and associated gene expression profiles in different CP entities.

P185 | Sleep impairment in adult patients with psoriasis

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Background: Psoriasis is a prevalent skin disease associated with symptoms (e.g. pruritus) and comorbidities (e.g. depression) that are known to affect sleep. Sleep is crucial for restoring and maintaining physiological functions and health. As of now, little is known about sleep quality and duration in patients with psoriasis patients.

Objectives: To investigate sleep characteristics and quality, and to identify sleep confounding factors, both clinical and psychological, in patients with psoriasis.

Methods: Our cross-sectional, questionnaire-based, case-controlled study included 334 consecutive psoriasis patients (response rate 86%, 286 patients) and 126 controls (response rate 82%, 103 controls). Measures included sleep quality [Pittsburgh Sleep Quality Index (PSQI)], psoriasis severity, pruritus intensity [visual analog scale (VAS) and Likert scale], severity of comorbidities, psychological variables (Hospital Anxiety and Depression Scale - HADS for the assessment of depressive mood, anxiety and psychological distress) and quality of life (skin disease-specific - Dermatology Life Quality

Index - DLQI, and generic - Short Form 12 - SF12). Analyses included group comparisons and regression analyses to identify independent predictors of sleep impairment.

Results: Overall, 59% of 286 patients with psoriasis were poor sleepers (PSQI > 5), compared with 34% of controls ($P < 0.001$). Self-reported sleep duration in patients was 1 hour shorter as compared to controls (median 6 vs. 7 hours, $P < 0.001$). Pruritus was highly prevalent, affecting 91% of patients over the course of their disease (point prevalence: 66%) with moderate average intensity (median 3.6 on the VAS). Patients with strong and very strong pruritus intensity on the Likert scale had more impaired overall sleep quality (total-PSQI) as compared to patients without pruritus ($P < 0.001$ for both). Psychological distress was the strongest predictor of sleep impairment in patients and controls. It was followed, in patients, by pruritus exacerbation at night, reflux, pruritus intensity (VAS) and age, altogether explaining 36% of the variance in overall sleep quality.

Conclusions: Sleep disturbances in patients with psoriasis are highly prevalent. Pruritus, especially exacerbating at night and of strong to very strong intensity, affects sleep quality in patients. Screening for psychological distress should be considered in patients with psoriasis. Reduction of severe and very severe pruritus may result in the improvement of sleep quality and, as a result, substantially contribute to improved quality of life and general health in patients with psoriasis.

P186 | Intense pruritus is linked to sleep disturbance across different chronic skin conditions

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Background: Pruritus is a frequent symptom in a wide variety of dermatoses. Chronic pruritus impairs many aspects of quality of life regardless of its etiology. Recently, a link between pruritus and sleep disturbances has been reported for psoriasis and atopic dermatitis. The relationship between pruritus and sleep impairment in many different skin conditions and the relationship between intensity of pruritus and sleep remain to be elucidated in detail.

Methods: Cross-sectional, questionnaire-based study among adult in- and out-patients of the Department of Dermatology and Allergy, Charité - Universitätsmedizin Berlin. In total, eight hundred patients with the following conditions returned completed questionnaires: chronic spontaneous urticaria (CSU, n = 143), chronic inducible urticaria (CINDU, n = 76), psoriasis (PSO, n = 138), atopic dermatitis (AD, n = 129), chronic prurigo (CPG, n = 75), primary cutaneous T-cell lymphoma (CTCL, n = 68), mastocytosis (n = 54), chronic pruritus on primarily unaltered skin (CP, n = 30), parapsoriasis en plaque (PeP, n = 29), and 58 patients with other diagnoses. Sixty-four patients with angioedema without wheals or pruritus were included as a control group. A dermatologist confirmed the diagnosis, and the patients received questionnaires, which included questions on demographic and clinical parameters (intensity of pruritus, Visual Analogue - and Likert - Scale, pruritus characteristics), and validated questionnaires for the assessment of sleep quality (the Pittsburgh Sleep Quality Index - PSQI, scale range of the overall-PSQI is 0 - 21, and the cut-off for chronic sleep disorder is 10 points), generic quality of life (QoL - SF-12), and itch-specific QoL (Itchy-QoL).

Results: The majority (56%) of patients reported that their itch intensity fluctuates over the course of the day, and in this group, itch attacks occurred most frequently in the evening (42%) and at night (32%). Fewer patients reported itch episodes in the morning (12%) or in the afternoon (6%). Overall sleep impairment in patients with strong pruritus (median PSQI = 10) and very strong pruritus (median PSQI = 11) was considerably higher as compared to controls and to patients without pruritus ($P < 0.001$ for both). Overall sleep impairment in patients with moderate pruritus (median PSQI = 7) was also higher as compared to patients without pruritus ($P < 0.001$) but not in comparison to controls ($P = 0.153$). There was no difference in overall sleep quality between controls (median PSQI = 6) vs. patients with dermatoses without pruritus (median PSQI = 6; $P = 0.264$), vs. patients with mild pruritus (median PSQI = 6; $P = 0.476$) and between patients without pruritus vs. mild pruritus ($P = 0.838$). In general, sleep impairment correlated with general quality of life impairment (mental health SF12, $r = -459$, $P < 0.001$; physical health SF12, $r = -366$, $P < 0.001$).

Conclusion: Overall sleep impairment, independent of the underlying cause and across all skin conditions investigated, was associated with the occurrence and intensity of pruritus. One-half of the patients with strong pruritus and even more than half of the patients with very strong pruritus have a chronic sleep disorder. Pruritus often intensifies in the evening and at night, which can explain its interference with sleep. Sleep is essential for restoration of many neurobiological functions, and its chronic deprivation may a have profound negative impact on health. Therefore, it is important to know, understand, and to treat factors that lead to sleep deprivation such as pruritus.

P187 | A comprehensive, case-controlled analysis of pruritus in psoriasis

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Background: Pruritus was recognized in recent years as an important driver of quality of life (QoL) impairment in patients with psoriasis.

Many detailed characteristics of pruritus in psoriasis, including its clinical correlations and burden, remain to be better characterized. We assessed detailed characteristics of pruritus in psoriasis, such as triggers, fluctuations of intensity, localization, along with the effect exerted by pruritus on patients' QoL, depression, anxiety, sex life, and suicidal thoughts in a large, international cohort of patients.

Methods: This case-controlled, observational, cross-sectional study included a total of 634 patients and 246 controls from Germany, Poland, and Russia. The severity of psoriasis was evaluated using the Psoriasis Area and Severity Index (PASI) and the body surface area (BSA). Clinical and demographic characteristics were collected by a physician during an interview and examination and self-reported by patients. The following validated questionnaires were used: Hospital Anxiety and Depression Scale - HADS, Dermatology Life Quality Index - DLQI, Short Form Survey - SF for generic QoL. Distribution patterns of skin lesions and pruritus were analyzed using recently developed software for body heat map analysis (Hawro et al., JAAD 2020Aug13;S0190-9622(20)32426-9). Additionally, for the first time, digital BSA assessments of skin lesions and pruritus were analyzed.

Results: Most patients (82%) had experiencing pruritus during their disease course, and 68% reported having current pruritus. The majority of patients (65%) described their pruritus as purely itchy, and their itch intensity was mild to moderate (median VAS [IQR]: 3.0 [1.5-5.0]). In contrast, patients who described their pruritus as painful (8%), burning (14%), and painful and burning (14%) had higher itch intensity, (6.0 [3.2-7.3], 5.0 [2.1-7.0], and 4.6 [2.3-7.0], respectively, all $P < 0.01$). In most patients, pruritus was localized to lesional skin areas. The scalp was the most frequent site of pruritus, even in the absence of lesions, and the most frequent non-pruritic lesions were located on the elbows and knees. Sweating (48%), followed by psychological tension (39%) were the most frequent factors exacerbating pruritus. Patients frequently reported impaired quality of life (47%) and sex-life (30%), due to their pruritus. Four percent of patients reported having suicidal ideations due to their pruritus. In up to one fourth of patients, anti-psoriatic therapies had little or no effect on pruritus (systemic non-biological treatment: 10%, biologicals: 15%, UV therapy: 20%, topical treatment: 24%).

Conclusion: Pruritus is a highly prevalent symptom of psoriasis and associated with impaired QoL, in some patients causing suicidal ideations. Painful and/or burning pruritus is of stronger intensity. The scalp is a particularly itch-susceptible area. Anti-psoriatic therapies are frequently insufficient to control pruritus; therefore, there is an urgent need for the development of effective anti-pruritic therapies. It is important to assess psoriasis patients for pruritus and to develop specific anti-pruritic therapies for patients with psoriasis.

Tumor Biology

P188 | RAD51 inhibition as therapeutic strategy for the treatment of metastatic melanoma

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Today, patients with BRAF mutated metastatic melanoma benefit from treatment with inhibitors of the hyperactivated MAPK pathway (MAPKi). However, the development of MAPKi resistance impairs the success of the therapy. As melanoma is known to be a cancer with high DNA damage, it is assumed that the maintenance of the remaining genomic stability is crucial. In fact, overexpression of some DNA repair genes has been demonstrated in melanoma cells with metastatic potential.

In this project we found a high basal expression of the DNA repair protein Rad51 in several metastatic melanoma cell lines compared to expression levels in noncancerous skin cells. Database analysis confirmed that particularly high RAD51 expression between the different DNA repair genes is significantly associated with worse survival of melanoma patients.

Furthermore, we show that RAD51 expression correlates directly with MAPK pathway activity, the most critical pathway for melanoma cells. Treatments with MAPKi can lower RAD51 levels at both mRNA and protein levels in treatment-naive melanoma cells, but not in melanoma cell lines with acquired resistance to MAPKi. Therefore, we treated the cells with novel small molecule RAD51 inhibitors and observed DNA damage, G2/M phase stop and subsequent apoptosis induction in these cells. In addition, co-targeting of RAD51 and MAPK led to synergistic reduction of melanoma cell viability and improved apoptosis induction in 2D and 3D in vitro melanoma models. Similar data were obtained in a xenograft mouse model.

We assume that DNA damage repair proteins such as RAD51 are crucial for maintaining genomic stability in metastatic melanoma cells and are therefore suitable targets for their treatment. We show that inhibition of RAD51 can enhance the effect of MAPKi treatment, both in therapy of naive melanoma cells and in melanoma cells with acquired resistance to MAPKi.

P189 | Functional melanoma cell heterogeneity is regulated by MITF-dependent cell-matrix interactions

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Phenotypic and functional cancer cell heterogeneity limits the efficacy of targeted and immuno-therapies. The transcription factor MITF is known to regulate melanoma cell plasticity and, consequently, response to drugs. However, the underlying mechanisms of this phenomenon remain incompletely understood. Here, we show that MITF levels control functional melanoma cell heterogeneity by fine-tuning the ability to contract the extracellular matrix, the maturation of focal adhesions and ROCK-mediated melanoma cell contractility. Modulation of MITF expression alters extracellular matrix organization, melanoma cell morphology and solid stress in three-dimensional melanoma spheroids, thereby accounting for spatial differences in cell cycle dynamics. Together, our data identify MITF as a master regulator of the melanoma micro-architecture and point towards novel targeting strategies for cancer cell heterogeneity.

P190 | The role of LKB1 in the development and progression of malignant melanoma

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Malignant melanoma is an aggressive form of skin cancer with a high metastatic rate. Although melanoma represents less than five percent of all skin cancer subtypes, it is responsible for 80 percent of skin cancer death. The tumor arises from melanocytes, which are melanin-producing neural crest-derived cells, located in the bottom layer of the epidermis.

LKB1 is a tumor suppressor and serine/threonine kinase which is able to activate numerous other kinases. It thereby regulates cellular processes such as cell proliferation, cell migration, cell metabolism and cell polarity. Because of these diverse responsibilities LKB1 is called “master kinase.” In various types of tumors, including malignant melanoma, LKB1 is shown to be significantly downregulated.

To investigate the role of LKB1 in melanomagenesis in more detail we generated a tissue micro-array (TMA) from tissue specimens of healthy skin, nevi, primary and metastatic melanoma. We analyzed the expression of LKB1 and other relevant proteins (e.g. AMPK), that are known to be affected by the expression level of LKB1. Moreover, we established melanoma cell lines with LKB1 overexpression and knockout. We will investigate if different LKB1 levels have an effect on downstream signaling cascades as well as cell migration, invasion and apoptosis resistance. Finally, we will study the metabolic effect of LKB1 expression in melanoma cell lines through the measurement of respiration, lactic acid production and glucose consumption.

P191 | Heparan sulfate dependent binding of melanoma cells to plasmatic von Willebrand factor attenuates melanoma metastasis

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The high metastatic potential of malignant melanoma is responsible for the bad prognosis and high mortality rate of patients. Intravasation of the disseminating melanoma cell into the vascular system is a hallmark of hematogenous metastasis. Once in the circulation, these melanoma cells can interact with different blood components such as platelets, immune cells, or plasma proteins.

Here, we report that different human and murine melanoma cell lines have distinct abilities to interact with plasmatic von Willebrand factor (VWF). VWF is a large multimeric glycoprotein and elevated plasma levels have previously been associated with tumor progression. Lack of the integrin binding motif or the heparan sulfate binding site in human recombinant VWF mutants abolished its deposition at the surface of melanoma cells. In addition, Fluorescence microscopy and superresolution microscopy further indicate that the binding of VWF to melanoma cells is synergistically dependent on integrins and heparan sulfate. Heparan sulfate is a highly sulfated glycosaminoglycan exposed at the plasma membrane of all mammalian cells. However, the length and the composition of the heparan sulfate chains depend on the cell type and are often altered in tumor cells. The biosynthesis of heparan sulfate is a complex process involving a series of different enzymes. Among those is exostosin-1 (EXT1) which is necessary for the polymerization of heparan sulfate chains. Knockdown of EXT1 by shRNA or gene deletion by CRISPR/Cas9 prevented the synthesis of heparan sulfate in human and murine melanoma cells. In functional assays, we found that the absence of heparan sulfate on the cell surface prevented VWF binding. By

microfluidic experiments mimicking melanoma cell extravasation, the presence of VWF attenuated the binding of flowing melanoma cells to the endothelium. The lack of heparan sulfate and thus abolished binding of VWF increased the number of endothelial bound melanoma cells, significantly. In vivo, murine melanoma model further confirmed that compared with wt B16F10 cells, HS abolished B16F10 cells formed more lung metastases. Further microfluidic experiments suggest that HS-bound plasmatic VWF interferes with melanoma cells exposed very late antigen-4. This prevents melanoma cell adhesion to the vascular endothelium via vascular cell adhesion molecule-1.

In conclusion and in line with previous animal experiments demonstrating increased lung metastases in VWF-deficient mice, our data point towards an anti-metastatic role of plasmatic VWF. Future in vivo experiments using VWF-deficient mice and melanoma cells with a modulated heparan sulfate biosynthesis will provide further insights into the pathophysiological relevance of heparan sulfate and VWF for tumor progression.

P192 | A transcriptome-wide isoform landscape of melanoma identifies gene isoforms associated with malignancy

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Transcriptomic patterns in primary melanomas or metastatic lesions may predict treatment response and prognosis. However, a complete picture of early transcriptomic features of tumor development and later tumor progression is still missing. More recently, genetic splice variants have become of central interest, as individual splice variants are differentially regulated in a number of cancers. Here, we analyze a transcriptomic data set of benign melanocytic lesions and primary melanomas (n = 80) for the expression-specific splice variants associated with each stage of melanoma development. Transcript level abundance is calculated from RNA-seq data with Kallisto aligner that utilizes a novel method of pseudoalignment for fast mapping of short reads to reference transcripts. By this means, a map for differentially expressed splice variants for melanoma versus benign melanocytic nevi was generated. Among the top genes with differentially expressed/used splice variants were RAB6B, MSR1 (Macrophage Scavenger Receptor 1), COL11A2, and CHEK1 (checkpoint 1 protein of intracellular stress signaling), all of which are known to be involved in cancer biology. Overall, benign lesions separated from malignant lesions using differentially expressed splice variants of different genes alone without using whole-gene expression patterns. The enriched gene ontology terms of differentially expressed splice variants showed involvement of translational elongation and other ribosomal components (upregulated) and type I interferon signaling (downregulated) in melanoma as compared to benign nevi. Interestingly, a large number of top switched isoforms had a high Jaccard index and significant Z score for 3' UTR mutations and switch associations. Taken together, a map of splice variants in melanoma is presented that differentiated between benign and malignant lesions and supported the role of translational mechanisms and interferon signaling as major drivers in melanoma biology.

P193 | "Retraction artefacts" in basal cell carcinomas do not result from fixation, but may be related to local tumor progression

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Basal cell carcinoma (BCC) arises most often in sun-damaged skin of elderly patients and grow locally aggressive without metastasizing. Frequent subtypes are nodular, morpheaform and superficial basal cell carcinoma. Histopathological characteristics of BCC are nests of basaloid keratinocytes originating from the epidermis that show peripheral palisading and are surrounded by a stroma of connective tissue rich of fibroblasts. A typical microscopic feature of BCC are empty clefts between the palisading tumor cells and tumor stroma known as "retraction artefacts" due to tissue fixation. However, confocal laser scanning microscopy (CLSM), which allows the direct microscopic visualisation of fresh tissue by reflectance and fluorescence signals revealed the same feature in native, non-fixated BCC.

To further investigate the nature of cleft formation in BCC we investigated 79 BCC by ex vivo CLSM.

The study was approved by the Ethical Committee at the Medical Faculty, Leipzig University (O93/18-ek).

Compared to conventional histology (hematoxylin &eosin staining in paraffin-embedded tissue), the clefts appeared identical in non-fixated, freshly excised BCC tissue investigated within minutes by CLSM.

Moreover, the clefts did not contain any material that could be stained with alcian blue, Masson-Goldner's trichrome stain or HA-binding protein, suggesting that they are either empty or filled with interstitial fluid.

Immunohistochemistry and fluorescence staining in ten BCCs of various subtypes was performed to investigate the related pathomechanism: Hyaluronidases (HYAL1 and 3) were expressed predominantly at the edge of palisading tumor cells at the clefts. Hyaluronan (HA) was found in the surrounding tumor stroma, but not within the clefts. In tumor regions, where clefting started, tumor-associated HYAL1 and 3 were concentrated and HA was degraded. Other hyaluronidases (TMEM2 and CEMIP) were homogeneously expressed in the whole tumor. Matrix metalloproteinases showed a more heterogeneous picture with stronger expression in the stroma (MMP9), the tumor (MMP1) or equal distribution in the tumor and stroma (MMP 14).

From these experiments we postulate that hyaluronidases in the palisading basaloid keratinocytes of BCC degrade HA of the extracellular matrix, and fibroblasts in the stroma subsequently contract the collageneous extracellular matrix leading to cleft formation.
Peritumoral clefting known from other solid tumors like prostate carcinoma, mamma carcinoma and oral squamous cell carcinomas correlates with more aggressive tumor growth and worse outcome. Obviously, peritumoral clefting in BCC is not a fixation artefact, but originates from HA degradation in the extracellular matrix by tumor cell HYAL1- and -3. It may be one factor mediating local progression by displacement of tissue.

P194 | Hit it hard enough 2.0: Improved MAPK pathway blockade in melanoma by ERK inhibitors

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The clinical availability of small molecule inhibitors that are specific for melanoma tumors which harbor a BRAF V600E mutation represents a significant breakthrough in the treatment of this disease.
Despite a dramatic anti-tumour activity and improved patient survival especially with combinational treatment with BRAF and MEK inhibitors, rapidly emerging resistance to these inhibitors, greatly limits their clinical benefit. A large number of different resistance mechanisms have already been described, yet common to many of them is a reactivation of the MAPK signalling pathway. The extracellular signal-regulated kinases 1 and 2 (ERK1/2) represent the central effectors of the MAPK signalling cascade. Based on that, the aim of this study was to asses a potential benefit of the ERK1/2-specific small molecule inhibitor Ravoxertinib (GDC0994) in the treatment of BRAF mutant melanoma cells with an acquired resistance to BRAF inhibitors or to the combination of BRAF and MEK inhibitors as well as the respective parental cells were tested. The ERK inhibitor ravoxertinib (GDC0994) shows in single treatment only limited antitumor activity. The combination of ERKi and BRAFi/MEKi causes growth inhibition and apoptosis in BRAF mutated melanoma cells irrespective of the sensitivity to BRAF or MEK inhibitors. Additional ERKi is effective especially in a chronic, long-term setting
Combinatorial treatment regimens including ERK1/2 inhibitors might be an attractive, novel therapeutic strategy in BRAF mutated melanoma cells.

P195 | Inhibiting the neural crest transcription factor SOX10 leads to cell cycle arrest and apoptosis in uveal melanoma cells

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Uveal melanoma (UM) is the most common intraocular malignancy in adults. Local disease can be effectively controlled by radiotherapy or enucleation; however, about 50% of all patients develop distant metastases predominantly in the liver and lung.
Development of melanocytes and other neural crest derived cells is controlled by the transcription factor SOX10, which is also expressed in cutaneous melanoma (CM). As shown previously, SOX10 inhibition decreases the invasion and proliferation and induces cell death in CM cells. Primary UM tumors also express SOX10, but its functional role is still unclear.
We detected high constitutive SOX10 gene and protein expression levels in the UM cell lines 92.1, Mel270, OMM1.5 and further examined the effects of SOX10 downregulation by RNA-mediated silencing. Cell viability was massively decreased already 24 h after siRNA transfection in UM cells but not in human melanocytes (HM). FACS-based analysis of cell cycle progression revealed a cell cycle arrest upon SOX10 downregulation. Lower levels of phospho-Rb and Cyclin D1 indicated that SOX10 inhibition led to an arrest in the G1 phase of the cell cycle. FACS and Western Blot analysis showed a strong induction of apoptosis and cleavage of caspases 9 and3, but not of caspase8, indicated that SOX10 inhibition promotes cell death via the intrinsic apoptosis pathway. Furthermore, higher levels of the DNA damage marker gamma-H2A.X were found after SOX10 inhibition. Expression analysis of known SOX10 target genes revealed that SOX10 inhibition downregulated microphthalmia-associated transcription factor (MITF), a master regulator of pigmentation in melanocytes, but not other targets as PMP2 and MIA. Similar to SOX10, MITF inhibition led to a cell cycle arrest in the G1 phase and induced apoptosis in UM cell lines 92.1 and Mel270. SOX10 inhibition and subsequent RNA sequencing revealed a differential expression of several genes involved in apoptosis and cell cycle regulation including antiapoptotic Bcl-2. In silico analysis of the Bcl]2 promoter region revealed putative SOX10 binding sites within a 1500 bp range upstream of the transcription start site, suggesting that Bcl]2 may be a putative SOX10 target gene, potentially connecting the oncogenic role of SOX10 to apoptosis induction.
Taken together, SOX10 inhibition leads to DNA damage, cell cycle arrest and cell death via the intrinsic apoptosis pathway in high-expressing UM cell lines and downregulates its target gene MITF. Thus, SOX10 may be a crucial factor for UM cell survival and therefore may offer novel therapeutic options for the treatment of UM.

P196 | Brn3a expression is epigenetically controlled by HDAC2 in melanocytes and melanoma

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Melanoma cells frequently express proteins and reactivate neural crest transcription factors to hijack oncogenic properties such as the ability to migrate and invade into surrounding tissues, resulting in metastatic spread of the disease. The neural crest transcription factor Brn3a is essential for proliferation and survival of melanoma cells. It is frequently expressed in melanoma and neural crest cells during embryogenesis, but not in normal adult melanocytes or benign nevi. The mechanisms underlying the aberrant expression of Brn3a in melanoma are unknown.

We investigated the epigenetic regulation of Brn3a in melanocytes and melanoma cell lines treated with DNA methyltransferase (DNMT), histone acetyltransferase (HAT) and histone deacetylase (HDAC) inhibitors. DNMT inhibition by decitabine and bisulfite sequencing revealed that Brn3a expression is not controlled by DNA methylation of its promoter region. HAT inhibition did not significantly alter Brn3a expression levels, whereas panHDAC inhibition by trichostatin A significantly increased its expression. Treatment with the isoform-specific HDAC inhibitor mocetinostat, but not with PCI-34051 also increased Brn3a expression levels in melanocytes and low-expressing melanoma cell lines suggesting that class I HDACs1, 2, and 3 and class IV HDAC11 were involved in the regulation of Brn3a expression. Transient silencing of HDACs1, 2, 3 and 11 by siRNAs revealed that specifically HDAC2 inhibition was able to increase Brn3a. ChIP-Seq analysis uncovered that HDAC2 inhibition specifically increased H3K27ac levels at a distal enhancer region of the Brn3a gene.

Altogether, our data suggest that HDAC2 is a key epigenetic regulator of Brn3a in melanocytes and melanoma cells, highlighting the importance of epigenetic mechanisms in regulating melanoma oncogenes.

P197 (OP01/02) | Interferon unresponsive melanomas resist CD8 T cell immunity but can be targeted with CD4 ACT and salvage virotherapy

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Melanoma is the deadliest form of skin cancer. Most primary melanomas are detected early and can be cured by surgical resection. However, a subset of patients develops an incurable, metastatic disease. The treatment of metastatic melanoma has been revolutionized by the introduction of immunotherapies such as adoptive T cell transfer (ACT) and immune checkpoint blockade, but frequent development of resistance remains a major problem. A bulk of recent evidence suggests that melanoma cells can acquire resistance through loss of interferon responsiveness. As these cytokines are also crucial for clearing viral infections, we hypothesized that oncolytic virotherapy could represent an ideal salvage strategy to overcome acquired resistance. To test the hypothesis, we generated Jak1-KO HCmel12 mouse melanoma cell lines with CRIPSR/Cas9. When mCherry or tagBFP expressing the Jak1-KO and mock transfected Jak1 wild-type melanoma cells were mixed to model cancer heterogeneity and the established tumors treated with anti-PD1 + anti-CTLA-4 immune checkpoint blockade, Jak1-KO cells were found to escape the therapy. Jak1-KO melanoma cells did not upregulate MHC-I after IFNγ or type I IFN treatment and CD8 + T cells failed to recognize the Jak1-KO melanoma cells in co-culture experiments in vitro. Salvage virotherapy using SFV VA7-eGFP oncolytic virus resulted in eradication of Jak1-KO population, infiltration of CD8 + T cells and significantly prolonged mouse survival. By carrying out ACT experiments we could mechanistically show that salvage virotherapy resulted in infiltration and activation of tumor specific CD8 + T cells turning “cold” tumors “hot” thus providing synergy with immune checkpoint blockade. The majority of the Jak1-KO melanomas could be eradicated by MHC-I independent T cell therapy by adoptively transferring Trp1 specific CD4 T cells, but one-third (3/9) of the tumors escaped the therapy. Of note, oncolytic virotherapy could eradicate large established type I IFN unresponsive melanomas also in Rag1-KO mice indicating the approach works in T cell independent fashion and could thus represent an attractive complementary salvage strategy in combination with CD4 T cell ACT. Finally, oncolytic infection of poorly type I IFN responsive human melanoma cell lines either with SFV VA7-eGFP or clinically approved T-VEC oncolytic virus resulted in highly efficient cell lysis despite interferon pre-treatment, thus supporting clinical applicability of the devised salvage virotherapy strategy. To best of our knowledge, this is the first report of successfully targeting acquired immunotherapy resistance arising through loss of interferon responsiveness with oncolytic viruses and CD4 T cell ACT. The presented

work has important implications for patient stratification and paves way for early clinical trials.

P198 | Immune checkpoint blockade needs Stat1-depending senescence pathways in cancer cells

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Immune checkpoint blockade (ICB) targets exhaustion-associated surface molecules using monoclonal antibodies, reactivates T cells and induces durable therapeutic stability in a variety of metastatic cancers. Cancer regression with ICB requires cancer cell killing but additional, unknown mechanisms are needed to establish the long-lasting tumor control in the responder patients. To analyze whether efficient ICB requires the induction of stable cell cycle arrest in the cancer cells we used a model of endogenously growing cancer and treated mice with ICB (mAbs to Programmed-Death-Ligand-1 and to Lymphocyte-Activation Gene 3) and adoptive transfer of tumor-associated antigen (TAA)-specific CD4 + T-helper-1 (TH1) cells. The therapy significantly increased life time, even in mice with pre-final cancer disease, restored a normal health status in the mice, partly destroyed the large cancers, and induced a p16Ink4a+ and Ki67- senescent phenotype in the residual cancer cells. The therapy was strictly dependent on an intact Stat1-signaling pathway and activation of p16Ink4a in the cancer cells. While TAA-TH1 cells migrated into cancers of either wild-type or Stat1-deficient cancers in comparable numbers, they failed to induce p16Ink4a+ and Ki67- senescent tumor cells. Stat1-deficient and Cdkn2a-deficient cancer were exclusively resistant to senescence induction, in vitro and in vivo, but fully susceptible to apoptosis and T cell-mediated killing. To determine whether a stable cell cycle arrest is also needed for ICB therapy of patients with melanomas, we analyzed metastatic melanomas that progressed within < 3 months during ICB and metastases that remained stable > 1 year. Progressing metastases had losses of senescence-inducing genes or ≥4-fold amplifications of senescence inhibitors. In contrast, such mutations were infrequent in melanoma metastases that regressed during ICB for > 1 year. Thus, cancer immune control with ICB requires, besides cancer cell killing, induction of a stable growth arrest, called cancer cell senescence.

P199 | Increased chlormethine induced DNA double stranded breaks in malignant T cells from mycosis fungoides skin lesions

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Background: Cutaneous T-cell lymphoma (CTCL) is a heterogeneous group of extranodal non-Hodgkin's lymphoma. Mycosis fungoides (MF) is the most common types of CTCL and considered as a malignancy of skin-resident T cells. Chlormethine (CL), also known as mechlorethamine or nitrogen mustard, is a synthetic agent with well-known alkylating capacity. Its topical application has a long tradition in dermatology, as CL-containing products have been used for the treatment of MF since 1940. Recently, a novel CL gel formulation has been approved for the treatment of skin lesions in MF adult patients.
Objective: Here we aim to study the impact of CL on malignant skin T cells regarding their susceptibility to treatment, proliferation, DNA double strand breaks and the expression of alkylated-nucleotides-excision genes.
Methods: Tumour blood or skin T cells were isolated by magnetic-activated cell (MACS) sorting. Susceptibility to CL exposure was evaluated by MTT assay. Proliferation and DNA double strand breaks upon CL exposure were detected by BrdU proliferation assay and flowcytometry for γH2AX Ser139. Expression of alkylated-nucleotides excision genes was measured by reverse transcription quantitative PCR (RT-qPCR).
Results: While CL exposure in vitro decreased time- and dose-dependently total blood Tcell viability, it did not statistically significantly influence neither blood nor skin T-cell proliferation. Of interest, when acting on skin T cells, CL induce DNA double stranded breaks predominately in the subpopulation of MF clonal malignant skin T cells but not the non-tumoral healthy T cells. Quantitative real-time PCR uncovered that several important genes involved in rescue of alkylated nucleotides were generally decreased in tumor T cells and CL exposure in vitro further significantly decreased the expression of FEN1 and BRCA2, two major alkylated-nucleotides-excision-repair genes.
Conclusion: This study sheds light on how CL affects malignant skin T cells from patients with MF and provides additional rationale for considering it as an early and valuable skin-directed treatment option for skin lymphoma.

P200 | A melanoma mutation panel for individualized treatment of melanoma cultures

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Melanoma is the most aggressive form of skin cancer. Its standard of care is treatment by surgery, chemotherapy, radiation and more recently immunotherapy and targeted therapy. However, response rates to single-agent immunotherapies are low and severe side effects often impair the treatment success of combined immunotherapies. For BRAF (~50%) and NRAS (~30%) mutated tumors, targeted therapies directed against the RAS-RAF-MEK-ERK pathway may be used. Relevant treatment responses are currently only observed for BRAF-mutant patients that reach response rates of up to 80%. However, the vast majority of patients show drug resistance months after beginning the treatment. Interestingly, most of these patients harbor activating mutations in other druggable pathways. Therefore, an individualized combinatorial treatment approach might help to overcome many of the current treatment failures. In this project we aim to identify treatment combinations based on individual mutational patterns. A pre-clinical in vitro model was set up using melanoma cultures as a 2D model and tumor microfragments as a 3D model. To select individual treatment combinations, a panel of 83 target genes was sequenced on a NextSeq high-throughput sequencing instrument (Illumina). Depending on the mutational profile, the biological effects of different drug combinations were tested to examine the optimal treatment strategy. Mutations were identified in BRAF, NRAS and NF1 as well as in PTEN, CDKN2A, ARID2 and ARID1B, and a number of other genes. Among substances used for targeted treatment were dabrafenib, trametinib, palbociclib, apitolisib and PKF118-310 (β-catenin inhibitor). Mutationally activated pathways showed response to pathway-targeted substances and combination therapy was more effective than individual substances in a number of cases. These analyses were performed in a 2D model using the IncuCyte system and partly with impedance spectroscopy in a 3D model. This approach may lead in future to more individualized treatment modalities for metastatic melanoma patients.

P201 | Blockade of programmed cell death protein 1 (PD-1) in Sézary syndrome reduces Th2 phenotype of non-tumoral T lymphocytes, but may enhance tumour proliferation

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Sézary syndrome (SS) is an aggressive leukemic variant of cutaneous T-cell lymphoma (L-CTCL) that arises from malignant clonally-derived skin homing CD4 + T cells. Based on advancements in our understanding of the mechanisms underlying L-CTCL, boosting the suppressed immune response emerges as a promising strategy in SS management. Immune checkpoint inhibitory molecules have already demonstrated efficacy in a wide spectrum of malignancies. Currently, agents targeting the programmed death-1 (PD-1) axis are under evaluation in LCTCL. Here, we investigated the expression of PD-1 and its ligands, PD-L1 and PD-L2 in blood and skin from patients with L-CTCL. We demonstrate that PD-1 expression is markedly increased on tumor T cells compared to non-tumor CD4 + T cells from SS patients and to CD4 + cells from healthy individuals. In contrast, PD-L1 shows decreased expression on tumor T cells, while PD-L2 expression is low without significant differences between these groups.

Functional PD-1 blockade in vitro resulted in reduced Th2 phenotype of non-tumor T lymphocytes, but enhanced the proliferation of tumor T cells from SS patients. Our study sheds some light on the PD-1 axis in both peripheral blood and skin compartments in SS patients, which may be relevant for the treatment of L-CTCL with immune checkpoint inhibitor.

P202 (OP01/03) | Acidosis promotes immune escape by interferon-γ-induced transcriptional induction of PD-L1 via the eIF4F-STAT1-PD-L1 axis on cancer cells

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Cancer cells express immune checkpoint proteins such as programmed death ligand-1 (PD-L1) to escape immune surveillance. Within the tumor microenvironment, PD-L1 expression is regulated by interferon gamma (IFN-γ) and hypoxia, and induces T-cell exhaustion or apoptosis. Thus, we aimed to uncover cancer cell specific PDL1 expression features to better predict anti-PD-1 or anti-PD-L1 monoclonal antibody (mAb) treatment success or failure. We investigated murine and human cancer cell lines, treated them with IFN-γ and/or acidic cell culture media and studied PD-L1 expression in vitro. Furthermore, we silenced the signal transducer and activator of transcription 1 (STAT1) and the eukaryotic initiation factor 4F (eIF4F) complex by specific inhibitors. In vivo, extracellular tumor pH (pHe) was neutralized by sodium bicarbonate treatment, as confirmed by non-invasive in vivo acido-chemical exchange saturation transfer magnetic resonance imaging (acido-CEST-MRI). Tumor tissue was evaluated for PD-L1 expression, macrophage and CD3 + T-cell recruitment. We found that acidosis significantly elevated IFN-γ-induced PD-L1 on cancer cells through increased expression and phosphorylation of STAT1 via the eIF4F complex. This phenomenon was exclusively observed in MC38 and CT26 adenocarcinoma cells which were responsive to anti-PD-L1 mAb treatment but not in non-responsive B16-F10 melanoma or 4T1 mammary carcinoma cells. Similarly, IFN-γ and acidosis synergistically promoted PD-L1 expression in human HCA-7 colony 29 adenocarcinoma, MCF-7 mammary carcinoma and U-87 MG glioma cells. Moreover, in immune-competent mice bearing MC38 tumors we found that neutralization of tumor acidosis by bicarbonate treatment resulted in enhanced immune cell recruitment and a remarkable reduction of the tumor volume. In addition, we observed increased immune cell PD-L1 expression within the tumors as a result of pHe neutralization. The functional role of both immune cell-derived IFN-γ and tumor acidosis for cancer cell PD-L1 induction and tumor immune escape was further demonstrated in IFN-γ-/- mice, which did neither reveal inducible PD-L1 expression nor a growth reduction of transplanted MC38 tumors. In anti-PD-L1 mAb nonresponsive tumors, sodium bicarbonate mediated pHe neutralization did not elevate tumor PD-L1 expression and failed to reduce B16-F10 and 4T1 tumor volume. In summary, conjoint IFN-γ- and acidosis-inducible PD-L1 expression on cancer cells represents a novel immune escape mechanism whereas pHe neutralization mediated PD-L1 expression on immune cells is associated with immunotherapy response.

P203 | Cold atmospheric plasma (CAP) for the selective control of tumor cells

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Plasma medicine has become an innovative area of research with great potential in recent years. Since the development of cold atmospheric plasma (CAP), new applications in medicine have become available. A relatively new field is Plasma Oncology. More and more studies on cell cultures and animal models have shown successful treatment of tumor cells with CAP. Larger clinical trials in which cancer patients are treated with CAP are not yet available, as the mechanisms of selective anti-tumor effects of CAP have not been sufficiently explored. Among the various CAP generated species, reactive oxygen species (ROS) and reactive nitrogen species (RNS) appear to be the major factors contributing to the selective anti-tumor effects of CAP by causing severe DNA double strand breaks or apoptosis in tumor cells rather than in normal cells.

For the treatment of tumor cells in vitro, we used a portable plasma device (miniFlatPlaSter) based on the Surface Micro Discharge (SMD) technology. The plasma is generated with the surrounding air through a range of microdischarges.

In our research, we observed dose-dependent effects on malignant melanoma cells by CAP generated with the SMD technique. CAP treatment for 2 min induced DNA damage and resulted in apoptosis of the melanoma cells. Interestingly, CAP caused apoptosis in only 9% of normal human epidermal melanocytes (NHEMs). Furthermore, a shorter CAP exposer of 1 min led to induction of senescence, which has been shown to be triggered by an accumulation of cytoplasmic Ca2 + . The effects of lower doses of CAP are of interest in tumor therapy because sub-lethal doses also act therapeutically without harming the surrounding healthy tissue.

Although the mechanisms of selective anti-tumor action of CAP are not yet completely understood, these findings contribute to a better

understanding of the molecular influence and mode of action of CAP on tumor cells but also on nonneoplastic cells.

P204 | Strong Hgf signaling in the microenvironment predisposes for the acquisition of Gnaq/11 mutations and promotes Gnaq/11 mediated tumor growth

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G-protein-coupled receptors (GPCRs) transduce their signals through interaction with heterotrimeric G-protein subunits (Gα, Gβ, and Gγ). The important role of GPCR-Gαq/11 signaling in melanocyte neoplasia became apparent with the discovery of somatic Gnaq mutations in blue melanocytic nevi in the skin and in uveal melanomas. These genes encode for the heterotrimeric G protein α subunits, Gαq and Gα11. Oncogenic driver mutations exclusively appear in the residues Q209 and R183, both leading to a constitutively active signaling in the Gαq pathway and its downstream effectors.

Our group has established the genetic Hgf-Cdk4R24C melanoma model, in which mice develop spontaneous skin melanomas that metastasize to the lymph nodes and lungs. Melanomagenesis can be accelerated by epicutaneous application of the carcinogen 7,12-dimethylbenzanthracene (DMBA). In an initial screen for somatic genomic mutations we found that oncogenic mutations in Gnaq/11 appear to be selected in 4 out of 10 primary Hgf-Cdk4R24C melanomas. We did not observe mutations in BRAF or NRAS. To substantiate this observation, we further characterized the mutational profile of spontaneous (n = 5) and DMBA (n = 9) induced Hgf-Cdk4R24C melanomas using a targeted next generation sequencing approach of PCR amplicons. All spontaneous tumors carried mutations in Gαq209 (40%) or Gα11209 (60%). Eight out of nine DMBA induced tumors carried either Gαq209 (33%) or Gα11209 (55%) mutations. We found no mutations in Gαq183. These initial results indicate that the genetic environment in Hgf-Cdk4R24C mice favors the acquisition of mutations in Gαq proteins.

To evaluate the impact of Hgf on proliferation and migration in vitro we transduced the primary melanocyte cell line melan-a with retroviruses encoding wild-type Gnaq or mutated GnaqQ209L. Using crystal violet assays and transwell migration assays we found that Hgf significantly increased cell proliferation and migration of melan-a GnaqQ209L cells in comparison to melan-a wild type Gnaq cells. Subsequent experiments in vivo revealed that the onset and progressive growth of melan-a GnaqQ209L tumors was significantly increased in Hgf-Cdk4R24C mice when compared to Cdk4R24C mice. Melan-a wild-type Gnaq cells did not form tumors in any mouse strain.

Our results show that strong Met signaling in the microenvironment of Hgf-Cdk4 melanomas predisposes for the acquisition of Gnaq/11 mutations and aberrant Gnaq/11 signaling promotes melanoma cell proliferation and migration. From our analyses we expect new general insights into the molecular mechanisms why and how mutant Gαq/11 signaling drives growth and metastasis of certain melanoma subtypes. We also hope to provide a basis for the development of novel therapeutic approaches targeting deregulated Gαq/11 signaling which is particularly important for patients with uveal melanoma.

P205 (OP05/04) | STAT5 is a therapeutically targetable vulnerability in leukemic cutaneous T-cell lymphoma

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Introduction: Cutaneous T-cell lymphomas (CTCL) are a heterogeneous group of lymphoproliferative disorders characterized by the expansion of mature malignant T-cells in the skin. Blood burden is a disease progression characteristic particular of leukemic disease types, such as Sézary syndrome (SS) and advanced leukemic mycosis fungoides (L-AMF). Despite the increasing amount of comprehensive sequencing studies alluding to numerous but infrequent genetic aberrations, driver mutations remain unclear. As a consequence, disease progression monitoring and new-targeted therapy options are lacking. Chromosome 17q gains encoding STAT3/5 genes were reported to occur in over 50% of CTCL cases, but it has not been elucidated if this translates into enhanced oncogenic STAT3 and/or STAT5 activity or if it can be targeted.

Methodology: We integrated pharmacologic interference studies and patient cancer genome landscape analysis to investigate the functional consequences of chromosome 17q gains, as well as the efficacy of the JAK-STAT pathway therapeutical blockage. For this purpose, we performed shallow whole-genome sequencing, as well as whole-exome sequencing of primary malignant cells from a cohort of four SS and two L-AMF patients.

Results: Our genomic study performed on a cohort of 4 Sézary and 2 L-AMF patients revealed copy number gains of chromosome 17q

in 5/6 patients, which correlated to increased expression of STAT3/5 mRNA and protein levels. These findings were supported by immunohistochemical analysis of skin biopsies showing STAT5 activation. Functional in-vitro testing of various JAK kinase and STAT inhibitors revealed pronounced sensitivity of CTCL cell lines and patient samples to STAT5 inhibitors. IQDMA, a yet unexplored inhibitor of STAT5 signaling, demonstrated exceptional selectivity for L-CTCL primary samples carrying 17q gains and is currently tested whether it is efficient in abolishing lymphoma growth in-vivo.

Conclusions: Taking into account findings from recent genomic studies, we conclude that chromosome 17q gains are a characteristic of advanced leukemic CTCL, in which particularly STAT5 might serve as a vulnerable node. Further preclinical development of specific STAT5 inhibitors and their clinical validation could provide new therapeutic opportunities for L-CTCL.

Keywords: CTCL; Sézary syndrome; Mycosis fungoides; STAT5, small molecule inhibitors.

P206 | Senescence enhances sensitivity to TRAIL and immune clearance by reinforcing early apoptotic features

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Senescence induction is becoming a relevant strategy in tumor treatment. Next to induction with therapeutic compounds (TIS - therapy induced senescence) senescence can be induced in tumor cells in vitro and in vivo via cytokine (IFNy & TNF) or TH1 cell treatment (CIS - cytokine induced senescence). While the majority of CIS cancer cells remains viable, ceases proliferation and shows SA-ß-Gal activity, the cells also accumulate features associated with early apoptotic events. The cells lose their original membrane composition indicated by an increased surface localization of negatively charged phospholipids. This is accompanied by increased expression of pro-apoptotic proteins (e.g. BID) and reduced mitochondrial membrane potential ($\Delta\psi m$) as well as changes in opening of the mitochondrial permeability transition pore (PTP), without changes in mitochondrial mass. This in turn compromises the retention of Cytochrome c, leading to a depletion of overall Cytochrome c protein. While Caspase 3 activity in CIS cancer cells (8.3% 5.7) is enhanced in comparison to non-senescent cancer cells (2.3% 1.9), we suspected that increased levels of anti-apoptotic protein Bcl-2 in CIS counteracts death induction in favor of senescence. Indeed, treatment of CIS cancer cells with Navitoclax (pan-Bcl-2 inhibitor) induced apoptosis preferentially in senescent cancer cells. We furthermore found, that the changes in cellular status after senescence induction lifted the resistance of cancer cells to TRAIL induced cell death. Here, we show that CIS and TIS tumor cells are susceptible to TRAIL induced cell death in comparison to non-senescent tumor cells. Further analysis showed that apoptosis induction in senescent cancer cells was a prerequisite to render them susceptible to phagocytosis by macrophages. Therefore, type I anti-cancer immune response can act as a relay between inducing a stable growth arrest in cancer cells and senescent cancer cell clearance by macrophages exploiting the acquired sensitivity to TRAIL of senescent cancer cells.

P207 | miR129-5p is a tumor-suppressor in BRAF(V600E) associated melanoma

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Background: Almost 60% of melanomas harbor a BRAF mutation, most frequently the V600E substitution, for which therapeutic BRAF inhibitors (BRAFi) have been developed such as Vemurafenib or Dabrafenib. However, patients rapidly develop resistance mechanisms towards these targeted therapies. Although these can be partially circumvented by inhibiting the ensuing kinase MEK, analysing and tackling BRAF resistance itself remains a reasonable goal. One novel approach is to analyse the involvement of microRNAs (miRNAs). They are small noncoding RNAs (20-22nt) with posttranscriptional regulatory functions, via suppressing protein translation or inducing mRNA degradation by guidance of the RNA induced silencing complex (RISC) to the 3 prime untranslated region (3`UTR) of target mRNAs.

Methods: We re-analyzed next generation sequencing (NGS) datasets (GSE94423, GSE98314) to identify candidate miRNAs induced upon BRAFi treatment. Data were validated by qRT-PCR. Analyzed cell lines: primary human wild-type melanocytes (NHEM), BRAF wild-type cells (SK-Mel-30, G361), BRAF mutation associated cell lines (WM9, WM902B, WM35, A375) and BRAFi resistant cell lines (WM9R, WM902BR, WM35R). We demonstrated the miR-129-5p tumor suppressor function by biological assays (proliferation, vitality and spheroid growth).

Results: Evaluation of next generation sequencing analysis (GSE94423) revealed that miR-129-5p expression is induced after BRAF(V600E) pathway inhibition in treatment sensitive but not in resistant A375 melanoma cell line. This data were validated by qRT-PCR with additional BRAF(V600E) associated cell lines (WM35, WM902B, WM9) and their corresponding resistant clones (WM35R, WM902BR, WM9R). Thus only cell lines with BRAF mutation, but not BRAFi resistant ones show raised miR-129-5p expression in response to BRAF or MEK inhibition. Overexpression of miR-129-5p expression as well as BRAFi treatment show decreased cell proliferation, whereas stabile inhibition of miR-129-5p in A375 cells lead to increased cell proliferation. Further, the functional block of miR-129-5p drives spheroid growth during treatment conditions by reduced BRAFi response. A putative target of miR-129-5p is RUNX1, which is a transcription factor associate with BRAFi resistance. After treatment with Vemurafenib the protein level of RUNX1 is reduced. miR-129-5p overexpression or inhibition inversely correlates with

RUNX1 protein. By luciferase assay we demonstrated that miR-129-5p binds directly to the RUNX1 3`UTR, inhibiting its protein translation.

Conclusion: miR-129-5p contributes BRAFi treatment response by targeting RUNX1.

P208 | The role of the citrate homeostasis in melanoma pathogenesis

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Membrane transport proteins are involved in the movement of ions and small molecules across biological membranes. Many different membrane transporters have been shown to facilitate cancer development, progression and drug resistance. A recent study of our collaborators Mycielska et al. showed that extracellular citrate is supplied to pancreatic cancer cells through a plasma membrane-specific variant of the mitochondrial citrate transporter pmCiC which is a member of the SLC25 mitochondrial transporter family. Metabolomic analysis revealed that citrate uptake by pmCiC broadly affected prostate and pancreatic cancer cell metabolism through citrate-dependent metabolic pathways. In particular Mycielska et al. showed that cancer cells are flexible in their choice of extracellular carbon donors. Switching to an extracellular citrate supply under hypoxic and low glucose conditions facilitated tumor progression.

These exciting findings prompted us to investigate whether pmCiC also plays a role in the pathogenesis of melanoma. Therefore, immunohistochemical analysis of pmCiC expression with a pmCiC-specific polyclonal antibody was performed utilizing tissue microarrays on benign nevi, primary tumors and metastatic melanoma. Positive pmCiC cytoplasmic expression was seen in 32 out of 60 (53%) metastatic melanoma samples whereas less than 2% (1 out of 56) of benign nevi stained positive for pmCiC. Primary melanoma also stained positive for pmCiC expression in more than 50% of the samples. Moreover pmCiC expression was detected in 14 melanoma cell lines whereas no expression was detected in primary human melanocytes. The goal of the research project is to analyze the role of pmCiC in melanoma pathogenesis.

P209 | Induction of apoptosis in cutaneous squamous cell carcinoma cell by celecoxib in combination with death receptor targeting is based on caspase activation and upregulation of proapoptotic Bcl-2 proteins Puma and Bad

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Background: Actinic keratosis (AK) derives from neoplastic epidermal keratinocytes and shows strongly increasing prevalence in fair-skinned populations worldwide. AK may proceed into malignant cutaneous squamous cell carcinoma (cSCC). Most significant risk factors are excessive UV irradiation as well as immunosuppression. The nonsteroidal anti-inflammatory drug and selective COX-2 inhibitor celecoxib has been suggested for treatment of cSCC/AK. Its mode of action, however, revealed largely elusive.

Materials and **Methods:** Celecoxib was applied in concentrations of 25, 50 and 100 μM in four cSCC cell lines (SCL-I, SCL-II, SCC-12 and SCC-13). TNF-related apoptosis-inducing ligand (TRAIL, Adipogen Biomol) was used at 50 ng/ml, and the agonistic CD95 antibody, CH-11 (Beckman-Coulter), was used at 100 ng/ml. Apoptosis was determined by propidium iodide staining and cell cycle analysis; cell viability was determined by calcein staining and flow cytometry. For cell proliferation, real-time cell analysis (RTCA, xCELLigence, Agilent) and WST-1 assay were used. Mitochondrial membrane potential and reactive oxygen species (ROS) were determined by flow cytometry, after staining with TMRM+ and H2DCF-DA, respectively. Expression of apoptosis-related proteins was determined by Western blotting using antibodies for caspase-3, -6, -7, -8, and -9, for caspase antagonists cFLIP (Flice-inhibitory protein), XIAP (chromosome X-linked inhibitor of apoptosis protein), survivin as well as for Bcl-2 family members Mcl-1, Bcl-w, Puma and Bad.

Results: Addressing the effects of celecoxib in CTCL cells, we show significant and dose-dependent antiproliferative effects in four cSCC cell lines. However at moderate concentrations (25 μM, 50 μM), celecoxib was unable to induce apoptosis or decrease cell viability. This limitation was overcome by combination of celecoxib with death receptor targeting either by TRAIL or by CD95 agonistic antibody. Thus, apoptosis was induced in up to 60% of treated cells, and cell viability was almost completely lost. This high efficiency was accompanied by loss of mitochondrial membrane potential (MMP) and production of reactive oxygen species (ROS). The combination with TRAIL resulted in complete activation of the proapoptotic caspase cascade (Csp-3, -6, -7, -8, and -9), in upregulation of the agonistic TRAIL receptor, DR5, in downregulation of characteristic antiapoptotic factors as survivin, XIAP, cFLIP, Mcl-1 and Bcl-w as well as in upregulation of the proapoptotic BH3-only proteins Puma and Bad. Caspase inhibition by a peptide inhibitor (QVD-Oph) completely abolished apoptosis induction.

Conclusions: Induction of apoptosis represents an important issue in tumor therapy. The finding that celecoxib was largely unable to induce apoptosis in cSCC cells may thus explain a limited efficiency. The response may be enhanced in combinations, and death ligands as TRAIL appear as highly suitable. Death ligands represent characteristic effectors of the immune system; they are produced by cytotoxic T lymphocytes and NK cells. Thus, the present data may also indicate that celecoxib can support the immune response against cSCC/AK, and combinations with checkpoint inhibitors recently approved for treatment of cSCC may be further considered.

P210 | Estrogen receptor beta stimulation as a possible novel therapeutic target for cutaneous T-cell lymphoma

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Introduction: Mycosis fungoides (MF) and Sezary syndrome are the most common lymphomas involving the skin. As other lymphomas cutaneous T-Cell lymphomas (CTCL) have greater incidence in males than females. It could already be shown that the androgen receptor (AR) and the estrogen receptor (ER) are expressed in immune cells and extra cutaneous lymphoid malignancies and that either blocking or stimulating these receptors has a protective function in lymphomagenesis. However, the endocrine contribution to the gender difference in primary cutaneous T-Cell lymphomas is unknown.
Material & **Methods:** In this present study we performed a screen of ten different compounds targeting androgen receptor or estrogen receptor signaling in four CTCL cell lines (Hut-78, MyLa, SeAx, HH) using MTS proliferation assays and lactate dehydrogenase (LDH) assays. To analyze the effects on cell cycle distribution we performed flow cytometry of Propidium Iodide (PI) stained cells. For hormone receptor detection in the investigated cell lines and to determine influence on proapoptotic pathways we performed qPCR and western blot analysis.
Results: We could show that blocking or stimulating AR or ERα signaling had no effect on cell proliferation. However, targeting ERβ with the selective synthetic ERβ agonists LY500307, ERB-041 and the phytoestrogen genistein significantly reduced cell proliferation of all four CTCL cell lines in a dose-dependent manner. Using qPCR and western blot analysis, we could show that all lymphoid cell lines express ERβ. ERα was only expressed in SeAx and HH cells. AR was not detectable in all four cell lines. Flow cytometry analysis demonstrated that LY500307 promotes apoptosis and elicits G2/M cell cycle arrest. Further, western blot analysis revealed that ER stimulation promoted several pathways related to apoptosis (e.g. upregulation of p53 and PARP cleavage).
Conclusion: Altogether, our preclinical results suggest that targeting ER with selective agonists might be potential novelty in the treatment of CTCL.

P211 | Disulfiram efficiently induces cell death in NF1 loss-of-function melanoma cells through a massive induction of reactive oxygen species

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Despite current advances in therapy and diagnostics, melanoma is a difficult to treat, aggressive skin cancer with a high mortality rate. The third most common subgroup in the genomic landscape of melanoma includes melanomas with aberrations in the NF1 gene (12-14%). Most mutations are loss-of-function (LoF) events. Since NF1 is a GTPase-activating protein known to down-regulate RAS activity through its intrinsic GTPase activity, NF1-LoF mutations are an alternative way to activate the canonical MAPK signaling pathway. Consequently, activation is critical for the RAS/ MAPK signaling pathway in melanoma. An effective targeted therapy is currently only available for BRAFV600 mutant melanomas, indicating the clinical need for novel treatment options, especially for metastatic NF1 LoF melanomas.
The long known anti-alcohol drug disulfiram shows antineoplastic effects mainly due to its key metabolite diethyldithiocarbamate (ET), a strong chelating agent for bivalent metal ions such as Cu2 + . We initially performed cell viability assays to investigate the efficacy of CuET on NF1 LoF melanoma cells. Actually, Cu2 + as monotherapy had no influence on cell viability. In contrast, treatment with ET, in particular, the combination with Cu2 + (CuET), showed a significant and complete inhibition of cell growth in NF1 LoF cells with a minimal effective dose of 250 nM. In addition, CuET persistently inhibited melanoma cell growth for 12 days after treating the cells for three days in a colony formation assay. Interestingly, the inhibitory effects achieved by CuET were Cu2 + dependent. Consequently, the effects of CuET on melanoma cells were completely reversible by the addition of an extracellular copper chelator, which prevented CuET formation. After treatment with CuET we measured a dramatic increase of intracellular Cu2 + in the melanoma cells, demonstrating that ET mediates the uptake of Cu2 + . The additional Cu2 + was localized in the nucleus of treated melanoma cells. Furthermore, the inhibitory effect was fully dependent on CuET-induced ROS production since adding the ROS scavenger N-acetylcysteine totally abolished the CuET mediated cytotoxicity.
Finally, we assessed the inhibitory and pro-apoptotic effects of CuET combined with the MEK inhibitor trametinib in order to improve the inadequate clinical effects of trametinib as monotherapy in BRAF wild-type melanoma cells.
We conclude from our results that the drug disulfiram could induce cell death in melanoma cells in general, but especially NF1 LoF melanoma cells, and add additional cytotoxicity to MEK inhibitors to obtain an effective therapy for this group of melanomas.

P212 | Sulforaphane: A novel anti-lymphangiogenic compound?

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Background: There is growing evidence that lymphatic vessels are linked to immune regulation, atherosclerosis, or metabolic and inflammatory diseases. In addition, the lymphatic vessels provide a route for tumor cells to metastasize. Therefore, influencing lymphangiogenesis is an interesting target in various pathological conditions. Recent studies suggest that Sulforaphane (SFN), a naturally occurring isothiocyanate derived from the consumption of cruciferous vegetables, such as broccoli and cabbage, may mediate part of their known antitumor effects by interfering with angiogenesis. We therefore examined the potential impact of SFN on cell proliferation in primary human lymphatic endothelial cells (LEC).

Methods: LEC were cultured in vitro and treated with or without SFN. Effects of SFN on proliferation, cell cycle progress, apoptosis and expression of the important endothelial receptors VEGFR-2 and -3 were analyzed mainly by BrdU-Assay, flow cytometry, quantification of histone-complexed DNA fragments and immunoblotting. In vitro angiogenesis was investigated using the Matrigel tube formation assay.

Results: We found that SFN inhibited cell proliferation in a concentration-dependent manner without any cytotoxic effects in LEC. Interestingly, these effects could not be abrogated by the addition of the pro-lymphangiogenic vascular endothelial growth factors VEGF-A and -C, indicating that SFN could influence angiogenic signaling pathways. Consistently, we demonstrated that SFN significantly inhibited VEGF receptors 2 and 3 protein expression. Additionally, we found that SFN induced apoptosis by activating PARP and cytochrome c release in LEC. Furthermore, we found that SFN induced S-phase arrest in LEC. In addition, we could demonstrate an inhibition of the formation of lymphatic capillary like structures by SFN treatment.

Conclusion: In conclusion, our pilot study provide for the first time evidence, that SFN may decrease lymphangiogenesis mainly by inhibition of the endothelial VEGFR-2 and 3 as well as apoptosis and inducing S-phase arrest.

P213 | Melanoma derived extracellular vesicles deliver miRNAs as molecular switches in macrophage activation

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Background: The tumor microenvironment is essential for tumor growth, metastasis and immune evasion. Recently it was shown that cancer cell derived extracellular vesicles (EVs) induce a tumor-promoting phenotype in tumor associated macrophages (TAMs). EVs are cell derived membrane vesicles including exosomes and micro vesicles. They are loaded with proteins, DNA, coding and non-coding RNAs e.g. miRNAs. A putative mechanism by which EVs modify macrophage phenotypes includes the transfer of miRNAs. However, the exact mediators and mechanisms particularly in melanoma are not known.

Aim: In this study, we investigated if melanoma cell derived EVs induce a tumor-promoting phenotype in macrophages and if so, whether they specifically contain certain miRNAs targeting the pathways which promote such a phenotypic change.

Methods: In this study, we examined the effects of melanoma derived exosomes on macrophage function and the underlying mechanisms. We performed next generation sequencing (NGS) to analyze melanoma EV induced macrophages phenotype and melanoma EV loaded miRNAs. Further we investigated the miRNA transport via EVs from tumor cells to macrophages and analyzed their biological functions.

Results: In vitro the treatment of M1 polarized THP-1 derived macrophages with melanoma EVs induce a proinflammatory and proangiogenic phenotype. By qRT-PCR analyses we revealed that melanoma EVs induce the expression of immunomodulatory genes like IL-6, TNFα, IL-1b, CD80 as well as angiogenesis factors IL-8 and VEGFA. Next generation sequencing analyses revealed enrichment for several miRNAs in melanoma EVs. We showed that this EV enriched miRNAs are delivered to macrophages and partially induces the observed tumor-promoting TAM phenotype. Especially for the miR-125b-5p we showed that it targets the lysosomal acid lipase A (LIPA) in macrophages, which in turn contributes to their phenotype switch and promotes macrophage survival.

Conclusion: Melanoma derived EVs deliver a pool of miRNAs into macrophages, thereby affecting the macrophage phenotype in a way, which could support tumor immune evasion and finally tumor progression.

P214 | Proapoptotic effects of ingenol mebutate (PEP005) in cutaneous T-cell lymphoma cells depend on activation of protein kinase C delta

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Background: Cutaneous T-cell lymphoma (CTCL) may develop a highly malignant phenotype in late phase, and patients may profit from innovative therapy. The plant extract ingenol mebutate (PEP005, Picato) has been approved for field treatment of actinic keratosis and was described to display proapoptotic activity also in neoplastic lymphocytes in vitro. While studies suggested that topical ingenol mebutate may be beneficial for patients with early stage CTCL, its mechanism of action is still elusive. Here, the activity of PEP005 in CTCL cells as well as its mode of action were investigated.
Material/**Methods:** Four CTCL cell lines (HH, HuT-78, MyLa and SeAx) were used as cell culture models. Apoptosis was determined by propidium iodide staining and cell cycle analysis, while cell viability was determined by calcein staining and flow cytometry. For cell proliferation, WST-1 assay was used. Mitochondrial membrane potential and reactive oxygen species (ROS) were determined by flow cytometry, after staining with TMRM+ and H2DCF-DA, respectively. Western blotting was used to determine the expression of protein factors involved in apoptosis control, as caspase-3, caspase-8, caspase-9, cFLIP (Flice-inhibitory protein), XIAP (chromosome X-linked inhibitor of apoptosis protein), survivin as well as protein kinase C (PKC) delta.
Results: When investigating the effects of ingenol mebutate on apoptosis induction in CTCL cells, HuT-78 (42% apoptosis) and HH (19% apoptosis) turned out as sensitive, while MyLa and SeAx were largely resistant. Induction of apoptosis came along with loss of cell viability and reduced cell proliferation. Production of reactive oxygen species (ROS) was seen in all cell lines in response to PEP005, independently of sensitivity, while loss of mitochondrial membrane potential (MMP) was found only in HuT-78. While activation of effector caspase-3 was restricted to sensitive cell lines, initiator caspase-8 processing was also seen in MyLa and SeAx. Concordantly with the activation of caspase-3, the caspase antagonistic proteins cFLIP, XIAP and survivin were downregulated by PEP005 in sensitive cells. Both inhibition of ROS by the antioxidant á-tocopherol and caspase inhibition by a peptide inhibitor (QVD-Oph) reduced apoptosis induction.
Finally, treatment with PEP005 resulted in significant downregulation of the proform of the isoform of proapoptotic PKC-delta (78 kDa), which is clearly indicative of its processing and its activation. Inhibition of PKC-delta by the selective inhibitor bisindolylmaleimide-1 resulted in recovery of MMP, reduced ROS production, reduced caspase-3 processing as well as inhibition of apoptosis and recovery of cell viability.
Conclusions: Due to the highly varying responses of CTCL cell lines, PEP005 may also affect only some CTCL tumors in patients while others may reveal resistance. Concerning the mode of action, PKC-delta appeared as central and as upstream of all other changes.

P215 | Evaluation of miR-142-3p and GARP as novel diagnostic biomarkers in melanoma patients

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Early detection and treatment of cancer significantly improves the 5 year overall survival of patients when compared to cancers detected at advanced stages. However, for many cancers, reliable and easily accessible diagnostic biomarkers, for the early diagnosis of cancer, remain few and far between. Therefore, there is an urgent clinical need to identify and to evaluate molecules unique to tumor pathogenesis for the discovery of novel diagnostic biomarkers.
One such molecule, a protein by the name of glycoprotein A repetitions predominant (GARP), is highly expressed on the surface of growing melanoma tumors and plays a key role in sustaining the immunosuppressive tumor microenvironment (TME). GARP is known to modulate the bioavailability and activation of TGF-beta and thus is involved in the regulation of peripheral immune responses. Previously, we have demonstrated that GARP is shed from the surface of tumor cells into the TME in a soluble form known as sGARP, which has strong regulatory and anti-inflammatory properties in vitro and in vivo. Specifically, sGARP leads to the induction of peripheral regulatory T cells (Treg) as well as the inhibition of tumor antigen-specific CD8 + T cells. Besides cancer cells, GARP is also expressed on the surface of activated Treg and platelets, which both exhibit key suppressive functions in the TME.
It has been observed in Treg, amongst others, that GARP expression is negatively regulated by the miRNA, miR-142-3p. miR-142-3p, is a known tumor suppressor, which inhibits cell proliferation and arrests cell cycling. Additionally, miR-142-3p expression has also been implicated in radiation sensitivity in various tumor cell types. Recently, we could show for the first time that GARP expression is also negatively regulated by miR-142-3p in melanoma cells. As GARP is preferentially upregulated on the surface of melanoma cells and regulatory immune cells, such as Treg and platelets, that aid in maintaining the immunosuppressive TME, GARP and its regulator, miR-142-3-p, present as promising potential biomarkers for malignant melanoma.
This study evaluated GARP and miR-142-3p as novel potential diagnostic biomarkers in malignant melanoma. We used easily accessible parameters, serum and plasma, derived from routine patient blood tests, and they were obtained from stage I and stage IV melanoma patients as well as healthy donors. To elucidate miR-142-3p and GARP mRNA levels, samples underwent RT-qPCR. sGARP protein levels were also determined by performing ELISAs. Preliminary results have indicated that miR-142-3p is differentially expressed in the serum and plasma of cancer patients compared to health donors, supporting the promise of miR-142-3p as a novel diagnostic biomarker for melanoma. Future studies will examine miR-142-3p and

GARP as potential predictive biomarkers for radiation resistance in melanoma patients.

P216 | Mcl-1 inhibition enhances the proapoptotic effects of vemurafenib in melanoma cells

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Background: Activation of the MAP kinase cascade via BRAF-MEK-ERK plays an essential role in melanoma. In recent years, the breakthrough in molecular targeted therapy has dramatically improved standard care of melanoma patients leading to approval of BRAF and MEK inhibitors (vemurafenib and trametinib) Nevertheless, inevitable emergence of drug resistance is still critically limiting the clinical efficiency. The antiapoptotic Bcl-2 family protein Mcl-1 is amplified in several cancer types including melanoma, and high Mcl-1 expression was correlated with tumor progression and resistance to BRAF/MEK inhibitors.

Materials and **Methods:** In BRAF-mutated melanoma cell lines A-375 and A-2058 as well as in BRAF-WT cell line MeWo, we investigated the effects of Mcl-1 inhibitor, S63845, in combination with vemurafenib and trametinib as well as the ERK inhibitor Sch-772984. Further, siRNA and miRNA strategies were applied for Mcl-1 downregulation. Apoptosis was determined by propidium iodide staining and cell cycle analysis, and cell viability was determined by calcein staining and flow cytometry. Mitochondrial membrane potential was determined by TMRM+ staining and flow cytometry, and activation of the proapoptotic Bcl-2 family member, Bax, was determined by Bax-NT (N-terminal) antibody.

Results: Whereas the effects of vemurafenib were varying in melanoma cell lines according to BRAF mutation, inhibition of Mcl-1 by S63845 alone induced apoptosis in all three melanoma cell lines (10-20%) associated with loss of cell viability. Of note, combination of S63845 with vemurafenib resulted in dramatically increased apoptosis in BRAF-mutated cell lines (A-375, 30% to 75%; A-2058, 18% to 60%) associated with almost complete loss of cell viability. Interestingly, also BRAFWT cell line MeWo showed some response to combined S63845/vemurafenib treatment (50% apoptosis). Abrogation of pERK by vemurafenib and enhanced Mcl-1 expression by S63845 were seen. Concerning the mode of action, activation of caspase-8/caspase-3, loss of mitochondrial membrane potential and activation of the proapoptotic Bcl-2 family member, Bax were shown. Apoptosis by vemurafenib was also enhanced by siRNA against Mcl-1 as well as by Mcl-1-targeting miRNAs (miR-193b and miR-339-3p). Partly in parallel effects were seen in combinations with trametinib and Sch-772984.

Conclusions: These data suggest Mcl-1 as an important antiapoptotic factor in melanoma, and its targeting may further improve present melanoma therapy.

P217 | Exploration of vascular activation and coagulation in metastatic melanoma patients treated with immune-checkpoint inhibition

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Background: The latest advances in targeting immune checkpoints opened a new promising era in management of metastatic melanoma patients. Immune checkpoint inhibitors (ICI) using anti-PD-1/ anti-CTLA4 antibodies have significantly extended progression-free and overall survival in patients with unresectable malignant melanoma stage IV patients. Despite these very encouraging results, about 50% of patients develop acquired resistance by 5 years and up to 59% of treated patients experience severe side effects under anti-PD-1 therapy. Therefore, there is an urgent but still unmet clinical need for the early identification of patients presenting a high risk of treatment failure in order to limit unnecessary exposure to immune-related adverse events. The effect of cancer on coagulation has been established for many years; however, recent investigations on coagulation pathways in cancer patients have suggested a crosstalk between coagulation and cancer with a direct role of thrombophilic markers in cancer progression. Novel biological functions of von Willebrand Factor (vWF) have been described, including a role in inflammation, angiogenesis, and metastasis formation. Compared to healthy donors, metastatic melanoma patients have higher systemic values of vWF, and tumor microvessels present with ultra large-VWF fibers (ULVWF), due to local inhibition of the VWF-degrading enzyme ADAMTS13. We also highlighted the particular role of tumor VEGF-A in endothelial activation leading to vWF secretion in tumor blood vessels, contributing thereby to hematogenic metastasis. Finally, growing pieces of evidence highlight a mechanistic implication of vWF in inflammation and immunothrombosis. We hypothesized that this link between coagulation and melanoma progression can also play a role in the response of metastatic melanoma patients to immunotherapy.

Methods: A melanoma biobank has been prospectively established. Blood materials and clinical data are collected from metastatic melanoma patients at stage IV/ III according to AJCC 2017 at each treatment infusion. 51 metastatic patients have been included and have completed 6 months of ICI and their first clinical evaluation. Among these, 36 have been analyzed at 2 time points. Von Willebrand factor and ADAMTS-13 have been measured at baseline, and 24 weeks

of therapy with established ELISA assays. Clinical parameters are recorded from routine management.

Results: In this prospective study on 51 melanoma patients we monitored the vWF levels during the course of therapy at 4 time-points (baseline, 6, 12 and 24 weeks). Non-responders patients tend to present with higher values of vWF at baseline (29 µg/ml vs. 33 µg/ml $P = 0.06$) and after 24 weeks of therapy (31 µg/ml vs. 36 µg/ ml $P = 0.02$), compared to patients presenting with a stable disease, or achieving a partial response or complete response (36 patients evaluated at 24 weeks). Patients presenting with a vWF above 34 µg/ml at baseline tend to present a shorter PFS in univariate analysis ($P = 0.050$; Hazard Ratio1, 94 [0.92-4.09]) (unpublished data).

Discussion: Our data provide evidence that elevated von Willebrand factor plasma levels, a reduced ADAMTS-13 activity, associate with poor prognosis. We expect to uncover new biomarkers and to pave the way for new potential targets implicated in the vascular system regulation for therapeutic interventions to increase the efficacy and safety of ICI in metastatic melanoma patients.

P218 | The aryl hydrocarbon receptor differentially modulates inflammatory responses in human and mouse melanoma cell lines

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Metastatic melanoma remains a deadly disease with a mortality rate of almost 50% despite the development of novel therapeutics. In the recent years, the role of inflammation in driving metastatic melanoma progression has become increasingly clear. In previous work, we have described the aryl hydrocarbon receptor (AHR), a ligand binding transcription factor of the basic helix-loop-helix family, as a driver of melanoma metastasis in a mouse model. Moreover, we were able to show that AHR knockout melanoma cells retain a more differentiated phenotype under inflammatory stimulation. In the current project, we analyze the influence of AHR on inflammatory responses in melanoma cells by focusing on the differential responses of human and mouse melanoma cells to adverse environmental conditions.

In initial experiments, we stimulated HCmel12 melanoma cells with the proinflammatory cytokine TNF-alpha and the high-affinity AHR ligand 6-formylindolo(3,2b)carbazole (FICZ) and detected an attenuation of the chemokines CCL2 and CCL5 in both qRT-PCR and ELISA compared to TNF-alpha alone. To gain a more profound insight into the transcriptomic impact of AHR activation under inflammatory conditions, we performed a 3' UTR RNA-seq analysis of HCmel12 cells treated with TNF-alpha and FICZ. In this, we again observed a decrease of NFkB-driven chemokine transcripts in the HCmel12 samples treated with both, TNF-alpha and FICZ, compared with only TNF-alpha. Interestingly, we instead detected an upregulation of interferon-stimulated genes in differential gene expression and gene set enrichment analyses. The immunomodulatory effects of FICZ were abrogated after CRISPR-mediated knockout of AHR in HCmel12 cells. Next, we analyzed a panel of human melanoma cell lines recapitulating the spectrum of differentiation states and driver mutations of cutaneous melanoma. Surprisingly, FICZ further increased the TNF-alpha induced expression of NFkB-driven chemokines.

Our work provides evidence for a crucial role of AHR in mediating inflammatory responses with opposing influence on human and mouse melanoma cells. This janus-faced role of the AHR on inflammatory reactions has also been demonstrated in models of autoinflammatory diseases. In our future work, we hope to unravel the molecular mechanism how AHR mediates these seemingly paradoxical effects.

P219 | Analysis and characterization of liver metastasis formation of murine melanoma cell lines

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Aims: Metastasis formation relies on the close interplay between disseminating tumor cells and the microenvironment of their target organs. In this process, tumor intrinsic mechanisms that promote adhesion, migration or survival at distant sites are decisively involved. In this study, we aim at deciphering malignant melanoma cell intrinsic mechanisms that control and facilitate hepatic metastasis.

Methods: To study the interaction of melanoma cells with hepatic endothelial cells (EC) an experimental liver colonization model was used to mimic the hematogenous spread of tumor cells. Five melanoma cell lines with different driver mutations were injected intrasplenically: B16F10 luc2, RET, D4M (Tyr:: CreER;BrafCA;Ptenlox/lox), HCMel12, derived from HGF-CDK4 mice, and WT31 cells (Tyr:: NrasQ61K/ °; INK4a-/-). Additionally, WT31 cells were also injected intravenously. To evaluate the metastatic behaviour, colonization efficiency, histological growth patterns, the differentiation of intra- and peritumoral ECs and extracellular matrix composition were characterized. RNA-sequencing of the melanoma cell lines was performed to identify patterns of gene expression and pathways associated with enhanced hepatic colonization.

Results: Profound differences in liver colonization were observed among the melanoma cell lines. While WT31 showed the highest efficiency to colonize the liver, B16F10 luc2 and RET also reliably induced liver metastasis on a lower level. D4M and HCMel12 showed a significantly lower efficiency to colonize the liver requiring injection of the highest cell numbers. Histologic analysis showed a pushing

type metastatic growth pattern of all cell lines. Besides, metastases of B16F10 luc2 and RET melanoma showed more intrametastatic necrosis as compared to WT31. The size of necrotic cores was larger in B16F10 luc2 melanoma in comparison to RET. Detailed analysis revealed corresponding patterns of vascularization. Metastases of WT31 cells were strongly vascularized, whereas B16F10 luc2 and RET metastases exhibited only few vessels, indicating that susceptibility to necrosis is related to the vascular density. EC exhibit a continuous phenotype in intratumoral areas and showed a preserved sinusoidal phenotype in peritumoral vessels. Intratumoral vessels also showed dense pericyte coverage and continuous perivascular Collagen 4 deposition indicating the formation of a basement membrane. Metastases of WT31, D4M and HCmel12 showed collagen 4 positive structures, i.e. empty sleeves, that were not associated with patent vessels indicating enhanced, abortive angiogenesis. RNA-sequencing of all cell lines revealed that B16F10 luc2, RET and WT31 show unidirectional regulation of 1995 genes in comparison to D4M and HCmel12 melanoma. Global analysis of these genes revealed strong regulation of pathways controlling angiogenesis, focal adhesions or actin cytoskeleton organization.

Conclusion: murine melanoma cell lines show great differences in the capability to induce liver metastasis as well as their vascularization and stromal cell composition. This indicates that melanoma-intrinsic mechanisms can control hepatic colonization and metastasis formation. Comparative gene expression analyses of these cells reveal pathways involving angiogenesis or cell-cell interactions that correlate with enhanced hepatotropism.

P220 | Establishment of RiboTag in melanoma cells

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As an immunogenic tumour, the progression and metastasis of melanoma is highly influenced by immune cells residing in the microenvironment and periphery. During melanoma genesis, both, immune and melanoma cells undergo an immunoediting process that includes interconnected phases such as elimination, equilibrium, and escape. In this context, recent evidence suggests that tissue-resident CD8 + memory T (TRM) cells play a critical role in melanoma surveillance.

Whereas the underlying mechanisms are insufficiently understood, the investigation of the genetic background of melanoma cells could help to find defining pathways of the melanoma immune equilibrium. To address this, we established the RiboTag approach (Sanz et al. 2009) in B16 melanoma cells and fused endogenous ribosomal protein L8 (Rpl8) and Rpl22 at the C-terminus with different protein tags (3xHA, 3xFlag, 3xV5), using a modified CRISPR/Cas9 approach (Schmid-Burgk et al. 2016). Using the RiboTag approach, we circumvented extensive tissue processing that is prone to introduce artefacts into translatome analysis and retrieved melanoma-specific translatomes via immunoprecipitation from complex tumour lesions. In the future, the application of the RiboTag technique to melanoma models gives us the opportunity to analyse intracellular mechanisms of heterogeneous melanoma lesions - not only on the level of the genome and transcriptome - but also on level of the translatome. The molecular dissection of intracellular responses upon immune pressure is a promising way to find new targets for immunotherapy approaches.

P221 | MCAM promotes melanoma growth and metastasis in a mouse melanoma model

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Metastatic progression of melanoma represents one of the most challenging hallmarks of the disease, accounting for the majority of the tumor-related mortality. Whereas the connection of ulceration with a higher risk for metastatic progression is a clinically well-known phenomenon, the importance of the ulceration-induced inflammatory responses are only recently shifting into focus. In earlier work, our group identified a mechanism how a neutrophil-rich inflammation induced by UV light drives angiotropic growth and metastasis in a mouse melanoma model. Early studies in search of biomarkers of melanoma detected a correlation between the expression of the melanoma cell adhesion molecule (MCAM, CD146) with disease progression and metastasis. Interestingly, MCAM is also strongly expressed by endothelial cells. In the current project, we aim to understand the molecular mechanisms of melanoma-endothelial cell interactions underlying angiotropism and metastatic dissemination.

Initially, we identified the expression of MCAM in the melanoma cell line HCmel12 with an increase after inflammatory stimulation with the cytokine TNF-alpha. To assess the MCAM-specific effect on metastatic tumor progression, we generated HCmel12 MCAM knockout cells via the CRISPR/Cas9 technique, which we validated via next generation sequencing. After intracutaneous transplantation of these cells, we observed a prolonged survival of mice transplanted with MCAM knockout clones compared to CRISPR control cells. Strikingly, we also detected a significant reduction in the number of pulmonary metastasis in the MCAM knockout cohort. Finally, we found an inhibited migration of MCAM knockout melanoma cells in co-culture with keratinocytes and endothelial cells in vitro.

Our data suggest a direct role for MCAM in the progression and metastasis of melanoma cells. In our further experiments, we aim to understand the precise mechanism of MCAM function in melanoma and analyze a therapeutic inhibition of MCAM in our mouse model.

P222 | Influence of the fatty acid composition of the tumor microenvironment on melanoma phenotype

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Lipid metabolism has been shown to play an important role in the development of cancer. In melanoma, the lineage addiction oncogene MITF regulates the metabolic enzyme stearoyl-CoA desaturase (SCD) which converts saturated fatty acids to mono-unsaturated fatty acids. The MITF-SCD axis regulates melanoma phenotypic plasticity and suppresses stress and inflammatory signaling. In MITF low melanoma cells SCD is downregulated resulting in an imbalance of saturated to unsaturated fatty acids which in turn leads to ER stress, inflammatory signaling and melanoma de-differentiation.
Here, we analyzed the effect of an imbalance of the saturation of fatty acids by specific SCD inhibitors or treatment with saturated fatty acids on human and murine MITF-dependent melanoma cell lines. Inhibition of SCD reduced melanoma cell proliferation and induced the expression of pro-inflammatory cytokines. Furthermore, we showed a connection between the cGAS-STING pathway and inflammatory signaling upon SCD inhibition. Moreover, changes in the fatty acid composition towards saturated fatty acids resulted in a strong phosphorylation of the IKK kinase TBK1. However, phosphorylation of TBK1 was independent of the cytosolic nucleic acid sensing pathways signaling via STING, MDA5 or RIG-I.
In the future we aim to unravel the exact mechanism of TBK1 phosphorylation and reveal the role of STING in this context and in the context of inflammation-induction. Our goal is to further understand the influence of the fatty acid composition of the tumor (and the microenvironment) on its phenotype and on possible treatment strategies of melanoma.

P223 | Identifying molecular mechanisms underlying MHC class I downregulation as a resistance mechanism to T cell-based immunotherapy in melanoma

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Significant breakthroughs in the treatment of melanoma have been achieved in the recent years by T cell-based immunotherapies. However, tumor cells frequently relapse from therapy by acquired resistance mechanisms such as loss of target antigen expression or presentation which abrogates immune recognition. Recently, we identified poor induction of MHC class I (MHC-I low) surface expression upon IFN-γ exposure as an immune evasion mechanism despite persistent antigen expression in our syngeneic mouse melanoma model. However, the underlying mechanisms causing MHC-I down-regulation are insufficiently understood. 3'mRNA sequencing revealed selective impairment of MHC class I gene induction, whereas other interferon-response genes were similar induced compared to controls. Using CRISPR/Cas9 we individually ablated the expression of tumor suppressor genes as well as transcriptional regulators for MHC class I in murine melanoma cells. We performed immunoblot analyses to confirm absence of protein expression, analyzed the impact of genetic loss variants on IFN-γ-induced MHC class I surface expression by flow cytometry and assayed recognition by T cells in vitro. We identified NLRC5 (NOD-like receptor CARD domain containing 5), a transcriptional regulator for MHC class I as the most promising candidate impairing MHC-I surface expression in our melanoma model. Ablation of NLRC5 expression in parental melanoma cell lines correlated with poorly induced MHC-I surface expression and accompanied reduced T cell recognition in vitro, which recapitulated the phenotype observed in our ACT-recurrent MHC-I low melanoma. So far, our results show that MHC class I down-regulation as an immune evasion mechanism to T cell-based immunotherapies could be provoked by loss of NLRC5 expression. To investigate whether an impairment of NLRC5 function is indeed the underlying mechanism in our spontaneously resistant melanoma model further experiments in the future are required. Identifying mechanisms and developing new approaches to reverse effects of MHC-I downregulation could be exploited to restore tumor immunogenicity and to optimize immunotherapy for cancer patients if this turns out to be a clinically relevant mechanism of immune escape.

P224 | Artesunate mediated cell growth inhibition and induction of Apoptosis in cutaneous T-cell lymphoma

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Despite the developments in recent years, the treatment options for Mycosis fungoides, the most prevalent cutaneous T-cell lymphoma are still limited.
Artesunate a semi-synthetic drug, which is derived from the Chinese herb Artemisia annua and which is used worldwide, in guideline-based therapy of resistant malaria has been reported to have anti-tumor activity.
To investigate the anti-tumoral potential of artesunate in Mycosis fungoides and Sézary Syndrome, we performed proliferation and cytotoxicity assays (BrdU, MTT, LDH) with the Mycosis fungoides cell line MyLa and the Sézary Syndrome cell line Hut-78. We were able to demonstrate, that artesunate suppresses cell proliferation with minor cytotoxicity in both cell lines. In FACS cell cycle analysis a significant increase in the sub-G0 phase was evident which could

be further specified as apoptotic cells by the cell death detection assay. Furthermore, we were able to measure increasing intracellular levels of ROS, a depletion of total GSH, an increase in mitochondrial Cytochrome C release and increasing Caspase 3 and 7 actitivity.
Beside these redox-mediated processes of programmed cell death, we were able to identify significant downregulation of c-myc a key regulator of cell growth and survival in mycosis fungoides.
These results show first evidence of an anti-tumoral effect of artesunate in cutaneous T-cell lymphoma. Artesunate is therefore a very interesting drug in mycosis fungoides, which should be followed up with further investigations to elucidate its potential for a future use in clinical practice.

P225 (OP05/01) | Role of DAMPs for immunoresistance towards anti-PD-1 immunotherapy in advanced melanoma

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The use of immune checkpoint inhibitors such as pembrolizumab has revolutionized prognosis and survival of patients with advanced melanoma. However, there is still an unmet need to understand and overcome the clinical challenge of immunoresistance, which limits the therapeutic benefit to a considerable subset of patients. Damage-associated molecular pattern-molecules (DAMPs) have been identified as central chemoattractants promoting tumor growth and metastasis.
In recent reports, secretion of DAMPs has been implicated with increased PDL1 expression on melanoma cells, which in turn may facilitate immunoevasion of melanoma cells and support progression of melanoma disease. In addition, first investigations identified a procoagulatory role of DAMPs. Hence, the importance of DAMPs for the development of immunoresistance in advanced melanoma as well as a possible link to procoagulatory DAMP-mediated effects should be further investigated.
DAMP serum concentrations of stage III-IV melanoma patients before and during pembrolizumab treatment using peripheral blood specimens were analyzed and correlated with individual therapeutic responses. Interestingly, increased DAMP levels correlated with non-response towards pembrolizumab treatment.
Microthrombi were identified in premetastatic lung tissue in a B16 melanoma mouse model and tissue analysis of DAMP expression was performed revealing increased DAMP expression of premetastatic lung tissue compared to controls. DAMP expression analysis of melanoma tissue in human samples of primary melanoma tissue as well as in comparison with a screening panel of various tumor entities from patient-derived melanoma mouse models (PDX) revealed a ubiquitous expression of DAMPs. Functional analysis of DAMPs regarding activation and interaction of coagulation-associated cells like platelets, endothelial cells or neutrophils underlined the role of DAMPs in promoting coagulation.
Taken together, serum DAMP levels are associated with immunoresistance towards anti-PD-1 immunotherapy of advanced melanoma. Moreover, DAMPs could promote immunoresistance via procoagulatory effects. Further in vivo- and in vitro investigations will be conducted to clarify this mechanism.

P226 | Distinct molecular signatures orchestrate tumor subpopulations in melanoma models mimicking tumor microenvironment and drug tolerance

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Dynamic heterogeneity is a leading cause of melanoma drug resistance. Slow cycling tumor cells are more drug resistant than rapidly proliferating cells. MITF is considered a master transcriptional regulator of highly proliferative but less invasive and slow cycling but highly invasive melanoma cells. Here, we aimed to decipher the molecular signature of this phenomenon. Cell cycle imaging of FUCCI-transduced 3D melanoma spheroids revealed two differentially cycling subpopulations: central slow cycling, MITF-low and peripheral rapidly proliferating, MITF-high cells. We isolated each subpopulation for RNA-sequencing. GSEA analysis revealed hallmarks of hypoxia, TNF-a and EMT as the top positively enriched pathways in the slow-cycling population. In contrast, cell cycle and proliferation-associated pathways such as hallmarks of E2F targets, G2/M checkpoint and MYC targets were negatively enriched in the slow-cycling population. Differentially expressed genes in 3D spheroids were compared with slow-cycling induced drug tolerant cells (IDTC). GSEA analysis of the common genes in these models showed similar enriched pathways. Survival analysis with the top 100 upregulated genes in the slow-cycling 3D spheroid center demonstrated a poor prognosis in Skin Cutaneous Melanoma (SKCM) patients (TCGA database). Similarly, higher expression of the top 20 common upregulated genes in the 3D spheroid and 2D IDTC models was associated with poor overall survival in SKCM patients. Overall, our study reveals a molecular gene signature of the slow-cycling melanoma cells in two melanoma models which mimic the tumor microenvironment or drug tolerance. This study provides insight in oncogenic pathways as potential therapeutic targets with targeted or immunotherapy.

Miscellaneous | P227 | Investigation of the molecular effects of a dexpanthenol-containing ointment and liquid in the treatment of radiodermatitis and mucositis in newly developed 3D skin models of both skin diseases

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Radiotherapy with ionizing radiation is a common treatment for various forms of cancer. Significant side effects of this therapy are radiodermatitis and mucositis, which can tremendously affect the quality of life of the patients. The adverse cutaneous effects of ionizing radiation depend on the damage caused by the generation of reactive oxygen species on the rapidly dividing cells of the basal layer and the underlying dermis. Early skin reactions to radiation therapy usually occur within days to weeks, while late changes can occur months to years after treatment. Radiation-related injuries generally include erythema, dry desquamation, hair loss, pruritus, and more severe reactions such as edema, fibrinous exudates, and the possibility of blistering. Many topical agents and specialized wound dressings are being used for the prevention and management of radiation-induced skin changes. This study aimed to investigate the acute molecular effects of a dexpanthenol-containing ointment and liquid on newly established three-dimensional (3D) full-thickness skin models depicting acute radiodermatitis and mucositis. While histological examination was performed to assess morphological characteristics, we utilized gene expression profiling using microarray and qRT-PCR analyses to identify molecular effects of the treatment with a dexpanthenol-containing ointment and liquid on day 7 after irradiating the skin models with 5 Gray (Gy). Gene expression profiling revealed an upregulation of genes that are associated with tissue remodeling and wound healing (e.g. MMP3, KRT7, HSPB8) as well as genes that are associated with the skin barrier (e.g. DEFB4A, S100A7A) in skin models of radiodermatitis and mucositis after treatment with a dexpanthenol-containing ointment and liquid in comparison to placebo and untreated controls.

In conclusion, a dexpanthenol-containing ointment and liquid showed beneficial effects on radiation-induced skin impairments in newly developed skin models of radiodermatitis and mucositis. Our findings confirm the potential of the these models as in vitro tools for the replacement of pharmacological in vivo studies regarding radiation-induced skin injuries.

P228 | Landscape of the nail fungal diversity in health and onychomycosis

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Onychomycosis (OM) is a common fungal nail infection. Current diagnostic tools, such as microscopy and in vitro culture, assume a single pathogen as its cause. Recent molecular diagnostics, however, indicate a general presence of multiple fungal pathogens in OM. Yet, an in-depth characterization of fungal diversity in nail infection and comparison to healthy nails is lacking. Here, we analysed the landscape of fungal diversity in healthy and onychomycotic nails by next-generation sequencing (NGS) of samples from clinically healthy toenails and from those with suspected OM. In addition to sequence analysis, samples underwent routine and molecular diagnosis for OM. Samples were classified as healthy or diseased, if OM was negative in all or positive in at least one diagnostic test, respectively. The final data set contained 55 individuals (17 controls and 38 confirmed cases) with a total of 324 operational-taxonomic units. In terms of richness and diversity per sample (alpha diversity) no significant differences between controls and confirmed cases were observed ($P < 0.05$. The variation of mycobial communities between the samples (beta diversity) showed a significant shift in the community structure between cases and controls ($P = 0.0096$). Within the case group, two distinct sub-clusters are present: Those with a high Trichophyton rubrum abundance, and those with an indistinguishable fungal beta diversity from healthy controls. Thus, we hypothesize that there are two types of OM, one pathogen driven, and one in which OM develops due to host- and/or environmental factors. Collectively, we here provide detailed insights into the communal composition of fungi in healthy and onychomycotic toenails.

P229 | Upregulation of MMP-3 is a key mechanism in calcium pantothenate-mediated wound healing after fractional ablative laser treatment

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Previous in vivo studies have indicated that matrix metalloproteinase-3 (MMP-3) is a key player in cutaneous wound healing. The aim of our in vitro study was to investigate the role of MMP-3 and its regulation by calcium pantothenate in wound healing processes at

the molecular level by using a laser-injured 3D skin model. We found that fractional ablative CO2 laser-injured skin models receiving aftercare treatment with calcium pantothenate exhibited an upregulation of MMP-3 expression at the protein level. To further understand the functional role of MMP-3 in wound healing, we established full-thickness 3D skin models using fibroblasts and keratinocytes with a MMP-3 knockdown that were irradiated with a fractional ablative Er:YAG laser to set superficial injuries with standardized dimensions and minimal thermal damage to the surrounding tissue. Performing histological analyses, skin models with MMP-3 knockdown exhibited a slower wound closure on day 3 after laser treatment compared to MMP-3 expressing controls. While MMP-3 expressing models showed a completed wound closure on day 5 after laser treatment, models comprising MMP-3 knockdown cells still exhibited defined lesions. A transcriptomic micro-array profiling detected an upregulation of cytokines and chemokines (e.g. IL-36B, CXCL17, IL-37, CXCL5), antimicrobial peptides (e.g. S100A7, S100A12), epidermal crosslinking enzymes (TSGM5) and differentiation markers (e.g. LOR, KRT1, FLG2) on day 3 after laser irradiation in skin models with MMP-3 knockdown in comparison to MMP-3-expressing control models. These data reflect the delayed wound healing and the decrease in epidermal keratinocyte proliferation. We also detected a downregulation of cathepsin V and MMP-10, both of which play a prominent role in wound healing processes.

Our data substantiate a key role of MMP-3 in wound healing processes. For the first time, we could show that calcium pantothenate exerts its clinical effects via a regulation of MMP-3.

P230 | Screening of novel therapeutic targets in wound healing

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Chronic, non-healing wounds are a huge health concern as they lead to a severe loss of live quality of affected patients and also to immense costs for the health care system. In an aging society, conditions that promote non-optimal wound healing, such as type two diabetes, will increase. Thus, also the number of patients with chronic wounds will certainly increase in the future. As the current treatment options are not satisfying, new treatment strategies need to be found.

Searching for new possible drug candidates, we performed a wound healing human skin organ culture (WHOC) which allows us to study wound healing for up to one week ex vivo. 141 candidates of the Selleckchem L3500 cherry pick inhibitor library are being screened in this model. To analyse their effect on wound healing, wound size parameters are determined using different imaging techniques. The wound area is assessed based on two-dimensional reflected light microscopy images. In addition, three-dimensional, depth-resolved tomograms were acquired using optical coherence tomography (OCT) to determine the wound volumes.

Within the first screening phase, we already found eight promising candidates which accelerated wound healing by at least 15% (relative wound area treated compared to untreated wounds after 7 days of incubation). These candidates will be further validated by histochemical analysis of the length and area of epithelial tongues as well as of the microscopic wound area and diameter. Immunohistochemical analyses will be performed to investigate how the inhibitors contributes to wound healing.

Since most of these drugs are already found in clinical trials for other diseases, we will presumably find new drug candidates for the treatment of chronic wounds among them.

P231 | Oral DMF targets HCA2-expressing skin cells in the Imiquimod mouse model

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Psoriasis is a chronic, immune-mediated disease affecting 2-3% of the world's population. The most frequently used first line therapy for moderate to severe psoriasis in Germany is oral dimethyl fumarate (DMF, marketed as Fumaderm® or Skilarence®). Monomethyl fumarate (MMF), the bioactive in vivo metabolite of the prodrug DMF is an agonist of the hydroxycarboxylic acid receptor 2 receptor (HCA2). HCA2 is a G-protein-coupled receptor for which the ketone β-hydroxybutyrate and the short chain fatty acid butyrate are known agonists. Nicotinic acid (NA) is another agonist of HCA2. Although HCA2-mediated effects of NA have been extensively analysed and despite its long use in psoriasis and the identification of HCA2's role in a common side effect of NA and DMF treatment, flush, the role of HCA2 for the therapeutic effect of oral DMF treatment in psoriasis is not yet known.

By using the Imiquimod (IMQ) model of psoriasis-like skin inflammation employing HCA2 expressing and deficient mice, the therapeutic effect of oral DMF treatment was confirmed to be dependent on this receptor. Treated with IMQ, HCA2-expressing mice showed a reduced epidermal thickness when exposed to oral DMF compared to vehicle treated mice. This effect was abrogated in HCA2-deficient mice. In these experiments oral DMF did not affect skin infiltrating immune cells but changed the mRNA expression of keratinocyte-derived pro-inflammatory cytokines, e.g. S100 proteins and Krt14. The use of bone marrow chimeras demonstrated that oral DMF treatment mediated its therapeutic effect through HCA2 expressed by radio-resistant cells, most likely keratinocytes. In vitro analysis showed that MMF treatment reduced the proliferation of keratinocytes.

In conclusion, this work showed that the HCA2-receptor mediated the therapeutic effect of oral DMF in psoriasis skin inflammation most likely by directly affecting the proliferation of keratinocytes.

P232 | Determination of biocompatibility and antimicrobial efficacy of a new ceramic micro-plasma source

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Aim: Cold atmospheric plasma (CAP) has become an interesting tool for biomedical application due to its high antimicrobial activity. Plasma devices differ in type (DBD, jet) and working gas (air, nitrogen). Therefore, the investigation of new plasma sources with regard to cellular reactions is of great importance. Here, a new ceramic micro-plasma device is presented, which is based on LTCC-technology (low temperature cofired ceramic). Biocompatibility and antimicrobial efficacy was analyzed in 3D-skin models as well as using a shell-less hen's egg test on the chick area vasculosa (HET-CAV).

Methods: The models were treated with CAP up to 30s. Morphological changes, cytotoxic effects, and pro-inflammatory reactions were investigated. For investigation of antimicrobial efficacy clinically relevant microorganisms were plated onto agar plates. After CAP treatment, agar plates were incubated at 37°C for 24 h under aerobic conditions. Furthermore, fertilized hen's eggs (HET-CAV model) were incubated for 72 h, transferred into petri dishes, and the vessel system CAV was treated directly with CAP for investigation of irritative effects (hemorrhage, vascular lysis, aggregation, lethality) up to 24 h. The HET-CAV model was co-cultivated with Pseudomonas aeruginosa for antimicrobial determination by visualization of autofluorescence.

Results: The generated plasma exhibited a good biocompatibility as well as antimicrobial efficacy. Prolonged treatment times did not lead to visible damage of skin models or enhanced secretion of pro-inflammatory cytokines. A strong antimicrobial effect was observed, which depended on the treatment time. Biocompatibility could be further confirmed by the HET-CAV model. In addition, antimicrobial effects could be verified by florescence reduction in the infected HETCAV model ex-ovo.

Conclusions: By in vitro and ex ovo investigations biocompatibility and antimicrobial efficacy of a ceramic micros-plasma device could be characterized. It could be shown that this plasma source may be suitable for treatment of infectious skin diseases.

P233 | Three-dimensional human skin models tattooed with black ink - cytotoxic and inflammatory effects

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Introduction: In the last decade, a growing popularity of tattoos was observed. In 2016 about 20% of the German population older than 14 years had one or more tattoos. Tattoo inks are suspensions of pigments dispersed in a liquid matrix like water, glycerine, or alcoholic derivatives. Additional ingredients are surfactants, fillers, thixotropic and binding agents, and preservatives, but they can also contain hazardous chemicals such as polycyclic aromatic hydrocarbons, primary aromatic amines, and metals. No regulations for the use of these inks in intradermal applications exist. Consequently, these inks have never been toxicologically assessed. The aim of this study was to establish a 3D skin model which can be tattooed to investigate the cytotoxic and inflammatory effect of tattooing in a complex cell environment.

Methods: A three-dimensional human skin model suitable for tattooing was developed. It consists of fibroblasts embedded in collagen as dermis and differentiated keratinocytes as epidermis. Using a rotary tattoo machine, the 3D skin models were tattooed with black ink. Tattooed 3D skin models were further cultivated for a period of 14 days. Cytotoxic and inflammatory effects were analysed by detection of lactate dehydrogenase and the cytokines interleukin-6, interleukin-8, and interleukin-1fÑ in the culture supernatants. Formalin fixed skin models were analysed histochemical by haematoxylin and eosin staining. Additionally, cytotoxicity of the black tattoo ink was analysed in a 2D model using HaCaT keratinocytes.

Results: Strong cytotoxic effects of the black ink were observed using the 2D model with HaCaT keratinocytes. High dilutions of the tattoo ink were necessary to obtain cell compatible conditions. After tattooing the 3D skin models the embedding of the black ink was clearly visible in the injured epidermal layer and the dermal layer using histochemical analysis. In the course of cultivation of the tattooed skin models, a distribution of the ink between the dermal and epidermal layer was observed. As expected, the insertion of the ink as a foreign matter into the skin model resulted in an inflammatory reaction of the skin model which subsided during the course of the experiment.

Conclusions: In this study, a 3D skin model was established which is robust and suitable for tattooing. Cytotoxic and inflammatory effects after tattooing could be observed. The developed skin model is a promising tool for investigating the mechanisms of skin reactions to tattooing and for evaluating different tattoo inks.

P234 | The atopic dermatitis microbiota decreases expression of filaggrin in a 3D skin equivalent

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Introduction
Loss of function mutations of the filaggrin gene (FLG) are the strongest known genetic risk factor for atopic dermatitis (AD). Filaggrin contributes to the mechanical strength and integrity of the stratum corneum, and its breakdown products form the natural moisturizing factor. A filaggrin deficiency can thus lead to a disrupted skin barrier. Approximately 10% of the northern Europeans carry a single FLG mutation, but only 40% develop AD. It is known that the skin of AD patients shows marked changes of the skin microbiome as compared to healthy individuals. Interestingly, healthy individuals with a single FLG null mutation exhibit shifts of the skin microbiota towards the changes seen in AD patients. However, the interaction of skin barrier function and the microbiota is still insufficiently understood. To analyze the impact on the skin barrier function on the microbiota and vice versa further, we started to analyze the influence of the whole skin microbiota of healthy individuals and AD patients on differentiation markers like filaggrin in a 3D skin equivalent.
Methods: Defined skin areas of probands were rinsed with a saline/detergent solution to harvest the microbiota. Subsequently, the rinsing solution containing the microbiota was sequentially centrifuged to remove host mediators and cellular debris. The isolated microbiota was stored at -80°C in a glycerol-pellet. These microbiota samples were then applied to the surface of 3D skin equivalents. After stimulation, expression of differentiation marker by the 3D skin equivalents were analyzed by real-time PCR and immunohistochemistry. The microbiota of AD patients was compared with localization-, gender- and age-matched microbiota samples of healthy control persons.
Results & **Conclusion:** We observed that treatment of the 3D skin equivalents with the AD-derived microbiota induced a decreased expression of the differentiation marker filaggrin compared to 3D skin equivalents treated with the microbiota derived from healthy individuals. This supports the hypothesis that the skin microbiota of AD patients has a direct influence on skin barrier function and may trigger the impairment of the cutaneous barrier.

P235 | MMP9-driven release of CXCL12 as a new therapy in wound healing

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CXCL12 (CXC-Motive-Chemokine 12; alias stromal cell-derived factor 1 (SDF-1)) is crucial for the regulation and orchestration of wound healing processes in the skin. The chemokine from the group of the CXC-motif chemokines is mainly produced by fibroblasts and endothelial cells in the skin. One of the most important functions, besides its involvement in angiogenesis and embryonal development, is to guide hematopoietic stem cells to the wounded area. Communication with the target cells takes place mainly via the CXCR4, partly also via the CXCR7, receptor. By binding to its receptors, CXCL12 provides hematopoietic stem cell activation, mobilization, homing and retention (Ma, 1999) as well as the attraction of lymphocytes (Bleul, 2013) & epithelial progenitor cells (EPC) (Xie, 2011). In diabetic wounds, it has been shown that the expression of CXCL12 is significantly disturbed and reduced and therefore its biological function during wound healing. This contributes to significantly disturbed healing of diabetic wounds, up to the development of non-healing chronic wounds.
In our study, we present an approach to improve CXCL12 levels within the wound environment through the application of CXCL12 by using biomaterials. Therefore, CXCL12 is covalently bound to biomaterials (PCL scaffolds and StarPEG hydrogels). The synthesis of the immobilized chemokine variant was achieved by expressed protein ligation. The protein C-terminus was synthesized by solid phase peptide synthesis and elongated by an MMP (matrix metalloproteinase) cleavage site as well as an anchor unit for immobilization. The N-terminus of the protein was generated by recombinant protein expression in E. coli and purified by using the IMPACT (intein mediated purification with an affinity chitin-binding tag) system, based on a C-terminal thioester prior to fragment ligation by native chemical ligation. The active conformation of the protein was regained by pulse rapid dilution refolding. By the introduced MMP cleavage site in the C-terminal linker sequence, the chemokine can be cleaved from the biomaterials by the MMPs present in the wound fluid.
First in vitro tests have shown that the (modified) CXCL12 leads to an increased migration of keratinocytes and increased viability of endothelial cells. In addition, the activation of the downstream signaling axis via the PI3K-Akt pathway could be demonstrated.
Furthermore, we demonstrate accelerated wound closure in an ex vivo wound healing assay. The CXCL12 was bound to PCL scaffolds and these were placed on ex vivo pig skin grafts. After 24 hours, a significantly improved growth of the epithelial tips could be observed. This effect could be reduced by blocking the CXCR4 receptors. Currently, application of StarPEG-hydrogels with releasable

CXCL12, is tested with respect to improvement of wound healing in a diabetic wound healing model in db/db mice.
So far, our experiments have shown first promising approaches that have to be validated in further experiments, especially the effectiveness of CXCL12 during diabetic wound healing in vivo.
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P236 | IgG autoantibody responses against desmoglein 3 and bullous pemphigoid 180 in lichen planus

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Lichen planus (LP) is a common, chronic relapsing inflammatory disorder of the skin and mucous membranes of unknown etiology. Our group has recently identified autoreactive Th1 and Th17 cell responses against desmoglein 3 (Dsg3) and bullous pemphigoid (BP) 180 in LP patients with mucosal and mucocutaneous involvement, respectively. We here analyzed a cohort of 98 LP patients (72 female and 26 male), 42 of whom showed skin lesions only, while 33 showed exclusively oral involvement and 23 had mucocutaneous lesions. Of note, 5 LP patients had anti-BP180 IgG and one patient with oral LP demonstrated anti-Dsg3 IgG autoantibodies. Among the 5 anti-BP180 IgG positive patients, 3 had mucocutaneous lesions and 2 showed only oral involvement. Direct immunofluorescence (DIF) was positive in 3 of the 4 anti-BP180 IgG positive patients and showed IgG and/or C3 deposits at the basal membrane zone. A female patient with long-standing oral lesions had serum IgG against Dsg3 and showed a refractory clinical course not responding to different immunosuppressive regimens. In light of this recalcitrant course and based on our findings revealing a possible pivotal role of IL-17 in LP pathogenesis, the patient received a compassionate use treatment with secukinumab (anti-IL 17A monoclonal antibody) 300 mg s.c. initially weekly, then monthly. This therapeutic approach led to a complete remission of the oral erosions after 12 weeks of treatment. Initially, there was no correlation between anti-Dsg3 IgG and clinical activity with anti-Dsg3 IgG ranging from 20 up to 70 relative units (RU)/ml. One month after the clinical improvement there was a decrease in anti-Dsg3 IgG and two months later a reincrease of anti-Dsg3 IgG without worsening of the oral lesions. Two patients with anti-BP180 IgG and extensive cutaneous LP and oral lesions showed rapid clinical improvement on the same regimen with secukinumab leading to a shift from inflammatory erythematous to post-inflammatory hyperpigmented skin lesions and a regression of oral lesions within 12 weeks. However, there was no correlation between clinical features and variable anti BP180 IgG serum concentrations. A fourth LP patient with long-standing, refractory ulcerative lesions of the tongue had anti-BP180 IgG and received a treatment with the anti-IL-23 antibody guselkumab, 200 mg s.c. initially monthly, later bi-monthly, leading to a dramatic improvement of the ulcerations within 6 months. Despite the improvement of the oral lesions, anti-BP180 IgG antibodies remained stable (53-55 RU/ml). In contrast, a female patient with anti-BP180 IgG positive LP pemphigoides (LPP) showed strong IgG deposits along the basal membrane by DIF. She had a chronic refractory course with lichenoid papules, bullae and generalized pruritus. Topical treatment with clobetasol propionate led to a prompt clinical regression of skin lesions and pruritus and was associated with a decrease in anti-BP180 IgG concentration, which reoccurred upon clinical relapse of LPP. In summary, IgG-mediated autoimmunity against cutaneous autoantigens in LP is less common than previously thought in light of the identification of autoreactive Th1 and Th17 cell responses. IgG autoantibodies seem to be associated with a chronic relapsing and, partly, refractory disease course but were, in contrast to LPP, not related to the clinical activity of LP. The relative contribution of autoreactive T cells versus IgG antibodies against cutaneous autoantigens in the pathogenesis of LP variants remains to be elucidated.

P237 | Inducible knockout of Has2 in murine skin leads to increased collagen deposition in resting skin and exacerbated allergic response in the tncb-model

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Hyaluronic (HA) acid is a main structural component of the extracellular matrix (ECM) and plays a key role in homeostasis and pathology like inflammation and repair. HA is synthesized by the three membrane-bound isoenzymes Has1-3, while Has2 synthesizes the majority of HA in the skin.
By using an inducible knock-out mouse model (Has2-/–UBC-creERT) we aim to uncover the physiological role of Has2 in adult organisms concerning putative structural changes in resting ECM as well as the role during acute inflammatory reactions. In this mouse model, Has2 is depleted by induction of cre-recombinase in mice of 8-10 weeks age and a reduction of the HA content in the skin by about 80% can be achieved. First, we examined the effect of HA reduction on the ECM composition. In the skin of wild-type mice, HA fills space between the collagen fibers in the ECM due to its hydrophilic and swelling properties. When analyzing trichrome-stained sections of the skin we noticed that the collagen in the dermis of Has2-/- mice was much more tightly packed. Collagen quantification by hydroxyproline assay, gene expression, protein analysis, and imaging

methods revealed significantly increased gene expression for type I and III collagens and higher collagen amounts per mg tissue. In addition, electron microscope analysis revealed an increase in collagen fibril diameter in Has2-/- mice.

Taken together, increased amounts of collagen fill up the space left by significantly reduced HA-deposition. Resulting effects on mechanical skin properties were revealed by atomic force microscope indentation measurements showing Has2-/- mouse skin to be softer than wild-type skin. It is concluded that the changes in ECM structure and composition cause the Has2-/- mice skin to be less stiff, suggesting that HA and its crosslinking components support an interconnected matrix with fibrillar ECM components to control mechanical properties of the skin.

To investigate the impact of decreased dermal and epidermal HA during inflammation we use a model of acute inflammation and delayed-type hypersensitivity (DTH) after sensitization and challenging skin with TNCB (2,4,6-Trinitrochlorobenzene). Preliminary data show a strongly increased DTH response in Has2 deficient mice suggesting that the reduction of Has2-derived high molecular weight HA is accompanied by increased immigration of inflammatory cells plus enhanced and prolonged inflammatory action of these cells. Further investigations will uncover the main cell types and cytokines that are involved in these processes depending on the HA content of the skin.

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P238 | The influence of the skin commensal bacterium Staphylococcus epidermidis on the epidermal barrier and inflammation

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The skin microbiota is a crucial component in maintaining cutaneous barrier function. It is known that the skin of patients with atopic dermatitis (AD) is characterized by a dysbiosis of the microbiota. This is reflected by a decreased microbial diversity and the abundance of specific potential pathogenic strains, in particular Staphylococcus (S.) aureus. Colonization with S. aureus is an associated characteristic in the pathogenesis of AD and is suggested as a promoter of inflammation. In contrast, Staphylococcus (S.) epidermidis is considered generally as an apathogenic commensal that exhibits beneficial effects in AD, e.g. by controlling the growth of S. aureus. However, S. epidermidis is also detectable in a high percentage in lesional skin of AD patients and some studies report that the presence of S. epidermidis was significantly higher in severe AD as compared to mild AD. Therefore, the aim of this study was to analyze the functional role of S. epidermidis concerning the skin barrier and inflammation. In particular, we hypothesized that different clinical strains of S. epidermidis may trigger the expression of AD-associated inflammatory mediators. To address this hypothesis we stimulated 3D organotypic skin equivalents with different S. epidermidis strains isolated from lesional skin of AD-patients and healthy control subjects. Realtime-PCR and ELISA analyses of AD-associated pro-inflammatory cytokines revealed a significantly induced expression of thymic stromal lymphopoietin (TSLP), IL-17c, TNF-alpha and IL-1beta in skin equivalents stimulated with AD-derived patient strains as compared to unstimulated controls. S. epidermidis derived from healthy controls also differentially induced expression of the above-mentioned cytokines. In contrast, the expression of the barrier-associated protein filaggrin was downregulated by S. epidermidis. Taken together these results indicate that S. epidermidis has the capacity to promote epidermal inflammation by induction of inflammatory mediators and inhibition of epidermal barrier proteins. These data warrant a more detailed analysis of the exact role of S. epidermidis in skin homeostasis, especially in the context of AD. This could help to optimize treatment strategies for patients suffering from AD.

P239 | Effects of RNase 7 on cutaneous RNA-induced inflammatory reactions

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RNase 7 is a ribonuclease which is constitutively expressed in human skin and exhibits antimicrobial and immunomodulatory properties. Expression of RNase 7 can be upregulated by endogenous and exogenous factors, such as cytokines, growth factors and microorganisms. RNase 7 is also a potent ribonuclease and is able to degrade host RNA. It is known that free host RNA, which is released upon cell damage and injury, induces inflammatory processes in keratinocytes characterized by an increased expression of proinflammatory cytokines. We hypothesize that the ribonuclease activity of RNase 7 may modulate this RNA-mediated inflammatory response by its capacity to degrade RNA.

To address this hypothesis we induced inflammatory processes in cultured primary keratinocytes by stimulation with the RNA-analogue poly(I:C). As readout parameter we measured the expression of different cytokines, especially of thymic stromal lymphopoietin (TSLP). TSLP plays an important role in the maturation of dendritic cells and contributes to inflammatory reactions mediated by keratinocytes and is considered as a proinflammatory marker in atopic dermatitis.

To evaluate the effects of RNase 7 on the RNA-induced TSLP expression in keratinocytes we stimulated the cells with poly(I:C) in the absence or presence of RNase 7. Stimulation with poly(I:C) caused a significant induction of TSLP expression. This induction was abolished when poly(I:C) were co-incubated with RNase 7. To examine the endogenous influence of RNase7, the keratinocytes

were treated with siRNA directed against RNase 7 to selectively down-regulate the expression of RNase 7. This revealed a significantly higher poly(I:C)-mediated induction of TSLP in keratinocytes treated with RNase 7-siRNA as compared to control keratinocytes. This is most likely caused by the RNase 7-mediated degradation of poly(I:C) which is reduced in the keratinocytes with decreased RNase 7 expression.

The ribonuclease activity of RNase 7 can be inhibited by the ribonuclease Inhibitor (RI). To analyze the endogenous influence of the RI, its expression in keratinocytes was downregulated by RI-specific siRNA and the cells were subsequently stimulated with poly(I:C). This resulted in a decreased poly(I:C)-mediated TSLP induction. These data suggest that the endogenous RI binds and blocks the ribonuclease activity of RNase 7 in keratinocytes leading to a decreased RNase 7-mediated degradation of poly(I:C). Hence, a down-regulation of the RI, as mediated by siRNA in our experiments, leads to increased concentrations of active RNase 7 which in turn promotes an enhanced degradation of poly(I:C) resulting in a decreased poly(I:C)-mediated TSLP induction. Interestingly, similar results as observed for TSLP were also seen for other poly(I:C)-induced proinflammatory cytokines such as IL-6 and IL-17c.

In summary, our data indicate that RNase 7 and the RI modulate the inflammatory reaction of keratinocytes upon RNA exposure. This may have important implications in inflammatory skin diseases such as atopic dermatitis where an increased expression of TSLP and other inflammatory mediators by RNA released from injured cells takes place.

P240 | Probing the morphogenic potential of human hair matrix keratinocytes in generating hair follicle organoids in vitro and ex vivo

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Androgenetic alopecia (AGA) is characterized by progressive miniaturization of terminal scalp hair follicle (HF) in androgen-sensitive scalp skin areas and their transformation into vellus HFs. Since AGA treatment with finasteride and/ or minoxidil often disappoints and never has more than temporary effects while hair transplantation is limited by the availability of sufficient occipital donor HFs, alternative technologies that could potentially generate HFs in vitro and cell-based therapies that inject suitable cell populations in order to retransform vellus into terminal HFs are being contemplated. Given that hair shaft production is controlled by coordinated epithelial-mesenchymal interactions between hair matrix keratinocytes (HMx) and dermal papilla fibroblasts (DP), we have investigated here the morphogenic potential of human HMx to generate HF organoids in vitro and ex vivo in human skin.

Human DP and HMx isolated from non-balding male HFs, as well as normal human adult epidermal keratinocytes (NHEK) (as control), were independently expanded in vitro before starting the co-culture of HMx or NHEK with DP spheroids. Both types of keratinocytes successfully generated HF organoids in vitro, although adult NHEK actually showed a higher success rate than HMx in our hands. Over 10 days of in vitro culture, both types of organoids showed a gradual decline in VERSICAN, NOGGIN and LEF1 mRNA expression, contrasted by an increase of IGF1, HGF, and TGFβ2 transcripts over time. In line with these results, both organoid types showed decrease in VERSICAN protein expression and alkaline phosphatase (AP) activity, suggesting that DP inductivity decrease over time. Interestingly though, HMx-DP organoids revealed higher transcript and protein expression for the pre-cortical hair matrix keratin, K85, and very low expression of ORS associated keratins, i.e. K6, K14 and K5. Instead, NHEK-DP organoids showed high expression of all these keratins. However, both organoid types remained negative for the inner root sheath keratin, K71.

Before placing organoids into human skin, we investigated the viability of HMx and DP in human skin ex vivo by injecting a mixture of labelled HMx and DP cells into 6 mm human skin punches. Upon injection into human skin, fluorescently labelled HMx and DP cells were viable and proliferated in the dermis of organ-cultured skin for at least three days after injection. However, after six days, injected cells no longer proliferated, but did not undergo apoptosis either, presumably reflecting a commitment to differentiation. When fluorescently labelled HMx-DP organoids were placed into human scalp skin ex vivo, HMx and DP did not undergo apoptosis during 10 days of organ culture. Within the organoids, DP expressed VERSICAN at protein level up to 3 days of organ culture, while HMx started to express K85 from days 6 of organ culture. No CK71 positive cells were observed ex vivo within the organoids.

Taken together, our data suggest that HMx have indeed morphogenic potential and that when co-culture with DP spheroids seem to be more committed to give rise to hair shaft lineage cells compared to NHEK. Although confirmatory evidence that HFs could be generated from HMx-DP organoids is required, our preliminary data potentially open up a new application for regenerative medicine technology in hair loss disorders.

P241 | Application of topical Sandalore increases epidermal dermcidin synthesis in organ-cultured human skin ex vivo

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Several olfactory receptors (ORs) are expressed in human skin, which have been shown to regulate skin pigmentation, barrier, and regeneration, as well as hair growth. In particular, we reported that the activation of OR2AT4 up-regulates the expression of the antimicrobial peptide (AMP) dermcidin (DCD) at gene and protein level in human scalp hair follicle epithelium ex vivo. Given that this chemosensory receptor is also highly expressed in the epidermis, we hypothesized that OR2AT4 may as well modulate intraepidermal antimicrobial peptide (AMP) production, and possibly regulate the skin microbiome by this means. We investigated this hypothesis by topically treating human skin ex vivo with Sandalore, a specific agonist of OR2AT4, and by quantifying the epidermal production of the AMPs dermcidin (DCD) and LL-37, and how microbial survival is affected by conditioned culture media (MTT assay). We indeed detected a significant up-regulation of DCD-positive cell number in the epidermis, and DCD secretion in the culture media of skin treated with Sandalore. Instead, the intraepidermal expression of LL-37 remained unaffected after stimulation with the OR2AT4-specific agonist. In line with the increased concentration of DCD in the culture medium, we also demonstrated that the survival of Staphylococcus epidermidis, Staphylococcus aureus, Cutibacterium acnes, Malassezia restricta and Malassezia globosa is modulated by treatment with the conditioned media from Sandalore-treated skin. In line with previous results, stimulation with Sandalore resulted in increased OR2AT4 expression in the epidermis. Therefore, Sandalore treatment or OR2AT4 stimulation deserves further exploration as adjuvant therapeutic option for skin conditions characterized by defective antimicrobial peptide production, and/or imbalance in microbial composition, or antimicrobial peptide production, such as atopic dermatitis.

P242 | In-vivo immunomodulatory effects after microinvasive skin treatment

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Background: Microinvasive techniques provide effective treatments for multiple skin conditions like elastosis, dyschromia, striae and scars. Having replaced ablative treatments, the current gold standard are modern techniques such as fractionated ablative lasers, radiofrequency, microneedling and the recently induced thermomechanical ablation (TMA). Although the common mechanism seems to be the induction of inflammation resulting in dermal collagen deposition, there are differences to be noted, that may lead to tailored treatment in the future. As these differences indicate diverging reaction patterns, knowledge of molecular mechanisms is essential, but only few studies about laser treatments exist. Being a new approach on microinvasive skin treatment, we investigated the immunomodulatory effects of TMA.

Methods: TMA was performed on 6 healthy study subjects (3 male, 3 female, 21-53 years, mean 38 years) using Tixel®; with a 16 ms double-pulse with 800 µm tip protrusion. Tissue fluid of treated and control areas was extracted using minimal invasive cutaneous microdialysis after 1 h and 24 h. In a multiplex assay analysis, inflammatory cytokines and growth factors were quantified using Luminex®; xMAP technology and descriptive statistics, using median comparison, were performed. Wilcoxon test was chosen and statistical significance was determined as $P < 0.05$. Results are presented as percentage change compared to controls.

Results: 1 h after TMA a significant increase of proinflammatory cytokines IL-1β (37.7%; $P > .05$), IL-6 (14.8%; $P > .05$), IL-8 (77.7%; $P < .01$), TNFα (83.9%; $P > .05$) and LIF (48.6%; $P > .05$) was measurable. 24 h post-TMA IL-1β (366.0%; <.05) and LIF (82.8%; $P > .05$; $P = .14$) showed progredient increase while IL-6 (-14%;$P = .14$), IL-8 (16.5%; $P = .74$) and TNFα (0%; $P = .23$) had decreased. Growth factors were upregulated after 24 h, except for FGF-2, PLGF-1 and VEGFD. VEGF-A (78.9%; $P < .01$; 52.2%; $P = .6$) and HGF (47.4%; $P < .01$; 47.2%; $P = .07$) had shown higher and significant upregulation after 1 h while PDGF, BDNF and EGF had shown downregulation. FGF-2 showed significant downregulation (-27.2%; $P < .01$; -22.1%; $P < .05$).

Discussion: The fast rise of IL-1, IL-6, TNFα and IL-8 directly after TMA reflects the inflammatory phase of wound healing. This indicates the induction of wound healing which is the desired effect. Also, the delayed and continuing upregulation of growth factors fits into the general mechanisms of wound healing. The downregulation of FGF-2 is an interesting result whose significance remains to be studied as the release is perhaps completely missing or beginning after more than 24 h.

Comparing our results to the previously described mechanisms of ablative and non-ablative lasers the smaller increase of TNFα and IL-8 has to be noted. TMA is known to induce less injury compared to the routinely used fractionated CO2-lasers. TNFα levels indicate lesser extent and shorter duration of acute inflammation compared to lasers while the fast decrease of IL-8 indicates reepithelialisation and restoration of the skin's integrity within 24 h. Therefore, risk for skin infection is lower after TMA compared to ablative CO2-Laser. Our results confirm the molecular basis of the clinically observable effect of TMA. Compared to the few available studies of other techniques we conclude that there are differences to be noted that need to be investigated further to allow for individualized treatment decisions. We further conclude that in the research of molecular effects of such techniques, cutaneous microdialysis is a legitimate alternative to skin biopsies, being less invasive and thus reducing risk of adverse events.

P243 | Role Dipeptidyl Peptidase 4 in skin homeostasis and repair

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DPP4/CD26 is expressed by fibroblasts in the Skin from E16.5 and onwards right at the time of lineage commitment of these cells and acquisition of a scarring phenotype after wounding. Inhibition of this Enzyme in small, splinted wound leads to reduced extracellular Matrix Deposition. We have deepened our understanding of the role of this molecule in the skin by studying its Expression and regulation in unwounded skin and in large wounds able to Regenerate hair follicles (so called wound induced hair follicle neogenesis, WIHN) using a combination of immunofluorescence, gene Expression Analysis, single cell RNA sequencing and flow cytometry. DPP4/Dpp4 is regulated during hair follicle cycle and in response to wound healing. Interestingly our study shows a non-universal expression of DPP4/Dpp4 on all fibroblasts populations as was previously assumed for adult skin. The transcriptional profile from Dpp4 + fibroblasts differs greatly from those without Dpp4 expression, especially in terms of activation of Wnt-Signaling, required for WIHN. In addition, single cell RNA sequencing shows a previously unknown early expression of Wnt-ligands in Dpp4 + Fibroblasts few hours after wounding. Furthermore, DPP4/Dpp4 Expression is not exclusive for fibroblasts but keratinocyte and immune cell Populations express the molecule/gene and show differential regulation during hair follicle cycle and upon wounding. So far, DPP4 seems to be involved in fine-tuning mesenchymal, epidermal and immune cell populations in terms of response to hair follicle cycle and hair follicle regeneration signals such as Wnt.

P244 | Evidence for a relation between ammonium ions and calcium ions of the skin surface

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Ammonium ions are little investigated cations which can be found at the skin surface. Besides sweat gland activity they might be due to activity of different epidermal enzymes. Among them are calcium-dependent transglutaminases which are responsible not only for cross linking of the cornified envelope proteins but also for the covalent linkage between lipids and cornified envelope in the extracellular compartment of the stratum corneum. A significant quantitative relation between ammonium ions as well as calcium ions might therefore be an indicator and a measure for transglutaminase activity in the upper skin. Therefore, the aim of the present study is to assess quantitatively ammonium ions along with calcium ions at the skin surface. Since the presence of hydrogen ions is a potential additional influence on the interaction between the ions skin surface pH also was assessed.

Overall 14 volunteers were included into the study after informed written consent. All measurements were performed under standardized room conditions. The areas of investigation were the median volar forearm portions of both sides. Determination of ammonium ions as well as of calcium ions was performed by rinsing the skin surface using ion free water and consecutive spectrophotometric determination. The pH of the skin surface was measured using a glass electrode. The statistical evaluation consisted after the determination of ammonium and calcium amounts per cm^2 skin surface of a correlation analysis as well as an exploratory regression analysis between the parameters.

The statistical evaluation revealed no relation between ammonium ions and calcium ions at the first sight (r = 0.300; P = 0.121). However, it could be concluded from the statistics that there were two different groups of similar size with regard to the ammonium levels. One group with values higher than 10 nmol/cm^2 that showed a significant linear correlation (r = 0.620; P = 0.018) between ammonium and calcium ions and a second group with ammonium values below 10 nmol/cm^2 that showed no linear correlation (r = −0.067; P = 0.397), but as a further regression analysis revealed, a quadratic or cubic relation (R^2=0.406; P = 0.057; R^2=0.552; P = 0.039) with mainly inverse characteristics. The comparison between ammonium ions and pH of the skin surface revealed an inverse significant correlation between both parameters (r = −0.388; P = 0.041). A similar result could be found between the pH of the skin surface and calcium ions (r = −0.383; P = 0.044).

The results obtained show significant relations among ammonium and calcium ions as well as among the ions and the pH of the skin surface. The finding of statistical relationships between ammonium and calcium ions suggests that at least a part of the results reflects the activity of an enzyme such as stratum corneum transglutaminase where calcium ions, enzyme activity and ammonium production are

linked with each other. Whether even both of the relations found between the ions represent the same phenomenon in two different functional states remains speculative. Additional investigations assessing the quantitative relation between ammonium ions, enzymatic activity such as transglutaminase activity and calcium ions in vitro allowing a better control of the parameters might reveal further insights into this topic. The finding of an inverse relation between both of the ions with the pH of the skin surface suggests binding to pH dependent acid groups or pH dependent complexation decreasing with decreasing pH and making the ions more available to extraction. Further studies to investigate ion binding and ion complexation in the upper skin are required.

P245 | Hair follicle chemosensation: TRPM5 signaling is required for anagen maintenance

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Transient Receptor Potential (TRP) ion channels comprise a functionally diverse group of molecules that function as cellular integrators of a plethora of physical and chemical stimuli; hence, they are considered as central players in chemosensation. Several TRP channels, such as TRPV1, TRPV3, and TRPV4, are expressed in the human hair follicle (HF), and the pharmacological activation of these TRP channels induces premature catagen regression in organ-cultured human HFs. Given that we had previously shown that olfactory receptors regulate human HF biology (Cheret et al., 2018), in the current study, we explored the role of another TRP channel, TRPM5, which is also activated by a wide range of extra and intracellular signals, ranging from gustatory agents and odorants to pheromones and acidic pH.

By immunofluorescence microscopy, human anagen scalp HF cryosections showed prominent TRPM5 expression in epithelial HF compartments, especially in the outer root sheath (ORS) keratinocytes. To probe the functional significance of TRPM5, microdissected human HFs were transfected with Accell TRPM5 siRNAs (or control scrambled oligonucleotides). Importantly, a significantly higher proportion of TRPM5-depleted HFs progressed into apoptosis-driven HF regression (catagen) than control HFs, which largely remained in anagen. This effect was associated with a significant reduction in the number of proliferating Ki-67 + cells in the anagen hair matrix and proximal bulb ORS, whereas the number of apoptotic TUNEL+ cells increased. Further, as assessed by qRT-PCR, transcript levels of the anagen-promoting factors LEF1 and IGF1 were significantly downregulated, whereas expressions of the catagen-inducing molecules TGFB2 and SFRP1 were significantly upregulated in siTRPM5-transfected HFs compared to controls.

We also manipulated TRPM5 activity using its best-known activators, 2-heptanone (2-Hep) and 2,5-dimethylpyrazine (DMP), and a selective inhibitor, triphenylphosphine oxide (TPPO). Similar to TRPM5 knockdown, inhibition of TRPM5 activity with TPPO promoted catagen and increased the hair cycle score, while TRPM5 activators 2-Hep and DMP maintained HFs in anagen at levels similar to or even greater than vehicle controls. Moreover, we also found a 10-15% increase in hair shaft elongation in both 2-Hep and DMP treated HFs compared to the control or TPPO treatments. Of further importance, TPPO treatment of HFs down-regulated transcription of anagen-promoting factors FGF7 and IGF1, whereas TRMP5 stimulation by 2-Hep reduced levels of catagen-inducing factors TGFB1/2 and increased AXIN2 expression, suggesting activation of the WNT signaling pathway.

Taken together, our findings demonstrate that TRPM5 signaling represents an important novel, therapeutically targetable control of human HF cycling that is "tonically" required for maintaining anagen and supporting HF keratinocyte proliferation in the ORS and hair matrix. This function of TRPM5 is quite unique in comparison to all other previously examined TRP ion channels, which actually inhibit hair matrix keratinocyte proliferation and induce catagen in human HFs ex vivo. This encourages one to explore, next, the nature of the unknown endogenous intrafollicular ligands, signals and types of receptor-mediated intra- and intercellular pathways that activate TRPM5 channels. Collectively, these data highlight TRPM5 as a new promising pharmacological target to modulate hair growth and treat hair disorders, such as various forms of alopecia or hirsutism.

P246 | Stratum corneum lipidomics analysis reveals altered ceramide profile in atopic dermatitis patients across body sites with correlated changes in skin microbiome

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Background: Atopic dermatitis (AD) is driven by the interplay between a dysfunctional epidermal barrier and a skewed cutaneous immune dysregulation. As part of the complex skin barrier dysfunction abnormalities in lipid organization and microbiome composition have been described. We set out to systematically investigate the composition of the stratum corneum lipidome, skin microbiome, and skin physiology parameters at three different body sites in patients with AD and healthy volunteers.

Methods: We analyzed tape strips from different body areas obtained from 10 adults with AD and 10 healthy volunteers matched for FLG mutation status for 361 skin lipid species using the Metabolon mass spectrometry platform. 16S rRNA data were available from all probands.

Results: Our study showed that the lipid composition differs significantly between body sites and between AD patients and healthy individuals. Ceramide species NS was significantly higher in AD patients compared to healthy volunteers and was also higher in AD patients with a FLG mutation compared to AD patients without a FLG mutation. The correlation analysis of skin lipid alterations with the

microbiome showed that Staphylococcus colonization in AD is positively correlated with ceramide subspecies AS, ADS, NS and NDS.
Conclusion: This is the first study to reveal site specific lipid alterations as well as correlations with the skin microbiome in AD.

P247 (OP03/03) | Modulation of inflammatory processes in the skin through regulation of macrophage activation by sulfated hyaluronan

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Hyaluronan (HA) is an essential component of the extracellular matrix and known to regulate inflammatory processes. Immunoregulatory properties of HA typically depend on its molecular size with high molecular weight HA (H-HA) being antiinflammatory while the supposed pro-inflammatory activity of low molecular weight HA (L-HA) is controversially discussed. However, therapeutic use of HHA in inflammatory conditions remains limited due to its degradation to L-HA and resultant loss of its anti-inflammatory functions. In this study, we show that artificially sulfated HA (sHA) possess anti-inflammatory properties independent of its size and downregulates inflammatory processes in skin superior to H-HA.
First, we assessed immunoregulatory effects of sHA (50 kDa) in comparison to HHA (1174 kDa), L-HA (50 kDa) on human inflammatory macrophages (inf-Ma) and analysed underlying molecular mechanisms using global quantitative proteomics, targeted assays and docking calculations. We observed significant down-regulation of inf-Ma activation by sHA that required rapid uptake of s-HA mediated by CD44, CD36 and Lox1. Induction of anti-oxidative proteins (SOD2, SOD3) and inhibition of transcription factor activation (pNFkB, pSTAT1, IRF5) resulted in reduced expression and release of pro-inflammatory cytokines (TNF, IL-12, MCP-1, IL-6) and upregulation of anti-inflammatory proteins (IL1RN, NMB). Skin ex vivo co-culture experiments showed that the reduced inf-Ma activation by sHA had an impact on the induction of pro-inflammatory signals in the skin. We, therefore, investigated the effect of sHA, H-HA and L-HA in a mouse model of acute skin inflammation. Administration of sHA reduced signs of skin inflammation (redness, scaling, epidermal thickness, immune cell infiltration) superior to H-HA. Monocytes/macrophages that we isolated from the inflamed tissue of sHA-treated skin showed reduced expression of inflammatory cytokines (IL-1b, TNF) while anti-inflammatory signals (IL-10, IL-1RA) were up-regulated. This demonstrates the capability of sHA to modulate pro-inflammatory macrophage functions in inflammatory conditions in vivo. Finally, we investigated the modulatory capacity of sHA on macrophages in pathological conditions such as obesity and diabetes. In a translational approach, we tested the impact of sHA on the healing of diabetic skin wounds using db/db mice, an established model for delayed diabetic wound healing. For this, we designed hydrogels that continuously release sHA over several days and compared those to hydrogels with no sHA release. We applied all hydrogels at day 3 post-wounding when inflammation in the wounds had already developed to test their therapeutic capacity. The sHA-releasing hydrogels improved defective tissue repair in the mice with reduced inflammation and augmented alternative macrophage activation (downregulation of IL-1b and up-regulation of IL-10, rental), increased vascularization (upregulation of VEGF, CD31) and accelerated new tissue formation (up-regulation of EGF, KI67, granulation tissue).
In sum, our data show that sHA independent of its molecular size modulates proinflammatory macrophage activity in favour of pro-resolution functions and that administration of sHA down-regulates inflammatory processes in vivo improving the outcome of different skin pathologies.

P248 | The German RECAP questionnaire: Linguistic validation and cognitive debriefing in German adults with self-reported atopic eczema and parents of affected children

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Background: Recap of atopic eczema (RECAP) is a patient-reported outcome measure (PROM) assessing eczema control. Long-term control of eczema is one of the four core outcome domains for atopic eczema trials. This instrument has been recently developed in the UK.
Objective: This study aimed to translate the English RECAP into German and test its content validity in a German population with self-reported atopic eczema.
Methods: A six-step procedure including two forward and one backward translations, two consensus decisions and an expert review was performed to obtain a German version of RECAP. We conducted semi-standardized cognitive interviews with adults with atopic eczema (n = 7) and parents having children affected by this disease (n = 5). A "think-aloud" method was used and aspects of comprehensibility, comprehensiveness and relevance according to the COnsensus-based Standards for the selection of health Measurement INstruments (COSMIN) criteria were examined. Interviews were coded using qualitative content analysis.
Results: No particular linguistic problems were encountered during forward-backward translation. Minor wording changes were made as required. The title was adjusted to a more familiar German term of the disease (which is "Neurodermitis"). The recall period was rephrased from "over the last week" to "over the last seven days" since there was a different cultural understanding of the time frame. Regarding content validity, the items of the German RECAP were

considered to be comprehensible, comprehensive and relevant for the participants and parents of affected children. The participants understood the instruction and considered the one-week recall period and the response options as appropriate.

Conclusions: A German version of RECAP that is linguistically equivalent to the original version is now available but further assessment of its measurement properties is needed.

P249 | Validation of the German Day-to-Day Impact of Vaginal Aging (DIVA) questionnaire in peri- and postmenopausal women

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Purpose: The Day-to-Day Impact of Vaginal Aging (DIVA) questionnaire is a validated patient-reported outcome measure (PROM) capturing the impacts of vaginal symptoms in postmenopausal women. We aimed to psychometrically validate the German version of the DIVA questionnaire.

Methods: Data were collected online and by paper-pencil. We ran confirmatory factor analyses to confirm the a priori four-factor structure of the DIVA. Internal consistency was calculated using Cronbach's alpha. Correlations with other outcome measures such as the Patient Health Questionnaire-4 (PHQ-4), the SF-12 SOEP (socio-economic panel) version and self-created anchor questions were calculated regarding convergent validity. Known groups regarding age, home country and disease severity were analyzed. Test-retest reliability after one week and responsiveness after four weeks were only descriptively assessed due to low sample sizes.

Results: One hundred and eighty-five postmenopausal women reporting vaginal complaints participated in the survey. The mode of administration did not influence the severity of vaginal symptoms. The four-factor structure of the DIVA could be confirmed and the model fit indicated sufficient structural validity. Furthermore, strong internal consistency in all of the DIVA domains was found. Regarding convergent validity, no hypothesis has failed completely. The results regarding known-groups validity were mostly in line with our a priori hypotheses. Descriptive evidence for sufficient test-retest reliability and responsiveness was given; however, the sample size for the assessment of those two measurement properties was low.

Conclusion: This study supports the excellent structural validity, internal consistency and construct validity of the German version of the DIVA questionnaire. It can be recommended for the assessment of the impacts of vaginal symptoms in postmenopausal women in future clinical GSM trials.

P250 | A novel strategy for preventing chemotherapy-induced permanent alopecia: PPARg signaling modulation protects from cyclophosphamide-induced bulge stem cell apoptosis and epithelial-mesenchymal transition ex vivo

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Permanent chemotherapy-induced alopecia (pCIA) has severe psychosocial repercussion on cancer patients, has increased in incidence, and remains a major unmet medical need in clinical oncology. Recently, we and other colleagues have shown that chemotherapeutic agents can deplete hair follicle epithelial stem cells (HFeSCs) through the induction of DNA damage and apoptosis. Here, we have explored the hypothesis that HFeSCs exposed to chemotherapy may also undergo pathological epithelial-mesenchymal transition (EMT), thus explaining the fibrotic phenotype of some cases of pCIA, and that pharmacological PPARγ stimulation may prevent this. To test these hypotheses, we assessed bulge K15 + HFeSCs activities after treatment of organ-cultured full-length hair follicles with the cyclophosphamide metabolite, 4HC (3 and 30 μM), which routinely induces reversible CIA in vivo, but occasionally also pCIA. Given the protective role of PPARγ signaling in human HFeSC biology that we had previously documented, we also examined whether the agonistic PPARγ modulator, NAC-GED-0507-Levo (NACGED- 0.01, 0.1 and 1 mM), protects human HFeSCs from 4HC-induced damage.

As expected, treatment of full-length HFs ex vivo with 4HC significantly induced HF cytotoxicity, as documented by increased LDH release, HF dystrophy, and premature catagen development, and apoptosis of K15 + bulge HFeSCs. Most importantly, 4HC also induced EMT in the bulge, as demonstrated by decreased expression of E-cadherin, upregulation of fibronectin expression, and vimentin+ cells. Importantly, double staining for K15 and vimentin revealed vimentin+K15 + cells in the bulge, indicating that HFeSCs are indeed undergoing EMT in HFs treated with 4HC.

Moreover, HF pre-treatment with NACGED slightly reduced 4HC-induced HF cytotoxicity and dystrophy, but - as expected - did not prevent premature catagen development. Strikingly, however, NACGED prevented 4HC-induced depletion of the bulge HFeSCs pool by suppressing their apoptosis. Finally, NACGED pre-treatment provided relative protection from EMT in bulge HFeSCs by antagonizing the 4HC-induced reduction of E-cadherin expression and the increase in vimentin+ cells within the bulge epithelium.

In summary, our data support the working hypothesis that chemotherapeutic agents induce pCIA by depleting HFeSCs via induction of both apoptosis and EMT, thus explaining why pCIA can have a scarring phenotype. We also show that HF organ culture provides an excellent surrogate ex vivo assay for the study and pharmacological manipulation of pCIA. That the toxicologically favorable, topically

applicable PPARγ modulator, NACGED, can antagonize these effects of chemotherapy in human scalp HFs ex vivo introduces a novel pharmacological strategy for preventing permanent hair loss in chemotherapy-treated cancer patients.

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