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ALLERGY

P001 | Raster-scan optoacoustic mesoscopy for precision assessment in allergy patch testing of the skin

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The differentiation between irritant and allergic skin reactions in epicutaneous patch testing is largely based on subjective clinical criteria and prone to a high intra- and interobserver variability. Dermatological imaging using Raster Scan Optoacoustic Mesoscopy (RSOM) allows three-dimensional assessment of microvascular reactions of the skin. For the first time, we investigated the potential of optoacoustic imaging to improve the precision of patch test evaluation by examining and analyzing a total of 69 test reactions and 48 healthy skin sections in 52 patients.

We identified several relevant models from the optoacoustic images and tested for their diagnostic potential. Linear discriminant analysis was applied and receiver operating characteristic (ROC) curves were calculated to identify optimal cut-off values and quantify test quality. With respect to the "number of vessel fragments" (mean 19.5 9.7 vs. 14.3 3.7; $P = 0.01$) and "ratio of low-to-high frequency signal" (mean 1.6 0.5 vs. 2.0 0.6, $P = 0.02$) we observed statistically significant differences between allergic and irritative test reactions. Regarding the differentiation of allergic and irritative test reactions, we achieved an area under the ROC curve (AUC) of 0.80 (95% CI 0.64-0.91). Using appropriate cut-off values the test method reached a sensitivity of 81% and a specificity of 63%.

The observations correlate most likely with differences in vascular physiology such as vasodilation and vessel tortuosity as well as edema. RSOM can be used for high-resolution imaging of skin allergic reactions. In addition, used as a complementary diagnostic tool, RSOM holds potential to improve precision of allergy patch testing.

P002 | Tolerance induction by prophylactic epicutaneous allergen-specific immunotherapy in a preclinical model of Hymenoptera venom-sensitized mice

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Introduction: Allergy to Hymenoptera venom (HV) is the second most common cause of IgE-mediated anaphylaxis. While subcutaneous HV-specific immunotherapy (HV-IT) shows high efficacy in inducing allergen tolerance, it is associated with potential severe systemic reactions. Utilizing an HV-allergic mouse model, we investigated whether epicutaneous HV-IT represents a safe and effective therapeutic alternative. Mice sensitized to one of the major allergens of either honeybee venom, Api m 1, or wasp venom, Ves v 5, were treated with different topically applied concentrations of the respective allergen and both clinical outcome and immunological changes were assessed.

Methods: Balb/c mice were topically treated for 4 weeks with different concentrations of Api m 1 (0, 0.25, 0.625, and 1 mg/ml) or Ves v 5 (0 and 1 mg/ml), solved in either PBS or microemulsion (ME). Subsequently, mice were sensitized intraperitoneally (i.p.) by 3 separate injections of 5 μg allergen and then challenged by an i.p. injection of 100 μg Api m 1 or 150 μg Ves v 5, respectively. Tolerance was assessed by measurement of rectal temperature. Allergen-specific IgE and IgG serum concentrations were determined by ELISA, and, T cell subsets from peripheral blood samples or isolated from lymph nodes and spleen one day after challenging were analyzed by either flow cytometry or ELISpot analysis.

Results: Mice receiving HV-IT with allergen doses solved in PBS showed a maximum rectal temperature drop of up to 5°C. In contrast, prophylactic treatment with allergen in ME, which was well-tolerated, led to a marked reduction in temperature drop and a substantially faster recovery in a dose-dependent manner. This was associated with increased production of allergen-specific IgG antibodies. Of note, no significant changes in frequencies of allergen-specific IL-5-, IL-10- and IFN-γ-secreting T cells as well as Foxp3^{+} regulatory T cells were noticed.

Conclusion: Epicutaneous HV-IT shows high efficacy preventing anaphylaxis in mice sensitized with either Api m 1 or Ves v 5. Tolerance induction was dependent on both the dose and formulation of allergen, and most likely due to the induction of allergen-specific IgG antibodies, while T cellular effects seem to be of less importance. Thus, epicutaneous IT might present a promising alternative to establish allergen tolerance in patients suffering from HV-allergy.

P003 (OP01/03) | Depletion of natural killer cells prevents allergen-induced intestinal and airway inflammation in a humanized mouse model of allergy

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Recently, we have developed a humanized mouse model of allergen-induced IgE-dependent gut and lung inflammation in PBMC-engrafted immunodeficient mice. As natural killer (NK) cells have been shown to promote allergen sensitization, type-2 immune responses and airway hyperreactivity, the aim of the present study was to investigate the impact of NK cells in this model. Therefore, NOD-scid-γc-/- mice were injected intraperitoneally with human PBMC or NK cell-depleted PBMC from highly sensitized birch or grass pollen allergic donors together with the respective allergen or with NaCl as control. After an additional allergen boost one week later, mice were challenged with the allergen rectally on day 21 and gut inflammation was monitored by video mini-endoscopy evaluating translucency, granularity, fibrin production, vascularity, and stool. Then, mice were further challenged intranasally on two subsequent days and airway inflammation was measured by invasive body plethysmography and by histology. Allergen-specific human IgE in mouse sera, if detectable after co-injection of the respective allergen, was reduced in mice being injected with NK cell-depleted PBMC compared to mice which received non-depleted PBMC. Additionally, allergen-induced IgE-dependent colitis, airway resistance and mucus-producing goblet cells were significantly inhibited in these mice. Importantly, infiltration of the colon and lung with human CD45^{+} cells was similar in all groups. These results demonstrate that allergen-specific intestinal and airway inflammation in PBMC-engrafted humanized mice can be diminished by depletion of NK cells prior to PBMC transfer, which may be of great interest for therapeutic intervention of allergic diseases.

P004 | Cutaneous mast cells are increased in patients with cholinergic urticaria

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Background: Cholinergic urticaria (CholU), a frequent form of chronic inducible urticaria, is characterized by the development of itchy wheals in response to physical exercise and passive warming. Mast cells (MCs) are the key effector cells in chronic urticaria including CholU. In patients with chronic spontaneous urticaria, MCs have been reported to be increased in lesional and non-lesional skin. Whether MCs are also increased in the skin of CholU patients is currently unknown.

Objective: To assess MC numbers in lesional and non-lesional skin of CholU patients in comparison with skin MCs numbers in healthy control subjects (HCs).

Materials and Methods: Biopsies of non-lesional and lesional skin were obtained from 13 CholU before and after pulse-controlled ergometry, respectively. MC numbers were assessed by quantitative histomorphometric analyses of Naphthol AS-Dichloroacetate (AS-D) or toluidine blue stained sections. Skin sections of HCs were used as controls.

Results: The non-lesional skin of CholU patients showed significantly higher numbers of AS-D-positive MCs as compared to the skin of HCs (48 ± 1.6 vs. 28 ± 1.2 per mm^2, $P < 0.05$). Similar results were obtained for toluidine blue-positive MCs (40 ± 1.0 vs. 24 ± 0.8 per mm^2, $P < 0.05$). Higher MC numbers in the non-lesional skin of CholU patients were observed in all dermal layers in both stainings. The biggest and most significant differences were detected in the papillary dermis and in the subcutis. The lesional skin of CholU patients showed higher numbers of MCs as compared to their non-lesional skin, but this difference was not statistically significant.

Conclusions: Our results suggest that MC cell numbers are increased in the skin of CholU patients. Further studies are needed to clarify if skin MC levels of CholU patients are linked to disease activity and if skin MC numbers decrease in response to effective treatment or spontaneous remission of CholU.

P005 | Human IL-10 DC facilitate cross-tolerance in birch pollen allergic patients with associated food allergy in vitro and in vivo

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The prevalence of type I allergies, including pollen-associated food allergies, has been increasing constantly in the last decades. 70% of birch pollen (Bet v 1 (Bet)) allergic patients suffer from at least one food allergy (e.g. hazelnut [Cor a 1] (Cor), carrot, apple), due to conserved structures of the associated allergens. Conventional specific immunotherapies can induce severe side effects and do mostly not affect the secondary food allergy. Hence, there is a high interest in the development of novel therapies.

In previous studies, we found that human tolerogenic interleukin-10-modulated dendritic cells (IL-10 DC) induce regulatory T cells (iTregs), which are anergic and have a high suppressive capacity. Here, we investigated the capacity of IL-10 DC to induce allergen-specific and cross-reactive iTregs in vitro in allergic patients with birch pollen and cross-reactive hazelnut allergies. Primary culture of $CD4^+$ T cells from allergic patients with syngenic unloaded or Bet-loaded IL-10 DC resulted in the induction of unspecific and Bet-specific anergic iTregs, respectively. However, restimulation of Bet-specific iTregs with syngenic Bet- or Cor-loaded mature DC resulted in a significantly increased or similar cell proliferation, compared to Bet-v-1-specific effector T cells. Importantly, in vitro suppressor assays revealed that Bet-specific iTreg exhibited a strong capacity to suppress syngenic Bet- or Cor-stimulated responder T cells, indicating induction of cross-tolerance by human IL-10 DC. In addition, Bet-specific iTreg inhibited birch-specific allergic inflammation in vivo in a humanized mouse model engrafted with PBMC from birch pollen allergic patients.

Recently, phase 1 clinical trials for different diseases showed that use of autologous tolerogenic DC is extremely safe with no severe side effects. We conclude from our data that IL-10 DC are highly suitable for (cross-)tolerance-inducing therapies in allergic diseases, in particular in pollen-associated food allergies.

P006 | Changes in immunoglobulin levels under real-life pollen exposure: Role of nasal IgA and IgG antibodies

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Background/Aim of study: In human, immunoglobulin A (IgA) is located on mucosal surfaces where it protects against pathogen invasion. IgG_4 antibodies have been implied as protective in allergy. To date, not much is known about the profile of total and Bet v 1-specific immunoglobulin responses to birch pollen, especially in the nose. The objectives of the study were (1) to compare local humoral immune responses, cross-sectionally (allergic rhinitis vs. healthy controls) and cross-seasonally (in vs. out of pollen season); (2) to assess the kinetics of the response under natural pollen exposure over the course of one year; (3) to link immune response profiles to the expression of nasal symptoms.

Methods: Airborne pollen concentrations were monitored daily using a Hirst-type volumetric pollen sampler. Symptoms were monitored daily by a symptom score app in two cohorts: (i) a pollen-sensitised allergic rhinitis (AR) cohort (n = 7) and (ii) a cohort of healthy, non-allergic volunteers (n = 8). Non-allergic subjects were defined both clinically as well as by serum IgE levels (ImmunoCAP ISAC 112). Immune monitoring was carried out over the full pollen season of 2015-2016 and included the determination of immunoglobulin isotypes (total and Bet v 1-specific), immunoglobulin free light chains (FLC), which mediate mast cell-dependent immune responses and proinflammatory cytokines & chemokines in nasal secretions. Data were analysed using multi-variate and time-series analyses to check for relationships between symptoms, immune mediators and airborne pollen concentrations.

Results: Directly following the birch pollen peak, Bet v 1-specific IgA and FLC levels were significantly higher in nasal secretions of non-atopic subjects as compared to AR patients. After the end of the main pollen season, nasal specific IgG_4 levels were significantly higher in AR patients than in controls. Cytokines that were found differentially expressed in nasal secretions were IL-33, eotaxin-2 and IL-1β. Total IgA levels, as well as Bet v 1-specific ones and FLCs were significantly increased inside the pollen season only in non-atopic subjects, when compared to out of season. In contrast, in AR patients, nasal total IgM, IgG_1, IgG_3 and IgG_4 levels were decreased.

Most strikingly, not only AR patients, but also non-allergic subjects exhibited nasal symptoms in the birch pollen season, which in all cases correlated with airborne birch pollen concentrations. Those non-allergic subjects who showed seasonal symptoms all lacked expression of nasal Bet v 1-specific IgG[4].

Conclusion: Nasal IgA and IgG antibodies could play an important role in the control of the local allergic immune response. Nasal non-allergic type Ig signatures are characterised by specific IgA/IgG4 antibodies. Airborne birch pollen does not only influence allergic patients but also non-allergic subjects.

P007 | ICOS drives TH22 involvement in atopic dermatitis

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Atopic dermatitis (AD) is characterized by excess TH22 activation. Inducible T cell co-stimulator (ICOS) is crucial for T-cell activation and differentiation. However, the role of ICOS in AD and its effect on TH22 T cells remain unclear. To assess the expression and effects of ICOS on TH22 cells and to characterize its role and relevance in AD, we quantified TH22 cells and IL-22 levels, as well as ICOS and ICOSL expression in AD patients and healthy controls (HCs). Then, we assessed the proliferation and the production of the TH22 chemokines CCR4 and CCR10 and of IL-22 by ICOSL-stimulated AD PBMCs as well as their effects on keratinocyte filaggrin production. Finally, we explored the link between ICOS-expressing TH22 cells and disease activity and IgE levels in AD patients. In AD patients, circulating TH22 cells, serum levels of IL-22, and IL-22-positive cells in lesional skin were all markedly increased. AD patients also showed higher levels of ICOS-expressing TH22 cells as well as ICOSL-expressing CD19^{+} B cells and CD14^{+} monocytes as compared to HCs. ICOSL increased the proliferation and the expression of CCR4 and CCR10, and of IL-22 in AD PBMCs. ICOSL-treatment also significantly increased the down-regulation of filaggrin expression by keratinocytes co-cultured with PBMCs from AD patients. Finally, blood levels of ICOS^{+} TH22 cells and ICOSL^{+} B cells in AD patients were correlated with disease activity as assessed by SCORAD and with total IgE levels. Our findings demonstrate that ICOS/ICOSL expression and effects are linked to TH22 skewing and the pathogenesis of AD, which suggests ICOSL and ICOS as well as TH22 cells and IL-22 as new and promising therapeutic targets.

P008 | Crosstalk between allergic and autoimmune reactions: clinically relevant type I diabetes prevented the development of allergic contact dermatitis in mice

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Among allergic skin disorders, the allergic contact dermatitis (ACD) is one of the most frequent occupational skin diseases, and leads to considerable impairment of live quality. ACD is a T cell-mediated inflammation of the skin which occurs after reexposure to the offending hapten. Currently, preventive and therapeutic strategies are avoidance of the allergen or treatment of clinical symptoms, respectively. Epidemiological studies noted a lower prevalence of ACD in individuals with autoimmune diseases like type I diabetes, psoriasis or rheumatoid arthritis as compared to healthy individuals.

However, the clinical relevance of this inverse correlation and the underlying immune mechanisms have not been evaluated so far. In our study, we wanted to confirm these results by analyzing the induction of contact hypersensitivity (CHS) reaction, which resembles the ACD in humans, in non-obese diabetes (NOD) mice spontaneously developing an autoimmune insulin-dependent diabetes mellitus (IDDM). Female NOD mice were considered diabetic when blood glucose levels showed two consecutive readings above 250 mg/dl. In order to induce the CHS reaction, a CD8^{+} Tc1-mediated cutaneous inflammation, the mice were epicutaneously sensitized with a contact sensitizer (e.g. the hapten TNCB), followed by an application of the hapten onto the ear to elicit the CHS reaction.

We compared the impact of the diabetic phenotype on the development of the CHS reaction in diabetic vs. non-diabetic NOD mice and also in non-obese resistant (NOR) animals as a further non-diabetic control strain. The experiments revealed that the existence of a clinically apparent diabetes in NOD mice protected from CHS development as demonstrated by a significantly reduced skin inflammation (diminished ear swelling, reduced cutaneous inflammatory infiltrate) as compared to non-diabetic NOD and NOR mice. In contrast, we did not observe an impaired hapten-specific CD8^{+} Tc1 cell response (T cell proliferation, Tc1-cytokine (IFN-γ, IL-2) production) in skin-draining lymph nodes or the spleen of diabetic mice. However, increased levels of IL-10 were detected in diabetic mice with reduced CHS reactions as compared to non-diabetic NOD and NOR animals with an unaffected allergic skin inflammation. Blocking of the IL-10 effect by injection an anti-IL-10- receptor antibody during CHS induction completely restored the development of the cutaneous allergic inflammation in diabetic animals, indicating a functional role of IL-10 for the impaired CHS in diabetic mice. Further experiments using PrimeFlow RNA assay for detecting RNA targets by flow cytometry gave further insights to the IL-10 producing cell type that prevents

the CHS in diabetic mice and revealed that CD4^{+}CD25^{+}Foxp3^{+} regulatory T cells show an elevated release of IL-10 in diabetic mice in comparison with non-diabetic mice.

In summary, our data indicate that the manifestation of an autoimmune disease like diabetes mellitus type I circumvents the development of CHS in mice and, therefore, confirmed the epidemiological data of a reduced susceptibility for ACD in patients suffering from autoimmune diseases. The identification of a novel link between the development of allergic and autoimmune diseases may result in new preventive strategies for inflammatory disorders.

P009 | Myeloid cell-specific expression of PAR2 and coagulation factors in patients with allergic contact dermatitis

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The allergic contact dermatitis (ACD) is a common occupational skin disease with a prevalence of 15 to 20% in Germany leading to distress of the allergic patients and high socio-economic costs. Current therapeutic options with topical glucocorticoids and calcineurin-inhibitors often fail to induce a satisfying improvement of the skin lesions. Despite progress in elucidating the pathogenesis of the ACD, the development of new targets for specific and effective therapeutic strategies is still at initial stages. Protease activated receptor (PAR) 2 is a G-Protein-coupled receptor that is activated by coagulation proteases, including the tissue factor (TF) ligands activated coagulation factors VII (FVIIa) and X (FXa) expressed by myeloid cells. In a murine model of trinitrochlorbenzene (TNCB)-induced contact hypersensitivity (CHS), mimicking the allergic contact dermatitis (ACD) in humans, we found that TF-PAR2 signaling in myeloid cells plays a crucial role in mediating allergic skin inflammation. However, little is known about cell-specific TF-PAR2 signaling in ACD. The aim of this translational pilot study was to analyze the expression of PAR2 and activating coagulation proteases in cutaneous lesions of ACD compared to non-lesional skin. Therefore, 15 patients with previously diagnosed contact allergies (nickel, colophony, p-phenylenediamine, chloro-methylisothiazolinone, p-toluenediamine) were recruited at the Allergy Unit of the Department of Dermatology at the University Medical Center Mainz. After informed consent of the patients, contact allergy was elicited using a patch test on the back of the patients with Vaseline as control. The clinical cutaneous reactions were read out at 48 and 72 hours after challenge. The plaster was removed at 48 hours after the challenge. At 72 hours after the challenge, a biopsy (diameter 6 mm) from the inflamed skin area and the control

site, respectively, was taken. The formation of a regular inflammatory reaction was verified by histological hematoxylin and eosin staining. Using immunofluorescent staining and widefield fluorescence microscopy, we analyzed the expression of PAR2 in CD45^{+}, CD11b^{+}, CD68^{+}, CD14^{+} and CD11c^{+} myeloid immune cells in a defined area of the epidermis and dermis, respectively.

We hope that our study contributes to the identification of novel therapeutics that may improve the treatment options for patients with cutaneous inflammatory disorders.

P010 | Nasal microbiome under natural pollen exposure conditions: a time series analysis in allergic rhinitis patients vs. health subjects

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Background: The incidence of allergic airway diseases has been increasing over the last decades. This has been partly attributed to higher or longer exposure to airborne pollen, which is a major cause for respiratory symptoms in sensitized patients. Imbalances and changes in mucosal microbiota parallel allergic disease outcome, with the cause effect relationship being undefined. We have recently shown that pollen harbor a distinctive microbiome. Since pollen-derived mediators and adjuvant factors, as well as pollen-associated microbiota, challenge the nasal epithelium, it stands to reason that pollen-associated microbiota also play a role in the pathophysiology of allergic rhinitis.

Our aim was to describe the changes in the nasal microbiome of healthy and allergic rhinitis (AR) patients, in comparison with pollen microbiome, as well as identify key microbes, both host- and pollen-derived, that are associated with AR under natural pollen exposure.

Methods: Over the course of one year, monthly or bi-weekly (within birch pollen season) swabs of the middle nasal meatus of eight AR patients and eight non-atopic volunteers were taken. From those, 16S rRNA hypervariable regions V1-V3 and V4 were sequenced using the Illumina MiSeq sequencing platform to study the microbial composition. During the birch pollen season, we additionally collected pollen samples from 60 birch trees from characterized sampling sites in Augsburg, Germany, for 16S sequencing of pollen-associated microbes.

Results: We observed that the individual nasal microbiome of non-allergic subjects did not change during or outside of the birch pollen season, whereas the alpha diversity of samples from AR patients decreased during the pollen season. Differences were also observed in a cross-sectional analysis of AR patients and healthy participants within and outside of the pollen season. Corynebacterium accolens was found to be more prevalent in non-allergics during the pollen season, whereas other Corynebacterium species were more

commonly found in allergics. Interestingly, Alcaligenes faecalis was found to be present in both cohorts but decreased during the pollen season.

Conclusion and outlook: In healthy subjects, the overall nasal microbiome seems to be stable independent of pollen exposure, when compared against the microbial diversity of sensitized patients which decreases during the pollen season. Comparison and characterization of nasal and pollen specific microbiomes in a multiomics approach could contribute to fathoming macro- and micro-environmental interactions in allergy.

P011 | FXII-inhibiting effects reduce due to cleavage of Mast cell chymase to C1 inhibitor.

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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 hallmark features of chronic inflammation is the release of preformed mediators including proteases such as tryptase and chymase from activated mast cells (MCs). MC proteases are known to cleave a large array of proteins, and contribute to the development of recurrent angioedema. In some patients, occurrence of angioedema has been proposed to be due to the cleavage of C1-inhibitor. Here, we assessed the effects of MCs proteases 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 reduced in size as shown by SDS-PAGE and coomassie staining. Using purified tryptase and chymase, we confirmed that chymase cleaves C1-Inhibitor. Chymase-cleaved C1-inhibitor showed reduced inhibition of FXIIa and kallikrein as shown by a chromogenic activity assay. These findings demonstrate that C1-Inhibitor is cleaved and that its inhibitory effects on FXIIa and kallikrein are reduced by the MC protease chymase. The release of chymase by activated mast cell may contribute to kallikrein-activation, bradykinin release, and angioedema formation in HAE patients via the cleavage of C1-inhibitor.

P012 | C57BL/6J and C57BL/6N mice show equivalent T helper cell profiles and strength of anaphylaxis in a murine food allergy model

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The inbred mouse strains C57BL/6J and C57BL/6N are widely used in various fields of murine immunological research. Although both strains have been derived from the same parent line, due to separation in the 1950s genetic alterations have developed independently and a large genomic screen identified numerous SNPs distinguishing these two strains. Significantly reduced ear swelling reaction in C57BL/6N animals compared to C57BL/6J mice in the cutaneous delayed-type hypersensitivity reaction (DTH) to dinitrofluorobenzene has been reported demonstrating functional differences in this disease model between these two mouse strains. Moreover, it has been shown that C57BL/6 strains purchased from different vendors exhibit varying microbiota composition in the gut leading to altered intestinal immune responses. We aimed to investigate the intestinal immune response in an established murine food allergy model in C57BL/6J and C57BL/6N mice. All animals were purchased from the same vendor and were subsequently housed in the same animal facility under special pathogen free conditions in one isolator. Mice were orally sensitized in parallel with the model antigen ovalbumin (OVA) two times per week for three consecutive weeks. After challenge with OVA, rectal temperature was recorded to quantify anaphylaxis. In addition, mesenterial lymph nodes and spleen as well as serum and feces were harvested. C57BL/6J and C57BL/6N mice displayed no significant differences in regard to strength of anaphylaxis as shown by a closely paralleled temperature decline. Analyzing T helper cell polarization in secondary lymphatic organs and serum IgE levels, C57BL/6J and C57BL/6N mice showed equivalent production of Th1 and Th2 hallmark cytokines as well as comparable IgE levels.

Taken together we could demonstrate that in contrast to the reported discrepancies in the immune response between C57BL/6J and C57BL/6N mice in the Th1-dominated cutaneous DTHR, in a Th2-driven food allergy model no differences in regard to strength of anaphylaxis and adaptive immunity could be observed.

P013 | Screening of skin commensals for potential therapeutical use in atopic dermatitis

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Atopic dermatitis (AD) is a chronic inflammatory skin disorder that affects up to 20% of children and 3% of adults in western countries. A defective skin barrier as well as an immune dysbalance plays crucial roles in this pathology, leading to Th2 biased immune responses. AD lesions show an increased susceptibility to cutaneous infections, especially with *S. aureus* during flares, stressing its central role in disease exacerbation. In contrast, skin commensals appear to play a positive role in maintaining microbial and immune homeostasis. For instance, *S. epidermidis*, an abundant coagulase-negative Staphylococcus species on healthy human skin, has been shown to reduce inflammation after injury and to promote antimicrobial peptides production. Therefore, we aimed at identifying beneficial skin commensal bacteria, with the ability to correct microbial dysbiosis

in atopic dermatitis that might be used as skin probiotics in prevention and therapy of AD. In an initial screening 25 skin commensals have been isolated from the arm fossa of healthy volunteers, most of them Staphylococci (*S. epidermidis*, *S. hominis*, *S. chromogenes*, *S. warneri*). Obtained strains were identified biochemically using API® Staph test strips or by mass spectroscopy. These candidate strains were then screened for their ability to inhibit growth of different *S. aureus* strains isolated from severe eczematous lesions. Different methods have been used to evaluate the inhibitory effects of living bacteria, dead bacteria or culture supernatants, such as agar well diffusion assays and growth kinetics monitoring. Supernatants showing inhibitory activity were furthermore analysed to determine the approximate molecular weight and further chemical properties of the inhibitory agent. Six candidate strains selected based on their inhibitory potential were also tested for their immune-modulatory activity on human PBMCs, human moDCs and mouse bone marrow derived DCs by flow cytometric analysis of maturation markers (CD83, HLA-DR) and quantification of cytokine production (IL10, IL12, IL13, TNFα).

P014 (OP05/03) | Skin barrier-disrupted FlgHrnr-deficient mice reveal strain- and sensitization-dependent susceptibility to the progression of the atopic march

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The risk to develop atopic diseases is strongly influenced by both genetic and environmental factors. Skin barrier dysfunction (e.g. by the loss of filaggrin (Flg) and hornerin (Hrnr)) is one of the most prominent predisposing factors for the development of allergic skin diseases, but also for posterior development of asthma (atopic march).

Here, we compare the progression of the atopic march using different antigen sensitization protocols in skin barrier-disrupted FlgHrnr-deficient (FlgHrnr^{-/-}) mice on different genetic background, namely C57BL/6 and Balb/c.

Our experimental atopic march model combines the induction of an AD-like phenotype by the topical application of MC903 (calcipotriol) together with allergen sensitization, either systemically by the application of OVA/Alum or epicutaneously by topical application of OVA/DBP (Dibutyl phthalate), which is followed by an allergen airway challenge with OVA-aerosol.

In general, skin barrier-disrupted FlgHrnr^{-/-} compared to wild-type mice show higher susceptibility to the induction of acute AD-like dermatitis, antigen-sensitization, and the proceeding of the atopic march, independent of treatment protocol and mouse strain used. In detail, MC903-treated FlgHrnr^{-/-} mice show worsened signs of AD-like dermatitis (stronger ear swelling response), a facilitated antigen-sensitization (higher levels of Th2-like immunoglobulins),

and exacerbated asthma-like features (increased BAL cell counts, especially eosinophils).

Comparing the routes of sensitization, systemic sensitization leads to a highly increased airway inflammation in C57BL/6, whereas these mice have much lower BAL cell count after epicutaneous sensitization in line with reduced IgE and OVA-IgG1 levels in this treatment regime. Controversially, Balb/c mice display similar airway inflammation using either sensitization protocols. Interestingly, compared to C57BL/6 mice, absolute BAL cell count in Balb/c mice is similar after epicutaneous sensitization, but much lower after systemic sensitization. However, IgE levels are comparable in both strains using the systemic protocol, but significantly higher in Balb/c mice during the epicutaneous treatment protocol.

Together, Th1-prone C57BL/6 mice are superior in inducing asthma-like feature compared to Th2-prone Balb/c mice, especially after systemic sensitization, whereas antigen sensitization is more prominent in Balb/c mice, even when done epicutaneously. This indicates that allergic immune responses are differentially regulated not only by the route of sensitization, but also by the genetic background and might hint to IgE-independent mechanisms for the induction of asthma-like features. This nicely reflects the situation seen in humans, where clinical manifestations vary among individuals with different atopic status.

Most importantly, skin barrier disruption in FlgHrnr-/- mice is the most robust factor for exaggerated progression of the atopic march, irrespective of the treatment scheme. However, further mechanistic insights into allergen sensitization in barrier-disrupted skin is required to better understand atopic disease progression possibly leading to innovative approaches preventing further disease development.

P015 | Skin microbiome diversity and transcriptome profiling in patients with atopic dermatitis

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Atopic dermatitis (AD) is the most common childhood inflammatory skin disease (up to 3% prevalence in adults and up to 15% in children), with a large unmet need for safer and more effective treatments. Early phase AD is characterized by a Th2 skewed immune response, but recent research shows additional Th1/Th17/Th22 immune profiles in acute AD exacerbations, together with epidermal hyperplasia and drastic decreases of epidermal differentiation markers. AD patients experience frequent cutaneous infections and

Staphylococcus aureus is commonly cultured from lesional AD skin. Several studies have investigated the microbial dysbiosis of inflamed AD skin lesions; however, detailed knowledge of host–microbiome interactions is still missing. The present study analysed skin microbiome diversity and corresponding host response in 7 adult AD patients and 8 healthy volunteers. Lesional, non-lesional and healthy skin microbiome samples have been collected by swabbing the antecubital area and then analyzed by 16S amplicon sequencing. Sampling started with acute AD flares and continued with follow-up sampling after one and two weeks of topical corticosteroid treatment. Additionally, nasal microbiomes were harvested and analyzed since the nasal mucosa may constitute a reservoir for skin microbes. Samples have been sequenced on an Illumina MiSeq platform and data were analyzed using the Rhea pipeline to estimate alpha and beta diversity as well as taxonomic binning. The host response was explored by RNA-seq of skin biopsies harvested from the antecubital areas. Differentially expressed genes were analyzed with a particular focus on chemical, physical and immunological skin barriers. In addition, various functional tests have been performed to investigate skin physiological parameters, including trans-epidermal water loss, skin pH and skin elasticity. Furthermore, sweat chloride levels have been investigated. Together, the acquired data will enable us to perform multifactorial analyses of host-microbe interactions in AD.

P016 | Double-edged role of IL-33 in human skin mast cells: reduced degranulation, strengthened proliferation and heightened histamine synthesis through p38 activation

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Background: Mast cells (MCs), the principal effector cells of IgE-mediated allergy, mature and are maintained by MC-supportive niches within tissues, of which IL-33 is one component. IL-33 as a member of IL-1 family, is released by epithelial cells as alarmin upon stress, e.g. after allergen contact. By virtue of their pronounced IL-33 receptor expression, MC-functions are important targets of IL-33 action, but details especially in chronic inflammation are poorly understood.

Methods: MCs were isolated from human skin tissue, and purified to homogeneity (>98%). To mimic chronic inflammatory milieu of skin disorders, MCs were cultured for 5 weeks either in stem cell factor (SCF) alone (100 ng/ml) or in SCF plus IL-33 (20 ng/ml) to imitate chronic exposure like in chronic inflammation. Gene expression was quantified by RT-qPCR, and histamine by an autoanalyzer-based technique. Immunoblot and flow-cytometry were utilized to discern signaling cascades. Kinase inhibitors and RNA-interference (RNAi) were employed to pinpoint the

pathway(s) underlying HDC/histamine overexpression following IL-33.

Results: Chronic exposure to IL-33 strengthened skin MC numbers through accelerated cell-cycle progression and restriction of cell death. Conversely, IL-33 reduced degranulation and dampened FcεRI expression, potentially as a feedback response to chronic "alarmin" exposure. Interestingly, the negative impact on histamine release was counterbalanced by amplified histamine production. We next explored the mechanism behind the heightened histamine accumulation. IL-33 induced swift phosphorylation of p38 and JNK (but not of ERK1/2 or AKT), and stimulated histidine decarboxylase expression in skin MCs. A combined strategy of pharmacological inhibition and kinase elimination by Accell®-facilitated RNAi revealed that histamine upregulation depends on p38, but not on JNK activity. To our knowledge, p38 is the first signaling intermediate rigorously linked to histamine biosynthesis.

Conclusion: Collectively, IL-33 exerts multifaceted effects on cutaneous MCs, strengthening their number and re-shaping functionality. Potentially the IL-33-skin MC axis leads to a balanced and lasting response, highlighting MCs as critical players in chronic skin inflammation.

P017 | Skin rashes induced by vemurafenib are caused by aryl hydrocarbon receptor antagonism

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Introduction: In recent years, BRAF protein kinase inhibitor vemurafenib has been successfully established for the treatment of advanced malignant melanoma. Despite its superior efficacy, the use of vemurafenib is limited by frequent inflammatory cutaneous adverse events that affect patients' quality of life and may lead to dose reduction or even cessation of anti-tumor therapy. To date, the molecular and cellular mechanisms of vemurafenib-induced rashes have remained largely elusive.

Material and Methods: To characterize vemurafenib-induced rashes we performed immunohistochemical and gene expression analysis of lesional skin sections of vemurafenib-treated patients. Lymphocyte activation tests (LAT) were conducted to detect vemurafenib-specific T cells. Furthermore, we stimulated T cells, keratinocytes and skin explants from healthy donors with different concentrations of vemurafenib and evaluated the gene expression profile on mRNA

and protein levels. Finally, the aryl hydrocarbon receptor antagonism was investigated using different cell-free protein interaction assays.

Results: Vemurafenib-induced skin rashes are characterized by a massive infiltration and clustering of T cells (CD4^{+} and CD8^{+}), CD68^{+} macrophages, mast cells as well as intraepidermal CD1a^{+} Langerhans cells, whereas eosinophils were not detected. We here demonstrate that vemurafenib inhibits the downstream signaling of the canonical pathway of aryl hydrocarbon receptor (AhR) in vitro, thereby inducing the expression of proinflammatory cytokines (e.g. TNF, IL1B) and chemokines (e.g. CCL5). In line with these results we observed an impaired expression of AhR regulated genes (e.g. CYP1A1) and an upregulation of the corresponding proinflammatory genes in vivo. Moreover, results of lymphocyte activation tests showed the absence of drug-specific T cells in respective patients.

Conclusions: Taken together, we obtained no hint of an underlying sensitization against vemurafenib but found evidence suggesting that vemurafenib enhances proinflammatory responses by inhibition of AhR signaling. Our findings contribute to our understanding of the central role of the AhR in skin inflammation and may point towards a potential role for topical AhR agonists in supportive cancer care.

P018 | FPR3 binding by processed lipocalin allergens mediates IL-12 downregulation and TH2 polarisation

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Background: Compared with TH1 immune responses to pathogenic viruses and bacteria, the induction mechanism of TH2 mediated allergic immune responses remains less understood. It is widely accepted that the absence or low concentration of interleukin (IL)-12 during antigen presentation to Th cells is a prime reason for the commitment of an immune response towards TH2. In an in vitro system, we verified polarisation of naïve CD4^{+} T cells towards TH2 by dendritic cells (DC) treated with the major dog allergen Can f 1 and towards TH1 by dendritic cells treated with the homologous but non-allergenic human tear lipocalin Lcn-1. Microarray data gained by comparison of DC treated with Can f 1 and Lcn-1 showed a significant difference in the expression of Formyl Peptide Receptor 3 (FPR3), which has been shown to inhibit LPS-induced IL-12 production in DC upon activation. We therefore hypothesized that binding of allergenic lipocalins or their processing products to FPR3 could be a mechanism for the induction of allergenic immune responses.

Methods: To test the hypothesis, we first examined whether lipocalins and FPR3 colocalize within the cells. In calcium mobilisation assays of FPR3 transfected HEK293 cells we investigated

whether allergenic Can f 1 and non-allergenic Lcn-1 or their peptides induce receptor activation of FPR3. Further, we performed gene silencing of FPR3 in DC prior to treatment with allergenic lipocalins and non-allergenic Lcn-1 monitoring IL-12 expression. Finally, FPR3-silenced DC were co-cultured with naïve CD4^{+}-T cells and supernatants were analysed for IFNγ and IL-13 contents.

Results: We demonstrate that FPR3 and lipocalins co-localize within the same vesicles. In calcium mobilisation assays we observed activation of FPR3 by Can f 1 N-terminal peptide and cathepsin S-digested Can f 1 and other allergenic lipocalins, but not with the non-allergenic homologous Lcn-1 peptide or its digestion products. Besides, silencing of FPR3 in human DC leads to higher IL-12 expression when treated with allergens. Moreover, naïve CD4^{+} T cells co-cultured with FPR3 silenced allergen treated DC exhibited a reduction in IL-13 expression while IFNγ production was unaltered.

Conclusion: Taken together we show that digestion products of allergenic lipocalins but not their non-allergenic homologues activate FPR3 and that diminished expression of FPR3 in human DC is accompanied by a higher IL-12 production and hampered TH2 polarisation. Thus, we here describe for the first time a molecular mechanism to induce allergic immune responses by at least 5 allergenic members of the lipocalin family.

P019 (OP04/04) | IL-10 is the driving force for successful tolerance induction in specific immunotherapy

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Background: Wasp sting-induced anaphylaxis is a paradigm of an IgE-mediated allergy. Allergen-specific immunotherapy (SIT) with purified venom preparations is highly successful reaching clinical efficacy of up to 95%. Tolerance induction during the early phase of SIT is believed to be mainly mediated by interleukin (IL)-10 secreted by regulatory CD4^{+}Foxp3^{+} T cells (Treg). However, in humans the investigation of cellular and molecular mechanisms of SIT is hampered by ethical and methodology constrains.

Objective: To study the functional role of IL-10 and Treg during SIT in a recently established mouse model of wasp venom allergy.

Materials and Methods: BALB/c mice were sensitized to wasp venom followed by SIT. The efficacy of SIT was investigated by a standardized wasp venom challenge; the reaction to the injected venom was measured by an anaphylaxis scoring system. To determine the functional role of IL-10 in SIT, a neutralizing anti-IL-10-receptor (IL-10R) antibody was repeatedly injected intraperitoneally. Moreover, we explored the impact of Treg function on the outcome of SIT in DEREG-BALB/c mice, in which Tregs were depleted by diphtheria

toxin injection. IL-10-producing cell populations within the spleen were monitored by intracellular staining followed by flow cytometry analysis.

Results: In analogy to the efficiency of SIT in wasp venom-allergic humans, SIT protected mice from venom-induced anaphylaxis. Successful tolerance induction was hampered by continuous blockade of IL-10 function via administration of a neutralizing anti-IL-10R antibody during SIT. Surprisingly, Treg depletion followed by immunotherapy did not show a disastrous effect on the outcome of SIT. Under these conditions, elimination of Treg was accompanied by a massive increase in IL-10-producing conventional CD4^{+}Foxp3^{−} T cells indicating that CD4^{+}Foxp3^{−} T cells might compensate for a rapid loss of Treg function. Again, this effect was abrogated by continuous IL-10R blockade, stressing the outstanding impact of IL-10 on tolerance induction by SIT.

Conclusions: IL-10 is the driving force for tolerance induction to the causative allergen in SIT. Loss of Treg function during the induction phase of SIT is at least in part compensated by IL-10-producing conventional CD4^{+} T cells resulting in protection from IgE-mediated anaphylaxis.

P020 | DRESS syndrome in a patient with metastatic malignant melanoma induced by targeted therapy with the BRAF inhibitors vemurafenib and dabrafenib

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Introduction: Mutations in the proto-oncogene BRAF have been found in approximately 50% of skin melanomas. Particularly, the increased activity of BRAF V600E is associated with an impaired regulation of cell growth, differentiation, and survival. BRAF inhibitors show high efficacy in suppressing tumor growth in patients with metastatic melanoma (MM) resulting in marked clinical improvement of the disease. However, adverse events including allergic reactions to BRAF inhibitors have been reported during the course of treatment. Here, we compared the drug-specific memory T cell responses to BRAF and MEK inhibitors of a MM-patient experiencing a BRAF inhibitor-induced severe drug reaction with eosinophilia and systemic symptoms (DRESS) with MM-patients tolerating a combination therapy with either vemurafenib and cobimetinib or dabrafenib and trametinib.

Methods: Peripheral blood mononuclear cells (PBMC) were isolated from i) a MM patient eight months after suffering from DRESS with generalized exanthema, erosive mucositis, facial edema, fever up to 38.5°C, eosinophilia, atypical lymphocytes, thrombocytopenia and elevated levels of transaminases while treated with vemurafenib, who in addition showed a flare-up when another BRAF inhibitor, dabrafenib, was applied due to an urgent treatment need, ii) MM-patients treated with BRAF and MEK inhibitors without an allergic drug reaction (n = 6), and iii) healthy control subjects (n = 6). All subjects lacked a history for allergy to sulfonamides. T cell responses to different concentrations of vemurafenib or dabrafenib and of the MEK inhibitors cobimetinib or trametinib were analyzed by ELISPOT analysis. Since BRAF inhibitors belong to the group of sulfonamides, we also investigated cotrimoxazole as a control antigen to check for cross-reactivity between the BRAF inhibitors and sulfonamides, as described elsewhere.

Results: Increased frequencies of granzyme B-positive T cells were observed after incubation of PBMC with either vemurafenib or dabrafenib in the MM-patient who experienced DRESS to these BRAF inhibitors. Notably, enhanced numbers of granzyme B-producing T cells were only detected after stimulation with low drug concentrations tested. Determining IFN-γ-, IL-5- or IL-10-secreting T cell subsets, and analyzing T cell populations after incubation with the MEK inhibitors cobimetinib or trametinib, marginal or no alterations in T cell frequencies were noticed. In addition, T cell reactions were also inducible by sulfonamides, which have been reported as BRAF inhibitor cross-reacting drugs.

Conclusion: BRAF inhibitor can cause severe drug reactions like DRESS, which are characterized by increased numbers of drug-specific, granzyme B-secreting T cells but only marginal or no alterations in other inflammatory T cell subsets. Further studies are needed to assess if this type of T cell response is specific for DRESS by BRAF inhibitors and can be utilized as a predictive biomarker for a risk of severe reactions to this group of drugs.

P021 | Murine mast cells express the anaphylatoxin receptor C3aR

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Background: Mast cells are tissue-resident cells, which exert crucial functions in defense against pathogens. They also play important roles in other innate as well as adaptive immune responses and in diseases such as allergies, autoimmune diseases and tumors. The complement system belongs to the innate immune system and consists of over thirty different components circulating in the blood. Specific cleavage products of the complement system, the anaphylatoxins C3a and C5a, serve to attract immune cells to sites of pathogen invasion. However, the interplay between mast cells and anaphylatoxins is only partially understood.

Methods: In order to investigate the expression of C3a receptor (C3aR) on different types of murine mast cells, we analyzed bone marrow-derived mast cells (BMMC) and peritoneal mast cells (PCMC)

from C57BL/6 and BALB/c mice. Development and maturation of the different mast cell types was assessed by flow cytometry and histochemical staining of cytospins. Expression of C3aR was investigated at different time points during culture using flow cytometry. C3aR expression was also explored in stimulated versus unstimulated cells. Finally, expression of C3aR was studied in vivo using skin sections of inflammatory murine skin, obtained upon induction of a bullous pemphigoid model, compared to normal skin using indirect immunofluorescence staining.

Results: We found that BMMC show a more immature phenotype compared to PCMC with respect to expression of the mast cell-specific markers FcεRIα and CD117. Upon culture with IL-3 and SCF, compared to IL-3 alone, BMMC developed a more mature phenotype. BMMC and PCMC of the two mouse strains all expressed C3aR. Expression of C3aR was already observed in early BMMC cultures. During culture over several weeks, the expression of C3aR increased along with the higher maturation status. Upon stimulation, there was no regulation of the expression of C3aR on BMMC. Skin sections of inflammatory and normal murine skin showed a comparable expression of C3aR on mast cells. However, we noted an increase of the total C3aR expression in inflammatory skin, indicating that other cells besides mast cells up-regulate C3aR during inflammation.

Conclusions: Taken together, we report on expression of C3aR on different types of murine mast cells. Our results serve to unravel the pathogenesis of inflammatory diseases and suggest exploring C3aR as therapeutic target in mast cell-associated disorders.

CELLULAR BIOLOGY

P022 | Insulin activates ADAMs via phosphatidylserine exposure

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"A disintegrin and metalloproteinases" (ADAMs) are able to shed and release other transmembrane proteins. The most prominent members are ADAM10 and ADAM17 which release, e.g., TNFα, TNF receptor or adhesion molecules like cadherins or fractalkine. ADAM10 is the major sheddase of epithelial cadherin and impaired regulation of protease activity leads to impaired cohesion of skin keratinocytes and wound healing complications. In this context, the proteases are involved in several inflammatory diseases such as eczematous dermatitis or psoriasis. Therefore, understanding the regulation of ADAM10 and ADAM17 activity is essential. In diabetic patients, altered skin wound healing is a common cause of morbidity and a higher risk of the development of psoriasis and cancer was described. However, the molecular mechanisms whereby diabetes alters these processes have not been elucidated. In this study, we investigated the role of insulin on ADAM shedding activity in the context of insulin resistance and liver cancer.

Here, we demonstrate that insulin stimulates ADAM10 and ADAM17 shedding activity via phosphatidylserine exposure in various cell types. At the outer cell membrane, negatively charged phosphatidylserine interacts with positively charged domains of ADAM proteases and enables the proteases to release different substrates such as TNF receptor 1 or fractalkine. We found that the underlying mechanism is mediated by the metabolic insulin signal pathway including PI3-kinase activation and AKT phosphorylation.

In insulin resistant cells, insulin-mediated phosphatidylserine-exposure and ADAM activation is inhibited which would suggest an increased sensitivity to apoptotic signals in insulin resistant cells of diabetic patients. This process might contribute to wound healing complications such as the diabetic foot syndrome.

On the other hand, in cancer cells insulin receptors and signaling are found to be elevated. In liver cells, we could show that activation of ADAM10 leads to an increased release of the adhesion molecule fractalkine and subsequently to a diminished monocyte adhesion. This process might contribute to an escape of the liver cancer cell from immune response. Overall, our presented data further elucidate the activation of the vitally important proteases ADAM10 and ADAM17.

P023 | The role of the plasma membrane for ADAM10-mediated prion protein release

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ADAM10, a prominent member of the "a disintegrin and metalloproteinase family", is responsible for ectodomain-shedding of many membrane-bound proteins. This proteolysis represents an essential mechanism in the regulation of physiological and pathophysiological cellular processes. The protease shows a very prominent expression in all epithelial tissues, especially in the epidermis. Moreover, ADAM10 plays a critical role during neurogenesis, in Notch signaling, and in the regulation of neuronal cell adhesion. Prion diseases such as Creutzfeldt-Jakob disease (CJD) are fatal, transmissible spongiform encephalopathies that affect humans and other animals. Even though the responsible prion protein is mainly expressed in cells of the neuronal system, the skin of CJD patients was found to contain both prion seeding activity and infectivity. Analysing skin samples is under consideration in diagnostic investigation. ADAM10 was described being the only protease responsible for the shedding of PrPC. However, the mechanism regulating this shedding process remains unclear. Recently, it was found that the plasma membrane fulfills important functions in controlling ADAM activity. It was proposed that the exposure of the negatively charged phospholipid

phosphatidylserine (PS) from the inner to the outer leaflet of the membrane contributes to the activation of ADAM10.

In this study, we set out to analyse the expression and the shedding mechanism of PrPC in epithelial cells and in neuronal cells with regard to the role of the plasma membrane as potential regulator.

Initially, HEK cells were used for PrPC transfection and for the analysis of the shedding mechanism.

We identified the ionophore ionomycin, the membrane fluidizer 2-phenylethanol and adenosine triphosphate (ATP) as suitable stimulants for the ADAM10-mediated PrPC shedding. To address the role of PrPC in keratinocytes we made use of HaCaT keratinocytes. Ionomycin was found to decrease the amount of full-length PrPC, however, the detection of soluble fragments in the supernatant proved to be very difficult. Thus, we next focused on the analysis of PrPC shedding in neuronal cells (N2a), which express high amounts of PrPC. Indeed, ATP was also found to increase the externalisation of PS and the shedding of PrPC in these murine neuroblastoma cells. The scramblase anoctamin-6 (ANO6/TMEM16F) is a calcium-activated chloride channel with a phospholipid scrambling domain. ANO6 with a mutated amino acid (D408G) has been described to be constitutively active due to increased calcium sensitivity. Transfection of this hyperactive ANO6-D408G led to enhanced PS exposure and PrPC shedding in N2a cells in the absence of any stimulus. Moreover, our experiments indicate that this correlates with decreased Fyn signalling and neurite outgrowth.

Taken together, we could show that several stimuli are able to induce the shedding of the cellular prion protein PrPC. Moreover, our data confirm that PS exposure is the decisive element in the ADAM10 activation process. Most likely, the latter also applies for ADAM10-meditated shedding in general. The functional relevance of ADAM10-meditated PrPC release and the role of PrPC in epithelial cells still have to be clarified in future studies.

P024 (OP03/03) | Visualizing scar development in living tissues reveals the collective migration of fibroblasts mediated by N-cadherin

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Scars are common pathologic manifestations to skin injuries, dermatologic syndromes and diseases. Our current knowledge of scar development derived from snap shot histological images, or 2D and 3D models that do not capture all features of scar development in vivo. How scars develop and why scar development is diverged across skin locations therefore remains unknown. The lack of suitable methods to directly visualize the dynamics of fibroblasts and

deposition of ECM is largely responsible for this gap, posing a major technical challenge for biologists and tissue engineers.

Here, we generate and characterize a live skin tissue model that develops dermal scars within 5 days (termed Scar-in-a-dish; SCAD), which closely resembles scar's composition, structure and cellular origin as occurs in vivo. When combining with genetic lineage tracing approaches, this system enables us to unravel and document the complexity and dynamics of scar development at single cell, lineage and tissue levels. By using multiphoton time-lapse imaging, and cell tracking coupled with in silicon analysis, we observed the diverged fibroblastic migration patterns in SCAD from dorsal skin and oral cavity, and leads to different scarring outcome. In the scarring back-skin SCAD, we find that activated fibroblasts operate in units that share cell trajectories and velocities. This collective migration is mediated by N-cadherin dependent cell-cell adhesion. Blocking N-cadherin inhibits swarming and reduces both wound contraction and scar formation. Our results define collective migration and N-Cadherin expression as pathologic fibroblast features of the wound response and raise promising opportunities to curtail scarring and contractures across a range of medical settings.

P025 | mTORC1—a potential player in the pathogenesis of acne inversa?

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Hidradenitis suppurativa (HS), also known as acne inversa (AI), is an inflammation of the sebaceous glands and terminal hair follicles that develops into painful nodules, which can result in abscesses and fistulae and is accompanied by severe pain and psychological stress for the patients.

The pathophysiology of AI has so far been only insufficiently understood. It is assumed that the IL-1β-IL-23/TH17/IL-17 pathway plays an important role in the pathogenesis of AI and appears to be preceded by psoriatic hyperplasia of the interfollicular epidermis. In previous studies we could show that the pharmacologically interesting mTORC1 (mammalian target of rapamycin complex 1) signalling cascade plays a decisive role in the pathogenesis of psoriasis and is activated by cytokines, which also play a role in the pathogenesis of AI. It is therefore conceivable that this signalling pathway also contributes to the pathogenesis and progression of AI.

We analyzed the expression and activation of different components of the mTORC1 signalling cascade in biopsies taken from different sites of AI lesions. While healthy skin only shows weak activation of the mTOR kinase in the granular layer and proliferative cells of the basal layer, the epidermis of AI nodules and fistular ducts displays massive mTOR activity. Similarly, we could detect strong activation of the mTORC1 target, the ribosomal protein S6 in suprabasal layers of lesional skin compared to healthy skin. First data points toward strong activation of the mTORC1 cascade at

sites of high inflammation and proliferation. Thus, we assume that inflammatory cytokines secreted by infiltrated immune cells activate mTORC1 signaling, resulting in increased epidermal proliferation and disturbed differentiation and thereby contribute to the pathogenesis of AI.

P026 | Function of LRRC8 volume-regulated anion channels during hypotonic stress response and differentiation of human keratinocytes

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The barrier function of the human epidermis is constantly challenged by environmental osmotic fluctuations and keratinocytes can become a direct target of osmotic stress. Cell swelling upon hypotonic stress is counteracted in mammalian cells by a compensatory mechanism called regulatory volume decrease (RVD) involving LRRC8 complexes, which act as volume-regulated anion channels (VRACs). We have recently provided first evidence that LRRC8A is also the essential VRAC subunit and involved in RVD in human keratinocytes. We could show that LRRC8A is preferentially expressed in basal and suprabasal epidermal layers, which declines in further differentiated layers. Moreover, isolated epidermal stem cells seem to differ in their LRRC8 subunit composition compared to transient amplifying keratinocytes and post-mitotic differentiated cells. By combining siRNA and CRISPR–Cas9-based knock-out studies with 2D and 3D cellular models, we revealed disturbed keratinocyte differentiation in absence of LRRC8A. In addition, inflamed skin shows altered LRRC8A expression. Taken together, this suggests that cell volume regulation by LRRC8A is an integral part of epidermal proliferation and differentiation and that LRRC8A is indispensable for proper epidermal stratification. Since disorders related to impaired epidermal barrier function can be aggravated by osmotic stress our findings provide a starting point to evaluate LRRC8A as a novel target to milden detrimental osmotic effects.

P027 | A DNA-repair independent pathomechanism in trichothiodystrophy

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The term trichothiodystrophy (TTD) was first coined by Price et al in 1980 to describe patients with sulphur deficient brittle hair, which was then later characterized as a marker for this complex disease. TTD is a rare autosomal recessive, multisystem disease

in which every organ of the body can be affected in particular the neuroectodermal tissues. Symptoms are ranging from delayed development, intellectual disability and progeroid symptoms like cachexia and recurrent infections and manifest at an early age. TTD is caused by mutations in the genes ERCC2 and GTF2H5 encoding for XPD and p8, respectively, the subunits of the transcription factor II H (TFIIH). TFIIH plays a dual role in the DNA-repair pathway nucleotide excision repair (NER) and the transcription process by RNA polymerases I and II. However, 50% of the TTD patients are identified with a form of TTD without DNA repair defect due to mutations in the genes GTF2E2 encoding for the β-subunit of the transcription factor II E (TFIIE), and C7orfl1 encoding for MPLKIP. While TFIIE is known to initiate the RNA Polymerase II transcription, the molecular function of the latter protein is unknown. TTD is a disease of accelerated ageing and its study could help in understanding physiological ageing.

This work investigates a DNA-repair-independent pathomechanism in both photosensitive and non-photosensitive TTD with the focus on a possible dysregulation in RNA polymerase I transcription and hence malfunction of the ribosomes. Key experiments present increased translation inaccuracy of the ribosomes in both photosensitive and non-photosensitive TTD cell lines. Inaccurate translation leads to the increased amount of misfolded proteins which activates the Unfolded Protein Response (UPR) of a disturbed proteome. In return UPR represses RNA polymerase I transcription. Moreover in our study we also show that the use of chemical chaperones like TUDCA (tauroursodeoxycholic acid) can rescue ER stress thus restoring the disturbed RNA polymerase I transcription. These findings can overall help us in understanding accelerated ageing and also imply possible therapeutic interventions for aging-associated disease.

P028 | Differential influence of individual human skin microbiota on the expression of innate defense genes

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Recently there is a growing body of literature that recognizes the significance of microbiota for the epithelium's homeostasis. The human skin is colonized by a specific diversity of microbes. The current understanding is that the majority of these microorganisms is harmless and can even provide protection against harmful effects. The interaction between the host and the skin microbiome can be viewed as a symbiosis. However, in some disease states, an altered balance of the microbiota can occur, a condition known as dysbiosis. These alterations may lead to disruption of the skin barrier and changes in the immune response, e.g. as seen in atopic dermatitis (AD) and psoriasis. This study aims to contribute to this growing area of research by exploring the native human cutaneous microbiota's influence on

cultured keratinocytes and 3D skin equivalents. In the first instance, methods of transferring the native human skin microbiota onto 3D skin equivalents had to be optimized. The samples were obtained by rinsing the study participant's forearm with saline solution to harvest the microbiota. Afterwards the solutions were sequentially centrifuged and stored in glycerol-containing buffer at -80°C. To assess the amount of cultivable microbes a specific amount of the mix was spread onto blood agar in addition to being transferred on 3D skin equivalents and monolayer keratinocytes. Subsequently, expression of specific innate defense factors (in particular cytokines, antimicrobial peptides) was analyzed by real-time PCR and ELISA. As a result we observed a microbiota-dependent differential gene expression of distinct innate defense factors. These data indicate that the individual microbiota composition has a profound influence on cutaneous innate defense.

P029 | Osteopontin Released from Mesenchymal Stem Cells Dampen Unrestrained M1 Macrophage Activation via TSG-6 and IL-1RA

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Macrophages play a crucial role in all phases of wound healing including inflammation, proliferation and remodeling. They occur within a spectrum ranging between two main phenotypes, classically activated pro-inflammatory type M1 macrophages and alternatively activated anti-inflammatory type M2 macrophages. The transition from pro-inflammatory M1 macrophages to anti-inflammatory M2 macrophages is severely disturbed in a variety of M1 macrophage dominated chronic wound disorders, like chronic venous leg ulcers, diabetic ulcers and pressure ulcers.

Mesenchymal stem cells (MSCs), beside their enormous regenerative potential, are endowed with the capacity to modulate immune cells, among them M1 macrophages, and in consequence, dampen unrestrained inflammation at the wound site. To better understand the molecular mechanism whereby MSCs dampen unrestrained activation of M1 macrophages under inflammatory conditions, we employed qPCR and microarray analysis and specific ELISAs from co-cultured MSCs and macrophages. Transcriptome analysis uncovered a known gene signature in MSCs which promote the conversion from M1 to M2 macrophages. These findings were confirmed by qPCR and specific ELISAs. Interestingly, we uncovered previously unreported molecular targets by which MSCs control the unrestrained activation of M1 macrophages. Among these new targets osteopontin, an important multi-domain protein with chemotactic and inflammation modulating properties, was identified. Osteopontin expression was significantly down-regulated in MSCs when co-cultured with pro-inflammatory M1 macrophages. Of note, the presence of osteopontin in MSC macrophage co-cultures significantly induced the anti-inflammatory cytokines like TNF-α-induced gene 6

(TSG-6) and interleukin-1 receptor antagonist (IL-1RA) in MSCs, both exerting their effects via dampening the transcription factor NFkB. Additionally, the silencing of osteopontin down-regulates TSG-6 expression. Both TSG-6 and IL-1RA were identified to be responsible for the switch from proinflammatory M1 to anti-inflammatory regenerative M2 macrophages as shown by qPCR and specific ELISAs. Our findings improved our knowledge on the action of MSCs applied for clinical use to the hostile pro-inflammatory microenvironment of chronic wounds in a therapeutic intent.

P030 | Mitochondrial DNA depletion leads to impaired proliferation, migration and contractile performance of dermal fibroblasts

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Fibroblasts play an important role in skin homeostasis where they orchestrate wound closure and scar formation but also contribute to pathologies occurring in aging. However, it is poorly understood how fibroblasts energetically manage these tasks. Here, we investigate how dermal fibroblasts depend on intact mitochondria and which consequences a defective respiratory chain might have.

To answer these questions, we developed two different approaches: wild-type dermal fibroblasts were isolated from newborn mice and treated with 10 mM sodium azide to block the respiratory chain. Furthermore, mice with fibroblast-specific accelerated accumulation of mtDNA deletions in fibroblasts were generated. For this purpose, we crossed mice that carry a targeted insertion of a dominant-negative mutant of the mitochondrial replicative helicase Twinkle with mice expressing a fibroblast-specific, tamoxifen-inducible Cre recombinase (Collagen Cre).

Our findings in vitro show that sodium azide treatment significantly reduces fibroblast proliferation. In addition, as revealed in a scratch-wound assay, fibroblast migration was significantly impaired by sodium azide treatment. Also, fibroblast–collagen interactions were disturbed as suggested from an altered collagen-lattice contraction assay when compared to controls. Together these findings indicate that key physiological functions of fibroblasts depend on proper mitochondrial activity. Furthermore, we analyzed the effect of the accumulation of mtDNA deletions in dermal fibroblasts by activating the Twinkle helicase in vitro. In Tamoxifen induced Collagen Cre positive fibroblasts mitochondrial DNA is depleted due to prior accumulation of mitochondrial DNA deletions, leading to a proliferative, contractile and migratory defect like the sodium azide effect. We can show that upon loss of mitochondrial DNA the fibroblasts slowly degrade the distinct complexes of the respiratory chain but most interestingly, they develop a pro-inflammatory phenotype in vitro. However, this does not have striking implications in vivo. Physiologically as well as in wound healing kinetics the Twinkle

mice show no obvious phenotype. This could be since the threshold of affected cells is balanced by cells that still have sufficient respiratory chain function and needs to be further investigated, also in other pathologies such as fibrosis or UV irradiation. It is well-established that during aging, tissues of mammals become mosaics of many normal and few cells with severe mitochondrial dysfunction. Future studies in gene modified Twinkle mice will help to better understand mitochondrial function and dysfunction in dermal fibroblasts.

P031 | Deletion of the autophagy gene Atg7 in K14-positive epithelial cells impairs the iron metabolism of murine ameloblasts

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Autophagy is activated in many cell types upon starvation, exposure to stress, or during differentiation. Autophagy-related genes such as Atg7 are essential for this process. In Atg7f/f K14-Cre mice, autophagy is blocked specifically in keratin K14-expressing cells including epidermal keratinocytes and precursor cells of skin appendage-associated epithelia. Here, we used this model, in comparison with wild-type mice, to investigate the role of Atg7 in ameloblasts, i.e. the cells that produce the enamel of teeth. The epithelium-specific deletion of Atg7 was compatible with the survival and enamel-forming capability of ameloblasts. However, Perls Prussian blue staining of sections through the maxillary jaw and incisors showed that the transport of iron from ameloblasts into the forming enamel was blocked in the absence of Atg7. While iron was deposited on the surface of normal enamel, it was aberrantly retained in enamel epithelial cells and subsequently taken up by macrophages in Atg7f/f K14-Cre mice. Due to the lack of iron in the enamel, the color of the incisors was changed from yellow to white. In conclusion, these results suggest that the autophagy regulator Atg7 plays an essential role in the iron metabolism and secretion of murine ameloblasts under homeostatic conditions.

P032 | Gene expression analysis and phylogenetic profiling of keratin K24

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Keratins are the main cytoskeletal proteins of epithelial cells. Most of the 54 keratin genes in the human genome are known to be expressed in a cell type- and tissue-specific manner, but some of the keratin genes have remained incompletely characterized. Here we determined the expression pattern of K24 which was recently proposed to be a differentiation marker of epidermal keratinocytes. Screening of publicly available microarray data showed that K24 is expressed at highest levels in the human cornea, placenta and salivary glands. RT-PCR and western blot analysis confirmed high expression levels of K14 in the cornea and low expression in the epidermis. By immunohistochemistry, K24 was detected in the suprabasal layers of the corneal epithelium and in epithelial cells of the salivary glands. Comparative genomics revealed conservation of K24 in terrestrial mammals and loss of K24 in cetaceans in which epithelia have adapted to the aquatic environment. These results suggest that K24 is not primarily an epidermal keratin but a component of the cytoskeleton in the corneal epithelium, salivary gland epithelium and other epithelia.

P033 | Inflammatory reactivity of reconstructed human epidermis to metal haptens by integration of TLR4-positive cells

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Allergic contact dermatitis is critically determined by the ability of haptens to mount an innate immune signal additionally to a directed T cell response. Reconstructed human epidermis (RhE) is widely employed as replacement assay to avoid animal experiments for safety evaluation of proinflammatory effects of new ingredients in industrial products. Unfortunately, RhE lacks responsiveness for metal haptens, the most relevant human contact allergens, raising concerns about their suitability as adequate assay system for allergen testing.

Here we investigated whether the defect of RhE might rely on a lack of functional TLR4 expression, which critically governs proinflammatory sensitivity to nickel and cobalt. By comparing RhE to reconstructed human full skin (RhS) we demonstrate that addition of dermal fibroblasts is sufficient to confer proinflammatory responsiveness for those metals. Unlike cultured epidermal keratinocytes, normal human fibroblasts expressed high levels of TLR4 mRNA and triggered production of IL-8 upon stimulation with nickel, cobalt or the TLR4 agonist LPS. Consistently, dermal isolates from RhS expressed considerable amounts of TLR4 mRNA whereas RhE or epidermis isolated from RhS or normal or inflammatory activated donor skin failed to express substantial TLR4 mRNA. Co-culture with human DCs similarly was able to license metal responsiveness of RhE, suggesting that integration of naturally TLR4 positive cells can compensate the defect of RhE. Our data suggest the use of RhS or RhE/DC co-culture models could circumvent current shortcomings of RhE concerning allergen risk assessment and may allow to combine benefits of complex and monoculture-based test systems in a single assay.

P034 | The role of RNase 7 as an immunomodulatory molecule during skin injury

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Keratinocytes are able to sense RNA from damaged cells via specific receptors (e.g. TLR-3) leading to the release of inflammatory cytokines such as TNF-alpha. It is known that the antimicrobial protein RNase 7 is the principal ribonuclease in human skin and is able to degrade RNA.

Stimulation of keratinocytes with polyinosinic-polycytidylic acid (poly I:C), a structural analogue of double-stranded RNA, showed increased RNase 7 and TNF-alpha expression. Addition of recombinant RNase 7 attenuated the poly I:C-mediated TNF-alpha release. These results suggest that an increased induction of RNase 7 expression during skin injury may control exaggerated RNA-mediated inflammation. To gain more insight into the role of RNase 7 in the context of RNA-induced inflammation we performed specific RNase 7-siRNA experiments. Downregulation of RNase 7 expression resulted in a significant higher induction of proinflammatory cytokines in keratinocytes treated with poly I:C. In line with these results, the release of proinflammatory cytokines in keratinocytes stimulated with UV-damaged necrotic HaCaT keratinocytes was increased by siRNA-mediated RNase 7 downregulation.

The ribonuclease activity of RNase 7 can be inhibited by the endogenous ribonuclease-inhibitor (RI). Keratinocytes with siRNA-mediated downregulation of RI expression demonstrated a decreased poly I:C-mediated induction of proinflammatory cytokines.

Taken together, these results indicate that RNase 7 in its function as a ribonuclease is able to degrade RNA released from damaged cells thereby controlling RNA-mediated inflammation during skin injury.

P035 | NF-kappaB c-Rel suppressed dermal fibroblasts display impaired fibrosis-associated cellular mechanisms

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Connective tissue diseases such as the autoimmune disease systemic sclerosis involve skin fibrosis with uncontrolled accumulation of extracellular matrix protein (ECM). Mechanistically, fibrosis involves proliferation of fibroblasts and TGF-β-dependent differentiation to myofibroblasts expressing alpha smooth muscle actin (αSMA), gaining enhanced contractility and increased ECM (mainly collagen I) production leading to higher tissue stiffness. Transcription factor NF-kappaB protein c-Rel has been associated with cell proliferation and fibrosis, i.e. c-Rel knockout mice reveal dampened fibrotic responses in liver, heart and skin. Beforehand, elevated c-Rel expression was shown for TGF-β-stimulated myofibroblast-like compared to homeostatic human dermal fibroblasts implicating a function for c-Rel in the fibrotic response.

Initially, c-Rel function was analyzed in a 3D fibroblast populated collagen matrix (FPCM) via siRNA-mediated suppression. Under homeostatic conditions (by direct detachment from the surface) c-Rel knockdown fibroblasts showed reduced contractility. However, under fibrotic conditions (stress-induced by later FPCM detachment and stimulated with TGF-β) c-Rel suppressed fibroblasts revealed only minor contractility changes. Thus, the impact of c-Rel on dermal fibroblast characteristics was analyzed more closely under homeostatic conditions. Furthermore, the migratory capability of c-Rel suppressed fibroblasts was reduced on collagen I coated surfaces. Although complete molecular mechanisms need to be elucidated fibrosis-associated processes are seemingly affected by c-Rel. With respect to our current insights, c-Rel may present a player in pre- or early fibrotic events.

P036 | A novel pathomechanism: are Cockayne syndrome and trichothiodystrophy ribosomopathies?

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The childhood premature aging diseases Cockayne syndrome (CS) and trichothiodystrophy (TTD) are attributed DNA-repair diseases as the affected genes code for DNA-repair proteins. However, no accumulating DNA-damage nor an elevated cancer incidence as a consequence of unrepaired DNA-damage has been reported in patients. The DNA-repair proteins are also involved in the key step of ribosomal biogenesis, transcription by RNA polymerase I. Here we investigated the cellular consequences of a disturbed ribosomal biogenesis and discovered that translational fidelity of the ribosomes of all patient cell lines tested is reduced. Ribosomes from patient cells are instable when isolated under stringent conditions. The resulting reduced accuracy of the translation process produces misfolded proteins that provoke endoplasmic reticulum stress and an unfolded protein response that in turn represses transcription of RNA polymerase I and ribosomal biogenesis. This vicious circle can be disrupted by pharmaceutical chaperones offering a treatment opportunity for the affected children. Interestingly this pathomechanism, identified in CS cells (Alupei et al, 2018), is also active in cells of TTD patients that also suffer from retarded growth, microcephaly and premature aging. Loss of proteostasis due to an inaccurate translation has not been described in human pathology before and offers one plausible explanation for the developmental problems and premature aging characterising these children.

P037 | TRADD regulates TNF complex I formation but is irrelevant for TNF-induced apoptosis

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Tumour necrosis factor (TNF) is known to induce a number of cellular responses such as pro-inflammatory, apoptotic and necroptotic signaling. Number of stress stimuli including UV light, bacteria or allergens as well as mechanical stress prompts rapid TNF expression in the skin. As the skin represents the first barrier of the organism, keratinocytes have developed well controlled mechanisms for regulation of the inflammatory responses. TNF ligation to TNFR1 results in formation of intracellular membrane-bound protein complex, named TNF complex I, which can activate NF-B and induce inflammation. Alternatively it can lead to formation of other intracellular complexes, namely complex IIa and complex IIb, which may induce both apoptotic (via caspase-8/caspase-3 activation) and necroptotic (via RIPK1/RIPK3/MLKL activation) cell death. FADD and TRADD are adaptor molecules with important role in TNF-induced apoptosis execution. TRADD specifically interacts with TNFR1 death domain and is required for the recruitment of other components of complex I. Moreover, in complex II TRADD can serve as docking station for FADD, which permits recruitment of caspase8, followed by activation of caspase cascade and execution of apoptosis. In this work we have used CRISPR technology to generate TRADD or FADD deficient HeLa cells. Here we show, that FADD deletion protected from TNF-induced apoptosis, when IAP antagonist or CHX were present. Intriguingly, under the same condition ablation of TRADD did not protect from TNF-induced apoptosis. Moreover in TRADD deficient cells TNF complex I composition was changed and only unmodified RIPK1 was recruited. In contrast, TNF complex IIb (Ripoptosome) demonstrated unchanged structure. These data suggest that TNF-induced apoptosis as well as the Ripoptosome formation may represent independent of complex I events. Taken together our data warrant future studies to dissect the impact of TNF dependent cell death and the role of adaptor molecules in a number of inflammatory diseases of the skin.

P038 | Combinatory treatment with IFN gamma and triamcinolone diversely influences normal and keloid fibroblasts—a potential keloid treatment?

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Imbalanced cell proliferation, extracellular matrix synthesis and degeneration as well as impaired wound healing can cause aberrant scarring. Stigmatization and physical restriction are the most severe impacts of such scarring on patients' lives. Although a broad variety of treatment regimens including conservative approaches like compression therapy, invasive approaches including cryotherapy, surgical procedures and laser ablation as well as combinatorial approaches with, e.g., glucocorticoids, chemotherapeutics and immunomodulators are used there is still a high recurrence rate of keloids. The aim of this study was to investigate the influence of IFNγ and/or triamcinolone on proliferation; cytokine, TIMP and MMP secretion; collagen type I synthesis and signal transduction in normal and keloidal fibroblasts. Three different donors of keloid fibroblasts and of normal fibroblasts were treated with different IFNγ and/or triamcinolone concentrations. Proliferation, collagen type I synthesis, cytokine as wells as MMP and TIMP secretion were time dependently analysed. Furthermore the influence on α-SMA, STAT 1, JNK and their respective phosphorylation were evaluated.

Our results show that during the observed time the proliferative potential in normal and keloidal fibroblasts was reduced after treatment with IFNγ or triamcinolone for 2d and 4d. The combinatory treatment showed a diverse influence in normal fibroblasts. After 2d the combinatory treatment showed an anti-proliferative influence whereas after 4d a pro-proliferative effect was observed in comparison with the mono-treatment with the active agents. In keloidal fibroblasts a clear additional anti-proliferative effect could be observed at both time points. Analysing the effect of both active agents and their combination on collagen type I synthesis revealed that in the used concentration range triamcinolone reduced collagen type I synthesis in normal fibroblasts after 4d, whereas it reduced collagen type I synthesis in keloidal cells already after 2d. IFNγ reduced the collagen type I synthesis in normal and keloidal cells at both time points. Analysis of different cytokines revealed that interleukin 6 and interleukin 8 secretion in normal fibroblasts was significantly reduced by all active agents and their combinations. In keloidal cultures only treatment with triamcinolone and the combinatory regimens showed reduced interleukin 6 secretion. On protein level clear cell specific differences were evident concerning α-SMA, JAK and STAT1.

The herein presented data suggest the combinatory application of IFNγ and triamcinolone as a promising therapy for hypertrophic scars.

P039 | Indocyangreen influences cell viability after irradiation with water-filtered nearinfrared—a new photodynamic therapy?

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Photodynamic therapy (PDT) is characterized by application of a photosensitive agent, its activation by a particular type of light and is mostly used in cancer therapy. In previous studies we could show

that water-filtered near-infrared (wIRA) influences wound healing and extracellular matrix generation differentially in normal and keloidal fibroblasts under hyperthermal conditions. 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 keloidal as well as melanoma cells under physiological as well as hyperthermal conditions. First of all we focussed on cell morphology and cell viability.

Normal fibroblasts, keloid fibroblasts and the melanoma cell line (A375) were pretreated for 4 hours with different indocyangreen concentrations. Thereafter the cultures were kept for 56 minutes at temperatures between 37°C and 46°C in a water-bath connected to a peristaltic pump. During this time the cultures were either kept light protected or exposed to 360 J/cm^2 generated by a wIRA irradiator (780 nm-1400 nm). Cell morphology and viability were monitored.

Our results show that increased temperature induced cell morphological changes in both cell species. Cultures that were additionally wIRA irradiated re-gained their initial morphology faster than light protected hyperthermally treated cultures. Furthermore it could be shown that cell viability as well as apoptosis induction was increased in indocyangreen pre-treated cultures after wIRA irradiation.

The herein presented data suggest wIRA in combination with indocyangreen and/ or heat as a promising therapy for hypertrophic scars and melanoma due to the observed cell viability reducing effect.

P040 | Disulfiram efficiently induces cell death in BRAF Wild-Type 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. BRAF Wild-Type melanoma comprises three major genomic subgroups (NRAS mutated, NF1 mutated and Triple Wild-Type) and accounts for approximately 55% of all cutaneous melanomas. An effective targeted therapy is currently only available for BRAFV600 mutant melanomas, indicating the clinical need for novel treatment options, especially for metastatic BRAF Wild-Type melanomas.

Since 1970, it has been reported that the anti-alcohol drug disulfiram has an antineoplastic effect. Its main metabolite is diethyldithiocarbamate (ET), a strong chelator for bivalent metal ions such as Cu2$^+$. Indeed, it was shown that ET in combination with Cu2$^+$ (CuET) provided antitumor effects in preclinical mouse models.

We initially performed cell viability assays to investigate the efficacy of CuET on BRAF Wild-Type 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 BRAF Wild-Type cells with a minimal effective dose of 125 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 in BRAF Wild-Type cells 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 could measure a dramatic increase of intracellular Cu2$^+$ in the melanoma cells, demonstrating that ET mediates the uptake of Cu2$^+$. The additional Cu2$^+$ was localized at the euchromatin 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.

A second mode of action of CuET could be the induction of protein stress by a proposed inhibition of the proteasome. Indeed, we were able to detect a rapid cytoplasmic accumulation of polyubiquitinated proteins in BRAF Wild-Type melanoma cells treated with CuET.

Finally, we assessed the inhibitory and cytotoxic 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 BRAF wild-type melanoma cells, and add additional cytotoxicity to MEK inhibitors to obtain an effective therapy for this group of melanomas.

P041 | Aberrant mTORC1 activity is regulated via TSC2 and transmits signals towards Stat3 to mediate the psoriatic differentiation defect

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To maintain homeostasis of the healthy epidermis, as outmost protective layer, keratinocytes are subjected to a tight control between proliferation in the basal layer and ordered differentiation and maturation to form the corneal layer. In inflammatory skin diseases such as psoriasis, this balance is disturbed leading to hyperproliferating keratinocytes that are unable to properly initiate the epidermal differentiation program. We previously found that aberrant activation of the Akt/mTORC1 cascade contributes to this defect. 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, mTOR 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 under these conditions and what downstream signaling molecules mediate the differentiation defect.

It is known from other tissues that mTORC1 is regulated through various pathways via specific phosphorylation of the tuberous sclerosis complex (TSC). By CRISPR genome editing, we knocked-out TSC2 in keratinocytes, which leads to 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. The generated cells also serve as an excellent tool to examine the downstream mediators of mTORC1. Specifically we identified Stat3 as a putative downstream target of mTOR activity by phosphorylating Ser727. Thus, inflammatory cytokines can mediate their physiological effect beyond the known activation of Janus kinases (Jaks). These results are especially interesting, as Jaks have recently drawn some attention as novel therapeutic targets for small molecule inhibitors. Therefore, these results suggest exploring combined inhibition of mTOR and Stat signalling as a novel therapeutic approach.

P042 | Automated immuno-histo-enzymatic investigation of measurable metabolic enzyme activity in cryosections of skin and epidermal equivalents

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Metabolic activity of cells within the skin is considered to depend on cell type, task, nutrient and oxygen availability, position, differentiation state, age of the individual, and other parameters. The existing methods relied on measurement of the enzymatic activity of samples extracted from pieces of tissue or on performing an enzymatic assay on tissue sections. A method that records multiple parameters in combination with measuring the enzymatic activity on a single cell level is however not known. We here report the development of an automated microscopy method that allows relating the individual enzymatic activity of single cells to immunohistochemical marker expression, but also to its position within the epidermis of the human skin or a 3D epidermal equivalent model.

We adapted an algorithm of the StrataQuest software (TissueGnostics) to automatically detect the epidermis based on nuclear density mapping and distance-based distinction between the basal and the first suprabasal epidermal layer, as well as the stratum

corneum on high power automated microscopy scans of skin sections. The sections were IF-stained for differentiation (KRT10, KRT14)- and proliferation markers (Ki67). The activity assay for the enzymes G6PD and GAPDH was performed with enzyme specific substrates and a tetrazolium based dye which allows relating the enzymatic activity to the chromogenic signal.

Results: The automated discovery of the basal layer of the epidermis based on nuclear fluorescence combined with bright field image analysis wrongly allocated less than five percent of Keratin 10 positive cells to the predicted area of the basal layer in duplicate skin sections (<500 cells per section) of three donors, a similar result was reached in organotypic epidermal equivalents. Next, we analyzed the enzymatic activity of G6PD within the predicted strata (basal, first suprabasal, all suprabasal, total epidermis) and cell shapes predicted from the nuclear distances in epidermal equivalents. The measured activity was significantly elevated from the basal to the "total suprabasal" but not the first suprabasal layer, reflecting the histologic confirmation of strong tetrazolium salt signal in the granular layer of the epidermis. We added 5 mM of Metformin, the pro-glycolytic anti diabetes drug to the organotypic cultures seven days or two days before cryosampling. We could detect a significant increase in measured G6PD activity within all individual layers in the samples that had received metformin two days before sampling, but this effect was not observed seven days after treatment.

In conclusion, we were able to establish an automated assay for epidermal image analysis that reliably identifies the basal and suprabasal strata of the human epidermis and of epidermal models. We also could prove responsiveness of a compatible enzyme activity assay that allows measurement of the most important metabolic enzymes to a commonly used drug that is not only used for treatment of diabetes but also the most investigated anti (skin) aging drug.

P043 | Differential expression of toll like receptors and antibiotic peptides by human keratinocytes

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Toll like receptors (TLR) and their target genes such as beta defensins (hBD), psoriasin or LL37 play an important role in psoriasis. In biopsies of psoriasis patients, TLR2, TLR4, TLR5 and TLR9 were found to be increased. Furthermore a shift within the different epidermal layers was reported, when psoriatic and normal skin samples were compared. An increased TLR mRNA expression has also been shown for inflammatory tissues. However, there are only few studies on the regulation of TLR in psoriasis or other autoimmune diseases with grossly diverging results.

Using sections of paraffin-embedded skin biopsy specimens, we could find significantly stronger signals of TLR4 expression in psoriatic skin

than in normal skin. TLR4 was found in all epidermal layers and no shift to the suprabasal layer as demonstrated by other authors. The expression of TLR2 was much weaker and detected primarily within the basal layer of the epidermis, not however within healthy skin.

RNA analysis showed no alterations in mRNA expression of TLR2, TLR4 or TLR9 in primary human keratinocytes and HaCaT cells (RT-PCR). When cells were treated with different amounts of specific stimuli (e.g. PGN, LPS, Zymosan), only Zymosan was able to stimulate TLR2 mRNA expression.

Biopsies of psoriatic skin are not easy to obtain and cell cultivation from psoriatic skin difficult and time-consuming. Therefore, primary human keratinocytes and HaCaT cells were used and treated with the proinflammatory cytokines TNF alpha, IL17A and IL22 to simulate an inflammatory environment. At different time points (6-72 h) the expression of hBD2, psoriasin and LL37 was analyzed using flow cytometry. While the expression of TLR2, TLR4 and TLR9 was not reproducibly altered, the antimicrobial peptides were clearly stimulated, hBD2 up to 6-fold after stimulation by TNF-alpha, psoriasin up to 5-fold (IL17A) and LL37 up to 3-fold (TNF-alpha). Stimulation was maximal after 48 hours. The administration of specific monoclonal IgG antibodies (adalimumab, secukinumab) abolished this stimulation to basal levels. Immunocytochemistry revealed a strong induction of hBD2 after LPS stimulation in HaCaT cells.

In conclusion, these data may help to supplement the current understanding of the regulation of psoriasis via TLR and its downstream targets like hBDs, LL37 and psoriasin. With regard to specific drug development, an experimental in vitro model for inflammatory diseases that is robust, cheap and easy to use would be useful.

CHEMOKINES/CYTOKINES

P044 | The role of EBI2 in skin inflammation

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Migration of leukocytes into the skin represents a critical step in development of inflammatory diseases of the skin. EBI2 and its ligand $7\alpha,25$-OHC direct immune cell localization in secondary lymphoid organs. CH25H and CYP7B1 hydroxylate cholesterol to $7\alpha,25$-OHC. We previously demonstrated a critical role of EBI2 for early transmigration of T cells into the CNS in EAE, the animal model for multiple sclerosis.

We analyze the impact of EBI2 expression in models skin inflammation such as the imiquimod-induced psoriasis-like dermatitis model or the TNCB-mediated contact hypersensitivity model (acute and memory). We use a reporter mouse line for EBI2 and we analyze expression of the enzymes responsible for synthesis of $7\alpha,25$-OHC in different cells in the skin in healthy and diseased animals.

Furthermore, we analyze EBI2 and the ligand generating enzyme expression in samples of psoriasis patients.

We found that EBI2 is highly expressed by inflammatory $\gamma\delta$ T cells, which mediate disease in the IMQ model. In line with this, the ligand-generating enzymes, CH25 hours and CYP7B1, are upregulated in the inflamed skin. Furthermore, EBI2-KO mice show a reduced accumulation of specific $\gamma\delta$ T cell subsets in the inflamed skin. Nonetheless, our preliminary data indicate that in acute models of CHS and of psoriasis-like dermatitis EBI2 does only play a minor role for the disease score. Since EBI2 is highly expressed on central memory T cells, we are currently investigating the role of EBI2 in these models in the memory phase.

Conclusions: EBI2 is highly and specifically expressed on IL-17 producing dermal $\gamma\delta$ T cells, nevertheless, the role of EBI2 in the acute models may be inferior or redundant mechanisms may take over the role of EBI2. Current experiments therefore are focussing on memory models of skin inflammation.

P045 | Efficacy of the anti-interleukin 17A monoclonal antibody secukinumab in acute generalized exanthematous pustulosis

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Introduction: Acute generalized exanthematous pustulosis (AGEP) is a potentially life-threatening drug reaction belonging to the group of SCARs (severe cutaneous acute reactions). Besides removal of the triggering drug current treatment recommendations encompass topical and/or in more severe cases systemic application of corticosteroids. Insights into the molecular mechanisms driving AGEP pathogenesis have been gained only recently. In particular, interleukin (IL)-17 seems to play a central role in the neutrophilic infiltration of the skin, thus presenting a potential target of novel and more efficient therapeutic approaches.

Aims: To evaluate the efficacy of the anti-IL-17A monoclonal antibody secukinumab in AGEP.

Methods: A 60-year-old patient developed fever, relative neutrophilia and a pustular rash few days after starting systemic treatment with terbinafine, administered for a fungal infection. Based on both medical history and clinical features, AGEP was suspected. Histopathological examination of a skin sample confirmed the diagnosis showing characteristic subcorneal pustules rich of neutrophils. Thus, terbinafine was discontinued and therapy with both topical and systemic steroids (prednisolone 1 mg/kg/day) was initiated. Three days later, since the patient showed no clinical response, a 300 mg single dose of secukinumab was administered subcutaneously. Both skin biopsies and peripheral blood were taken at distinct time points, before and after secukinumab application. Histochemical stainings of skin samples were performed for CD4^{+} and CD8^{+} T lymphocytes, CD15^{+}

neutrophils and IL-17 positive cells. Furthermore, different subpopulations of peripheral blood T cells, stimulated by terbinafine, were identified by their cytokine secretion profile utilizing ELISPOT assay

Results: Twenty-four hours after secukinumab administration, body temperature had dropped and collarette-shaped scaling typical of the resolution phase started to replace the pustular lesions on trunk and limbs. Furthermore, a marked decrease of dermal CD4^{+} T cell infiltrates as well as CD15^{+} neutrophils infiltrating the epidermis was observed. Thus, prednisolone was quickly tapered and stopped within 3 days. One week later the patient had completely recovered. T cell analysis by ELISPOT assay revealed enhanced frequencies of granzyme B-positive, terbinafine-reactive T cells both before applying secukinumab and several weeks after disease resolution, thus confirming terbinafine induced AGEP. Of note, numbers of IL-17-secreting T cells were not increased, neither before nor after secukinumab treatment, underlining that neutrophils rather than T lymphocytes are the source of this cytokine. In addition, no augmented percentages of T cells releasing IFN-γ, IL-5 or IL-10 were noticed.

Conclusion: Our results confirm that drug-specific T cells as well as neutrophils are of special importance in the pathogenesis of AGEP. We here demonstrate, that the anti-IL-17 antibody secukinumab shows high clinical efficacy presumably by resolving IL-17-mediated neutrophil recruitment to the skin, and thus presents a promising pathogenesis-targeted alternative to systemic corticosteroids for treating AGEP, especially in patients suffering from a recalcitrant course.

P046 (OP03/05) | IL-17E (IL-25) enhances innate immune responses during murine skin inflammation

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IL-17E (IL-25) is a member of the IL-17 cytokine family involved in the promotion of type 2 immune responses. Despite that, IL-17E has been recently reported to be upregulated in distinct skin inflammatory diseases such as psoriasis, atopic and contact dermatitis. In this study, we assessed the role played by IL-17E in skin inflammation in mice. Subcutaneous injection of IL-17E in the back skin of Balb/c mice induced a marked skin inflammation, characterized by the expression of innate immune response genes including the neutrophil chemoattractant Cxcl1 and genes involved in type I interferon/NFB signaling. Flow cytometry analysis revealed a significant increase in the number of leucocytes infiltrating the skin, with a skewing of the cell infiltrate towards the preferential recruitment of neutrophils to the detriment of T lymphocytes. Noteworthily, IL-17E transcripts were rapidly upregulated

in murine psoriasiform inflammation induced by tape-stripping and imiquimod. Genetic deletion of IL-17E or IL-17E neutralization with monoclonal antibody ameliorated the skin inflammation with reduction in neutrophil, NK and macrophage infiltration as assessed by t-SNE-guided multiparameter flow cytometry analysis. In addition, imiquimod-treated IL17e^{−/−} mice displayed significantly reduced number and size of scales compared to wild-type mice. Finally, IL-17E was found to be up-regulated in other human neutrophil-rich inflammatory skin diseases besides psoriasis, such as pyoderma gangrenosum and acute generalized exanthematous pustulosis. Our data demonstrate a novel role for IL-17E in skin inflammation, which is unrelated to the development of type 2 immune reactions. We propose that IL-17E is an important common denominator of chronic skin inflammation promoting innate immune cell recruitment and activation.

P047 | The IL-31-producing circulating T cells subset represents a unique population of CLA[+] CRTH2^{+} CCR4^{+} effector memory T cells

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Recent findings underscore an important role of IL-31/IL-31RA signalling in pruritus. The novel TH2-derived cytokine interleukin-31 (IL-31) has been implicated in the pathophysiology of atopic dermatitis (AD) and induces pruritus via a synergistic cooperation of dysregulated immune cells and stimulated sensory neurons. In particular, the clinical efficacy of the IL-31RA-targeting antibody nemolizumab in treating itch in atopic dermatitis patients emphasized the importance of the IL-31/ IL-31RA pathway. Although type 2 memory T cells (TH2) are considered to be the major source of IL-31 production, the phenotype IL-31-producing T cells is poorly characterized. In the present study, we established a reliable protocol for intracellular IL-31 staining in re-stimulated T cells and investigated the detailed phenotype of IL-31-producing T cells in AD patients (n = 30) and healthy volunteers. First, we observed that IL-31 is predominantly produced by TH2-polarized CRTH2^{+} T cells co-expressing low levels of IL-4 and IL-13. Intracellular IL-31 is nearly absent in polarized TH1 or TH17 cells. Stimulation with the alarmin IL-33 enhances the production of IL-31 in TH2 cells. In depth examination of the chemokine receptor repertoire indicated that IL-31-producing T cells express in addition to the already reported skin-homing marker CLA, further skin-homing receptors such as CCR4, CCR10, but are negative for CXCR3 or CCR6.

Interestingly, the abundance of circulating IL-31^{+} T cells (CD62L^{+}) as well as the overall IL-31 production by T cells is increased in AD patients with AD in comparison with healthy volunteers. Taken together our findings indicate that IL-31^{+} T cells may represent a unique population of skin-homing type 2 memory T cells that play a role in atopic

inflammation. In the absence of reliable tools to measure serum or plasma levels of human IL-31, analysis of the frequency of circulating IL-31-producing T cells may offer an opportunity to select or stratify patient cohorts during clinical studies investigating IL-31/IL-31RA-targeting drugs and IL-31-dependent T cell-triggered pruritus.

P048 | IL-17A and IL-17E disparately affect the basic functions of keratinocytes and may contribute to different aspects of the pathophysiology of psoriasis.

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The epidermis is a multilayered epithelium that is constantly renewed throughout life. A balance between basal cell proliferation and suprabasal cell differentiation maintains the homeostasis of the epidermis. A disturbance of this tightly regulated process is observed in certain skin diseases such as psoriasis. Psoriasis is a frequent chronic inflammatory skin disorder characterized by red scaly lesions caused by hyperproliferating keratinocytes that do not execute differentiation program properly. As of now, neutralization of the IL-23/Th17 pathway, and in particular IL-17A, is the most effective therapy for this disease. We have recently shown that IL-17E, an isoform of IL-17A, is also highly overexpressed in lesional psoriatic skin. The aim of this study is to address the influence of IL-17E on the basic functions of keratinocytes and, as a consequence, the homeostasis of the epidermis.

IL-17E is mainly produced by keratinocytes, while the infiltrating immune cells are the major source of IL-17A in the psoriatic lesional skin. Given that keratinocytes express subunits of the IL-17E receptor (IL-17RB and IL-17RA) as well as IL-17A receptor (IL-17RC and IL-17RA) they may represent a target of both cytokines. We analyzed the influence of both cytokines on keratinocyte proliferation in 2D cultures with two different approaches. First, we used WST-1 proliferation assay and we detected around 37% increase in metabolic activity when cells were cultured in the presence of IL-17E, but not IL-17A. Further evaluation of proliferation was accomplished with crystal violet staining and the obtained results indicated that IL-17E significantly increased cell number by more than 2.3× after 72 hours of cell culture. In this test, we could also observe the 1.6× increase in cell proliferation after IL-17A stimulation. Consistently, increased number of Ki67-positive keratinocytes was measured in 3D epidermal models cultured in the presence of IL-17E. Next, we investigated the effect of both cytokines on the terminal differentiation process of keratinocytes. Surprisingly, we detected that IL-17E and IL-17A upregulate the early differentiation marker involucrin and downregulate of the late differentiation marker filaggrin. However, while the IL-17A inhibited expression of all the other differentiation markers (i.e. keratin 10, desmocollin 1 and loricrin), the addition of IL-17E resulted in increased expression of these proteins. We obtained similar results at both mRNA level in 2D cultures and at protein level with immunohistochemical staining of 3D cultures.

Taken together, our results indicate that IL-17E is a more potent inducer of keratinocyte proliferation than IL-17A. At the same time, both cytokines affect strongly the differentiation of keratinocytes in a distinctive manner and the observed effect depends on the presence of calcium ions. Thus, our data point at the combine role of IL-17E and IL-17A on the structure and function of human epidermis what may be directly responsible for the features characterizing the psoriatic plaque.

CLINICAL RESEARCH

P049 | Digital assessment of hidradenitis suppurativa disease activity

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Background: Hidradenitis suppurativa (HS) is a chronic inflammatory skin disease affecting up to 2% of European adults. Disease activity is commonly assessed by counting of inflammatory nodules, abscesses and fistulas. Thus, it is time consuming and subject to inter-rater variability.

Objectives: To assess HS disease activity by means of automated, digital image analyses.

Methods: Digital images of axillary and inguinogenital HS were collected in a clinical routine setting, using smartphones and a CE medical device certified skin imaging platform. Photos were automatically normalized for illumination and color. Image characteristics such as an erythema-score and image complexity were calculated for all photos comparing affected and unaffected skin. Parameters were used to calculate the HS Activity and Severity Score (HiSASS) and correlated with the average Physician Global Assessment (PGA) of each picture provided by 3 independent dermatologists. Follow-up images were used to evaluate disease activity over time.

Results: 226 photos of 150 HS-affected skin areas (52% axillary, 48% inguinogenital) and 33 non-affected controls were analyzed. HiSASS correlated significantly with PGA scores ($P = 0.000$). Further, HiSASS clustered disease activity into three categories similar to the Hurley grading system: HiSASSave mild (PGA-0/1): 0.7-1.3, moderate (PGA-2/3): 1.5-1.6 and severe disease (PGA-4/5): 1.8-1.9. Additionally, the HiSASS allowed for a dynamic assessment of disease activity over time: Increasing HiSASS in the follow-up group (n = 30; baseline: HiSASSave = 1.6, follow-up: HiSASSave = 1.9) indicated disease worsening and correlated significantly with increasing PGA scores ($P = 0.009$).

Conclusion: Normalized mobile phone images could allow a fast, reliable, dynamic and reproducible disease severity assessment in HS patients.

P050 | Impact of Prior Treatment History on Efficacy of Risankizumab Compared with Placebo in Patients with Moderate-to-Severe Plaque Psoriasis: Integrated Analyses from Three Phase 3 Trials

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Introduction: Interleukin-23 (IL-23), an important regulator of multiple effector cytokines, plays a pivotal role in the development and maintenance of psoriatic lesions. Risankizumab (RZB) is a humanized IgG1 monoclonal antibody that selectively inhibits IL-23 by binding to its p19 subunit. The superior efficacy of RZB compared with placebo (PBO) and ustekinumab as well as its acceptable safety and tolerability profile have been demonstrated in three phase 3 randomized, double-blind, PBO- and active-comparator-controlled trials. The objective of this analysis is to report the integrated efficacy of RZB compared with PBO by prior treatment history in patients (pts) with moderate-to-severe plaque psoriasis.

Material and Methods: Data from three phase 3 studies (IMMhance, UltIMMa-1, and UltIMMa-2) in pts with moderate-to-severe plaque psoriasis were integrated over the 16-week (wk) PBO-controlled period. Pts stratified by weight and prior TNFi exposure at randomization received either 150 mg RZB (N = 1005) at wks 0 and 4 or matching PBO (N = 300). The consistency of PASI 90 and sPGA clear or almost clear (sPGA 0/1) responses at wk 16 was assessed in subgroups of pts by prior psoriasis treatment history. The reason for prior treatment discontinuation was generally pt-reported. Missing data were imputed as non-responders. Treatment comparisons were conducted by Cochran-Mantel-Haenszel test stratified by study, baseline weight (≤100 kg vs. >100 kg), and prior exposure to TNFi (0 vs. ≥1).

Results: Baseline demographics and disease characteristics were comparable between the two treatment arms. Mean age was 48.1 years and mean weight was 90.8 kg; 70.3% of pts were male. Mean baseline PASI and BSA were 20.3 and 26.1%, respectively. A history of diagnosed or suspected PsA was reported in 30.9% of pts. Of the 1305 pts included in this integrated analyses, 19.7% were naïve to all treatment except topical therapy and 30.0% were naïve to systemic therapy. Prior psoriasis therapy received included phototherapy (31.2%), photochemotherapy (8.7%), non-biologic systemic therapy (47.8%), and biologic therapy (44.8%). TNFi therapy was reported in 28.1% of pts, while 29.6% received non-TNFi biologic therapy. A total of 218 pts reported failure of ≥1 prior biologic therapy; a majority was failure of at least one prior TNFi therapy. At wk 16, RZB-treated pts achieved significantly higher PASI 90 and sPGA 0/1 response rates compared with PBO-treated pts (P < 0.001 for both endpoints across all subgroups), regardless of previous psoriasis treatment history. The efficacy of RZB in each of the subpopulations was comparable to the overall efficacy in the pooled population. **Discussion**: Treatment with RZB was associated with superior efficacy compared with PBO in adult pts with moderate-to-severe plaque psoriasis, regardless of previous psoriasis treatment history, including prior biologic failure.

P051 | Efficacy and safety of Risankizumab compared with adalimumab in patients with moderate-to-severe plaque psoriasis: Results from the Phase 3 IMMvent trial

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Interleukin-23 (IL-23), an important regulator of multiple effector cytokines, plays a key role in the development and maintenance of psoriatic lesions. Risankizumab (RZB) is a humanized IgG1 monoclonal antibody that selectively inhibits IL-23 by binding to its p19 subunit. The objective of the phase 3 IMMvent trial was to investigate the efficacy and safety of RZB compared with originator adalimumab (ADA) in patients (pts) with moderate-to-severe plaque psoriasis.

Baseline pt demographics and disease characteristics were similar between the two treatment arms. Mean age was 46.2 years and mean weight was 90.1 kg (27.9% of pts were over 100 kg); 69.8% of pts were male. Prior TNFi therapy was reported in 14.7% of pts (previous ADA exposure was not allowed), while 29.4% of pts received non-TNFi biologic therapy. All ranked endpoints were achieved (P < 0.001). At wk 16, RZB-treated pts achieved significantly higher PASI 90 (72.4%) and sPGA0/1 (83.7%) response rates compared with ADA-treated pts (47.4%; 60.2%). PASI 100 was achieved by 39.9% and 23.0% of RZB- and ADA-treated pts, respectively. Among ADA-treated pts achieving PASI 50 to <PASI 90 at wk 16, 66.0% of pts switching to RZB achieved PASI 90 response at wk 44 compared with 21.4% of pts continuing on ADA. Furthermore, 39.6% of pts switching to RZB achieved PASI 100 versus 7.1% of pts continuing on ADA. Treatment-emergent adverse event (TEAE) rates were comparable across treatment groups in the two randomized phases. The most frequently reported TEAE was viral upper respiratory tract infection.

Treatment with RZB was associated with greater clinical responses compared with ADA in adult pts with moderate-to-severe plaque psoriasis. In ADA-treated pts achieving PASI 50 to <PASI 90 at wk 16, switching to RZB resulted in superior efficacy compared with continued ADA treatment. The rates of AEs leading to discontinuation of study drug, serious AEs, and severe AEs were generally low and the frequency of AEs remained stable over time. No additional safety concerns were identified in pts who switched from ADA to RZB.

Methods: IMMvent (N = 605) was a phase 3 randomized, double-blind, active controlled study evaluating the efficacy and safety of RZB compared with ADA in adult pts with moderate-to-severe plaque psoriasis. Pts were stratified by weight and prior TNFi-exposure and randomized 1:1 to receive 150 mg RZB (N = 301, week [wk] 0, 4, 16, and 28) or ADA (N = 304, 80 mg at wk 0, 40 mg every other week [eow] from wk 1). At wk 16, ADA-treated pts achieving PASI 90 response continued on eow ADA, while non-responders (<PASI 50) were switched to RZB (wk 16, 20, and 32). ADA-treated pts achieving PASI 50 to <PASI 90 at wk 16 were re-randomized 1:1 to either continue eow ADA (N = 56) or switch to RZB (N = 53, wk 16, 20, and 32). Co-primary endpoints for Part A were PASI 90 and sPGA 0/1 at wk 16. The primary endpoint for Part B was PASI 90 at wk 44. Ranked secondary endpoints included PASI 75 (wk 16) and PASI 100 (wk 16 and 44). Missing data were imputed as nonresponders.

CLINICAL RESEARCH

P052 | Efficacy of Risankizumab compared with placebo across subgroups in patients with moderate-to-severe plaque psoriasis: integrated analyses from three phase 3 trials

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Introduction: Interleukin-23 (IL-23) plays a key role in the development and maintenance of psoriatic lesions by regulating multiple effector cytokines. Risankizumab (RZB) is a humanized IgG1 monoclonal antibody that selectively inhibits IL-23 by binding to its p19 subunit. The superior efficacy of RZB compared with placebo (PBO) as well as acceptable safety and tolerability profile have been demonstrated in three independent phase 3 randomized, double-blind, PBO-controlled trials. The objective of this analysis was to evaluate the integrated efficacy of RZB compared with PBO across subgroups of patients (pts) with moderate-to-severe plaque psoriasis.

Material and Methods: Data from three phase 3 studies in pts with moderate-to-severe plaque psoriasis were integrated over the 16-week (wk) PBO-controlled period. Pts stratified by weight and prior TNFi-exposure at randomization received either 150 mg RZB (N = 1005) at wks 0 and 4 or matched PBO (N = 300). Co-primary efficacy endpoints assessed for consistency at wk 16 across subgroups were PASI 90 and sPGA 0/1 responses. Missing data were imputed as non-responders. Treatment comparisons were conducted by Cochran-Mantel-Haenszel test stratified by study, baseline weight (Ü100 kg vs. >100 kg), and prior exposure to TNFi (0 vs. 1).

Results: Among 1305 pts included in this integrated analysis, baseline demographics and disease characteristics were generally similar between the two treatment arms. Mean age was 48.1 years and mean weight was 90.8 kg; 70.3% of pts were male. Mean baseline PASI and BSA were of 20.3 and 26.1%, respectively. Median baseline PASI score was 18.0, while baseline sPGA was moderate in 80.2% of pts. A history of diagnosed or suspected PsA was reported in 30.9% of pts. At wk 16, RZB-treated pts achieved significantly higher PASI 90 and sPGA 0/1 response rates compared with PBO-treated pts (P < 0.001 for both endpoints across all subgroups), regardless of baseline demographics or disease characteristics. The efficacy of RZB in each of the subpopulations was comparable to the overall efficacy in the pooled population. Discussion: Treatment with RZB was associated with superior efficacy compared with PBO in adult pts with moderate-to-severe plaque psoriasis, regardless of baseline demographics or disease characteristics. Each of the subpopulations was comparable to the overall efficacy in the pooled population.

P053 | Optical coherence tomography as a novel intravital method for human hair follicle morphological analyses

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The visualisation of live human scalp hair follicles (HFs) is of great interest to hair transplant surgeons, clinicians who wish to utilise these mini-organ for regenerative medicine purposes and in preclinical hair research. However, HFs typically have to be fixed for histology (and thereby killed) to discern their anatomy in sufficient detail. Here, we introduce label-free optical coherence tomography (OCT), using a Thorlabs Ganymede Series Spectral Domain system, as an intravital method for obtaining 2D or 3D high-resolution images of viable human microdissected HFs at a previously unreported level of anatomical detail. Using OCT, it is possible to distinguish macroscopically each HF stage, i.e. anagen, early, mid or late catagen, in live microdissected HFs. Up to now, this was only possible with light microscopy by staining microdissected HFs with the vital dye methylene blue. However, as this method requires that HFs are removed from the culture medium, the analysis of ex vivo HFs over the organ culture period is very laborious and induces unnecessary stress upon

the HFs. Instead, OCT images can be obtained without labelling the HFs and in a very short time-frame. Therefore, this technique allows stress-free temporal analysis of morphological changes to the HF during ex vivo culture periods. Moreover, key HF compartments (dermal papilla, outer root sheath, inner root sheath, connective tissue sheath, hair shaft) and even distinctive cell populations, such as HF pigmentary unit melanocytes, can be visualised in live HFs. As a result, OCT is a powerful intravital method to obtain important first indications on whether a specific substance affects growth, and possibly pigmentation or hair shaft structure, in treated live HFs ex vivo.

P054 | T-cell responses against autoantigens of the skin in patients with lichen sclerosus

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Introduction: Lichen sclerosus (LS) is a rare inflammatory skin disorder affecting mainly mucous membranes of the anogenital area, although others parts of the skin can also be involved. Its pathogenesis is still unclear. Previous studies suggested a pivotal role of pathogenic T cells mainly shifted towards an interferon-gamma (IFN-γ)-producing T helper (Th) 1 phenotype. Like lichen planus, the histopathology of LS shows an interface dermatitis, with linear arrangement of T cells along the basement membrane zone (BMZ), suggesting a potential T cellular recognition of autoantigens in the epidermis or BMZ.

Objectives: To characterize the T cell response against autoantigens involved in the pathogenesis of autoimmune blistering disease (AIBD) in patients with LS and to evaluate differences in the T cell response between genital and extragenital LS.

Materials & Methods: A total of 14 patients with LS, confirmed by histopathology, and 11 healthy controls (HC, age- and sex-matched), that were consecutively referred to our department, were included. Peripheral blood mononuclear cells (PBMC) were isolated and co-cultured in vitro with the bullous pemphigoid (BP) 180 antigen (NH2- or COOH-terminal ectodomains), desmoglein 3 (Dsg3) and collagen VII (Col VII). Subsequently, the frequency of antigen-specific T cells producing IFN-γ (Th1), interleukin (IL)-5 (Th2) and IL-17A (Th17) was determined by ELISpot assay in patients and HC. Furthermore, paraffin-embedded skin sections from LS patients were stained for CD3, CD4, T-bet, GATA3 and IL-17A in order to analyze the profile of the cutaneous inflammatory T cell infiltrate.

Results: The IFN-γ secretion by peripheral T cells stimulated with the NH2-terminal or COOH terminal domain of BP180 was significantly higher in LS patients compared to HC (NH2: $P = 0.03$; COOH: $P = 0.05$). No significant differences were observed between LS patients and HC with respect to Dsg3 and Col VII. The frequency of IL-5- and IL 17-secreting T cells did not show differences between LS patients and HC with regard to all the antigens tested. The observed

peripheral blood Th1 cell response against BP180 was paralleled by a shift towards CD4$^+$ T cells in the inflammatory infiltrate found in LS lesions (mean ratio CD4/CD8 = 1.27) showing a predominance of T-bet$^+$ (Th1) T cells (mean ratio T-bet/GATA3 = 1.30).

Conclusion: We identified BP180 as an autoantigen of LS which may play a role in the pathogenesis of LS, both genital and extragenital. The absence of a significant T-cell response against Dsg3 and Col VII supports the specificity of this finding. Whether BP180 is involved in the developmental phase of LS pathogenesis or rather in perpetuating, chronic inflammatory stage is yet to be elucidated.

P055 (OP02/01) | Clinical response to JAK inhibition in patients with familial chilblain lupus and TREX1 mutation

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Background: Familial chilblain lupus (FCL) a monogenic autosomal dominant form of cutaneous lupus erythematosus in most cases based on mutations in three prime repair exonuclease 1 (TREX1). FCL presents in early childhood with cold-induced painful erythematous infiltrates leading to mutilation and is associated with variable systemic involvement resembled by an elevated type I interferon (IFN) signature in skin and blood. Treatment is currently insufficient. TREX1 is a cytosolic DNase anchored in the outer nuclear membrane and safeguards the cell against innate immune activation by degrading short DNA metabolites derived from the nucleus that leak into the cytosol. In TREX1-deficient cells, self DNA accumulates in the cytosol and stimulates the cyclic GMP-AMP synthase-stimulator of interferon pathway leading to induction of type I IFN. Inappropriate chronic type I IFN activation can break immune tolerance and promote autoimmunity. In keeping with this, patients with FCL exhibit constitutive upregulation of IFN-stimulated genes (ISGs) and proteins in blood and skin. The effector functions of type I IFN are mediated by the interferon-α/β receptor (IFNAR) which signals via the Janus kinase/signal transducers and activators of transcription (JAK/STAT) pathway.

Objective: To evaluate the clinical response to the Janus kinase inhibitor baricitinib in familial chilblain lupus and assess the effect of cold on patient fibroblasts.

Results: In 3 adult patients with FCL we initiated treatment with baricitinib 4 mg daily for 3 months. All 3 patients showed a significant reduction of cutaneous lupus lesions measured by revised cutaneous lupus area and severity index (R-CLASI). In blood we observed a decrease of the systemic type I IFN signature. One patient had complete pain relief and in two patients pain associated with joint inflammation was partially reduced. No severe adverse reactions were reported.

Exposure of patient fibroblast to cold induced a stress response, enhanced senescence and induction of type I IFN induced genes in vitro. These findings delineate a mechanism by which cold a well-known trigger factor of chilblain lupus can enhance a cell-intrinsic ISG response in TREX1-deficient cells.

Conclusion: Our findings demonstrate the therapeutic efficacy of JAK inhibition in a monogenic form of lupus and give mechanistic insight into the process of disease exacerbation by cold in TREX1-deficient cells. This may be relevant for implicating JAK inhibition as a therapeutic option also in multifactorial cutaneous lupus erythematosus and other interferonopathies.

P056 | Pre-treatment progression dynamics of melanoma determines response to anti PD-1 immunotherapy

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Purpose: Immune checkpoint inhibition with PD-1 antibodies has greatly increased the prognosis of patients with advanced melanoma. Clinical experience suggests that patients responding to immunotherapy predominantly harbor slow-growing metastases rather than fast-growing metastases. Currently, data on indirect markers like lactate dehydrogenase (LDH) or extent of tumor burden support this assumption. This study is the first to assess metastatic growth rate as prognostic marker in melanoma patients treated with anti-PD-1 antibodies.

Patients and Methods: Diameters of RECIST target lesions were measured in two independent cohorts of altogether 179 melanoma patients treated with the anti-PD-1 antibodies nivolumab or pembrolizumab. The largest target lesion at baseline was identified. Pre-treatment growth rate was calculated as the difference of the largest target lesion's diameter between baseline and the last pre-baseline staging, divided by the time difference between these staging assessments (mm per month). Kaplan–Meier survival curves and univariate as well as multivariate Cox regression analysis were used to examine the prognostic value.

Results: Patients with pre-treatment metastatic growth rate >3.9 mm/month exhibited significantly impaired overall survival (OS) compared with the remaining patients (nivolumab group: 1-year OS: 40.0% vs. 92.9%; pembrolizumab group: 34.0% vs. 84.2%, both $P < 0.000001$). Metastatic growth rate also correlated with response rates. Patients showing an objective response or stable disease showed significantly lower growth rates than patients with progressive disease. Multivariate Cox regression analysis revealed that growth rate was independently associated with OS and predicted OS better than LDH or tumor burden.

Conclusion: Pre-treatment metastatic growth rate could be utilized to identify patients being not suitable for anti-PD-1 immune

checkpoint inhibition and should be further assessed for guiding therapeutic decisions.

P057 | Occurrence of autoimmune thyroiditis predicts response and favorable progression-free survival of melanoma patients receiving PD-1 antibodies

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Purpose: Immune checkpoint inhibition with PD-1 antibodies has the capability to induce long-lasting responses in melanoma patients. In the vast majority of patients, treatment-related adverse events (AEs) are manageable and severity is mild to moderate. Auto immune thyroiditis is considered one of the most commonly occurring AEs in anti-PD-1 monotherapy. However, its effect on efficacy is still unknown. Moreover, data on response rates and progression-free survival (PFS) are contradictory. The aim of this study was to investigate best objective response and PFS in accordance with immune related AEs (irAEs) in melanoma patients receiving PD-1 antibodies as monotherapy.

Patients and Methods: IrAEs were assessed in 158 patients starting treatment with the anti-PD-1 antibody pembrolizumab at University Hospital Tubingen, Germany between June 2014 and February 2017. Safety data were collected continuously during the treatment period using questionnaires and laboratory tests. Treatment-related irAEs were graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events (CTCAE), version 4.03. Survival analyzes were conducted using Kaplan-Meier estimator. PFS was assessed in all patients. Best objective response (complete or partial response) and best disease control rates (response or stable disease) were compared using two-sided Fisher exact test.

Results: Thirty-eight (24.1%) of the 158 patients experienced treatment-related irAEs of any grade. Compared with the remaining patients, they had a significant longer median PFS (not reached [NR], 95% confidence interval [CI] 13.4 months-NR vs. 3.2, 95% CI 3.0-3.5 months, $P < 0.0001$). Best objective response was 68% vs. 30% (odds ratio [OR] 5.0, 95% CI 2.1-12.1, $P < 0.0001$) and disease control rate was 82% vs. 41% (OR 6.4, 95% CI 2.5-18.8, $P < 0.0001$). Most patients experienced grade 1 or 2 toxicity (66%, grade 3: 24%, grade 4: 11%, considering patients with irAEs only). Administration of systemic corticosteroids or end of treatment due to irAE toxicity did not influence PFS significantly ($P = 0.29$ and $P = 0.24$, respectively). Baseline factors predicting occurrence of irAEs included lung metastasis, metastasis restricted to less

than 5 sites, primary melanoma localization on the trunk, and absolute eosinophil count ≥150/μL.

Occurrence of thyroiditis during treatment was also associated with favorable PFS (NR, 95% CI 13.4 months-NR vs. 3.3 months, 95% CI 3.0-6.1 months, P = 0.00028). Most patients experienced low grade thyroiditis (grade 1-2: 82%, grade 3: 14%, grade 4: 5%). Fifty-five percent of therapy related cases of thyroiditis occurred within the first 6 weeks of treatment and 73% occurred within the first 12 weeks. Best objective response rates were 73% vs. 34% (OR 5.1, 95% CI 1.7-17.0, P = 0.00085) and disease control rate was 86% vs. 45% (OR 7.7, 95% CI 2.1-42.8, P = 0.00035).

Conclusion: irAEs are strongly associated with response to and favorable PFS under therapy with PD-1 antibody pembrolizumab. Even autoimmune thyroiditis, although commonly presenting with asymptomatic courses and laboratory findings only, seems to be related with improved response rates. Monitoring laboratory changes of thyroid stimulating hormone (TSH) and the free thyroid hormones fT3 and fT4, especially during the first 6 weeks of treatment, will help identifying patients who are likely to respond to anti-PD-1 immune checkpoint inhibition.

P058 | Multiparametric characterization of T and B cell subsets in pemphigus vulgaris

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Pemphigus vulgaris (PV) is a rare, potentially life-threatening autoimmune bullous disease characterized by flaccid blisters and erosions of the skin and mucous membranes. Pathophysiologically, the underlying loss of epidermal adhesion is caused by IgG autoantibodies (auto-ab) against the desmosomal adhesion proteins, desmoglein (Dsg) 3 and Dsg1, on epidermal keratinocytes. Data strongly suggest that PV is mediated by a close interaction of autoreactive T and B cells. The production of antigen-specific, pathogenic IgG auto-ab in PV depends on distinct Dsg3-specific CD4$^+$ T cell subsets producing a variety of cytokines that are crucial for the activation of autoreactive B cells. In this regard, only little is known about the frequencies of different T follicular helper (Tfh) cell subpopulations and T regulatory follicular (Tfr) cells owning opposite roles in regulating germinal center responses in PV, since Tfr cells have been shown to inhibit B cell responses mediated by Tfh cells. Thus, Tfh cell activity might be enhanced due to an impaired Tfr cell compartment resulting in the expansion of autoreactive B cells and increased auto-ab production at certain stages of PV.

To get further insights into the immunopathogenesis of PV, we analyzed T and B cell subsets in peripheral blood of clinically well-defined PV patients (n = 40) at different phases of the disease (active, chronic,

in remission) to assess alterations in their cellular compartment. A cohort of age- and sex-matched healthy individuals included in the study served as control (HC; n = 20). Peripheral blood mononuclear cells were isolated from blood of patients and HC for multiparametric flow cytometric analysis and distinct T helper (Th), Tfh and Tfr cell populations were determined by the expression of surface markers, i.e. CD3, CD4, CD45RA, CXCR3, CXCR5, CCR6, CD25, and CD127. Furthermore, we dissected peripheral B cells in eight subpopulations comprising total (CD19$^+$), naïve (CD19$^+$CD27$^-$IgD$^+$), memory (CD19$^+$CD27$^+$CD38$^-$), transitional (CD19$^+$CD27$^-$CD24^{++}CD38^{++}), marginal zone (CD19$^+$CD27$^+$IgD$^+$IgM$^+$), non-class-switched memory (CD19$^+$CD27$^+$CD38$^-$IgD$^+$IgM$^+$), class-switched memory B cells (CD19$^+$CD27$^+$IgD$^-$IgM$^-$CD38$^{+/-}$), and plasma cells/plasma blasts (CD19$^+$CD27$^+$IgM$^-$IgD$^-$CD38^{++}). Furthermore, in order to identify autoreactive B cell subsets we used fluorochrome-labeled Dsg3.

Thus, the assessment of cellular alterations during different stages of PV provides pivotal information to better understand the role of B and T cell subsets in the pathophysiology of pemphigus. Furthermore, these results could contribute to the identification of biomarkers potentially indicating autoreactive immune responses which precede a clinical relapse in PV patients.

P059 | Bullous pemphigoid with the picture of acquired reactive perforating dermatosis

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Bullous pemphigoid (BP) is the most common autoimmune blistering disorder. Besides skin blistering the disease manifests with diverse clinical presentations, which can be nonbullous. Recently, 4 patients with BP with co-existence of clinical features resembling acquired reactive perforating dermatosis (ARPD) were published. All 4 patients had type 2 diabetes. They initially presented with generalized papules and nodules with a central keratotic plaque, features characteristic of ARPD. Only at a later time point did they develop blisters, prompting diagnostics for autoimmune blistering disorders and finally confirming the presence of autoantibodies at the DEJZ. We here describe 5 previously unreported patients with BP, which clinically and histologically resembled ARPD at first presentation. Unlike the already published work, we performed diagnostics for blistering autoimmune disorders at first patient presentation in our Department. This was before the patients had developed blisters, thus they could be considered to be in the prodromal BP phase.

Contrasting the typical BP histopathology we found only a neutrophilic infiltrate and no eosinophils in the patients' skin. In addition, 2/5 patients carried autoantibodies only against non-NC16A

regions on BP180. IgE autoantibodies are associated with increased BP severity. We analysed the sera for IgE autoantibodies to BP180 and BP230 and found low-level BP230 IgE-autoantibodies in 2 patients, while one patient had low level BP180 and BP230 IgE-autoantibodies. These findings support a distinct pathogenetic background of this BP presentation than in classical BP.

Although the sample size is small, our results highlight ARPD as a clinical and histopathological pattern of nonbullous BP. Since therapeutic regimens in BP patients are different than in ARPD, in patients with this clinical picture BP diagnostics should be initiated already at first presentation.

P060 | Effect of skin neutral pH versus basic emollient application on skin microbiome and physiology in atopic dermatitis patients and healthy controls

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Background: A rather acidic pH of 5.5, termed skin neutral, is part of a functional skin barrier. Previous studies have found a slightly higher skin pH in atopic eczema (AE) patients. In addition, AE patients typically suffer from disturbed skin barrier and exhibit skin microbiome dysbiosis, marked by high abundance of *Staphylococcus aureus*. However, it is not known if a higher skin pH in AE patients is a cause, or a result, of microbiome dysbiosis.

Aim: We investigated the effects of the application of emollients with either skin neutral pH (5.5) or basic pH (8.5) on skin microbiome, also considering skin physiology and AD related clinical parameters.

Study design/Methods: A double blinded study was conducted over 8 weeks with 6 AE patients and 6 gender and age matched healthy controls (HE). An emollient was applied twice daily with two different pH levels: 5.5 and 8.5, each on a different, randomized, body side. Once a week, skin swabs were taken for microbiome analysis, skin physiology was investigated (TEWL, hydration and pH) and severity scores for AE (SCORAD, local SCORAD, EASI) were assessed. Microbiome frequencies were obtained by 16S (region V1-V3) NGS sequencing, and analyzed for changes in alpha-diversity, beta-diversity and in particular changes in frequency of Staphylococcus species.

Results: At baseline, AE patients had significantly higher skin TEWL and significantly lower skin hydration, whereas no difference in pH was detected between AE and HE in this study. Furthermore, no significant changes over time in skin physiology (pH, TEWL or hydration), or in AE related clinical scores, were observed for AE or HE, with no difference between the pH level of the emollients.

At baseline, beta-diversity analysis showed a difference in global microbiome between AE and HE, where the main difference between the groups was the increased abundance of *S. aureus* in AE. Interestingly, whereas for HE controls no change in beta-diversity global microbiome was observed over the time period of 8 weeks, using emollients with either pH 5.5 or 8.5; beta-diversity analysis in AE patients showed a shift in global microbiome composition over time, independently of the pH level of the emollient. Moreover, microbiome richness showed a significant increase over time in part of both AE patients and HE controls, independent of the pH level of the emollient. The increase in richness over time was associated with a lower baseline skin pH and a lower baseline microbiome richness. Further analysis of the microbiome changes on the different taxonomic levels is ongoing.

Conclusion: To best of our knowledge, this is the first data on frequently tracked skin microbiome over a time course of 8 weeks, investigating the influence of emollients. We hypothesize that the beta-diversity shift in global microbiome observed only in AE patients might be due to a higher susceptibility for external factors, whereas skin of healthy controls seems to stabilize microbiome over time. This opens new avenues for targeted intervention on the skin microbiome level in AE patients. The pH level of the emollient used did not show a significant difference on the microbiome changes, and the microenvironment of the skin was not significantly altered by any of the study emollients. The possibility of 'restoring' the skin's homeostasis—using personalized emollients—is an important tool in the therapy of AE and merits further investigation.

P061 | CD23 is a biomarker of clinical response to IgE-specific immunoadsorption in patients with severe atopic eczema

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IgE-specific immunoadsorption (IA) can be an effective treatment for severe forms of atopic eczema (AE). However, the underlying mechanisms as well as biomarkers predicting the therapeutic outcome are not yet known. Here, we investigated the impact of IA on cellular level and clinical outcome. Ten patients with severe AD (SCORAD > 40) and highly elevated IgE levels (IgE > 2000 IU/mL) were included in this study. Every patient received a total of ten IgE-specific IA sessions that were performed in three intervals with IgE-specific IA on three or four consecutive days. A follow-up examination was performed four weeks after the last session. All patients were comprehensively characterized during and after IA therapy by assessment of cytokines in the serum, analyzing bacterial skin colonization as well as collecting skin biopsies for next generation sequencing (NGS). Frequencies of different immune cell populations and their Fc receptor expression profiles were monitored by flow cytometry at the beginning and the end of the first

and last interval as well as at a follow-up appointment four weeks after the last IgE-specific IA to analyze potential long-term effects. As expected, dramatically decreased IgE levels were detected directly after IA. IgE levels were partly restored overnight, but still showed an efficient reduction after each session. However, IgE levels were completely restored four weeks after the last IA, while a subgroup of patients (n = 5/10) still showed clinical response (SCORAD40). Flow cytometric analysis of circulating immune cells revealed strong regulation of CD23, the low affinity IgE receptor, on B cells by IA with downregulation of CD23 in responders at follow-up, while non-responders did not down-regulate CD23 on long term. Thus, our data indicate that CD23 levels on B cells are mechanistically involved in the mode of action of IgE-specific IA, and that CD23 is a biomarker of clinical response.

P062 | microbIEM—Microbiome analysis tool with automated decision making and user-friendly graphical interface

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Background: In the last years, there has been an enormous boost of microbiome studies which generated high amounts of amplicon sequencing data along with a high number of meta-information such as treatment information, time points and sample descriptions. Because microbiome studies are often performed by researchers with limited computational experience, the analysis and interpretation of microbiome data remains a big challenge and is a time consuming step and therefore offers many pitfalls. For these reasons, there is a great need for computational tools for helping non-experts during the analysis process including pre-processing of OTUs, diversity analysis and contamination detection or removal. This especially holds true for tissues that are particularly challenging to analyse, such as human skin where the yield of microbes is rather low compared to other tissues.

Aims: Here, we aimed to generate a tool, microbIEM, where experts in bioinformatics but also biological experts, who perform microbiome studies, worked intensively together to establish a tool that allows to manage the filtering of species, removal of biological, technical and other types of contamination in a sensitive and automated way.

Methods: microbIEM is an in-house tool which is implemented in scripting language R and which uses a Java-based frontend to remove operational taxonomic unit table (OTU Table) based on filter that account for the total and relative amount of reads or by considering negative and positive controls. microbIEM also allows the definition and omission of low quality samples by using a binning approach where the most reasonable threshold to exclude low-sequenced samples. Furthermore, an option to filtering for artefacts (technical filtering) and for relevant OTUs (biological filtering) is provided. All filters can be used either fully or semi-automatized. In contrast to other tools, microbIEM can be easily used by non-experts but results can be also compared to QIIME or IMNGS by providing input files. Furthermore, it includes the possibility to compare results to state-of-the art tools such as RHEA.

Results: microbIEM allows to filter for relevant OTUs and samples, generates overview tables of amount of reads in total per OTU and on species level which were lost in each filtering step. Furthermore, microbIEM allows computing alpha- and beta-diversity efficiently by selecting single or group of samples of interest based on easy and dynamical manipulating of them. Therefore changes over time and for individual treatments can be explored and detected much faster and easier. In the future, we will aim at including various methods to compare the microbiome composition also with human gene expression and especially of highly correlating genes, which should allow generating novel hypothesis in a faster manner and more understandable way.

SUMMARY AND OUTLOOK

P063 | Autologous skin-derived ABCB5^{+} mesenchymal stem cells as advanced-therapy medicinal product to treat chronic venous ulcers

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Background: The prevalence of chronic venous ulcers (CVUs) is on the rise and so is the demand for effective treatments since a significant number of CVUs fail to heal or do recur. As chronic wounds, CVUs appear arrested in a prolonged inflammatory state. Dermal mesenchymal stem cells expressing ATP-binding cassette subfamily B member 5 (ABCB5^{+} MSCs) have been shown to display distinct immunomodulatory properties involving several pathways including programmed cell death protein 1 and superoxide dismutase 3. In addition, ABCB5^{+} MSCs have capacities that would become relevant during later (proliferative) stages of wound healing, such as pro-angiogenic paracrine signaling and a certain endothelial trans-differentiation potential. These characteristics make ABCB5^{+} MSCs a promising candidate for cell-based therapy of non-healing CVUs.

Objective: To test the efficacy and safety of ex vivo expanded autologous skin-derived ABCB5^{+} MSCs as advanced-therapy medicinal product for the treatment of non-healing CVUs in a first-in-human trial.

Methods: In this ongoing phase I/IIa clinical trial, patients with non-healing CVU receive a single topical application of 510E5 autologous

ABCB5$^+$ MSCs per cm^2 wound area. Wound size, wound quality, pain, and health-related quality of life are assessed before and during 12 weeks after cell application. To ensure that the ulcer has indeed been arrested despite standard medical care in a chronic state before treatment, two predefined criteria regarding the pretreatment wound size development were set: Patients who meet one of these criteria, i.e. enlargement >25% or reduction >50% during the 6-20 week phase of ex vivo MSC expansion, are excluded from efficacy evaluation.

Results: Nine patients (3 males/6 females, median age 77 years, median body weight 88.6 kg, median BMI 31 kg/m^2) have been treated so far, 6 of whom were eligible for efficacy evaluation. In these patients, the wound size markedly decreased from baseline to week 12, leading to a median wound size reduction of 63.4% (range 32.1%-100%). Moreover, wound size reduction was accompanied by an early occurring pain relief and life quality improvements. Most of the wound closure was achieved during the first 6 weeks following cell application. In all patients, the therapy was well tolerated without any side effects.

Conclusions: Single topical application of autologous ABCB5$^+$ MSCs facilitated wound healing of conventionally non-curable CVUs. The results confirm ABCB5$^+$ MSCs as a promising therapeutic candidate for the treatment of therapy-resistant CVUs, thus bearing the potential to make a significant contribution towards the serious unmet medical need of patients suffering from non-curable CVUs.

P064 | Hidradenitis suppurativa leads to a profound impairment in quality of life

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Objectives: Hidradenitis suppurativa (HS; also designated as acne inversa) is a chronic disease leading to painful and destructing skin alterations of axillary, inguinal, gluteal, and perianal sites. The primary works by the Jemec and the Matusiak groups many years ago demonstrated adverse effects of HS on the quality of life. The objectives of our study were (i) to characterize the quality of life of the patients currently afflicted with HS, (ii) to identify life quality aspects that are most affected, and (iii) to detect parameters that are associated with the impairment of the quality of life in these patients.

Methods: This was a prospective study involving 460 patients suffering from HS. The following data were analyzed: quality-of-life data (Dermatology Life Quality Index, DLQI questionnaire), demographic, anamnestic, clinical, and labor parameters.

Results: The mean +/− SD DLQI score was 13+/− 8. Approximately 40% and 20% of HS patients declared moderate (DLQI > 10 and ≤20) and severe (DLQI > 20) impairment of life quality, respectively. Strongest alterations were associated with the presence of symptoms like pain, soreness, stinging or itching and to restrictions in

self-feeling. The impairment of life quality positively correlated with clinical disease severity as assessed by Sartorius score and Hurley score. Furthermore, DLQI was associated with the number of involved body regions, in particular the number of regions containing nodules or fistulas. Patients with moderate/severe impairment of life quality more frequently reported about skin alteration at inguinal and gluteal sites. Moreover, such patients significantly more often suffered from back pain, in particular in neck and lower back. We also observed a moderate significant association between DLQI and BMI.

Conclusion: HS continuously causes a profound impairment of the quality of life, which was even higher than for previously studied other skin diseases. The degree of the impairment was associated not only with the extent and localization of skin alterations and pain but also with HS comorbidity. Thus, DLQI should be taken into account when selecting the appropriate therapy.

P065 (OP05/02) | Nummular eczema—a distinct clinical entity with overlapping features of both, psoriasis and atopic eczema

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Background: Nummular eczema (NE) is a chronic inflammatory skin disease that is characterized by multiple, pruritic, discoid-shaped eczematous lesions.

Objectives: We sought to better understand the disease characteristics of NE and compare it to Atopic eczema (AE) and psoriasis.

Methods: A total of 31 patients with NE, 40 patients with AE and 65 patients with psoriasis confirmed by clinical and histological evaluations were included in this study. We compared clinical, histopathological and immunohistochemical as well as RNA sequencing patterns between these groups.

Results: The atopic characteristics, serum IgE levels and blood eosinophils were highly significantly elevated in AE (median total IgE: 627 kU/L; eosinophilia: 71%) compared to NE (105 kU/L; 32%), while there was no significant difference between NE and psoriasis (67.4 kU/L; 19%), although both diseases presented higher IgE levels compared to healthy controls (27.9 kU/L). As expected, colonization with *Staphylococcus aureus* (SA) on lesional skin was most pronounced in AE (85%). However, NE (52%) was significantly more often colonized by SA than psoriasis (17%).

Histologically, lesional epidermis showed greater acanthosis in NE (322 μm) than in AE (230 μm), but was less pronounced compared to psoriasis (484 μm). Significant intralesional neutrophilic infiltration was more often present in NE (52%) compared to AE (8%), while there was no difference considering intralesional eosinophils between AE and NE. Consistent with the clinical and histological data, immunohistochemistry revealed a higher expression of neutrophil elastase (14 vs. 2 cells/HPF) and Ki67 (188 vs. 129 cells/HPF) and a lower expression of the Fcε-receptor (106 vs. 52 cells/ HPF) in NE

compared to AE. Furthermore, RNA sequencing revealed a significant upregulation of the IL-17 pathway in NE, but not in AE.

Conclusion: Clinical and histological evidence suggest that NE is a distinct clinical entity rather than a variant of AE with overlapping features of both AE and psoriasis.

P066 | A novel approach for Hidradenitis suppurativa scoring

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Hidradenitis suppurativa (HS)/acne inversa (AI) is a chronic, recurrent, immune-mediated disease characterized by deep inflammatory nodules, abscesses, fistulas, and undermined scars in skin areas bearing apocrine glands, like intertriginous folds (Sabat et al; Hautarzt. 2017 Dec;68(12) 999-1006). Estimating the severity of the disease and subsequently the efficacy of treatments can be confusing. Several scores have been developed, often lacking consideration of aspects of the disease. Recently, a consensus score, International HS Severity Score System 4 (IHS4), has been developed based on number of typical skin lesions (Zouboulis et al; Br J Dermatol. 2017 Nov;177(5):1401-1409). However, the patient's opinion is not captured. HS leads to strong impairment of the quality of life of the patients resulting to DLQI score 13.28 (Schneider-Burrus et al; unpublished data). Considering this aspect, we proposed a new approach of HS activity scoring implementing the DLQI to the clinical estimation of inflammatory lesions (IL) based on IHS4 score. According to Hidradenitis Activity and Severity Index, HS can be graded to mild (1-4 ILs and 0-10 DLQI), moderate (5-10 ILs or 11-20 DLQI) and severe (>10 ILs or 21-30 DLQI) (Kokolakis, Sabat; JAMA Dermatol. 2018 Aug 1;154(8):971-972). Relying on this grading a new therapy algorithm has been proposed. Henceforth, approximately 200 HS patients will be evaluated to estimate HS severity according to HASI score before and after conservative or surgical treatment. Besides the characterization of the population, a correlation with established HS scores will be performed.

DERMATO-ENDOCRINOLOGY

P067 | The alpha7 nicotinic acetylcholine receptor agonist PHA-543613 has antifibrotic activity in vivo in the scleroderma mouse model

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Our previous results demonstrate that the serotonin receptor antagonist tropisetron inhibited transforming growth factor (TGF)-beta1-induced collagen synthesis in human dermal fibroblasts (HDFs). Tropisetron mediated its anti-collagen activity via alpha7 nicotinic acetylcholine receptors (alpha7nAchR) in HDFs acting as a partial alpha7nAchR agonist. Moreover our in vivo data disclose that tropisetron not only reduced but also prevented experimentally induced skin fibrosis in the bleomycin (BLM) mouse model of scleroderma. To further elucidate the role of the alpha7nAchR in fibrosis, we tested PHA-543613, a full agonist of the alpha7nAchR for its antifibrotic potency in the BLM mouse model of scleroderma. We treated C3H mice for 21 days with BLM alone or in combination with PHA-543613 versus controls. Moreover, in a therapeutic approach we first induced fibrotic conditions in mouse skin using BLM for 21 days and afterwards applied the alpha7nAchR agonist for further 14 days. Our results disclosed an induction of murine collagen type I (COL I alpha I), alpha-smooth muscle actin (alpha-SMA) and connective tissue growth factor (CTGF) expression in mouse skin after BLM treatment alone. PHA-543613 given simultaneously significantly attenuated BLM-induced mRNA expression of COL I alpha I, alpha-SMA and CTGF as shown by quantitative RT-PCR. Interestingly, the alpha7nAchR dramatically improved fibrosis when added in a therapeutic fashion post BLM treatment. Real-time RT-PCR results disclosed a significant reduction of COL I alpha I, COL I alpha II, COL III alpha I, as well as alpha-SMA, fibronectin 1 (FN1) and CTGF in mRNA expression in mouse skin treated with PHA-543613 in comparison with animals treated with NaCl. In accordance, at protein level we found a clear accumulation of collagen type I in mouse skin treated with BLM alone as analysed by pepsin digestion of the skin tissue followed by SDS-PAGE and Coomassie blue staining. In contrast skin samples injected with PHA-543613 in a preventive as well as therapeutic fashion had significantly lower collagen type I deposition compared to BLM-treated skin. These results were also confirmed by histological analyses which disclosed a thickening of the dermis in skin exposed to BLM and a reduction of the dermal thickness after treatment with the alpha7nAchR agonist PHA-543613 in both the preventive and therapeutic approach. Finally, we tested the substance PHA-543613 for its effects also in vitro in HDFs. Therefore, HDFs were treated with the profibrotic factor TGF-beta1 alone and in comparison with PHA-543613 and another full alpha7nAchR agonist AR-R17779. Using quantitative RT-PCR, we could find a strong suppressive effect of both agonists on TGF-beta1-induced collagen type I and III, as well as alpha-SMA, FN1 and CTGF mRNA expression. Mechanistically, PHA-543613 and AR-R17779 elicit their anti-TGF-beta1 effect on collagen synthesis via modulation of specific microRNAs (miRNAs), such as the profibrotic miR-21 and the antifibrotic miR-29a. In detail, TGF-beta1 highly induced the expression of profibrotic miR-21 in HDFs. In contrast, PHA-543613 as well as AR-R17779 attenuated the TGF-beta1-induced increase in mRNA amounts of the profibrotic miR-21 and accordingly induced the mRNA expression levels of the antifibrotic miR-29a. In summary the alpha7nAchR agonist PHA-543613 is a novel potent antifibrotic agent, which further underlines the role of the alpha7nAchR in skin fibrosis. Experiments with alpha7nAchR deficient animals lacking

this gene are in preparation in our laboratory to further clarify the role of this receptor in fibrosclerotic diseases.

P068 | High fat diet exacerbates skin inflammation independent of obesity: Saturated fatty acids as key players

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Epidemiological evidence has linked obesity to the risk and severity of various inflammatory disorders, including type II diabetes, cardiovascular diseases, hepatic steatosis, asthma, neurodegeneration, inflammatory bowel disease, arteriosclerosis, and psoriasis. Consequently, interactions between the adipose tissue, metabolism and the immune system are postulated to be of importance in the pathogenesis of obesity-associated inflammatory diseases. In obesity, hypertrophic adipocytes secret high amounts of adipocytokines resulting in low-grade inflammation amplified by infiltrating pro-inflammatory macrophages, oxidative stress, hypoxia and lipolysis. It is known, that these chronic pro-inflammatory conditions support the development of type II diabetes and cardiovascular diseases, while mechanisms of obesity-related exacerbation of chronic inflammatory disorders are still unclear.

In the present study positive correlation of waist-to hip-ratio to disease severity in plaque type psoriasis patients was confirmed. Consistently, high fat diet induced obese mice develop a more pronounced psoriasis-like skin inflammation. Obesity per se did not alter the pro-inflammatory status of skin and immune cells, but rather renders them more susceptible to pro-inflammatory stimuli.

Free fatty acid (FFA)-serum-levels were identified as an central obesity-associated parameter affecting disease severity. Importantly, an increase of FFAs in healthy, lean mice alone was sufficient to induce an exacerbation of psoriasiform inflammation. Consequently, reduction of nutritional saturated fatty acids (SFAs) alone diminished the psoriatic phenotype in obese mice.

Mechanistic studies revealed that SFAs alone did not affect the pro-inflammatory immune response of myeloid cells but render them more susceptible to pro-inflammatory stimuli. In detail, SFAs sensitize DCs resulting in augmented secretion of TH1/TH17-instructive cytokines upon pro-inflammatory stimulation resulting in amplification of TH1/TH17 immune responses. Similarly, SFAs sensitize macrophages to an increased inflammatory response in answer to pro-inflammatory stimuli which in turn augments the activation of keratinocytes in an IL-1β dependent manner.

In summary, we uncover nutritional SFAs as major risk factors for the amplification of skin inflammation, independent of obesity-related parameters, like fat mass extension, adipocytokines and glucose homeostasis. Thus, our findings open new perspectives for adjuvant dietary measures accompanying anti-inflammatory psoriasis therapies in lean and obese patients.

P069 | New insights into the effects of melatonin on human melanogenesis

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Melatonin (N-acetyl-5-methoxytryptamine) is a ubiquitous physiological mediator that is found throughout the evolutionary scale of animals and also in plants. In mammals, the pineal gland secretes melatonin into the blood circulation to exert a range of well-documented physiological functions. Functionally, this indoleamine has an extensive repertoire of biological activities such as oncostatic, anti-oxidative, anti-inflammatory and anti-apoptotic ones. In this study, we performed an in vitro study on the role of melatonin on melanogenesis employing MNT-1/Sk-Mel-28 melanoma cells as a tool. Based on our expression analysis we detected both MT1 and MT2 in MNT-1 cells, and these G-protein-coupled receptors with seven transmembrane domains bind melatonin and its antagonists with high affinity. Thus, we treated MNT-1 cells with the MT antagonist luzindole (for MT1/MT2) and 4-phenyl-2-propionamidotetralin (4-P-PDOT) (selective for MT2) for 72 hours prior to stimulation with melatonin. We found prominent differences in the cellular growth ratio compared to control samples treated either with melatonin (10^{-9}-10^{-3} M) or with the MT antagonists. Subsequent analysis revealed significant regulation of melanin, both for melatonin itself and when MT1 or MT2 receptors were inhibited. These observations were supported by electron paramagnetic resonance spectroscopy which allowed quantification of melanin within the melanosomes of the cells. A similar pattern of regulation by melatonin and its antagonists was noticed for activity of tyrosinase, the key regulatory enzyme of melanogenesis. In addition to this, we tested the melanogenic inhibitor (kojic acid) and stimulator (alpha-melanocyte-stimulating hormone or forskolin). Experiments performed using these modulators in comparison with luzindole or 4-P-PDOT revealed that melatonin acts as a potent regulator of melanogenesis.

P070 (OP04/02) | The secretome of skin cancer cells activates mTOR in healthy keratinocytes to convert them into tumorigenic cells

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The secretome of cells has been defined as a class of proteins consisting of extracellular matrix and secreted proteins, such as cytokines, chemokines, growth factors and proteases. 3000 genes encode proteins that are secreted; these are almost 11% of all human genes. While most proteins are secreted by glands like the pancreas or salivary gland, nevertheless in the skin the secreted protein fraction ranges between 10 and 20%. The secretome is an attractive reservoir of druggable proteins, 15% of all FDA-approved drugs target secreted proteins. The secretome of cancer cells has recently been the object of many oncoproteomic studies, as it represents a pool of putative tumor biomarkers and pharmaceutical targets. Nevertheless, it is still not known how or if the secretome is affecting the surrounding healthy cells during tumorigenesis. Skin cancer is the most common type of cancer amongst Caucasians, with up to 3 million non-melanoma skin cancer (NMSC) cases per year worldwide, and one in every three cancer-diagnosis is a skin cancer. NMSC arises from keratinocytes, and based on the cell types, it can be divided into basal cell carcinoma (BCC) or squamous cell carcinoma (SCC). To identify the secretome of healthy human keratinocytes and NMSC cells, we employed the following approach: We cultured human keratinocytes (HaCaT) and A431 cancer cells in serum-free medium for 24 hours and collected the cells and the culture medium (CM) containing the secretome. Both fractions were analyzed by tandem mass spectrometry. After quantification, we determined the proteins enriched in the CM vs. the cell pellet and analyzed the data bioinformatically. We characterized 620 proteins enriched in the CM of HaCaT cells and 603 in the CM of A431. In a second experiment, we incubated HaCaT keratinocytes for 24 hours with the CM of A431 cancer cells and analyzed the transcriptome by next-generation sequencing. We identified more than 1.000 significantly up- or down-regulated genes in the transcriptome of HaCaT cells incubated with the CM of A431 cells compared to control HaCaT cells. We found tumor suppressor genes like TP53, CDKN2A and PTEN to be down-regulated in keratinocytes incubated with the secretome of A431 compared to normal incubated HaCaTs, while the mRNA of proto-oncogenes like MYC, EGFR and MET were up-regulated. On protein level, we confirmed an increased expression of MYC and MET in HaCaT cells incubated with A431 secretome compared with control HaCaTs, and an increased phosphorylation of EGFR, but a decreased phosphorylation of the tumor suppressor protein PTEN. To investigate cell migration and proliferation, we did a wound healing experiment and revealed that HaCaT cells treated with CM of A43 showed a faster gap closure than control cells. Interestingly, we found a significantly increase of activated mammalian target of rapamycin (mTOR) complex and one of its most prominent downstream targets S6 ribosomal protein in A431-CM treated HaCaT cells compared to nontreated control cells. Proteins regulating mTOR are often overexpressed in cancer activating the mTOR complex to keep the cancer machinery running. Further, we inhibited mTOR by rapamycin and showed that an inhibition of mTOR results in a delayed gap closure in HaCaT cells incubated with A431-CM. In conclusion, the results demonstrate that the secretome of cancer cells exerts an important impact on the transcriptome of healthy cells and the mTOR complex plays a major role in converting healthy cells into tumorigenic cells.

P071 (OP06/04) | Breaking epidermal stem cell quiescence in cutaneous wound healing

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Skin aging is a complex biological phenomenon characterized by multiple morphological and physiological changes. Consequently, skin becomes fragile and vulnerable, is prone to non-healing ulcers and is more susceptible to irritating environmental factors and allergens. Using comprehensive whole genome expression profiling, we previously showed, that Wnt signaling is significantly downregulated in aged human skin of both genders. In this study we aimed to investigate the expression of Wnt receptor, fzd7 (frizzled 7) in epidermal stem cells, whether fzd7 affects epidermal stem cell homeostasis and—if so—whether this may impact on overall wound healing. Epidermal bulge and basal stem cells were isolated from the epidermis of young (6-8 weeks) and aged (22-24 months) wild-type mice and after staining for CD34 and α6 integrin (ITGA6) were analysed by means of FACS sorting. The bulge stem cell population (ITGA6high/CD34high cells) was significantly reduced in old mice as compared to young mice ($P < 0.01$). mRNA and protein expression levels of fzd7 and corresponding downstream effectors showed a significant downregulation of JUN, RhoA, cttnb1 ($P < 0.01$) as well as of cdc42 ($P < 0.001$) in the old epidermal bulge stem cells. Fzd7 expression was higher in the basal stem cells (ITGA6high) as opposed to more differentiated epidermal cells expressing less α6 integrin (ITGA6low) and its expression was significantly reduced in old basal stem cells. Interestingly, high expression of fzd7 significantly regulated number and morphology of epidermal basal and bulge stem cell colonies grown in vitro as observed by means of crystal violet staining. Lentiviral transduction of old basal stem cells with fzd7, remarkably induced the expression of stem cell markers as detected by FACS, implying that induced fzd7 expression at least in part rejuvenated old epidermal stem cells. Of note, fzd7 expression was significantly changed in the epidermis during wound healing of young mice. In order to further elucidate the role of fzd7 expression and how its expression correlates with stem cell activation in cutaneous trauma, a newly established pTRE-fzd7[+] K5tTA mouse model was employed for functional wound healing assays. These mice are characterized by high expression of fzd7 in the basal stem cell layer of the epidermis. Taken together, fzd7 was found to play a crucial role in epidermal stem cell homeostasis. Downregulation of fzd7 as

occurring with age most likely contributes to epidermal aging due to reduced self-renewal of epidermal stem cells. It will be interesting, whether this, in consequence, may promote age-associated wound healing disorders.

P072 | Prolylcarboxypeptidase—an emerging player in immune-mediated inflammatory skin diseases?

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Prolylcarboxypeptidase (PRCP) is s serine protease that regulates a number of hormonal pathways including the proopiomelanocortin system. Accordingly, PRCP metabolizes alpha-MSH (1-13) into biologically inactive alpha-MSH (1-12). Alpha-MSH (1-13) has a variety of biological functions in the skin, such as regulation of pigment formation, exocrine activity, inflammatory reactions and immunomodulation. As alpha-MSH has strong anti-inflammatory and immunomodulatory actions we hypothesized that PRCP due to de-activation of the biological activity of alpha-MSH could play role in the pathogenesis of the immune-mediated skin diseases. As a first step towards answering this question we examined the expression of PRCP in various cutaneous cell types at RNA and protein level in vitro. PRCP transcripts were detected in normal human melanocytes, normal human keratinocytes and human dermal fibroblasts (HDFs) from different donors as shown by endpoint reverse transcriptase-polymerase chain reaction (RT-PCR). However, Western immunoblotting revealed expression of PRCP protein only in HDFs but not in the other cell types. Here, PRCP could be visualized as a granular cytoplasmic staining as shown by immunofluorescence analysis. As detected by liquid chromatography coupled with mass spectrometry, HDFs treated with alpha-MSH formed alpha-MSH (1-12) in conditioned media suggesting expression and secretion of active PRCP. Interestingly, quantitative real-time RT-PCR analysis further revealed a time-dependent upregulatory effect of both tumor necrosis factor and ultraviolet A irradiation but not interleukin-1beta on PRCP mRNA in HDFs. However, Western immunoblotting of total cell lysates and detection of PRCP in cell culture supernatants employing ELISA did not reveal upregulation of this enzyme in HDFs exposed to these stressors. Immunohistochemical studies are currently underway to determine the expression pattern of PRCP in normal and diseased human skin in situ. In sum, our data provide first evidence of cell type-specific expression of PRCP in human skin types. Further studies have to assess the relevance of these findings for cutaneous biology.

DERMATOPATHOLOGY

P073 | Analysis of the diagnostic value and practicability of serration pattern analysis by direct immunofluorescence microscopy in pemphigoid diseases

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Pemphigoid diseases (PDs) are characterized by circulating and tissue bound autoantibodies against structural proteins of the dermal-epidermal junction (DEJ). The diagnostic gold standard of PDs is direct immunofluorescence microscopy (IFM) which shows linear immunodeposits (IgG, IgA, and/or C3) at the DEJ in perilesional skin biopsies. More than a decade ago, the concept of serration pattern analysis (SPA) has been proposed in PDs describing two patterns of antibody deposition along the DEJ characterized by different undulation of the immunodeposits, i.e. nand u-serrated. A u-serrated pattern shows arches open at the top resembling "growing grass" and is only seen with deposition of type VII collagen-specific immunodeposits found in epidermolysis bullosa acquisita (EBA) and bullous systemic lupus erythematosus. SPA is of particular importance in EBA since serum autoantibodies can only be detected in about half of the EBA patients. The aim of the present study was to determine the portion of PD patients in whom the serration pattern could be identified by direct IFM and optimal thickness of sections. Reports of all direct IFM of skin biopsies compatible with PD between January 2014 to June 2016 (n = 226, 6 μm) were analyzed. All biopsies without SPA or an undetermined pattern (n = 83) were then reexamined in 6 and 4 μm sections. In 74.3% (168/226) of biopsies, a pattern was recognized including 94.6% (159/168) with an n- and 5.4% (9/168) with a u-serrated pattern. Pattern detection frequency was 74.3% and 66.8% in 4 μm and 6 μm sections, respectively. Overall, a serration pattern was determined in 78.3% of bullous pemphigoid, 50% of mucous membrane pemphigoid, 60% of anti-p200 pemphigoid, 66.7% of linear IgA disease, and importantly, in 100% of EBA patients. In none of the PD patients where SPA remained undetermined and serum was available (n = 44), antibodies against type VII collagen were detected by various approaches. No difference between the detection frequencies of the serration pattern, irrespective of n- or u-pattern, was seen with regard to the age and sex of patients and biopsy site. In conclusion, while the sensitivity of SPA by routine direct IFM in non-EBA PDs was 73.3%, the sensitivity and specificity in EBA reached 100%.

In summary, SPA can be performed on both 4 μm and 6 μm sections. The serration pattern could be determined in all EBA and 73.3% of all other PDs. U-serrated pattern was always recognized at the first time, which implicates that no u-serrated pattern remains undetected. Serration pattern analysis is easy to implement if the

specific laboratory already performs direct IFM and increases the possibility to diagnose EBA patients, especially in a seronegative situation.

P074 | JAK1/3-inhibition preserves epidermal morphology in full thickness 3D skin models of atopic dermatitis and psoriasis

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Background: Janus kinase (JAK) inhibition may be a promising new treatment modality for inflammatory (skin) diseases. However, little is known about direct effects of kinase inhibitors on keratinocyte differentiation and function as well as skin barrier formation.

Objective: Our aim was to address the direct impact of kinase inhibition of the JAK1/3 pathways by tofacitinib on keratinocyte immune function and barrier formation in atopic dermatitis (AD) and psoriasis.

Methods: 3D skin equivalents of both diseases were developed and concurrently pretreated with tofacitinib. To induce AD, 3D skin equivalents were stimulated with recombinant human IL-4 and IL-13. Psoriasis-like conditions were induced by incubation with IL-17A, IL-22 and tumor necrosis factor α (TNFα). The activation of signal transducer and activator of transcription (STAT) 1, STAT3 and STAT6 was assessed by western blot analysis. Microarray analysis and quantitative real-time PCR were used for gene expression analysis.

Results: Tofacitinib pretreatment preserved epidermal morphology and reduced STAT3 and STAT6 phosphorylation of AD-like and STAT3 phosphorylation of psoriasis-like culture conditions in 3D skin models compared to sham-controls. Filaggrin expression was fully maintained in the AD-like models, but only partially in psoriasis-like conditions after pretreatment with tofacitinib. In addition, tofacitinib upregulated DSC1, FLG and KRT1. Using gene expression analysis, downregulation of POSTN and IL24 was observed in AD-like conditions whereas downregulation of IL20 and IL1B was observed in psoriasis-like conditions.

Conclusion: JAK1/3 inhibition counteracted cytokine-induced AD- and psoriasis-like epidermal morphology and enhanced keratinocyte differentiation in 3D skin models. This effect was more pronounced in the AD-like models compared to the psoriasis-like 3D skin models.

P075 | Ex vivo confocal laser scanning microscopy: an innovative method for direct immunofluorescence of cutaneous vasculitis

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Ex vivo confocal laser scanning microscopy (ex vivo CLSM) offers an innovative diagnostic approach in dermatology. It provides vertical scanning of the skin samples and allows examination of all skin layers with a resolution close to conventional histology. In addition to assessment of general morphology, it enables fluorescence detection in the tissue and offers an alternative method to conventional fluorescence detection systems in the diagnosis of skin lesions.

Diagnosis of cutaneous vasculitis is based on clinical, histological and direct immunofluorescence (DIF) findings. Conventional DIF examination provides evidence of tissue bond antibodies along the vessel wall using fluorescence microscope, which is of great importance in Henoch-Schonlein purpura. Ex vivo CLSM has been shown to be helpful in fluorescence immunohistochemistry examination of melanoma; however, its use in the diagnosis of cutaneous vasculitis has not been published yet. In this study, we aimed to assess the applicability of ex vivo CLSM in the diagnosis of cutaneous vasculitis and compare its diagnostic accuracy with conventional DIF.

82 sections of 49 vasculitis patients with relevant DIF findings were examined with ex vivo CLSM following the same staining protocol as conventional DIF examination using FITC-labeled anti-human antibodies. Two ex vivo CLSM specialists, one of them trained in histology and the other in direct immunofluorescence techniques, assessed the obtained ex vivo CLSM images. DIF showed immunoreactivity of vessels with IgG, IgM, IgA, C3 and Fibrinogen in 2.0%, 49.9%, 12.2%, 59.2% and 44.9% of the patients, respectively. Ex vivo CLSM detected positive vessels with the same antibodies in 2.0%, 38.8%, 8.2%, 42.9% and 36.7% of the patients, respectively. Detection rate of positive subepidermal vessels was significantly higher in DIF examination as compared to ex vivo CLSM ($P < 0.05$). Positive dermal vessels were identified in a higher number of patients using ex vivo CLSM as compared to DIF; however, the difference was not statistically significant.

In conclusion, ex vivo CLSM could identify specific binding of the antibodies in subepidermal as well as dermal vessels and showed a comparable performance to conventional DIF in diagnosing vasculitis. This study offers the first view on the possibilities of detecting skin-bond antibodies with the use of ex vivo CLSM.

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P076 (OP01/02) | The role of HIF1a in skin: trying to preserve epidermal homeostasis during mitochondrial dysfunction

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During aging, skin changes functionally and morphologically due to intrinsic and extrinsic factors such as environmental features. Accumulation of mitochondrial DNA (mtDNA) deletions has been related to skin aging; however, whether this causally contributes to aging related skin alterations and through which molecular mechanisms is still unclear.

To further understand these mechanisms, we have previously generated a mouse model in which mtDNA deletions and mtDNA depletion occurs only in the epidermis (K320E-TwinkleEpi). For that purpose, we expressed a mutated form of the mitochondrial helicase TWINKLE in Keratin 14-expressing tissues such as the basal layer of the epidermis. Due to its high proliferation rate, those mice showed low amounts of deletions but a dramatic mtDNA depletion, leading to an imbalanced stoichiometry of mitochondrial respiratory chain complexes. We had demonstrated before that the mitochondrial respiratory chain is dispensable for epidermal proliferation and differentiation (Baris et al., 2011). In agreement, K320E-TwinkleEpi mice showed no developmental or differentiation defects in back skin, however they featured a severe inflammatory phenotype in ventral skin and died between postnatal days 5 and 8 (Weiland et al., 2018). Importantly, K320E-TwinkleEpi mice showed low glucose levels and high lactate levels in blood, pointing to lactic acidosis as the most probable cause of perinatal death. Moreover, transgenic mice had upregulated expression of the transcription factor Hypoxia Inducible Factor 1 alpha (HIF1a).

HIF1a plays a crucial role in hypoxia, promoting a metabolic switch from oxidative phosphorylation to glycolysis as an alternative source of energy and also promoting angiogenesis to restore oxygen supply. It has been described that HIF1a is constitutively expressed in the basal epidermal layer, however, its absence in epidermal keratinocytes (HIF1aEKO) did not show skin dysfunction until the mice were 6 months, when they featured age-like skin defects together with basement membrane disturbances (Rezvani et al., 2011). Therefore, we hypothesized that in the absence of HIF1a lactate production in K320E-TwinkleEpi mice will be prevented avoiding perinatal death. To prove this, we generated mice expressing K320ETwinkle in the absence of HIF1a in the epidermal compartment (TwinkleHIFepi).

By knocking-out HIF1a we could restore glucose and lactate levels and no differentiation defects were found in the skin of those mice at birth. HIF1a target genes related with glycolysis, such as Pdk4, Hk2 and Slc2a1, that were upregulated in K320E-TwinkleEpi mice, were normalized in TwinkleHIFepi mice. The expression of Hk2 and Slc2a1 was even lower compared to the controls. Similarly, the proangiogenic HIF1a target genes, Vegfa and Adm, were also upregulated in K320E-TwinkleEpi mice but normalized again in TwinkleHIFepi mice to levels comparable to control mice. However, TwinkleHIFepi mice died already at postnatal day 0 to 1.

The analysis of HIF1a target genes related with inflammation revealed an increased expression of the pro-inflammatory cytokine Il1b and the vasodilator Nos2 in TwinkleHIFepi but not in K320E-TwinkleEpi mice, suggesting that this response could contribute to the early death of the mice.

In conclusion, HIF1a is necessary for the metabolic and proangiogenic phenotype of the basal epidermal layer. Thus, it may play an important role in keratinocytes preventing skin aging features derived from mtDNA deletions and mitochondrial dysfunction during aging.

P077 | Radiation dermatitis: treatment with cold atmospheric pressure plasma and pathomechanistic assessments in mice

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About 95% of all cancer patients receiving a radiotherapy develop a radiation dermatitis caused by radiation damage of skin tissue leading to erythema, hair loss, 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), an ionized mixture of gases, provides an innovative therapy option that exerts antiseptic and anti-inflammatory properties and supports tissue regeneration as well. Therefore, this study aims to assess the clinical course and the molecular pathomechanism of a radiation dermatitis as well as their modulation by treatment with CAP. For this purpose, an acute radiation dermatitis is induced in a nude mouse model and the course of the disease is monitored closely using a scoring system and objective measures such as hyperspectral imaging and laser scanning microscopy. After identifying the optimal radiation dose to induce a moderate radiation dermatitis (score 2.5) the effected skin will be treated with CAP using a dielectric barrier discharge plasma source and compared to a non-treated control group. Skin biopsies will be taken for immunohistochemistry and transcriptome analyses. Molecular analyses of treated and untreated tissue will help to understand the pathomechanisms of a radiation dermatitis and how these are modulated by CAP. As CAP has been shown to support wound healing and regenerative processes without causing any relevant side effects, we expect it to also reduce the severity of radiation dermatitis and hence CAP may help to reduce the acute side effects of a radiotherapy in cancer patients allowing an uninterrupted treatment.

P078 | The power of neural networks to detect cutaneous basal cell carcinomas in histological sections

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Basal cell carcinomas (BCCs) represent the most common malignancy in humans. Due to their high numbers, pathologists have limited time for the judgement of histologic sections of this tumor entity. Digital pathology is a tool to improve and simplify histologic diagnoses in terms of safety, quality and efficiency. The next step in digital pathology will be to further improve these features by providing diagnosis suggestions to the pathologists. This can be achieved using artificial intelligence: machine learning methods. The aim of the study was to investigate, whether it is possible to detect basal cell carcinomas in anonymized histologic sections using state-of-the-art machine learning methods.

Slides of normal skin and skin sections containing BCCs were stained with hematoxylin and eosin (H/E). We implemented a convolutional neural network (CNN), which was trained to classify H/E sections as 'contains BCC' or 'does not contain BCC'. Standard statistical parameters like accuracy, sensitivity and specificity were calculated.

The CNN was able to decide with an accuracy of 84%, whether an H/E stained section showed normal skin or a skin section containing a BCC. Therefore, we propose that a CNN is a proper method to detect BCCs in histological, H/E stained sections and might be also capable of distinguishing different cutaneous lesions from each other. CNNs have the potential to aid pathologists in decisions about malignancy and further tracking of a lesion.

P079 | IL23 differentially regulates cytokine profile of Th17 subsets leading to deviation of protective and pathogenic Th17 cells

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Th17 cell plasticity has two poles: a protective subset expressing IL-17 and IL-10 and a pathogenic Th17 subpopulation secreting IL-17 and IFN-gamma as well as IL-22 and GM-CSF. While the latter one is implicated in the pathogenesis of psoriasis, regulatory factors driving Th subsets into one or the other direction are largely unknown. IL-23 is a cytokine that is known to drive differentiation and expansion of Th17 cells. Memory T cells in the skin of psoriasis patients are hypothesized to express IL-23R and readily secrete inflammatory cytokines like IL-17, IL-22, IFN-gamma and TNF-alpha upon IL-23 exposure. The aim of our study was to characterize, the role of IL-23 in shaping pathogenic versus protective human Th17 cells.

Therefore, we analyzed the cytokine profile of human CD4^{+} T cells as well as memory CD4^{+} T cells that were stimulated in the presence of IL-23 or under IL-23 neutralizing conditions. Moreover, a potential effect of IL-23 not only on effector function of Th17 cells, but also on Th17 differentiation was assessed by in vitro polarization of naïve T cells in the presence of IL-23 or under IL-23 neutralizing conditions. Cytokines were measured by ELISA and by intracellular cytokine staining for FACS analysis to assess cytokine co-expression at single cell level.

We observed slightly decreased IL-17 as well as IFN-gamma level while IL-10 was upregulated under IL-23 neutralizing conditions. We could show that IL-23 has a differentially regulating effect at the cytokine profile of Th17 cells shaping a pathogenic Th17 phenotype, while neutralization of IL-23 reversed this effect emphasizing the high potential of this target for therapeutic approaches in Th17 driven diseases.

P080 (OP02/04) | Transit amplifying cells, the key players of epidermal renewal, are expanded in psoriatic lesions and show profound transcriptome and partly associated methylome alterations.

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Despite decades of intensive research, the primary molecular processes leading to the cutaneous alterations in psoriasis are still incompletely understood. The knowledge gained by most studies of psoriatic skin is often a priori limited by the use of whole skin biopsies.

In our study, we focused on transit amplifying cells (TA cells), constituting the primary proliferating keratinocyte subpopulation in the epidermis. These cells were isolated from psoriatic lesions and healthy control skin and subjected to an integrated multiomics analysis.

Our analyses revealed that already in TA cells derived from non-lesional psoriatic skin, 1.7% of human protein-coding genes were affected by altered methylation compared to healthy skin TA cells. Interestingly, only <1% of these methylation changes were associated with respective transcriptional changes, suggesting external triggers not present in non-lesional skin to be essential for the development of psoriatic lesions.

In psoriatic lesions the numbers of TA cells but not of epidermal stem cells were strongly elevated and methylation changes were strongly increased concerning more than 15% of protein-coding genes. Surprisingly, the majority of these alterations represented hypermethylations. Compared to non-lesional skin, TA cells from persisting lesions comprised 22-fold more transcriptional changes, and 17% thereof were associated with respective methylation changes. In line with these data, ~40% of proteins associated with lesional TA cells were not present in TA cells derived from non-lesional and healthy skin.

Furthermore, we identified the hallmark psoriatic skin markers S100A9 and keratin 16 to be active already in the TA cell subtype.

In summary, our data suggest that TA cells, known to mediate epidermal renewal and psoriatic hyperproliferation, are altered at the epigenetic level already in the nonlesional skin of psoriasis patients. The occurence of psoriatic lesions is associated not only with an increase of these cells but also with a strongly altered transcriptome that already comprises features characteristic for the suprabasal psoriatic epidermis. Our results furthermore imply that longterm persistence of psoriatic lesions may affect the epigenetic pattern of keratinocyte subtypes.

P081 | IL-17 pathway in hidradenitis suppurativa

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Hidradenitis suppurativa (HS), also known as acne inversa, is a chronic inflammatory disease characterized by painful skin lesions with destruction of skin architecture and purulent exudate. Despite the high burden for the patients, pathogenetic pathways underlying HS remain obscure. As IL-17 is a key player in different chronic inflammatory conditions, we wondered if pathways involving IL-17 family members are also active in HS. Expression studies in lesional skin of respective patients indicated massive levels of IL-17A and IL-17F, which even exceeded those in lesional skin of psoriasis patients. While expression of IL-17C and IL-17E was also increased in HS lesions compared to healthy skin, levels did not reach those in psoriasis. IL-17B and IL-17D were even downregulated in HS lesions compared to healthy skin. Flow cytometry-based separation of immune cell populations present in lesional HS skin revealed IL-17A and IL-17F production specifically by CD4$^+$ T cells. Detailed examination of CD4$^+$ cells identified the CD25$^-$/CCR4$^+$/CCR10$^-$/CCR6$^+$/CXCR3$^-$ subpopulation, which clearly populates the HS skin, as main IL-17 producers.

The effects of IL-17A/F on cells are mediated by a transmembrane receptor complex composed of IL-17RA and IL-17RC. Expression of receptor components in lesional skin was upregulated compared to that in perilesional skin of HS patients. Cell-specific studies assigned their expression mainly to keratinocytes and dermal fibroblasts, while the expression in dermal endothelial cells was rather low. To shed light on the concrete pathways IL-17A/F is involved in HS, we combined transcriptome analysis of HS skin and functional studies in vitro. Cutaneous RNA-seq and knowledge-based expression analyses allowed the systems biological creation of a correlation network for the HS skin. Specific elements of the IL-17 pathways (including both induction and effects of IL-17) were substantiated by investigating the generation/function of IL-17-producing CD4$^+$ cells under different conditions and the effects of IL-17 on keratinocytes and dermal fibroblasts. Results revealed the promotion of neutrophilic infiltration as major function of IL-17 in HS skin, and that this function is specifically enhanced in the presence of another key player in HS, TNF-a. This therefore suggests IL-17 as being a crucial factor of the purulent exudation seen in HS and a potential therapeutic target for this condition.

P082 | Non-invasive diagnostic approaches for psoriasis and lupus erythematosus

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Background: Both scarring alopecias and psoriasis can cause diagnostic challenges in cases of minimal disease activity or in certain anatomical localisations such as the palmoplantar area. We aimed to explore non-invasive techniques which can add meaningful molecular information to support clinical diagnostic.

Methods: Here we report the diagnostic value of two non-invasive techniques, plucked hair follicles and epidermal sampling by tape stripping. 5-10 hair follicles from the scalp of patients with CDLE, psoriasis as well as healthy volunteers were plucked, cryosectioned, followed by RNA isolation. Hair follicle gene expression was analysed by microarray and quantitative real time PCR. Tape stripping was performed (10 consecutive CuDerm discs) from lesional psoriatic and eczematous skin and from other erythrosquamous diseases as well as from non-lesional and healthy skin.

Results: A number of diagnostic features of CDLE can be linked to differentially expressed mRNA in epidermal material from plucked hair and these include genes related to apoptotic cell death, interferon signature, complement activation and CD8^{+} T cell immune responses. The expression profiles of plucked hair follicles parallels published expression profiles of CDLE full biopsy lesions. Our tape stripping results show that IL-36γ is a reliable biomarker for the diagnosis of psoriasis. Its high expression in psoriatic lesional skin allowed differentiation in clinically challenging cases, where even skin biopsy failed to provide diagnostic certainty.

Conclusions: Our results suggest that information obtained from plucked hair follicles may be sufficient to diagnose scalp LE. However, this hypothesis has to be further validated in routine clinical settings. Quantification of IL-36γ levels in tape stripping samples is a highly specific diagnostic tool to identify psoriasis. As non-invasive diagnostic methods, both hair follicle analysis and tape stripping can complement or even replace more invasive techniques in the future and facilitate early diagnosis.

EPIDEMIOLOGY

P083 | Exposed children – protected parents: Shading of playgrounds as an intervention field of skin cancer and dermatological prevention

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For the prevention of skin damage and skin cancer from UV radiation, childhood represents a critical period of life. For children playgrounds are major resources for physical activity and social interaction. However, children in playgrounds are often exposed to sunlight. Against this background, it is surprising that sun exposure and sun protection on playgrounds have internationally hardly been investigated so far. The present PLAyground Study MAnnheim ("PLASMA") addresses this research gap, by evaluating and quantifying the availability of shade in the total playground area and the main play area for children of 144 playgrounds in the German city Mannheim by innovative GIS technique (using GPS data, Google Maps, Google maps Calculator and Google Street View). In addition, associations with spatial characteristics (neighbourhood context, population density, social deprivation) were investigated by using Hierarchical Linear Modelling. The proportion of shaded area in the total playground area averaged 41.33% 0.27%, while the main play area for children was, on average, only 22.26% 21.10% in the shade. In many cases, children play totally unprotected under direct sun exposure while parents and educators are in the shade. These findings were independent of socio-spatial characteristics.

P084 | Sunless tanning products: Healthy alternative or risky addition to tanning bed use?

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Introduction: Sunless tanning products (STPs) are often promoted as "healthy alternative" to indoor and outdoor tanning. However, STP use may entail indirect risks, because individuals using STPs may tend to think that they have a darker skin tone, which in turn may result in risky tanning behavior, because they think their (overestimated) skin tone allows them to spend longer on tanning beds or in the sun without using sunscreen. We aimed to investigate whether STP use is more common among tanning bed users (overestimation hypothesis) or whether STP users have a higher skin cancer risk and a higher risk awareness (healthy alternative hypothesis).

Methods: We used data from wave 2 of the National Cancer Aid Monitoring on Sunbed Use (NCAM), a nationwide representative cross-sectional sample (n = 3000, aged 14-45, 48.6% female) interviewed via telephone. Differences between STP users and nonusers regarding the abovementioned aspects were identified using chi-square-test.

Results: Tanning bed users showed a higher prevalence of STP use than past and never users (16.1% vs. 9.6% vs. 5.8%, P < 0.05). Although STP users had a higher skin cancer risk based on individual characteristics, they were less likely to have participated in a skin cancer screen. In addition, they were less risk aware regarding the risks of tanning beds than nonusers of STPs.

Conclusion: We found a parallel use of STPs and tanning beds which supports the overestimation hypothesis. As mentioned above, this can have severe health consequences, because the "fake tan" of STPs may lead to an overestimation of the individual's skin type, which may result in risky tanning behavior. The higher skin cancer risk of STP users may support the healthy alternative hypothesis; however, STP users were less risk aware, which contradicts this hypothesis. The results underline the importance of target group-specific prevention to promote as safe use of STPs.

P085 | How important are attractiveness-related motives for tanning behavior? Findings from a nationwide representative survey

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Background: Previous studies showed that attractiveness-related motives for tanning (ARM) are an important aspect for tanning behavior. We aimed to analyze ARM in a large representative sample with regard to sociodemographic characteristics and the perceived risk of ultraviolet radiation (UVR).

Methods: We used data from the nationwide representative National Cancer Aid Monitoring on Sunbed Use (NCAM; wave 3), which includes 3000 participants aged 14-45 years. We explored associations of ARM with various sociodemographic characteristics, tanning behavior, and risk perception of UVR.

Results: ARM were more likely to be relevant for participants aged 18-35, participants with immigrant background, participants with a medium level of education, and participants without a partner. Those who highly agreed with ARM showed a lower risk perception regarding UVR, were more likely to tan indoor and outdoor, and had a higher sunburn frequency in summer. In predicting indoor and outdoor tanning behavior, we found the highest association for ARM (OR = 3.56 and OR = 10.24, both $P < 0.001$), while sociodemographic characteristics and risk perception played a rather minor role as factors preventing from UVR exposure.

Discussion: The NCAM showed that persons who tan with the aim to increase their attractiveness are more likely to have a lower risk awareness regarding UVR and to show risky tanning behavior. Therewith, they have a greater risk for developing skin cancer. Instead of only emphasizing risks of UVR, future prevention measures should also focus on the reduction of the positive evaluation of tanned appearance and therewith a change in Western beauty ideals.

P086 | Frequency and comorbidities of eczema in an elderly population in Germany: results from augur

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Background & Objective: There is a lack of knowledge on the epidemiology of inflammatory skin diseases in the elderly. A recent review (1) discovered only few studies on the prevalence of atopic eczema (AE) or hand eczema (HE) in the aged population. In addition, co-morbidities of eczema in the elderly have been insufficiently investigated thus far. Therefore, the aim of this study was to investigate the prevalence of atopic and hand eczema and associated comorbidities in an elderly mobile population in Germany.

Material & Methods: We analysed baseline data of the AugUR study (2), a cohort study in an elderly population conducted in Regensburg and surrounding areas (n = 1133; 45% female, median age: 76.7), with a focus on disease frequencies and the role of relevant comorbidities.

To assess differences between persons with and without AE or HE, Pearson's chi-square test or Fisher's exact test was used. Analyses were performed using SAS.

Results: Among the1133 persons analysed, 3.3% (95%-confidence interval (CI) 2.31-4.47) reported a previous diagnosis of AE (59% female). Frequency of AE was highest in the group aged 85-89 years (4.4%, 95%-CI 1.22-10.99) and lowest in those aged 75-79 years (2.4%, 95%-CI 1.72-4.43).

16.2% of participants with AE reported a diagnosis of asthma as compared to 6.4% without AE ($P = 0.033$). Rheumatism (24.3% vs. 13.2%, $P = 0.053$) and psoriasis (13.5% vs. 5.3%, $P = 0.0497$) tended to be more prevalent in patients with AE.

2.7% (95%-CI: 1.79-3.76) of study participants reported a previous diagnosis of HE (43% female). Frequency of HE was highest in the group aged 90-95 years (3.9%, 95%-CI 0.10-19.64), lowest in those aged 80-84 years (0.5%, 95%-CI 0.01-2.67). Psoriasis was associated with HE (16.7% vs. 5.3%, $P = 0.0219$) and rheumatism tended to be more prevalent in participants with HE (26.7% vs. 13.2%, $P = 0.0523$).

There were no further significant associations with atopic or relevant metabolic disorders for AE or HE.

Discussion: To our knowledge this is the first population-based study on atopic and hand eczema in highly aged individuals. The disease frequencies we found are lower than previously reported estimates. Wolkewitz et al. (3) reported a lifetime AE prevalence of 4.3% in an elderly population in Germany. Thyssen et al. (4) calculated a median weighted lifetime HE prevalence of 15% in a systematic review. More men than women reported hand eczema in our study, which was surprising. The association of AE and asthma is well established.

The associations between HE or AE and psoriasis might be results of misclassification bias, but recent research indicates that there may be also a psoriasis-eczema disease spectrum.

Epidemiological studies on skin diseases in the elderly with validated diagnoses and long-term follow-up observation are warranted.

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P087 | Comparing keratinocyte carcinoma in high-risk outdoor professions and a control group

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Background: Main risk factor for keratinocyte carcinoma (KC), the most common cancer worldwide, is solar ultraviolet radiation (UVR), which has led to the recognition of KC as occupational disease in Germany. However, outdoor occupations are very diverse with substantial differences in UVR exposure.

Objective: To compare KC and associated protective behavior in different high-risk outdoor professions and indoor workers as a control group.

Methods: Cross-sectional study among mountain guides, gardeners and farmers as well as office workers (control group) using a full body skin examination by board certified dermatologists and self-filled questionnaire on UVR awareness and exposure as well as protective behavior.

Results: A total of 348 outdoor workers (38.8% farmer, 35.3% gardener, 25.9% mountain guides) and 215 office workers in Bavaria, Southern Germany participated in the study between March and September 2017 (46.9% women, 46.9 13.8 years).

Significant differences of UVR exposure as well as preventive behavior were seen between the indoor and outdoor but also between the three outdoor professions (indoor workers 61.4%, mountain guides 57.8%, farmers 31.9%, gardeners 27.6%). KC incl. actinic keratosis was diagnosed in 33.3% of mountain guides, 27.4% of farmers, 19.5% of gardeners and in 5.6% of indoor workers with significant differences between the outdoor professions and mountain guides at highest compared to farmers (OR = 2.6, 95% CI = 1.2-5.7).

Conclusion: Different outdoor professions carry different risks for KC and show different risk behavior and awareness. Future prevention and awareness strategies therefore should be tailored to the specific needs and requirements of the respective target population.

P088 | Treatment of moderate-to-severe atopic dermatitis (AD) after the approval of the first biologic agent: results from the german national registry TREATgermany

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TREATgermany collects comparative real life clinical data on patients with moderate-to-severe AD to generate information on the effectiveness and safety of therapies under real-life conditions. These include disease severity scores and patient-reported outcomes using validated measurement instruments. Here, we describe the results of treatment modalities, with a special focus on patients who received dupilumab, and satisfaction of patients included in the registry from June 2016 until August 2018.

A total of 500 patients were recruited. At baseline, the mean objective SCORAD was 40.014.8 and the mean EASI was 14.611.2. The mean number of completely controlled weeks was 2.23.3 out of 12.

49.2% of the patients had received one or more systemic medications (excluding systemic corticosteroids) prior to baseline with cyclosporine being the most frequently used drug (40.2%). 7.6% of the patients had received dupilumab until baseline. 212 of the patients had at least one follow-up visit (3 months after baseline). At V2, the

oSCORAD decreased to 32.615.3 and the EASI to 10.510.4, respectively. The number of completely controlled weeks increased to 3.34.1.

Dupilumab was prescribed in 24.8% of the patients during the course of the registry. Of these, 48.4% had at least one follow-up visit. Treatment with Dupilumab significantly reduced the mean oSCORAD from 38.419.0 to 24.414.5, the EASI from 17.414.8 to 7.39.6 and improved quality of life (DLQI at prescription: 9.36.9, at follow-up: 4.82.2) (*P* < 0.001). Moreover, the period of completely controlled weeks increased from 2.53.9 to 6.24.7 (*P* < 0.001).

This analysis from TREATgermany provides valuable information about treatment effectiveness and preferences of adults with moderate-to-severe AD in Germany. As the registry continues, more comparative real-world evidence on immunomodulatory therapies will become available. It remains to be seen whether long term efficacy and safety can be achieved by biologic treatment.

GENETICS

P089 | Evaluation of genetic variants in new-onset hidradenitis suppurativa patients by exome sequencing

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Background: Hidradenitis suppurativa (HS) is a chronic inflammatory skin disease affecting up to 2% of adults. Recently, autosomal dominant genetic aberrations in the gamma-secretase pathway have been identified in HS families. Little is known about genetic variants in new-onset HS patients.

Objectives: To identify genetic aberrations in pedigrees of new-onset HS.

Methods: Saliva samples of HS patients as well as their unaffected parents were collected and subject to whole exome sequencing (Illumina HiSeq 4000). Data were analyzed for Mendelian transmission patterns and de novo mutations. In silico prediction tools such as CADD and FATHMM were used to predict the functional importance of potential disease-associated variants.

Results: Four families with one affected child and two healthy parents, as well as one family with two affected, two un-affected children and their healthy parents were analyzed. Five out of six HS patients were male (83%). On average, HS patients were 38 (26-46) years old and reported an average disease duration of 12 (3-25) years. Patients had an average body mass index of 28.8 (22-34). None of the patients ever had severe acne. 50% were smokers. Reported co-morbidities were psoriasis in one patient, arterial hypertension in one HS patient, and Crohn's disease in another.

Sequencing revealed an average of 33.8M (SD 6.1) reads per sample, representing an average coverage of over 60X in target regions, with over 85% of bases having at least 30X coverage. Herein we present an analysis of variants identified in the gamma secretase pathway as well as novel variants identified in other pathways. Natural language processing was used to identify EGFR, AHR, TLR2, and NFkB signaling as potential targets involved in HS pathogenesis. To our knowledge, this is one of the first studies to use an exome sequence approach to evaluate genetic variants in HS.

P090 | Genetic characterization of acral melanoma using a targeted next-generation sequencing panel

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Introduction: Acral lentiginous melanoma (ALM) is one of four major histological subtypes of cutaneous melanoma (1). ALM represents an aggressive melanoma subtype with only a minority of tumors harboring known therapeutic relevant mutations and therefore limited therapeutic options.

Method and material: 50 samples from 26 male and 24 female patients with the diagnosis of ALM were analyzed with a 29 'targeted next-generation-sequencing' panel in the Department of Dermatology, University Hospital Essen, Germany.

Tumor tissue was identified and DNA with sufficient quality for genetic analysis isolated after macrodissection using the QIAamp® DNA-Mini-Kit. The study was performed in accordance with the guidelines of the ethics committee of the medical faculty of the University Duisburg-Essen.

The sequencing and data analysis were done on an Illumina MiSeq® sequencer.

Results: In 26 cases the tumor was located volar, in 10 dorsal, in 13 in other locations such as subungual, volar/dorsal foot edge, interdigital, and in 1 tumor the exact location was unknown.

After exclusion of known SNPs, mutations in protein coding gene domains were found in 41 samples (82%). In 9 cases (18%) no mutations were identified, and tumors classified as wild-type.

A BRAF mutation was identified in 15 (30%) samples, NRAS mutations in 14 (28%) samples, NF1 mutations in 7 (14%) samples, and KIT mutations in 3 (6%) samples. Tumors situated on volar sites presented a higher frequency of NRAS and NF1 mutations compared to dorsal sites, 30.7% (8/26) vs. 20% (2/10) and 15.3% (4/26) vs. 10% (1/10) respectively. Mutations in TERT promoter were observed in 14 (28%) samples, seven co-harboring BRAF mutations. TERT

promoter mutations were more frequent in tumors on dorsal sites 42% (6/14) than in tumors arising on volar sites 28.5% (4/14).

Other rare mutations were found in the following genes: GNAQ, ARID1A, ARID2, IDH, PTEN, PIK3CA, SMARCA4, EZH2, BAP1, WT1, and TP53.

Discussion: The frequency of mutations identified in the BRAF, NRAS, and KIT gene is less than what is described in the literature (2-4). MAPK activating mutations (i.e. NF1, RAS, KIT, BRAF) were identified in 64% of tumors, leaving 36% of samples where mutations activating this pathway remain unknown. The relatively high frequency of TERT promoter mutations identified (28%) was unexpected and needs to be further investigated. Our data indicate that the location of tumor development influences the genetic fingerprint of ALMs. ALMs arising from dorsal sites should be distinguished from those on volar sites, demonstrating a different genetic pathogenesis and probably biological behavior. Further experiments are planned to better characterize the genetic alterations leading to acral melanoma, in particular in tumors where no activating mutation was identified.

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P091 | Exome sequencing of CD18 hypomorphic PL/J mice reveals novel nonsynonymous mutations in psoriasis associated genes

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Previously, we uncovered a hypomorphic mutation in the CD18 gene coding for the common chain of the β2 integrins, to be responsible for a severely reduced expression of 5% of CD18 wild-type expression. This CD18 hypomorphic mutation leads—if backcrossed to the PL/J mouse strain—to a psoriasiform dermatitis which proved to be very valuable to elucidate the polygenic base of psoriasis. In contrast, the same CD18 hypomorphic mutation on the C57BL/6J background did not demonstrate this psoriasiform phenotype. Employing a genome-wide linkage analysis and congenic approach, two major loci were identified on chromosome 10 as contributing to the development

of psoriasiform dermatitis under the condition of low CD18 expression in CD18 hypomorphic PL/J mice. However, the responsible gene mutations have not yet been fully elucidated. To identify mutations specific to recessive susceptibility for PL/J alleles or a dominant suppressing C57BL/6J inheritance, we have performed whole exome sequencing of three CD18 hypo PL/J and three CD18 hypo C57BL/6J mice strains. This analysis revealed 25 719 to 563 single-nucleotide variants in the coding region of PL/J hypo compared to C57BL/6J mice. We have detected 339 non-synonymous mutations on chromosome 10 in all three CD18 hypomorphic PL/J mice, but not in the CD18 hypomorphic C57BL/6J mice. Moreover, we extracted 267 locus-specific mutations in PL/J hypo mice. Our data indicate that different combinations of mutations in disease-causing genes most likely contribute to the precipitation of the psoriasiform phenotype. We are currently exploring the possible role of these locus-specific mutations in psoriasiform disease observed in CD18 hypomorphic mice. In conclusion, these studies will further empower our understanding of psoriasis and related autoimmune disorders.

P092 | Desmoplakin gene variants and risk for skin and heart diseases: usefulness of a functional biochemical assay

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Background: Desmoplakin (DSP) anchors intermediate filament (IF) networks to the desmosomes that are cell-cell adhesion complexes. Depending on the cell type, DSP binds to different kinds of IFs, keratins (Ks) in simple and stratified epithelia and class III IF proteins desmin in cardiomyocytes and vimentin in meningiomal and certain arachnoidal cells. DSP gene variants have been associated with heterogeneous inherited diseases that variably result in arrhythmogenic cardiomyopathy, skin and hair defects. However, many aspects of the genotype-phenotype relationship remain poorly understood. We here analyzed eight amino acid substitutions that are located within the IF-binding region of the carboxyl (C) terminus of DSP and were identified in human patients. We investigated their effects on the interaction of DSP with various Ks and type III IF proteins to determine if they could be correlated with the reported clinical phenotype(s).

Methods: The recombinant IF proteins were expressed in bacteria and purified to apparent homogeneity. They were then dialyzed in buffer conditions favoring their hetero- or homo-dimerization for Ks or vimentin and desmin, respectively, and immobilized on nitrocellulose membranes. The proteins enhanced green fluorescent protein (EGFP), as control, and EGFP-DSP C-terminus, with or without amino acid substitutions, were expressed in transfected human epithelial kidney 293T cells. The soluble fraction of these cells was

used for fluorescence overlay binding assays. All experiments were performed in triplicate and independently repeated at least 3 times. **Results**: Using a fluorescence-based overlay assay, we found that four single amino acid substitutions in the C-terminus of DSP, p.(Gln2295His), p.(Arg2366Cys), p.(Gly2375Arg) and p.(Ala2655Asp) systematically diminished its binding to all tested IF proteins, K1/K10, K5/K14, K8/K18, vimentin and desmin. Therefore, these mutations could be pathogenic. However, a survey of the published clinical phenotypes associated with inherited variants, which specifically alter the sequence of the DSP C-terminus, indicates that they are exceptionally pathogenic in a heterozygous state. The other analyzed substitutions p.(Glu2343Lys) and p. (Arg2639Gln), or p.(Asp2512Tyr) and p.(Arg2541Ser) had weak or no significant impact, respectively, suggesting that they are not pathogenic. Truncation of the C-extremity of the DSP C-terminus reduced its binding to all IF proteins. Our data explain the clinical phenotypes observed with homozygous and compound heterozygous DSP variants that reduce the IF-binding activity of DSP.

Conclusions: In vitro analysis of the IF-binding properties of the DSP C-terminus variants is helpful to corroborate genetic-based prediction models. Our findings indicate that DSP interacts with Ks and desmin with the same set of binding sites. Therefore, it is likely that homozygous or compound heterozygous DSP variants, which reduce the IF-binding activity, will be systematically pathogenic in both the skin and the heart.

P093 | Gene editing as a therapeutic option for epidermolytic ichthyosis

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Epidermolytic ichthyosis (EI) is a skin fragility disorder caused by heterozygous mutations in KRT1 or KRT10. Keratins K10 and K1 polymerise to build the intermediate filament (IF) cytoskeleton of epidermal suprabasal keratinocytes. Mutant keratins integrate into this, resulting in fragility and collapse upon mild stress, leading to IF aggregate formation and epidermal blistering at birth. This progresses to highly disfiguring hyperkeratotic plaques in adulthood that are frequently colonised by bacteria and crack. Therapeutic options are scarce, primarily relying on application of antibiotics, to prevent bacterial colonisation, and topical emollients, to soften hyperkeratotic regions. As EI is difficult to treat and currently lacks a cure, there is an acute need for novel therapies. Heterozygous parents of patients with recessive EI and heterozygous K10 knockout mice express only one KRT10 allele. As they are phenotypically unaffected, these cases demonstrate that one KRT10 allele is sufficient

to support normal skin function. Elimination of mutant keratins in EI patients should therefore result in curation of the disease.

Transcription activator-like effector nucleases (TALENs) are designer nucleases that can be used to introduce frameshift mutations and inactivate targeted genes. TALENs can be efficiently constructed to specifically target many genomic regions with few design parameters, while off-target effects are rare.

TALENs were designed to specifically target a region of KRT10, upstream of a PTC known to induce knockout. These proved efficient at gene disruption (42% efficiency) without selection. Mutant KRT10 knockout was confirmed at the RNA and protein level in isolated modified clones. Following this, reversion of the cytoskeleton fragility phenotype associated with EI was observed following immunofluorescent analysis of differentiated monolayers, concurrent with immunofluorescent and electron microscopy examination of murine xenografts. Off-target activity was not observed at 22 predicted sites via next-generation sequencing (NGS), confirming the safety of this approach.

These studies demonstrate the development of an ex vivo gene editing therapy for EI, using TALENs to knockout dominant-negative mutant KRT10 alleles in keratinocytes to permanently cure the disease. We aim to take a skin biopsy from an EI patient, isolate, grow and treat KSCs with TALENs to phenotypically correct cells prior to grafting these onto the patient's skin as an effective ex vivo therapy for 95.5% of dominant-negative EI cases.

P094 | Generation and characterization of a SNAP29 knockout in immortalized human fibroblasts using CRISPR/Cas9

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Loss-of-function mutations in the SNAP29 gene have been discovered to be the cause of the rare autosomal-recessive neurocutaneous human CEDNIK (Cerebral Dysgenesis, Neuropathy, Ichthyosis, Keratoderma) syndrome. SNAP29 is a SNARE (Soluble NSF Attachment Protein (SNAP) Receptor) protein involved in membrane fusion and thereby epidermal differentiation as well as the formation of primary cilia and autophagy. Due to the small collective of available patients, we formerly generated a total as well as a keratinocyte-specific Snap29 knockout mouse line to study the molecular roles of SNAP29. Loss of SNAP29 resulted in epidermal hyperproliferation, abnormal keratinocyte differentiation and impaired epidermal barrier formation. In order to further examine the function of SNAP29 in autophagy and intercellular transport processes, we applied the CRISPR/Cas9 technique to generate a human SNAP29 knockout cell line. CRISPR/Cas9 is a very potent technique allowing site-specific gene knockout. Using lentiviral transduction rather than mere plasmid transfection which was much less efficient, we were able to

generate a homozygous SNAP29 knockout in immortalized human MRC5Vi fibroblasts. Already in cell culture these cells showed morphological atypia and a reduced proliferation rate compared to wild-type cells. Sequence analysis revealed a homozygous mutation in exon 1 of SNAP29—a deletion of 28 bp and subsequent substitution with 5 bp leading to a frameshift and early truncation after 45 amino acids with only the first 13 amino acids unchanged from the wild-type protein. SNAP29 mRNA and protein expression analyses including immunofluorescence labeling as well as enhanced autophagy and endoplasmatic reticulum stress detection via Western blotting of LC3B-I, LC3B-II, p62/SQSTM1, and CHOP levels will complete the primary characterization of the knockout cells. We now have a unique tool at hand to further study the molecular roles of SNAP29 and showed that an essential gene for life development is dispensable on a cellular level.

P095 | Enhanced susceptibility to poly(I:C) in SMARCAD1 deficient fibroblasts from patients with Huriez Syndrome

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Autosomal dominant Huriez syndrome is characterized by scleroatrophy of the fingers and toes, palmoplantar keratoderma, and susceptibility to cutaneous squamous cell carcinoma (CSCC). The disease is based on heterozygous mutations in the skin-specific isoform of SWI/SNF-Related, Matrix-Associated Actin-Dependent Regulator Of Chromatin, Subfamily A, Containing DEAD/H Box 1 (SMARCAD1) leading to haploinsufficiency.

SMARCAD1 plays a role in the DNA double strand repair. SMARCAD1-deficient keratinocytes and fibroblasts exhibited impaired repair of DNA double strand breaks, proliferated slowly and were prone to a senescent phenotype.

As DNA damage and senescence induction have been linked to the type I interferon (IFN) pathway, we analysed the expression of type I IFN-stimulated genes in SMARCAD1-deficient fibroblasts.

Cultured SMARCAD1-deficient fibroblasts did not show elevated mRNA expression of type I IFN-stimulated genes even when exposed to UV-irradiation.

Instead, stimulation of the cells with the viral mimic poly I:C resulted in an enhanced induction of type I IFN. Further expression analysis showed significant upregulation of toll like receptor 3 (TLR3). TLR3 is a pattern recognition receptor of the innate immune system leading to type I IFN upon ligation with double stranded RNA. Interestingly, TLR3 upregulation and associated susceptibility to poly I:C has been described in systemic sclerosis. These data suggest that there might be a common mechanism of inflammation induced fibrosis, and

warrant further investigation of DNA damage associated immune responses.

P096 (OP03/06) | Exome-wide association study reveals DOK2 as novel atopic dermatitis susceptibility gene harbouring low frequency risk variants

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Atopic dermatitis (AD) is a common multifactorial inflammatory skin disease with a strong hereditary component. Previous association studies of AD have identified over 30 genetic susceptibility loci of common frequency (MAF ≥ 5%), but only a few were resolved to be functional variants. In the current study we systematically evaluated the contribution of coding and low frequency variants to AD susceptibility using the exome chip for 1913 German AD patients and 14 295 controls followed by replication in 4379 AD cases and 11 724 controls of European descent. Single variant and gene-based association analyses identified DOK2 (OR = 0.64; p lead SNP = 5.74×10^{-11}; p DOK2 = 6.19×10^{-14}) as novel genome-wide significant AD susceptibility gene harbouring numerous missense variants of low (MAF < 5%) and rare frequency (MAF < 1%). Sequence and structural analyses showed that risk variants in DOK2 in combination with a novel missense AD risk variant in CD200R1 (OR = 1.16; $P = 2.38 \times 10^{-7}$) together may affect tyrosine phosphorylation sites in DOK2 and CD200R1 important for anti-inflammatory signalling and thus linking these two genes with an inherited reduced anti-inflammatory response in AD.

P097 | RNA Profiling of Bullous Pemphigoid Patient Skin

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Bullous Pemphigoid (BP) is the most common autoimmune skin blistering disease, with a high prevalence in the ageing population. Although it is understood that local autoinflammatory processes lead to the formation of the characteristic blistering phenotype at the dermal-epidermal junction (DEJ), and some modulatory factors have been identified, a comprehensive transcriptomic study in BP patient skin has not yet been performed.

To understand the processes leading to effector cell recruitment and blister formation in BP, we analysed the transcriptome of 14 BP patients at perilesional (>2 cm from lesion) and matching, unaffected biopsy sites along with 9 age- and sex-matched control biopsies. This analysis was complemented by the addition 15 near-lesional (<2 cm from lesion) biopsies, representing a more acute stage of the disease.

Gene ontology enrichment analyses indicated a clear pro-inflammatory shift in BP patient skin, increasing with near-lesional proximity as well as disease associations of the expression profiles with multiple sclerosis, peeling skin syndrome and multiple mitochondrial disorders. Intriguingly, no difference could be observed between nonlesional and perilesional biopsy of BP patients, suggesting that non-lesional skin of BP patients is already in an inflammatory state, despite the absence of lesions. Coupled with the pro-inflammatory shift in perilesional and near-lesional biopsies, an inversely proportional expression change of mitochondrial protein coding genes, both of nuclear and mitochondrial encoding, was observed, despite an in-silico predicted influx of pro-inflammatory cells and increased energy demand. Previously, we have provided evidence for dysfunctional mitochondria as a putative disease mechanism of BP, with the current findings matching this hypothesis.

Furthermore, we performed RNA variant calling to identify intra-patient, somatic mutations that may aide disease development. Genes harbouring deleterious somatic mutations in lesional proximity included ITGA6 and DST (BP230), the former critical for DEJ anchoring, the latter one of the two known BP antigens. Among others, further deleterious variants were found in immunomodulatory genes, such as FRP1 and LRR1.

In light of these findings, we suggest that somatic mutations may play a role in weakening skin adhesion and/or promote inflammation locally, thereby driving the disease towards blister formation, possibly in conjunction with dysfunctional mitochondria further contributing to the inflammation.

P098 | Mitochondrial mutations in pemphigus vulgaris

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Background: Pemphigus vulgaris is an autoimmune skin blistering disease with autoantibodies directed against Desmoglein 3 (and 1). While genetic determinants involved in the development of PV have been investigated in genome-wide association studies, mitochondrial genetic determinants enhancing the susceptibility to PV or protecting from developing the disease remain poorly explored. The mitochondrion is an exceptional organelle in eukaryotic cells. It is the key energy producer of cells, and it has its own genome, replication and transcription machinery. Mitochondrial dysfunction has been reported to contribute to a wide range of disorders. Their role in PV has been hinted by the existence of antimitochondrial antibodies in the sera of PV-patients.

Objective: In this study we investigated the mitochondrial genome of PV patients in order to elucidate mitochondrial polymorphisms associated with an in- or decreased risk of developing PV and their potential impact on cellular functions.

Methods: We sequenced the mitochondrial genome of 216 German PV-patients and 425 sex-, age- and ethnicity-matched controls using a next-generation sequencing approach on the MiSeq Illumina platform. The sequencing data were compared against the revised Cambridge Reference Sequence (rCRS) of human mtDNA (GenBank NC_012920.1) and SNP-wise and gene-wise analysis was performed.

Results: We found significantly associated variants in the ATP6 gene. ATP6 encodes the subunit 6 of the mitochondrial ATP synthase (complex V), which is responsible for the final step of oxidative phosphorylation in the electron transport chain. Particularly, we found a heteroplasmic mutation, occurring in 10.3% of the PV-patients and none of the control samples ($P < 0.0001$). Further studies will elucidate the exact influence of this mutation on cellular functions.

Conclusions: This study shows that mitochondrial mutations are associated with PV and might be pathomechanistically involved in the occurrence of the disease. In this study, we found a mutation in the ATP6-gene, which is associated with PV.

IMMUNOLOGY

P099 | Sodium chloride is an ionic checkpoint for human 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.

P100 | The leukocyte compartment in subcutaneous white adipose tissue—an immunological compartment apart from the skin?

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In contrast to the well-characterized leukocytic compartment in human skin, relatively little is known about the one residing in the fat layer beneath. To address this issue, we collected thigh-liposuction-derived subcutaneous white adipose tissue (SWAT; n = 14) from healthy young women, normal skin (n = 8) and blood (n = 8), isolated the leukocytes from these compartments, characterized them by FACS and subjected them to cytokine stimulation assays. In addition, we performed in situ multicolor immunofluorescence stainings. We found major differences between SWAT and skin leukocytes: while T cells outnumbered mononuclear phagocytes in the skin, macrophages constituted the largest population (>50%) among SWAT leukocytes, followed by T cells (ca. 25%) and several minor leukocyte subsets such as natural killer cells, dendritic cells, type 2 innate lymphoid cells and eosinophils). These leukocytes were essentially confined to connective tissue septa separating individual lobules of adipose tissue. The majority of SWAT-macrophages exhibited an M2 phenotype. Most SWAT-T cells, composed of about equal proportions of type 1- and type 2-polarized cells, belonged to the memory subset and half of them were tissue-resident (mostly CD69^{+}CD103^{+}). Our data constitute the basis for further experiments investigating features and functions of SWAT-leukocytes under physiologic and pathologic conditions and studying the interplay with their symbionts.

P101 | Analysis of immune cell populations in systemic sclerosis

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Systemic sclerosis (scleroderma) is a chronic and severe autoimmune disease with unknown etiology and is associated by the progressive development of fibrosis in the skin and internal organs such as lungs, heart, kidney and the gastrointestinal tract. Cutaneous fibrosis is characterized by disproportionate accumulation of collagens and other extracellular matrix substances. However, involvement of innate immune cells and the sequence of inflammatory events in the early phase of the disease have not been addressed so far. For this purpose, we studied a murine model of spontaneous systemic sclerosis: the fos-related antigen-2 mouse (fra-2). These mice overexpress fra-2 under MHCI promotor control and spontaneously develop vasculopathy, inflammation and fibrosis of the skin and internal organs to dyspnea at week 15-17. Starting at week 12 mice show massive dermal and pulmonary fibrosis leading as demonstrated by a significant enhancement of dermal thickness (H&E) and a pronounced cutaneous collagen accumulation (Goldner's

trichrome, hydroxyproline assay). In Fra-2 mice we observed the inflammatory response during week 5-9 of life. Flow cytometric analysis of the skin demonstrated an early cellular infiltrate which peaked at week 5 mainly consisting of $CD45^+CD11b^+$ immune cells. Further characterization revealed activated myeloid cells ($CD11b^+MHCII^+$) and inflammatory monocytes ($CD11b^+Ly6C^+$) as major skin infiltrate. Further characterization indicated terminally differentiated macrophages, consisting of $CD206^+CD301b^+$ and TNF-α^+ $CD11b^+$ myeloid cells in the skin. Furthermore, we found an increased frequencies of $CD45^+CD11b^+$ immune cells at week 5-9 and of inflammatory monocytes $CD11b^+Ly6C^+$ in the blood at week 3.

Besides the myeloid cell infiltration, Fra-2 mice exhibited enhanced frequencies of $CD4^+$ T cells compared to $CD8^+$ T cells in the skin. However, Fra-2 mice displayed reduced percentages of $CD4^+Foxp3^+$ regulatory T cells in the skin, lymph nodes and lung.

Our findings determine a massive myeloid and lymphoid infiltration and reduced Treg frequencies contributing to fibrosis development in Fra-2 mice and may attend as targets for novel therapeutic strategies in fibrosis.

P102 | Relevance of neutrophil extracellular traps during vessel damage in skin-limited IgA immune complex vasculitis

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Immune complex vasculitis (ICV) is a vascular inflammation that mainly affects small blood vessels. Initial steps are formation of immune complexes (IC) in the vessel, followed by neutrophil (PMN) and IC interaction, accumulation, deposition, activation and ensuing destruction of vessel wall. It is unknown how and which cytotoxic components cause the vessel damage. Stimulated PMN produce extracellular structures called neutrophil extracellular traps (NET). NETs are filaments of decondensed chromatin associated with proteins like histones, elastase and myeloperoxidases (MPO) which can have a cytotoxic effect to the endothelium. NETosis is stimulated by several molecules, like ICs, but the relevance in importance in skin-limited IgA ICV is unknown. The objective was to investigate the impact of NETs from human PMN on vessel damage during skin-limited IgA ICV in vivo, in vitro and ex vivo. We confirmed that PMN of skin-limited IgA ICV patients are able to release high amounts of NET after stimulation with IgA immune complexes. Accompanying, we found serum molecules IL-6 and S100A8/S100A9 prestimulating the PMN to react even enhanced. Additionally we were able to prove the presence of highly cytotoxic NET structures in lesion tissue from skin-limited IgA ICV patients and were able to reconstruct the in vivo conditions with

a shear-flow system showing the cytotoxic capacity of NET. In our mouse model a degradation of DNA after inducing vasculitis resulted in an improved disease outcome. These findings indicate the importance of PMN and NET during the evolvement of vessel damage in skin-limited IgA ICV.

P103 | The Yersinia outer protein M (YopM) reduces keratinocyte hyperproliferation and neutrophil infiltration psoriasis-like inflammation in mice

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Psoriasis is an inflammatory skin disease affecting around 2%-4% of the western European population. Patients suffer from red, itchy and scaly plaques and an impaired quality of life. Several treatment options are available, but with higher efficacy often toxicity and/or therapy costs are rising.

The Yersinia outer protein M (YopM) was the first bacterial effector protein discovered to be a cell-penetrating peptide (CPP), meaning it is able to autonomously enter eukaryotic cells independent of the T3 secretion system. It has been demonstrated that recombinant YopM (rYopM) down-regulated the expression of pro-inflammatory cytokines, like TNF-alpha or IL-1beta, after penetration of the host cell. Current theories about the mechanism of action focus on the inhibition of caspase-1 activation by either binding directly to pro-caspase-1 or to the scaffolding protein IQGAP1 and the downstream inhibition of the formation of the NLRP3 inflammasome. Hence, rYopM might be a promising molecule for further investigation in the context of local therapies for inflammatory skin diseases like psoriasis.

Subcutaneous injection of full-length rYopM already showed promising results in ongoing imiquimod (IMQ)-induced psoriasis-like skin inflammation in mice. To narrow down the functional groups of the protein a truncated version of the protein was generated where only the two N-terminal α-helices (essential for translocation) and the first three leucine-rich repeats (LRRs) are present, called rYopMLRR1-3. As a negative control a construct wherein only the first three LRRs are missing was used, termed rYopMΔNLS1. To elicit psoriasis-like inflammation in mice, the animals were creamed daily with 62.5 mg of Aldara, containing 5% IMQ on the shaved lower back for 8 consecutive days. Following disease onset the mice were additionally injected with 50 µg rYopM, rYopMLRR1-3, rYopMΔNLS1 or PBS as control on a daily basis. Throughout the experiment the clinical score of the mice was determined and on the final day of the experiment skin samples were taken for histological examination, RNA analysis and protein quantification. The results clearly showed a reduced clinical score and scratching behavior of the rYopM and

rYopMLRR1-3 treated animals, which was reflected in the reduced epidermal thickness as assessed by hematoxylin & eosin staining. Furthermore, keratinocyte proliferation was examined by the means of Ki-67 stainings and was markedly decreased in these groups resulting in lower levels of acanthosis. The quantification of inflammatory protein levels in the skin revealed reduced concentrations of pro-inflammatory and psoriasis-associated cytokines, like IL-17A, TNF-alpha or IL-1beta, as well as neutrophil-attracting chemokines, like CXCL1 and CXCL5. This finding was also confirmed by RNA-seq analysis, suggesting a similar mode of action of rYopM and rYopM-LRR1-3. Consequently, rYopM and rYopMLRR1-3 treatment significantly impaired neutrophil immigration into affected skin areas. Therefore, the anti-inflammatory capacity of rYopM treatment in psoriasis-like skin inflammation has clearly been shown. In addition, the essential part of rYopM for mediating this effect was narrowed down to the first three leucine-rich-repeats.

To sum this up, YopM is a promising target for further analysis in the context of local treatment of inflammatory skin diseases.

P104 (OP02/06) | Disruption of the eye's immune privilege in mice with 4-1BB overexpression in basal keratinocytes

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The 4-1BB protein (also called CD137 and TNFRS9) belongs to the tumor necrosis factor receptor superfamily (TNFRS), and has a crucial role as a costimulatory molecule in a variety of immune processes. It is overexpressed upon cell activation. The overall effect of 4-1BB/4-1BB ligand signaling is an enhanced inflammatory response. To investigate the in vivo relevance of this signaling pathway in more detail we generated a mouse model with overexpression of 4-1BB under control of the keratin-14 (K-14) promoter. At the age of 4 months K14-4-1BB transgenic (tg) exhibit clinical signs of a pruritic skin disease resembling atopic dermatitis with inflammation at the ears, snout and neck. Surprisingly, besides severe skin inflammation, K14-4-1BB tg mice spontaneously develop uveitis and anterior cataract starting at the age of 3 weeks, which is associated with the infiltration of immune cells into the eye, finally resulting in blindness. Notably, we detected an enhanced 4-1BB expression in the areas of transgenic eyes that express K14. However, 4-1BB was never present in eyes from wild-type mice. Interestingly, by performing a whole mRNA array analysis we were able to demonstrate the overexpression of genes related to the presence of immune cells in the eye of K14-4-1BB tg mice, whereas these mRNAs were absent in wild-type controls. Furthermore, immunofluorescent staining as well as qPCR and FACS analysis was used to confirm the array data, revealing that the immune cell infiltrate mainly consisted of MHC-II[+]F4/80[+] macrophages. In support of this, chemokines involved in macrophage homing to inflammatory tissues, such as CCL2 and CCL3, were highly upregulated in the eyes of K14-4-1BB tg mice compared to wild-type controls. Moreover, is well known that inflammation characterized by the presence of macrophages might induce epithelial–mesenchymal transition (EMT). EMT is a process in which the epithelial cells are gradually losing their epithelial phenotype while acquiring a mesenchymal phenotype, thus becoming more proliferative and motile. The lens epithelial monolayer is a tissue prone to undergo EMT upon several stimuli. Accordingly, EMT of the lens epithelial cells is a crucial pathological mechanism associated with alterations of the physiological crystalline structure and thus, with a progressive opacification of the lens. EMT has therefore direct implications on anterior cataract formation. Strikingly, we observed that the cells proliferating in the anterior chamber of K14-4-1BB tg mice are highly positive for several mesenchymal markers like alpha-smooth muscle actin (α-SMA), Collagen I and Fibronectin while they show a reduced expression of epithelial markers such as E-cadherin. Hence, we demonstrated the presence of an EMT occurring in the lens epithelial cells in the anterior chamber of K14-4-1BB tg mice leading to the development of anterior sub-capsular cataract. This suggests that the disruption of the immune-privilege and the infiltration of macrophages into the anterior chamber of the eye from K14-4-1BB tg mice might elicit the EMT, probably by causing a break of the anterior capsule and a pro-inflammatory environment, finally resulting in the development of cataract. Together, these data strongly suggest that 4-1BB signaling is critically involved in the development of uveitis and anterior cataract and might play an important role in disrupting the immune privilege of the eye.

P105 | Clinical signs of epithelial surface disruption impact pain and sexual Health in Patients with moderate-to-severe genital psoriasis

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Introduction: In genital psoriasis, cutaneous and mucosal lesions of the genital area can present with erosions, fissures and ulcers, clinical signs of epithelial surface disruption. This post-hoc subset analysis evaluated the impact of these signs on pain and sexual activity in patients with moderate-to-severe genital psoriasis and the effect of ixekizumab (IXE) treatment in this subgroup of patients.

Methods:

Patients with moderate-to-severe genital psoriasis from a double-blind, randomised, placebo-controlled phase 3 study, receiving either placebo or IXE 160 mg at week0, followed by IXE 80 mg every 2 weeks (IXEQ2W), were analysed for presence of genital erosions, fissures and/or ulcers at baseline through week 12. In this analysis these signs were correlated with patient-reported outcomes (PROs) on pain and sexual activity using generalised linear models and the

effect of treatment on primary and secondary outcome measures in this subpopulation was analysed.

Results: At baseline, 38% (n = 57) of patients presented with genital erosions, fissures and/or ulcers. This subset had significantly higher scores in the Genital Psoriasis Symptoms Scale (GPSS) total (P = 0.018), the GPSS Pain (P = 0.013) and the GPSS Discomfort (P = 0.043), with significantly higher mean and summary scores for genital pain, stinging and burning (P = 0.025) compared to patients without fissures/erosions/ulcers. This subgroup also differed significantly in the static Physician Global Assessment of Genitalia (sPGA-G) score (P = 0.002). The differences in GPSS total and GPSS for pain, stinging and burning between the subgroups with and without epithelial surface disruption were also significant when analyzing the subpopulation with an overall body surface area (BSA) involvement <10%. Evaluation of how often genital psoriasis limited frequency of sexual activity at baseline confirmed that patients without fissures/erosions/ulcers were more likely to not be limited sexually by their disease (odds ratio [OR] 4.17 vs. 3.33) with an overall 25% greater OR over the 12 week observation period. Improvement of genital erosions, fissures and/or ulcers in response to treatment with IXEQ2W was paralleled by reduced severity in pain and sexual health-related PROs.

Conclusion: These data emphasize the distinct phenotype of genital psoriasis that can present with erosions, fissures and/or ulcers, clinical features that contribute to disease severity and impact genital pain and sexual health in patients with moderate-to-severe genital psoriasis even in the absence of higher BSA involvement. Treatment with IXEQ2W improved genital psoriasis severity, genital pain and sexual health.

P106 | No reactivation of tuberculosis in Patients with latent tuberculosis infection while on ixekizumab treatment: a report from 11 clinical studies

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Introduction & Objectives: While TNF-blocking therapies are associated with an increased risk of active tuberculosis (TB), recent data indicate that IL-17 inhibition might not be critical in latent tuberculosis infection (LTBI) immunity. Ixekizumab (IXE), an IgG4 monoclonal antibody that selectively binds IL-17A, was shown to be a safe and effective treatment in patients with chronic plaque psoriasis. This integrated safety analysis assessed the occurrence, course and outcome of treatment-emergent LTBI in patients with chronic plaque psoriasis under treatment with IXE.

Methods & Materials: This analysis included safety data for 5730 patients (with 13479 patient-years of IXE exposure) from 11 psoriasis clinical trials, including the UNCOVER Studies 1, 2 and 3, who received at least one dose of IXE. Per protocol, patients with

a history of previously treated TB could be enrolled in the studies, as well as patients with LTBI (positive tuberculin skin test [TST] or QuantiFERON®-TB Gold test [QFT] without evidence of active TB) at screening who started prophylactic treatment for LTBI at least 4 weeks prior to enrolment. Patients with newly diagnosed LTBI (positive TST or QFT), after randomisation at study visits up to 52 weeks, were discontinued from the studies. Since no events of active TB in patients with LTBI in the UNCOVER studies were observed up to Week 52, the protocol was amended to allow patients with new onset of LTBI in the absence of active TB at Week 52 or later to remain in the study and continue treatment with IXE.

Results: In total, 72 (1.3%) patients with treatment-emergent LTBI while on IXE treatment were identified with 56 patients being reported before Week 52 leading to discontinuation from the study, while 16 patients showed positive TST or QFT results at Week 52 or later. Subsequently, 14 patients remained in the study and continued IXE treatment and two patients discontinued the study. In 12 patients, LTBI-specific therapy with isoniazid (INH; n = 8), rifampicin (n = 1) or a combination of rifampicin and INH (n = 3) was initiated. One of these patients treated with INH/RIF developed INH-dependent elevated liver enzymes and in this patient LTBI-specific therapy was discontinued after 12 weeks. Two patients did not receive any LTBI-specific therapy and continued IXE treatment for 8 and 12 weeks, respectively. Reasons for stopping IXE were loss to follow-up and completion of study participation. None of the patients developed active TB.

Conclusion: This integrated safety analysis of new onset of LTBI under continuous IXE therapy, whether or not receiving LTBI-specific therapy, in patients with chronic plaque psoriasis showed no evidence of active TB. Acknowledging the limitations of this analysis due to the small number of events and short observation periods, these data contribute to the growing evidence for a low risk of IL-17 neutralising antibodies for the development of active TB compared to TNF-blocking therapies.

P107 | Preclinical efficacy of NLRP3 small molecule inflammasome inhibitors: Implications for future treatment of autoinflammatory syndromes.

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Background: Systemic autoinflammatory diseases (SAIDs) are characterized by abnormally increased inflammation affecting different organs. They are mediated predominantly by the cells and molecules

of the innate immune system. Inflammasome activation represents the critical pathogenic mechanism shared by most SAIDs. Current treatment strategies are limited to downstream cytokine blockade. Specific inflammasome inhibitors are not available so far.

Methods: To address this unmet medical need, we performed a high content screening of more than 60.000 small molecules from the compound collection of the "Leibniz- Forschungsinstitut für Molekulare Pharmakologie (FMP)", which includes the ChemBioNet and LOPAC®1280 libraries as well as donations of academic chemists, FDA approved drugs (Selleck library) and a natural product collection from AnalytiCon Discovery.

For the primary screen, we used a fluorescent murine inflammasome reporter cell line to detect ASC speck formation, a marker of inflammasome activation. Compounds were selected based on their inhibitory capacity on ASC speck formation, as observed by automated fluorescence microscopy, and IL-1ß release after activation with canonical NLPR3-inflammasome inducers ATP and nigericin. The 10 most potent and druggable hit compounds were tested in peripheral blood mononuclear cells (PBMCs) obtained from venous blood of patients with Schnitzler syndrome (N = 9), familial Mediterranean fever, FMF (N = 9) and from unmatched healthy donors (N = 18). In vitro effect on cellular IL-1ß release was measured by ELISA.

Results: Selected compounds proved to efficiently inhibit the secretion of IL-1ß in PBMCs from both patients and healthy controls in a dose dependent manner, validating their inhibitory capacities in human and murine cellular assays. Among these compounds were known anti-inflammatory drugs such as auranofin and a VEGFR tyrosine kinase inhibitor. The median inhibitory capacity upon stimulation with lipopolysaccharide (LPS) and ATP at 10 μM ranged between 50% and 80% with IC50s in the low μM region. A similar inhibitory profile could be observed for the previously reported inflammasome inhibitor MCC950, which was included in our assays as a reference substance. Moreover, compounds had similar efficacy in inflammasome inhibition PBMCs obtained from patients and healthy controls.

Conclusion: Based on our results in murine and human cells in vitro, small molecule inflammasome inhibitors may complement current treatment options for SAIDs in the future.

P108 | 12/15-lipoxygenase counteracts and resolves pemphigoid disease-like dermatitis

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Resolution is the active, programmed termination of tissue inflammation and the restoration of tissue homeostasis. Although this concept is mainly derived from observation made in mouse models of peracute peritonitis, it has been suggested that failing resolution is responsible for the emergence of chronic tissue inflammation. 12/15-lipoxygenase (12/15-LO) is a key enzyme for the biosynthesis of specialized pro-resolving lipid mediators (SPMs) and it is believed to be a major orchestrator of resolution. The role of 12/15-LO in the regulation of dermatitis, including pemphigoid diseases, which are characterized by production of autoantibodies targeting the structural proteins of the skin, is, however, unknown. We have therefore addressed the role of 12/15-LO in the antibody transfer bullous pemphigoid (BP)-like EBA mouse model, using the 12/15-LO deficient (Alox15$^{-/-}$) mice. In this model, pemphigoid disease-like dermatitis was pronouncedly aggravated and prolonged in Alox15$^{-/-}$ mice in comparison with wild-type mice. Moreover, elevated levels of docosahexaenoic acid-derived SPMs, including 10,17-DiHDHA, 17(S)-HDHA, and 14(S)-HDHA were found in lesional skin of wild-type mice, but not in the skin of Alox15$^{-/-}$ mice, indicating, that 12/15-LO counteracts skin inflammation and is involved in its resolution via the biosynthesis of SPMs. Herein, 12/15-LO expression was induced in skin infiltrating PMNs, suggesting that PMNs participate in both initiation and termination of skin inflammation in this model. Moreover, In line with a similar role of 12/15-LO in the human situation, we have found its upregulation in lesional skin of bullous pemphigoid patients in comparison with skin of normal healthy controls. Our results provide direct evidence that the concept of resolution is valid for skin and its failure may be critically involved in the emergence of chronic skin inflammation.

P109 (OP05/01) | IgG of scleroderma patients establishes a proinflammatory and profibrotic phenotype in monocyte-like THP-1 cells

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Functional IgG autoantibodies against diverse G protein-coupled receptors (GPCRs), i.e., antibodies with agonistic or antagonistic activity at these receptors, are abundant in human serum. The serum levels of specific anti-GPCR autoantibodies are altered under certain medical conditions, such as in systemic sclerosis (SSc). Autoantibodies against the GPCRs angiotensin II receptor 1 (AT1) and endothelin receptor A (ETA), e.g., are both elevated in most SSc patients compared to healthy controls, and their serum levels closely correlate with disease activity. Anti-AT1 and anti-ETA autoantibodies presumably promote the pathogenesis of SSc, among others, by directly activating monocytes to release CXCL8 and CCL18. However, the molecular mechanisms IgG of SSc patients engages to induce the release of CXCL8 and CCL18 from monocyte have remained elusive. Furthermore, it is unknown whether SSc-IgG specifically induces

the release of CXCL8 and CCL18 or whether it in parallel elicits the release of other mediators, possibly promoting or suppressing the emergence of SSc and its sequelae.

We have utilized monocyte-like THP-1 cells to profile the effect of SSc-IgG on monocytic cells on the transcriptional level as well as on the (phospho-)protein level. SSc-IgG shifted the secretome of THP-1 cells towards a proinflammatory and profibrotic phenotype. On the phospho-protein level, SSc-IgG markedly activated the kinases ERK, AKT, RSK, and p70 S6 kinase.

Detailed examination of the molecular mechanisms regulating the induction of CXCL8 and CCL18 by SSc-IgG revealed a differential regulation of the two chemokines with the induction of CXCL8 largely depending on the TAK/IKK-β/NF-B pathway and of CCL18, in contrast, depending on AP-1.

Our results suggest that SSc-IgG by direct actions on monocytes significantly contributes to the generation of the proinflammatory–profibrotic tissue milieu characteristic for SSc. Furthermore, our data suggest that the release of CXCL8 and CCL18 can be selectively inhibited in SSc patients.

P110 | Differential impact of PI3K isoforms on neutrophil activation pathways

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Neutrophils, in addition to their role in host defense, are key players in the pathogenesis of inflammatory skin diseases, such as the pemphigoid disease epidermolysis bullosa acquisita (EBA). In EBA activation of neutrophils is initiated by their binding to the skin-bound immune complexes (IC) via activating Fc gamma receptors. Binding of the Fc gamma receptors to IC triggers signal transduction pathways in neutrophils leading to the release of proteases and reactive oxygen species. In general, PI3 kinase (PI3K) has been identified as a key molecule in the signaling pathways of neutrophils. Currently, four isoforms of the PI3K are known: PI3Kα, β, γ and δ, which differ in their cellular functions and tissue distribution. The respective contribution of each PI3K isoforms to distinct neutrophil functions, as well as the consequences of PI3K-subcall inhibition in the context of inflammatory skin diseases, have so far not been addressed in depth. To address this knowledge gap, we investigated the role of all known PI3K isoforms in different features of neutrophils that can trigger disease pathogenesis by using eight PI3K inhibitors that vary in their specific inhibitory activity to the different PI3K isoforms. Decreased IL-8-induced neutrophil chemotaxis was observed for blockade of either PI3K isoform. In contrast, the spreading of IC-activated neutrophils was only decreased after inhibition of the PI3Kα and δ isoforms. Moreover, the release of reactive oxygen species from IC-activated neutrophils strongly depends on the PI3Kβ and δ isoforms. Formation of neutrophil extracellular traps (NETs) after IC stimulation was

only decreased if the δ isoform was inhibited. Additionally, an inhibitor of all isoforms reduced the release of cytokines, especially IL-6 and TNF, whereas specific inhibition of single subunits did not have any effects on IC-induced cytokine release from neutrophils. None of the inhibitors showed toxic effects in the used concentrations. To investigate a possible in vivo effect, and to evaluate the potential therapeutic use of (topical) PI3K-targeted treatment in pemphigoid diseases, we applied 7 specific PI3K inhibitors in the antibody transfer-induced EBA using oral as well as topical treatment options. In oral treatment, inhibitors of the PI3Kα, β and γ significantly impaired clinical disease manifestation in experimental EBA, while the used PI3Kδ-specific inhibitors had no significant effect. Of note, topical application of PI3Kα and γ inhibitors (TGX-221 and Alpelisib) was effective in treatment of experimental EBA, representing a new therapeutic approach for the development of well tolerable and specific treatment options of immune-complex-induced neutrophil dependent autoimmune diseases like EBA.

P111 | Skin-resident CD49a^{+} ILC1s in Imiquimod-induced Psoriasiform Dermatitis

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Innate lymphoid cells (ILCs) are an essential component of the immune system, mediating tissue homeostasis and inflammation. Group 1 ILCs, consisting of conventional NK cells and ILC1s are T-bet dependent and produce the Th1 cytokines interferon (IFN)-γ and tumor necrosis factor (TNF)-α. Besides, ILC1s display a unique pattern of chemokine receptors and adhesion molecules (e.g. CD49a, CXCR6, CD69, CD103) distinct from conventional NK cells, which are among others, important in maintaining their tissue-resident position.

As the functional properties of ILC1s in the skin remain poorly defined, we were keen to delineate how this subset contributes to chronic inflammatory skin conditions such as psoriasis. To this end, the well-established mouse model of imiquimod-induced psoriasiform dermatitis was applied. Topical treatment of C57Bl/6N mice with 5% imiquimod cream over a period of two to seven days, led to a significant accumulation of skin-resident CD49a^{+} ILC1s. Intriguingly, this subset was a major source of TNF-α with an overall contribution of 40% among TNF-α-producing lymphocytes. IFN-γ production by skin-resident ILC1s, however, could not be detected. Furthermore and in line with previous results obtained in the liver, skin-resident ILC1s were characterized by the expression of the chemokine receptor CXCR6 and the hyaluronan receptor CD44.

Since ILCs share many common regulatory pathways with T helper cells, we investigated whether the CD49a^{+} subset also accumulated in ear-draining lymph nodes (dLNs). Indeed, we observed a significant increase of CD49a^{+} ILC1s in imiquimod-treated mice. In accordance

with the data obtained in the skin, ILC1s from dLNs produced TNF-α upon restimulation, but lacked IFN-γ.

Taken together, our data indicate that skin-resident ILC1s promote skin inflammation by substantially contributing to TNF-α production in a mouse model of psoriasis.

P112 | Diversity of CD4^{+} blood T-cell clonality predicts longer survival with CTLA4 or PD-1 checkpoint inhibition in advanced melanoma

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Recognition of cancer antigens drive clonal expansion of cancer-reactive T cells, which is reflected by restricted T-cell receptor (TCR) repertoires. It has been believed that T-cell repertoires are restricted only in tumor-infiltrating lymphocytes (TILs) in cancer patients. To understand how tumor escapes anti-tumor immunity, we analyzed tumor-associated T-cell repertoires from patients with advanced melanoma upon the therapeutic blockade of cytotoxic T-lymphocyte-associated protein 4 (CTLA4) or programmed cell death 1 (PD-1) using TCR Vβ-gene spectratyping. Surprisingly, TCR repertoires in the blood of melanoma patients showed variable levels of restriction in CD4^{+}, extensive restrictions in CD8^{+} T cells, and contained CD4^{+} and CD8^{+} T-cell clones before the start of immunotherapy. The circulating clones were enriched in TILs, indicating that melanoma-reactive T-cell clones can be detectable in the blood. Moreover, a greater degree of clonal diversification in especially CD4^{+} blood T cells before immunotherapy correlated with long-term survival upon CTLA4 or PD-1 inhibition. In patients who developed severe immune-related adverse events (irAEs), TCR spectratypes became more restricted during CTLA4 blockade, suggesting that newly expanded T-cell responses contributed to irAEs. Collectively, our data demonstrate that diversity of T-cell clones in the circulation may reflect the immune status of anti-melanoma responses and thus provide a rationale for predicting anti-tumor responses with checkpoint inhibitors using patient's blood.

P113 | Development of antigen specific immunotherapy against melanoma with TRP1 targeted CD4^{+} T cell transfer.

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Introduction: Immunotherapy is emerging as the standard treatment modality along with surgery, radiotherapy, and chemotherapy. Immune responses against tumor cells have typically been attributed to CD8 T cells; however, the importance of CD4 T cells in anti-tumor immunity is gaining prominence. It is long known that CD4 T cells can target tumor cells directly by cytolytic mechanisms or indirectly by helping antitumor CD8 T cells (Borst et al., 2018; Muranski et al., 2008; Quezada et al., 2010). However, it is still debated whether CD4 T cells can recognize their cognate antigen directly on tumor cells. In the current work, we hypothesize that tumor-specific CD4 T cells control the growth of melanoma through direct cytolytic activity involving direct recognition of its cognate antigen on tumor cells.

Materials and Methods: Adenovirus and Modified vaccinia Ankara vectors were engineered to ectopically express GP100 or Pmel (CD8 T cell) and TRP1 (CD4 T cell) epitopes. HCmel12 melanoma cells derived from HGF-CDK4R24C mice were used and Trp1 genetic loss variants as well as Class II transactivator (CIITA) loss variants of HCmel12 melanoma cells were generated by CRISPR–Cas9 gene targeting technology. Our adoptive T cell therapy involved chemotherapeutic preconditioning with cyclophosphamide followed by transfer of Trp1 antigen-specific CD4 T cells, an adenoviral vaccine and 3 peritumoral doses of poly I:C and CpG. The mice additionally received an MVA booster vaccine 14 days post adoptive T cell transfer.

Results: We show that similar to transfer with tumor antigen-specific Pmel CD8 T cells, transfer with tumor antigen-specific Trp1 CD4 T cell therapy can result in a stable disease, complete regression or escape of HCmel12 melanomas. However, anti-tumor responses with CD4 T cells were less pronounced and expansion of Trp1 CD4 T cells was clearly less efficient than the Pmel CD8 T cells. Importantly, even in the absence of endogenous CD8 T cells, CD4 T cells provide some tumor control indicating that CD4 T cells can have a direct anti-tumor effect. We further show that HCmel12 Trp1^{−/−} (antigen loss) and CIITA-/- (MHC-II deficient) melanoma cells cannot be recognized by Trp1 CD4 T cells in vitro indicating that the CD4 T cells need to recognize their cognate antigen presented in MHC-II. However, while HCmel12 Trp1^{−/−} tumors barely respond to CD4 T cell therapy in vivo, HCmel12 CIITA-/- tumors do partly respond to this therapy.

Conclusion: We have successfully established the adoptive T cell therapy protocol involving Trp1 antigen-specific CD4 T cells and show that both antigen presentation and expression is important for the therapeutic effect of CD4 T cells. Future studies are directed towards understanding the phenotype of anti-tumor CD4 T cells mechanism by which they orchestrate the anti-tumor immune response.

P114 (OP01/05) | Antigen specific adoptive CD4 T cell transfer immunotherapy against melanoma causes reactive neutrophil response

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Introduction: Previously we developed an adoptive T cell transfer (ACT) immunotherapy protocol involving CD8 T cells that target the melanocytic antigen gp100. We observed a reactive increase in the number of immunosuppressive neutrophils in the blood, lymph nodes and tumors within days after ACT. Inhibition of the HGF receptor c-Met impaired this reactive increase and enhanced CD8 T cell expansion and therapy efficacy (Glodde et al., 2017). As it has become increasingly apparent that subsets of CD4 T cells also take part in anti-tumor immunity (Muranski et al., 2008; Quezada et al., 2010), we want to advance our understanding of the role of CD4 T cells in cancer immunity and study their interaction with neutrophils. **Materials and Methods**: We established an ACT protocol with CD4 T cells that specifically target the melanocytic antigen TRP1. The ACT is complemented by chemotherapeutic lymphodepleting pre-conditioning, an adenoviral vaccine and peritumoral injections of poly(I:C) and CpG to stimulate innate immunity. Analysis of T cells and neutrophils is primarily done via flow cytometry and immuno-histochemistry. In order to investigate the interactions between tumor specific CD4 T cells and neutrophils locally in the tumor microenvironment, CatchupIVM-red mice will be used, a mouse line with tdTomato[+] neutrophils (Hasenberg et al., 2015). BFP-expressing HCmel12 tumor cells will be transplanted into these mice. When a palpable tumor is present, ACT with GFP-expressing TRP1-specific CD4 T cells will be provided. Intravital 2-photon microscopy will be utilized to study interactions between CD4 T cells, tumors and neutrophils and at the same time track neutrophil and CD4 T cell density and movement within the tumor microenvironment.
Results: We observed that adoptively transferred CD4 T cells could control tumor growth for some time, however, after an initial tumor regression phase, tumors relapsed. Strikingly, a significant increase of neutrophils was found after CD4 ACT in both peripheral blood and tumor-draining lymph nodes. We hypothesized that, similar to CD8 T cells, the c-Met inhibitor capmatinib can reduce the reactive increase of neutrophils, resulting in enhanced CD4 T cell expansion. Therefore, an initial experiment was performed in which HCmel12 melanoma bearing wild-type mice were treated with our CD4 ACT therapy. In half of these mice capmatinib was administered every 12 hours for 5 consecutive days, starting on the day of T cell transfer. Interestingly, combination therapy of CD4 ACT with c-Met inhibition did not preclude tumor relapse as compared to CD4 ACT treatment only. However, flow cytometry analysis of peripheral blood revealed a strong increase of transferred CD4 effector T cells in a subset of mice that had received capmatinib.

Conclusion: Our findings indicate that, similar to CD8 cells, also CD4 T cell immunotherapy causes a reactive increase of neutrophils. Preliminary data suggest that, at least in a subset of mice, CD4 ACT benefits from combination therapy with a c-Met inhibitor, resulting in improved T cell immunity. Future studies will be directed towards dissecting T cell and neutrophil interaction, density and motility in the tumor microenvironment.

P115 | Psoriatic regulatory T cells are impaired in their suppressive activity and respond differently to IL-33

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Interleukin 33 (IL-33) is a unique cytokine with regard to its structure, localization and function. Initially described as an alarmin, released by cells following cell damage, IL-33 has been shown to fulfill numerous functions in various infections and inflammatory diseases by modulating both the innate and the adaptive immune system. But IL-33 can also act in an immunosuppressive fashion by inducing regulatory T cells (Treg). We observed that disruption of the skin barrier by tape stripping in mice induced IL-33. In addition, the contact hypersensitivity (CHS) was found suppressed which was due to the induction of Treg by IL-33. Accordingly, blockade of IL-33 enhanced CHS, whereas injection of IL-33 suppressed CHS via induction of Treg. Enhanced IL-33 levels can be found both in the skin and the serum of psoriatic patients. On the other hand, in psoriasis Treg appear to be impaired in their suppressive function. Hence, we studied whether "psoriatic" Treg are impaired in their response to IL-33, utilizing the imiquimod (IMQ)-induced psoriasis like model. IL-33 expression was elevated in both skin and serum of IMQ-treated mice, as demonstrated by in situ immunofluorescence staining and ELISA. To study whether the activity of Treg is influenced by the enhanced levels of IL-33 in IMQ-mice, CD4^{+}CD25^{+} Treg were obtained from IMQ or untreated mice. Cells were injected into naïve mice which were sensitized 24 hours thereafter. Ear challenge was significantly reduced in mice receiving Treg from untreated mice in comparison with positive control mice which were sensitized and challenged only. In contrast, no suppression was observed upon injection of Treg obtained from IMQ-mice, suggesting that in IMQ mice Treg are impaired in their function. To study whether the response of Treg to IL-33 is altered in IMQ-mice, Treg from untreated or IMQ-mice were incubated with IL-33 for 24 hours and injected into naïve mice which were sensitized thereafter. The suppressive capacity of Treg obtained from WT mice could be further enhanced by IL-33, whereas Treg from IMQ mice appeared to respond to IL-33 not at all. Similar observations were made in humans. In an in vitro suppression assay Treg isolated from blood of psoriatic patients were significantly inhibited in their suppressive activity, when compared to Treg obtained from healthy volunteers. Flow cytometry analysis of CD4^{+} T cells obtained from

healthy volunteers revealed a strong increase of Foxp3 expression in response to IL-33 treatment, whereas T cells from psoriatic patients did not respond to IL-33. Together this study confirms that in psoriasis Treg are impaired in their function. This may be associated with an altered response to IL-33. Future studies have to elucidate the mechanisms which are responsible for this altered behavior of Treg in psoriasis and in the IMQ-model.

Conclusion: A high frequency of $CD14^+$ $CEACAM^+$ monocytes is associated with poor prognosis in patients with advanced melanoma. Significant differences between patients and HD were found regarding the phenotype of peripheral monocytes. We hypothesise that $CD14^+$ $CEACAM^+$ monocytes support tumour progression and suppress anti-tumour immunity, but underlying mechanisms have to be further elucidated.

P116 (OP04/05) | Identification of a new monocyte subpopulation with prognostic significance in the peripheral blood of patients with advanced melanoma

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Background: The presence of distinct leukocyte populations can facilitate or interfere with benefit from immune checkpoint blockade (ICB) in melanoma. Three different human monocyte subpopulations have been characterised based on CD14 and CD16 epitope load: classical ($CD14^+CD16^-$), intermediate ($CD14^+CD16^+$) and non-classical ($CD14^{low}CD16^+$) monocytes. High classical monocyte frequencies have recently been detected a positive prognostic marker for response to anti-PD1- immunotherapy and improved overall survival in patients suffering from malignant melanoma. In this study, we performed a comparative analysis of monocyte subsets and characterized a previously poorly described monocyte subpopulation ($CD14^+CEACAM^+$) from peripheral blood of patients with metastatic melanoma.

Methods: Peripheral blood mononuclear cells (PBMC) were obtained from patients with metastatic melanoma (n = 20) and healthy donors (HD) (n = 20) after informed consent. Phenotypic characterisation of monocytes was performed by multi-colour flow cytometry. Cellular responses of monocyte subtypes towards pro-inflammatory stimuli (LPS, IFN-gamma and GM-CSF) were determined by intracellular staining for TNF-alpha and IL-1 beta.

Results: We detected an inverse correlation of the percentage of $CD14^+CEACAM^+$ cells and patient survival, as patients with a cell count above the median showed inferior survival compared to those below. Patients $CD14^+CEACAM^+$ monocytes display a significantly enhanced CD64 expression compared to corresponding cells of HD. PD-L1 surface expression is slightly reduced in patient monocyte subpopulations compared to HD cell fractions. The frequency of IL-1 beta+ cells (out of total $CD14^+$ cells) for all subpopulations is increased in patients. Protein levels of IL-1 beta+ are upregulated in the intermediate/non-classical monocyte fraction compared to $CD14^+$ $CEACAM^+$ cells and classical monocytes, independent of the stimulating agent, for both patients and HD.

P117 | The transfection of natural killer T cells with a tumor-antigen specific chimeric antigen receptor (CAR) for melanoma immunotherapy

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After the huge success of chimeric antigen receptor (CAR) T cells in hematological malignancies, its efficacy is currently evaluated in different solid tumors including melanoma. However, first results were not as compelling as for hematological malignancies, due to the fact that CAR-T cells need to cope with several challenges when used in solid tumors. One obstacle is defined by the occurrence of immune escape mechanisms developed by tumor cells to by-pass immune recognition, e.g. downregulation or loss of the target antigen. Natural killer T cells (NKT cells) represent an effective cell subpopulation in pathogen and tumor cell defense. An important advantage over conventional T cells is their pronounced intrinsic anti-tumor activity. Thus, NKT cells transfected with a CAR, which recognizes a tumor-specific surface antigen, could attack tumor cells antigen-specifically via the CAR, as well as through their intrinsic anti-tumor activity. In this way, CAR-NKT cells could be active, even in case of loss of the target antigen.

For the experiments, NKT cells were MACS-isolated from PBMCs, expanded over 10 days and were then electroporated with mRNA encoding a CAR specific for the melanoma surface antigen CSPG4. CAR-expression on the cell surface of NKT cells was analyzed and their in-vitro functionality, i.e. cytokine secretion and cytotoxicity, was evaluated after stimulation with melanoma cells and compared to that of conventional CAR-T cells. In addition, the intrinsic cytolytic ability of the CAR-transfected NKT cells was examined after stimulation with alpha-GalCer-loaded target cells.

About 2% NKT cells could be isolated out of PBMCs, leading to an approximately tenfold increase through expansion. CAR-staining after transfection revealed that NKT cells were efficiently transfected. CAR-NKT cells produced antigen-specifically IFNγ and TNFα upon stimulation with melanoma cells. However, cytokine secretion levels of CAR-NKT cells were in general lower compared to conventional CAR-T cells. Specific cytotoxicity towards melanoma cells

was similar in both cell populations with CAR-NKT cells showing a trend towards higher lysis. Importantly, CAR-NKT cells were still able to kill target cells through their intrinsic cytolytic activity after CAR-transfection.

Taken together, it is feasible to generate CAR-NKT cells via mRNA-electroporation. The in-vitro cytotoxicity of CAR-NKT cells is similar compared to conventional CART cells, while CAR-NKT cells maintained their intrinsic lytic capacity after receptor transfection. The next step will be the in-vivo functionality analysis in appropriate melanoma mouse models.

P118 | The direct identification of neoantigens via mass spectrometry in malignant melanoma

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Background: Finding targets that are only present on tumors for CD8^{+} T cell-based therapies remains a challenge. Somatic mutations can generate neoantigens, leading to the presentation of new peptide-HLA-class I complexes on the cell surface, which are promising tumor specific targets. The aim of this project is to establish and optimize the direct identification of HLA peptides in malignant melanoma cell lines and tissue samples via mass spectrometry. This will allow the search for neoantigens, potentially building the foundation for a personalized vaccination strategy to induce targeted T cell responses.

Methods: First, the isolation of the peptides in complex with their HLA molecule is performed using an antibody against HLA class I molecules covalently coupled to protein A Sepharose beads. Afterwards the peptides are separated from the HLA molecules and the peptide fraction is analyzed by nano-LC-MS/MS (nanoscale liquid chromatography coupled to tandem mass spectrometry). De novo sequences are determined from the raw MS/MS data and aligned with databases. HLA class I binding prediction is performed for sequences found in the databases, identifying peptides that are presented with a high probability.

Results: We were able to identify up to 2000 HLA peptides in four different cell lines (SK-Mel 28, SK-Mel 5, DG-75 and JY) and one melanoma metastasis (MS 160.2). Furthermore, we observe a significant portion of peptides with high-quality MS/MS spectra and high de novo scores, from which the peptide sequences can be predicted with high reliability. A large fraction of these cryptic peptides, which cannot be identified in a large protein database, are predicted to bind the sample specific HLA alleles. For the identification of the cryptic HLA peptides from non-canonical reading frames or non-coding RNA regions, we performed a search in the RefSeq transcriptome database translated in silico in the three forward open reading frames. Almost every cryptic peptide can be mapped to a 5′UTR-RNA region, 3′UTR-RNA region, coding out of frame or non-coding RNA.

Conclusion: The HLA peptide isolation was established and we are able to identify cryptic peptides in all our samples. The high de novo scores, the positive HLA binding prediction and the determination of their origin makes a large fraction of cryptic peptides likely to be actually presented via HLA class I molecules. Further studies on the nature and therapeutic potential of cryptic peptides and detectable neoantigens are warranted.

P119 | Screening identifies novel treatment targets for pemphigus

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Pemphigus vulgaris (PV) is a severe autoimmune bullous dermatosis where autoantibodies against desmoglein (Dsg) 3, and in some cases additionally Dsg1, cause skin blistering. The treatment options are not considered satisfactory yet. In recent years, it was shown that intracellular signalling cascades play an important role in the pathogenesis of the disease (e.g. the p38 MAPK pathway). Therefore, targeting cell signalling might be a new therapeutic option for pemphigus patients. However, comprehensive insights into anti-Dsg1/3-induced signalling are so far lacking. To address this knowledge gap, and to potentially identify novel therapeutic targets for the treatment of pemphigus, a comprehensive screening was performed using a chemical library composed of intracellular pathway inhibitors in the anti-Dsg3-induced Dsg3-internalisation assay with HaCaT cells. Of 141 inhibitors 20 were identified in the initial screening. Using the keratinocyte dissociation assay, where fragmentation of keratinocytes is induced by pemphigus patient IgG, 6 compounds inhibited pemphigus-IgG induced fragmentation in both HaCaT cells and normal human keratinocytes. BIRB 796, an inhibitor of the p38 MAPK, was among these 6 compounds. As inhibition of p38 MAPK had been reported earlier to contribute to reduce blistering in pemphigus, this validated the screening approach. Next, we aimed to validate the compounds in experimental models that are closer to the human pathology. For this we employed the human skin organ culture model, and induced intraepidermal blistering by injecting an anti-Dsg1/3 single-chain variable fragment in absence or presence of the 6 pathway inhibitors at 3 different concentrations (1, 0.1, and 0.01 mM, respectively). Subsequently, the epidermal split formation was evaluated in a quantitative manner and compared between specimens treated with and without inhibitor (positive control). We show a reduced split formation in sections treated with BIRB 796 and four other compounds. In conclusion, our results confirm the importance of the p38 MAPK pathway in mediating blistering in pemphigus, and in addition, we identify four novel, so far unknown target molecules contributing to anti-Dsg1/3 induced blistering.

Interestingly, these 4 molecules are among a single pathway which is independent of the p38 MAPK pathway. (Topical) Application of compounds targeting this pathway seems to be a good treatment option, especially to bridge the time to remission in patients treated with anti-CD20 antibodies.

using ELISpot assay. Monitoring of autoreactive T cells holds major promise as a helpful tool for disease evaluation. Moreover, the identified Dsg3-peptide-specific T cells are potential targets of ongoing novel therapeutic approaches aimed at down-regulating pathogenic T cells in pemphigus.

P120 | Monitoring of autoreactive T cells in patients with pemphigus

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Introduction: Pemphigus encompasses a group of life-threatening skin diseases including the clinically most common variants, pemphigus vulgaris (PV) and pemphigus foliaceus (PF) which are associated with IgG autoantibodies against desmoglein (Dsg) 1 and Dsg3, respectively, leading to blisters of the skin and/or mucous membranes. Loss of tolerance to Dsg on the CD4$^+$ T cell level and close association of PV with the human leukocyte antigen (HLA) class II alleles, HLA-DRB1*04:02 and HLA-DQB1*05:03, are crucial for the development of autoreactive T cells leading to the initiation and perpetuation of the autoantibody response. Thus, detection of autoreactive T cells in pemphigus is of particular interest to monitor disease progression and to evaluate the use of autoreactive T cells as potential therapeutic target in pemphigus.

Methods: A total of 20 patients with pemphigus (PV: n = 15; PF: n = 5), were included as well as 20 age- and sex-matched healthy controls. Peripheral blood mononuclear cells (PBMC) were isolated from peripheral blood and ex vivo stimulated with Dsg3 or Dsg1 recombinant proteins to induce autoreactive T cell activation. The autoreactive T helper cell profile was subsequently characterized by ELISpot assay which determine the frequency of Th1 cells secreting IFN-γ and Th2 cells secreting IL-5. In a subgroup of patients with PV, reactivity of autoreactive T cells against distinct immunodominant Dsg3 peptides was further analysed by ELISpot and 3H-thymidine incorporation assay.

Results: In both PV and PF, autoreactive Dsg-specific T cell response were predominantly of the Th2 type as significantly elevated numbers of Dsg1- and Dsg3-specific T cells secreting IL-5 were observed compared to HC (Dsg1: $P = 0.001$; Dsg3: $P < 0.001$). Moreover, in PV, Dsg3-specific Th2 cells showed a moderate correlation with serum anti-Dsg3 IgG ($r = 0.485$; $P = 0.036$) while autoreactive Th2 cells were also present in anti-Dsg3 IgG seronegative patients suggesting a general persistence of autoreactive T cells in peripheral blood. In addition, Dsg3-specific autoreactive T cells recognized a set of immunodominant Dsg3-epitopes in the majority (7/8) of the analysed HLA-DRB1*04:02 positive PV patients as determined by ELISpot assay or by 3H-thymidine incorporation assay.

Conclusion: We here demonstrate the presence and persistence of autoreactive Dsg3-specific Th2 cells in patients with pemphigus

P121 | Divergent glucocorticoid responsiveness of psoriatic vs. non-psoriatic human Th17 cells

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Glucocorticoids potently inhibit T helper (Th) cell type 1 memory responses but often fail to control memory Th17 cells. Nevertheless, the responsiveness of human Th17 cells to glucocorticoids depends on the pathophysiological context. Here, we studied the effect of glucocorticoids on Th17 and Th1 responses using peripheral blood mononuclear cells (PBMCs) from patients with psoriasis, a steroid-sensitive Th17-/Th1-driven disease, and from non-psoriatic subjects. PBMCs were stimulated with Candida albicans and had their supernatants harvested for cytokine analyses. We found that Th1 responses, as measured by means of IFN-γ secretion, were inhibited by glucocorticoids in both the psoriatic and control PBMC cultures. In turn, IL-17 levels in the supernatants of psoriatic PBMCs were significantly decreased, in sharp contrast to sustained IL-17 secretion observed in control PBMCs. Together, our study suggests that Th17 memory responses of psoriatic patients are intrinsically different from those of non-psoriatic individuals in their responsiveness to glucocorticoids.

P122 | Culprit drugs induce specific IL-36 overexpression in acute generalized exanthematous pustulosis

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Acute generalized exanthematous pustulosis (AGEP) is a severe adverse drug reaction of the skin which is characterized by acute formation of sterile pustules on erythematous skin, fever and peripheral blood neutrophilia. Although an involvement of drug-specific T cells and increased secretion of IL-8 has been reported,

the physiopathology of AGEP and mechanism of neutrophilic skin inflammation remains incompletely understood.

In our studies, we could identify IL-36 as a possible key player in the pathogenesis of AGEP. Using gene expression profiling, quantitative Real Time PCR and immunohistochemistry, we found elevated levels of IL-36α and IL-36γ in the skin of AGEP patients, when compared to normal skin and to maculo-papular rash, the classical type of drug-induced skin reaction. The major source of IL-36 was found to be keratinocytes and macrophages. Notably, the majority of IL-36 expressing keratinocytes were localized close to the subcorneal/intraepidermal pustules.

In vitro, the causative drug specifically induced IL-36γ release, either directly by peripheral blood mononuclear cells (PBMC) from AGEP patients, or indirectly by autologous keratinocytes in the presence of PBMC. Using a co-culture model, we observed that high levels of IL-8 gene expression occurred exclusively in patients' PBMC and not in keratinocytes in response to culprit drug exposure. Interestingly enough, upregulation of IL-8 gene expression in AGEP patients' PBMC was abrogated by IL-36Ra, suggesting that IL-8 expression in PBMC is dependent on PBMC- and/or keratinocyte-derived IL-36. Strikingly, treatment of a pure monocyte population induced IL-36γ secretion. This was specific for AGEP and involved the sensing of drug/albumin complex as a danger signal by the Toll-like receptor 4. Our results suggest that IL-36γ play a crucial role in the pathogenesis of AGEP. A direct activation of peripheral blood monocytes by culprit drugs could be shown for the first time, suggesting an important impact of the innate immune system in AGEP pathogenesis. These findings may help to understand the complex immune reactions occurring in these patients and open up new relevant research avenues to explore in the field of cutaneous adverse drug reactions.

P123 (OP01/06) | From coagulation to contact hypersensitivity—PAR2 signaling in cutaneous inflammation

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Exogenous and endogenous proteases influence cutaneous immunity by controlling inflammation, edema, itch and pain. The G protein-coupled protease activated receptor (PAR) 2 is activated by coagulation proteases, including the tissue factor (TF) ligands activated coagulation factors VII (FVIIa) and X (FXa) that are circulating in the blood, but are also expressed by myeloid cells. Protease signaling through PAR 2 plays a central role in cutaneous inflammatory diseases, but which proteases contribute to contact hypersensitivity (CHS), the mouse model of the allergic contact dermatitis in humans, is incompletely understood. The aim of this study was to analyze how cell-type specific proteolytic PAR2 activation by coagulation proteases mediates immune-hemostatic system crosstalks in cutaneous inflammation. We showed that antibody-mediated TF inhibition during both, the induction and effector phase of CHS, attenuated cutaneous inflammation (ear swelling, inflammatory infiltrate) and hapten-specific T cell response (T cell proliferation and Tc1 immune response). Complete PAR2 insensitivity to proteolytic cleavage by a specific receptor mutation (PAR2 mutant mouse strain R38E) significantly inhibited early (8 hours) and late (24 hours) stage of the allergen-specific CHS reaction. PAR is mostly expressed by keratinocytes and sensory afferent neurons. Experiments with cell-specific knockout mice unexpectedly identified PAR2^{+}LysM^{+} myeloid cells, but not PAR2^{+}CD11c^{+} dendritic cells, as functionally relevant orchestrators during the early effector phase of the allergic skin reaction. This was reflected by a high expression of PAR2 and TF on CD11b^{+} myeloid cells, including Ly6c^{+}CD64^{+}CCR2^{+} and inflammatory Ly6C^{+}CCR2^{+} monocytic cells, in the skin and the skin-draining lymph nodes at 8 hours after the challenge. PAR2 mutant mouse models with resistance to FXa and epidermal proteases, i.e. matriptase and prostasin, cleavage (PAR2 G37I mice) or resistance to skin proteases alone (PAR2 K36E mice) exhibited attenuated CHS responses at 8 hours after the challenge, but only mice resistant to FXa-cleavage remained protected from CHS in the later phase. Our study demonstrates that PAR2 signaling in myeloid cells plays a crucial role in allergic skin inflammation and is controlled by a complex network of proteases. Our findings may provide novel targets in the topical and systemic treatment of cutaneous inflammatory diseases.

P124 (OP05/04) | Mast cells modulate viral-specific CD8 T cell activation during Herpes simplex viral infection

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Mast cells (MCs), strategically localized at mucosal surfaces, provide first-line defense against pathogens and shape innate and adaptive immune responses. Recent studies have shown that human MCs are involved in pathogenic responses to several viruses including respiratory syncytial virus, human immunodeficiency virus and dengue virus. Furthermore, MCs are present at sites of active viral replication, are long-lived, and are resistant to cytotoxic effects of viruses. Here, we investigated the role of MCs during human herpes simplex virus 1 (HSV-1) infection in vitro. Incubation with HSV-1 resulted in infection rates of up to 50% in human MCs (hMCs) isolated from skin biopsies in a dose and time-dependent manner. HSV replicated in MCs and progeny virions were released from infected MCs. In addition, HSV infection resulted in the downregulation of hMC IgE receptor FceRI and the upregulation of their extracellular activation markers. HSV infection did not induce MC degranulation or cytokine production. In addition, we assessed the ability of MCs

to activate CD8 T cells by using an in vitro HSV-1 murine infection model. Interestingly, HSV-infected murine bone marrow-derived MCs induced HSV-specific CD8 T cell upregulation of extracellular activation markers such as CD25, CD69 and CD44 as well as IFN-g and TNF-a production. Our data suggest that skin MCs contribute to HSV infections in humans. Specifically, MCs may function as non-professional antigen-presenting cells and directly modulate CD8 T cells responses during HSV infection. A better understanding of the role of MCs during HSV infections may allow us to develop new therapeutic options to improve antiviral host responses.

P125 | Autoantigen CHD4 forms immune stimulatory complexes with endogenous DNA

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Background: Dermatomyositis (DM) is an idiopathic inflammatory myopathy belonging to the spectrum of autoimmune connective tissue diseases. DM patients present with anti-nuclear antibodies against Chromodomain-helicase-DNA-binding protein 4 (CHD4).

Objective: The impact of CHD4 on the immunogenicity of endogenous DNA is investigated by measuring the type I Interferon (IFN) pathway activation in immortalized keratinocytes (HaCaTs).

Methods: Immunohistochemical staining of CHD4 in patient skin samples from five different skin diseases to quantify the amount of autoantigen. The immune stimulatory capacity of CHD4-DNA-complexes was compared to the one of DNA alone by measuring the concentration of secreted CXCL10 using ELISA and by mRNA-seq to investigate the differences in expression of all type I IFN-inducible genes in HaCaTs. Surface plasmon resonance and fluorescence microscopy were used to determine the binding affinity from DNA to CHD4 and to show the complexes visually, respectively.

Results: CHD4 is increased in DM and two other autoimmune connective tissue diseases. CHD4 tightly binds endogenous DNA with a KD of 0.195 nM ± 0.0586 nM and forms visible complexes which have an immune stimulatory property. HaCaTs stimulated with CHD4 and endogenous DNA together show a significantly higher amount of CXCL10 in the supernatant than DNA alone, RNA-seq shows amplification of the expression for most IFN-regulated genes, e.g. OASL, OAS3, STAT1, and MX2.

Conclusion: We can show that CHD4 is able to form immune stimulatory complexes that are able to active the type I IFN pathway. Concluding this mechanism could contribute to the sustainment of the pro-inflammatory vicious cycle in DM skin lesions and play a role in their pathogenesis.

P126 (OP05/06) | GARP dependent induction of Treg by platelets: implications for malignant melanoma?

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Platelets are the central cells mediating hemostasis at the site of injury. Furthermore platelets are important modulators of the innate and adaptive immunity through their interaction with immune cells. In case of infection, platelets get activated and are able to modulate the inflammatory process. However, detailed information about the platelet–leukocyte interaction in inflammation is still limited.

Recently, we were able to show that the soluble form of Glycoprotein A repetitions predominant (sGARP) has strong regulatory and anti-inflammatory properties in vitro and in vivo. GARP, first described on platelets and as an activation marker on the surface of activated regulatory T cells (Treg), is known to modulate the bioavailability of TGF-beta and is therefore involved in the regulation of the peripheral immune responses. sGARP leads to induction of peripheral Treg as well as to inhibition of tumor antigen-specific CD8^{+} T cells as shown before. In the present study, we analyzed the role of GARP expressing platelets in melanoma and its potential as a prognostic marker in more detail.

We were able to detect sGARP in the supernatant of activated platelets. In coculture, platelets inhibited dose dependently the proliferation and cytokine production of CD4^{+} T cells, while inducing a strong Foxp3 expression and a suppressive capacity. Using a blocking anti-GARP Ab in the co-culture, we were able to reverse these effects. Additionally we analyzed the platelet count, GARP expression on platelets and sGARP levels in serum of melanoma patients at different stages of disease in comparison with healthy donors. Here, patients with stable disease showed a decreased platelet count compared to those patients with progression of disease.

In conclusion, our data give evidence that platelets are capable to induce peripheral Treg (pTreg) in a GARP-dependent manner. Through the induction of Treg via GARP expression and sGARP shedding, platelets could be of importance in melanoma where poor prognosis and metastasis are associated with elevated numbers of circulating platelets (thrombocytosis).

P127 | Pathogenicity of anti-desmoglein 3 IgA1 autoantibodies is Fc-dependent in IgA pemphigus

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IgA pemphigus is an autoimmune blistering skin disease characterized by tissue bound and circulating IgA, but not IgG, autoantibodies (autoAbs) against epidermal cadherins, particularly desmogleins (Dsgs) or desmocollins (Dscs). Patients typically present with vesiculopustular skin eruptions, intraepidermal acantholysis and prominent neutrophilic infiltrates. To facilitate dissection of pathomechanisms involved, we applied antibody phage display to genetically clone anti-Dsg3 monoclonal antibodies (mAbs) from an IgA pemphigus vulgaris (IgA PV) patient, as confirmed by direct immunofluorescence (IF) microscopy and high serum anti-Dsg3 IgA reactivity. No Dsg1 or Dsc reactivity was detected. We isolated three Dsg3-specific IgA mAbs, in form of single-chain variable fragments (scFvs). As expected, these scFvs bound to Dsg3 by ELISA and IF on Dsg-transfected cells. Sera from the same patient, from unrelated active IgA PV patients and from active IgG PV patients led to decreased binding of our scFvs in inhibition ELISAs, indicating that they bind to similar or identical Dsg3 epitopes as serum-derived polyclonal IgA/IgG autoAbs. The biological validity of our mAbs was further exemplified by dose-dependent cell sheet dissociation of cultured human keratinocytes and pathogenicity in human skin organ culture. These assays were conducted under concurrent elimination of Dsg1 by exfoliative toxin A, because anti-Dsg1 IgA/IgG reactivity was absent in our patient. Our findings indicated that in IgA PV the anti-Dsg3 IgA mAbs alone are necessary but insufficient for disease induction. Additional Fc-dependent cellular mechanisms potentially involved in tissue pathology were therefore studied by an in vitro human skin cryosection model, using recombinant full anti-Dsg3 mAbs of different isotypes. Incubation of cryosections with a full anti-Dsg3-IgA1 mAb and human peripheral polymorphonuclear cells led to intraepidermal acantholysis and typical pathology, whereas the same mAb in the IgG1 format was ineffective in the same assay. We here present conclusive in vitro data showing that anti-Dsg3 IgA1-Fc:FcalphaR interaction is mediating epidermal pathogenicity in IgA pemphigus, without requiring concurrent inactivation of Dsg1 by autoAbs. Blocking the interaction of anti-desmosomal IgA and FcalphaR-bearing cells may be effective future treatment for this difficult-to-treat skin disorder.

P128 | GPR15L is upregulated in murine models of psoriasis in an IL-23-independent manner but its cognate receptor GPR15 does not regulate skin disease

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Background: GPR15 has been implicated in the pathogenesis of T cell-driven inflammation of the skin and the gut. Expression levels of GPR15 ligand GPR15L are increased in psoriatic skin and considered as potential biomarker for the treatment response to anti-IL-17 antibody therapies. However, the significance of the GPR15L/GPR15 for the pathogenesis of psoriasis and the mechanisms regulating GPR15L expression in psoriatic skin are still elusive.

Methods: We addressed the role of the GPR15L/GPR15 in two mouse models of psoriasis, the Aldara(TM)-induced psoriasiform dermatitis (AIPD) and the IL-23-induced dermatitis model. In both models, we charted the expression levels of GPR15 and GPR15L in the skin and assessed the significance of GPR15L/GPR15 using $Gpr15^{-/-}$ mice.

Results: GPR15L levels were increased in the AIPD model, but not in the IL-23-induced dermatitis model. Deficiency in Gpr15 did not alter the course of disease neither in the AIPD, nor in the IL-23-induced dermatitis model. In neither model, deficiency in Gpr15 modulated the hallmarks of psoriasis on the histopathological or the molecular level. Despite the induction of GPR15L in the AIPD model, GPR15^{+} cells did not accumulate in the skin.

Conclusions: GPR15L expression is induced in psoriasiform dermatitis, but the activation of the IL-23/IL-17 axis alone is not sufficient for its induction. This restricts the potential use of GPR15L levels in the skin as biomarker for the treatment response to anti-IL-17 antibody therapy. Overall, our results exclude a significant role of GPR15 in the pathogenesis of psoriasiform dermatitis. As a corollary, GPR15L does not modulate psoriasiform dermatitis or employs GPR15-independent mechanisms.

P129 | The Ornithodoros moubata soft-tick-derived lipocalin Coversin abrogates skin inflammation in a murine model of pemphigoid diseases by sequestering complement factor C5 and leukotriene B4

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Background: Pemphigoid diseases (PDs) are a group of autoimmune blistering skin diseases driven by the deposition of autoantibodies at the dermal-epidermal junction and the subsequent recruitment of granulocytes. Previously, a critical role for the ligand/receptor pairs leukotriene B4 (LTB4)/BLT1 and the activated complement C5 fragment (C5a)/C5aR1 was uncovered for the eruption of skin inflammation in the antibody transfer bullous pemphigoid-like epidermolysis bullosa acquisita (BP-like EBA) mouse model. The Ornithodoros moubata soft-tick-derived lipocalin Coversin in parallel sequesters C5 and LTB4, while its mutant L-Coversin only sequesters LTB4.

Methods: Using the antibody transfer BP-like EBA model and human materials from BP and EBA patients we have addressed the potential of both compounds as therapeutics in PDs.

Results: Coversin and L-Coversin dose-dependently attenuated disease when administered prophylactically. Herein, due to its dual

inhibitory ability, Coversin, was more potent in suppressing disease than L-Coversin, Coversin also reversed established skin inflammation when tested in a therapeutic regimen. LTB4 and C5a were both present in the blister fluid of BP patients in concentrations chemoattracting human granulocytes. In line with a contribution of LTB4 to the recruitment of granulocytes in PDs, its receptor BLT1 was mainly expressed on granulocytes in perilesional skin of BP and EBA patients. In contrast, C5aR1 was predominantly expressed on keratinocytes in perilesional skin of BP and EBA patients.

Conclusions: Collectively, our results highlight both Coversin and L-Coversin as potential drugs for the treatment of PDs. However, simultaneous inhibition of C5a and LTB4 through Coversin may be superior to sole inhibition of LTB4 through L-Coversin. Our results encourage initiating clinical trials on the therapeutic potential of Coversin and L-Coversin in PDs.

P130 (OP06/01) | Effect of phosphodiesterase-4 inhibition on skin and mucosal lesions in experimental anti-laminin 332 mucous membrane pemphigoid

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The autoimmune bullous disease mucous membrane pemphigoid (MMP) is characterized by autoantibodies against the dermal-epidermal-junction and a predominant mucosal involvement. Reactivity against laminin 332 (Lam332), a heterotrimer consisting of 3 laminin chains, is found in one third of MMP patients. Major clinical and immunopathological characteristics of the human disease, including both mucosal and skin lesions, are recapitulated in the recently established antibody transfer model for anti-Lam332 MMP in adult mice. As the treatment of MMP patients still relies on high-dose corticosteroids, there is a high unmet need for new and more specific therapies. Phosphodiesterase-4 (PDE4) inhibition has previously been shown to be effective in patients with psoriasis vulgaris and Behcet's disease. Here, in the anti-Lam332 MMP mouse model, a specific PDE4 inhibitor, roflumilast, was applied in a prophylactic approach, using 5 mg/kg/day p.o. In two independent and blinded experiments, roflumilast significantly reduced oral lesions compared to vehicle-treated mice as quantified by endoscopy (P = 0.029), whereas, interestingly, a significant increase in skin lesions was observed (P < 0.0001). In line, in lesional biopsies, the number of inflammatory cells was significantly decreased in the buccal mucosa, but not in the skin, of roflumilast-treated compared to vehicle-treated mice (P = 0.007, P = 0.1523). A significant decrease of oral lesions could also be seen, when the PDE4 inhibitor was applied in a therapeutic approach (P = 0.016), i.e., when lesions had already developed. To investigate the differential effect

of the PDE4 inhibitor observed in the prophylactic approach, a transcriptome analysis by NGS of affected skin and buccal mucosa from roflumilast-treated anti-Lam332 MMP mice (n = 5), vehicle-treated anti-Lam332 MMP mice (n = 5) and mice injected with non-pathogenic rabbit IgG (n = 3) was performed. Differential gene analysis revealed Chil3, Ly9, IL18 bp, integrin alpha-L, and TNF to be upregulated in lesional skin of untreated mice with anti-Lam332 MMP. Among these inflammatory mediators, TNF showed a central role by STRING analysis. In the buccal mucosa of roflumilast-treated mice with anti-Lam332 MMP, Aldh1a2, Ryr1, Tceal7, and Il22ra2 were upregulated. Further validation by Gen Set Variation Analysis, qPCR, and Western Blotting will help to corroborate these findings and uncover pathways responsible for the differential effect of PDE4 inhibition on antibody-mediated tissue destruction in skin and mucosa. Furthermore, our data support PDE4 inhibition as potential novel therapy for mucous membrane lesions in patients with MMP.

P131 | Interaction of tight junction downregulation and staphylococci-dominated dysbiosis in the context of atopic dermatitis

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Atopic dermatitis (AD) is a common, chronic, relapsing inflammatory skin disease and is estimated to affect 10-20% of children and 2-10% of adults in the western world. AD is characterized by dry and scaly skin, which periodically exhibits severe, itchy eczema typically on the face, scalp and limbs. Skin barrier defects and dysbiosis of the skin microbiota are hallmarks of AD pathogenesis, but their functional interaction still needs to be investigated. Regarding this, the microbial dysbiosis is predominantly characterized by an increased colonization with *Staphylococcus aureus* (*S. aureus*) and may be enabled by barrier disruption indicating an interdependence of cutaneous bacteria and skin. Tight junctions (TJs) in the skin are close contacts between the plasma membranes of neighboring cells and are known to be important for barrier function, cell proliferation and differentiation. Murine and human studies indicate an essential function of some of them in maintaining skin barrier function in the context of AD. However, little is known about the function of their interplay with *S. aureus*.

Here, we investigated how skin barrier impairment contributes to the development of AD, focusing on the microbial shift towards *S. aureus* dominance in a mouse model. We found that barrier impairment, induced by repetitive tape stripping of the back skin of mice leads to a significant downregulation of several TJ proteins such as claudin-1, claudin-10, occludin and ZO-1 after 6 or 18 hours

detected by RT-PCR ex vivo. Furthermore, an upregulation of *S. aureus*-associated pro inflammatory cytokines, e.g. IL-1 beta, TNF-alpha and IL-6, was detected at the same time points. First analyses of the skin microbiome before and after tape stripping contribute to this finding by pointing to a predominance of staphylococci after tape stripping. We applied this concept to the contact hypersensitivity model to FiTC, a type 2 immune prone model mimicking the acute phase of AD. As a result, previous tape stripped mice showed a significant higher ear swelling than non-tape stripped mice. Additional, the tape stripped mice showed an increased Th2-dominated immune response associated by an increased CD3^{+}CD4^{+} cell population as well as increased IL-4 and IL-13 levels compared to the non-tape stripped controls.

These findings demonstrate the establishment of a model of skin barrier disruption by tape stripping which can be applied to study molecular mechanisms of AD pathophysiology. These results also indicate an important role of the downregulation of TJs contributing to a staphylococci-dominated dysbiosis of the skin and AD development. To conclude, this model and findings should be used to develop treatment strategies for patients suffer from this disease.

P132 (OP01/01) | The C5a/C5aR1 axis is critically involved in the induction of auto-antibody response in Epidermolysis bullosa acquisita

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Epidermolysis bullosa acquisita (EBA) is a rare, chronic subepidermal blistering disease caused by auto-antibodies (aAbs) against collagen type VII (COL7). COL7 is a major component of anchoring proteins, which are responsible for stability of the epidermal-dermal junction in the skin. IgG aAbs can identify and interact with a part of murine COL7, which has sequence homology to the von-Willebrand-factor A-like domain 2 (vWFA2). Previously, we could show in an Ab transfer model of EBA that a deficiency of the G protein-coupled receptor C5a receptor 1 (C5aR1) protects the mice from disease progress, which demonstrate the critical role of C5aR1 in the effector phase. Moreover, we showed an important regulatory cross-talk between C5aR1 and Fcγ-receptors (FcγR). The FcγR translate Ab responses into cellular answers. Previous publication reported that EBA is mostly FcγRIV-dependent, but we found a dependency also on FcγRIII in an active mouse model of EBA.

We immunized EBA-susceptible (B6.s) and EBA-non-susceptible (B6.j) wild-type (wt) mice, B6.s C5aR1^{−/−} mice and B6.s

FcγRIIb^{−/−} mice with vWFA2 and Titermax (1:1). Biweekly for eight weeks we scored the clinical phenotype of the mice and took blood samples for ROS analysis, COL7 epitope mapping, IgG subclass-assays and cytokine assays. We purified COL7-specific aAbs of the blood serum and quantified the IgG subclasses. Furthermore we investigated the cytokine levels of pro-inflammatory markers during the course of disease. Finally, we analyzed the IgG Fc-glycosylation, which defines additionally the pro- or anti-inflammatory properties of IgG Abs. Moreover we harvested spleen, draining lymph nodes and affected skin tissue for phenotypic and functional cellular analyses.

After 4-6 weeks B6.s wt mice developed first clinical symptoms, whereas C5aR1^{−/−} on B6.s background were completely protected from EBA. We found in B6.s C5aR1^{−/−} mice significantly lower serum levels of COL7 specific pro-inflammatory IgG2b and IgG2c and a significantly higher frequency of anti-inflammatory COL7 specific IgG1 aAbs. Additionally we analyzed the glycosylation of IgG1 and found significantly decreased levels of pro-inflammatory agalactosylated aAbs, whereas the level of anti-inflammatory highly galactosylated IgG1 was elevated in C5aR1^{−/−} sera in comparison with wt sera. To analyze the interaction of IgG with different FcγR we used a ROS-assay in which neutrophils got activated with aAbs from EBA mice. Here we showed that the activity of neutrophils dependents on both FcγRIII and FcγRIV, in contrast to data published with the rabbit-to-mouse Ab transfer model which only depends on the activity of FcγRIV.

Furthermore we investigated the pro-inflammatory cytokine pattern in serum and we showed that B6.s wt mice showed during week 6 and 8 elevated levels of IL6, IL12 and IL17a, all important cytokines which promote the migration of antigen presenting cells to site of inflammation, compared to B6.s C5aR1^{−/−} mice. Interestingly, we found also that B6.s C5aR1 mice expressed significantly higher levels of IL-10 than B6.s wt mice. Both observations pointing into less pro-inflammatory environment in C5aR1^{−/−} mice. Our findings identify the C5a/C5aR1 axis as a critical driver of EBA. Mechanistically, with these results the C5a/C5aR1 axis seems to be important for the induction of pathogenic aAbs and drives the Fc glycosylation towards a proinflammatory Ab type. Moreover in this context we showed the relevance of FcγRIII for the first time in an active model of EBA.

P133 | Alterations in the cutaneous microbiome in the course of allogeneic hematopoietic stem cell transplantation

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The success of allogeneic hematopoietic stem cell transplantation (HSCT) remains limited due to severe side-effects, such as infections and graft versus host disease (GVHD). Recent studies suggest that dysbiosis of intestinal microbes is associated with an increased risk of GVHD and poor outcome, while the role of the cutaneous microbiome remains elusive. We obtained patient material (peripheral blood, skin scales, stool and skin biopsies) at 5 time points before myeloablative conditioning and up to one year after HSCT (n = 20).

The cutaneous and intestinal microbiome is analyzed with 16S rRNA and whole metagenome sequencing. In vivo interactions of bacteria with immune cells are monitored by combining monoclonal antibodies with fluorescent in situ hybridization (FISH). Bacterial numbers/mm2 and distance calculations from CD45^{+} and HLA-DR^{+} cells are assessed via StrataQuest Analysis Software.

16S sequencing was established from healthy stool and skin samples and showed an expected mixture of commensal bacteria. Visualization of bacteria via 16S rRNA FISH in HSCT patients revealed a decrease in bacteria/mm² skin in the epidermis as well as the upper (<500 μm) and lower (>500 μm) dermis at day 0 and day 14 after transplantation. At day 100 bacterial numbers were comparable to baseline before transplantation. Although often in close contact with CD45^{+} cells, no intracellular bacteria were observed.

This study gives us the unique possibility to examine the repopulation kinetics and crosstalk between the immune system and the residing microbiome. Furthermore, we will establish risk profiles for GVHD development and occurrence of infections based on the individual skin and gut microbiome.

P134 (OP06/06) | Transcriptional profiling reveals factors for long-term survival of skin-resident T cells in hematopoietic stem cell recipients

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Myeloablative conditioning preceding allogeneic hematopoietic stem cell transplantation (HSCT) presents a unique situation in the human system to compare survival capacities of central and peripheral T cells. To determine factors mediating long-term tissue residency, we analyzed T cells isolated from peripheral blood and skin of patients receiving HSCT by flow cytometry and low-input RNA sequencing. Samples taken in full myeloablation and central immunosuppression (day 0) were compared to serial samples from before start of therapies until 1 year after transplantation. Additionally, long-term skin-residency was assessed in skin sections of patients receiving sex-mismatched donor cells by X/Y fluorescence in situ hybridization.

While >90% of peripheral blood T cells were eliminated by day 0, merely 50% of skin T cells were affected by myeloablative conditioning therapy. Notably, CD69^{+} and CD103^{+} αβ memory T cells remained stable and functionally competent populations in epidermal and dermal tissues, with recipient T cells still constituting >30% of total T cells 2 years after transplantation. These skin-resident cell subsets down-regulated tissue egress molecules while enhancing transcription of tissue retention genes, maturation markers and pro-inflammatory cytokines. Instead of a glucose-based metabolism common for effector T cells, skin cells at day 0 showed upregulation of lipid scavenger receptors, likely relying on exogenous free fatty acid uptake for longterm survival.

Our results combine data of a unique clinical setting with in-depth cell profiling and imaging techniques. Thus, we were able to identify long-lived and radio-resistant T cells and their distinct survival program in human skin.

P135 (OP06/03) | IL-9-producing T helper cells are a subpopulation of PPAR-gamma+ TH2 cells

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γTH1, TH2, and TH17 cells are well-defined proinflammatory T helper (TH) cell lineages in humans. Whether IL-9-producing "TH9" cells constitute a bona fide T cell lineage remains debated, despite their first description 10 years ago. Our previous work identified IL-9-producing TH cells as a subpopulation of TH2 cells expressing IL-9 transiently post activation. In fact, IL-9-producing TH cells were found to show key TH2-lineage-defining properties: They express TH2-lineage-defining cytokines (IL-5, IL-13), chemokine receptors (CCR4^{+}/CCR8^{+}), and transcription factors (GATA3) when analyzed irrespective of activation status. Here, we investigated the transcriptional program that differentiates IL-9^{+} TH2 cells from "conventional" TH2 cells that lack IL-9 expression. To this end, we performed transcriptional profiling before and after activation of different human TH cell subsets. We found that IL-9^{+} TH2 cells specifically express high levels of the transcription factor PPAR-gamma. Accordingly, PPAR-gamma was strongly induced in naive TH cells by priming with IL-4 and TGF-β ("TH9" priming), just as IL-9 itself. Functional importance of PPAR-gamma for IL-9 expression was confirmed by pharmacological antagonism or gene silencing of PPAR-γ. PPAR-γ inhibition reduced IL-9 production in TH2 cells while leaving production of other cytokines in TH2, TH1, and TH17 cells largely unaffected. In human skin disease, we found high numbers of IL-9^{+} TH2 cells in acute but not

chronic allergic skin inflammation and these numbers correlated with the presence of PPAR-γ+ cells. Correspondingly, antagonism of PPAR-γ in T cells isolated from acute allergic contact dermatitis resulted in specific downregulation of IL-9. Taken together, these findings suggest PPAR-γ as novel regulator of IL-9 in TH2 cells and identify TGF-β as a key factor inducing PPAR-γ in human TH2 cells. Our findings in humans are in line with recent findings in murine models of allergy and parasite infection where PPAR-g emerged as a driver of pathogenic TH2 inflammation.

P136 | Obesity drives chronicity of psoriatic skin inflammation

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The inflammatory skin disease psoriasis is often characterized by comorbidities with obesity as the most prevalent. Epidemiological studies clearly associate obesity as an aggravating factor for skin inflammation in psoriasis but mechanisms mediating this are unknown. The aim of the present study was to investigate how obesity alters skin immune responses.

To this end, a diet-induced obesity model was established by feeding of male C57Bl/6J mice with high-fat diet (HFD, 60% fat). To investigate the effect of obesity on Th17/Th1-mediated skin inflammation, a mouse model of 2,4,6-trinitrochlorobenzene (TNCB) contact hypersensitivity (CHS) was used. Challenging sensitized mice resulted in a significant increase of the chronic phase of skin inflammation in obese mice. Characterization of the immune response after the TNCB challenge revealed a significant elevation of IFNg, IL-17 and TNF in draining lymph nodes (dLN) and an increase of IL-17, IL-1beta, IL-6, and TNF in serum of HFD-fed mice in comparison with the controls. In the skin we found an elevation of IL-17 expression and further experiments showed that dermal TCRlow gamma/delta T cells were the major source of IL-17.

Expression analyses in adipose tissue showed a significant increase of IL-12, IL-6, TNF, iNOS and CCL2 in obese mice, suggesting a possible accumulation of M1 macrophages in this tissue. Furthermore, expression of TNF, IL-6, iNOS, CCL2, and macrophage numbers were also significantly elevated in TNCB-CHS skin of obese mice. Finally, macrophage depletion resulted in the abrogation of the increased chronic phase of skin inflammation. Next, we aimed to investigate the role of gut microbiota. Administration of antibiotics during the diet resulted in an abrogation of increased skin inflammation due to obesity with no difference in the numbers of gamma/delta T cells in the skin between obese and lean mice.

Taken together, our data indicate that obesity drives chronicity of psoriatic skin inflammation by increasing macrophages and IL-17 producing Gamma/delta T cells in the skin. Interestingly, this occurs with involvement of gut microbiota, which warrants further investigations.

P137 | The G protein-coupled receptor GPR15 suppresses pemphigoid disease-like dermatitis

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The G protein-coupled receptor GPR15 (GPR15) has been implicated in the orchestration of colitis by regulating the migration of T effector and T regulatory cells. Herein, depending on the precise setting, GPR15 exerts pro- or anti-inflammatory net effects in the gut. In the skin, GPR15 was shown to mediate recruitment of dendritic epidermal T cells (DETCs) during early postnatal development. Once in the epidermis, self-renewing DETCs participate in wound healing and tissue repair. More recently an antimicrobial peptide C10ORF99, a cognate ligand for GPR15, now designated as "GPR15L" was identified. GPR15L was found to be expressed in the skin upon its challenge with the toll-like receptor agonist imiquimod and during allogenic skin transplantation. Despite the indirect evidence suggesting a contribution for GPR15/GPR15L to skin homeostasis, no mechanistic studies examining the role of this receptor-ligand pair in cutaneous inflammation were performed.

We have therefore addressed the significance of GPR15-GPR15L signalling in autoantibody driven skin inflammation, using the antibody transfer model of bullous pemphigoid-like epidermolysis bullosa acquisita (BP-like EBA). Interestingly, GPR15 was predominantly expressed in the skin under homeostatic conditions. Genetic deficiency of GPR15 significantly aggravated disease in this model, suggesting a protective role for GPR15 in autoantibody-induced skin inflammation. Investigating possible mechanisms mediating the protective effects of GPR15 in the skin, we noted that Gpr15$^{-/-}$ mice bear significant deficiency in DETCs both under homeostatic and inflammatory conditions. Given the protective role of DETCs in the skin, their deficiency is therefore consistent with the exacerbation of skin inflammation in Gpr15$^{-/-}$ mice. In line with the antimicrobial properties of the GPR15 ligand we assessed its expression levels in the skin, and compared the microbiome of wild-type (WT) and Gpr15$^{-/-}$ mice in healthy and EBA affected skin. GPR15L was markedly upregulated in lesional skin of both WT and Gpr15$^{-/-}$ mice, but its increase did not alter the alpha- and beta-diversity of the skin microbiota in diseased mice. In contrast, differences in the microbial composition were observed between Gpr15$^{-/-}$ and their co-housed littermates WT controls under homeostatic conditions, further highlighting the role of GPR15 in controlling skin microbiome.

Collectively, our results uncover a previously unappreciated counterregulatory role of GPR15 in antibody-induced skin inflammation in mice, likely mediated by DETCs.

P138 (OP03/01) | Environmental antigens may promote cross-activation of CD8^{+} T cells against melanocytes in psoriasis

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Psoriasis vulgaris is an HLA-C*06:02-associated T-cell mediated autoimmune skin disease, in which environmental factors play important roles. Using the Vα3S1/Vβ13S1 T-cell receptor (TCR) from a pathogenic epidermal psoriatic CD8^{+} T-cell clone, we had shown that HLA-C*06:02 mediates an autoimmune response against melanocytes through presentation of a peptide autoantigen from ADAMTS-like protein 5 (ADAMTSL5). TCRs, however, are polyspecific. They do not recognize specific antigens but are ligated by multiple peptides sharing certain amino acid motifs.

To investigate how environmental factors contribute to psoriasis onset, we screened for potential environmental TCR ligands using the conserved amino acid motif of the Vα3S1/Vβ13S1 TCR that may cross-activate the Vα3S1/Vβ13S1 TCR and thus initiate the psoriatic autoimmune response. In silica data base search and peptide stimulation experiments identified 24 peptides which were presented by HLA-C*06:02 and ligated the Vα3S1/Vβ13S1 TCR. These candidate antigens included epitopes from *C. trachomatis*, *M. tuberculosis*, the alcohol-fermenting yeast Saccharomyces cerevisiae, coffee, wheat, and, various skin and gut microbiota. Interestingly, several candidate factors had formerly been proposed as potential environmental psoriasis triggers. To further assess their actual relevance to clinic and autoimmune responses, we evaluated antigen-driven CD8^{+} T-cell activation by stimulating peripheral blood mononuclear cells from psoriasis patients (PvPs) and healthy controls (HCs) with these peptides. 3H-Thymidine incorporation and the activation-induced CD137 expression on CD8^{+} T cells serve as readout of antigen-specific activation. The strongest CD8^{+} T-cell activation from PvPs was induced by wheat peptides and moreover, the wheat responses statistically correlated with those to the autoantigenic ADAMTSL5 peptide. As reported, a substantial number of psoriasis patients were positive for gluten-specific IgG and IgA antibodies. Indeed, T-cell activation by wheat was stronger in patient with anti-gluten antibody. Together, our data demonstrate that an autoreactive TCR, which mediates a pathogenic HLA-C*06:02-restricted autoimmune response of CD8^{+} T cells against melanocytes in psoriasis, also recognize diverse environmental peptides. This study provides a conceptual model where environmental antigens cross-activate autoreactive T cells to exert an HLA-restricted autoimmune response, which may develop novel strategy for prevention of T-cell mediated autoimmune disease.

P139 (OP06/05) | Integrin aE (CD103) regulates dermal innate lymphoid cells type 2 in contact hypersensitivity reactions

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Dermal Innate-like Lymphoid Cells (dILC2s) are increasingly recognized for their key role in development of allergies and eczematous skin diseases. Integrin aE (CD103), expressed especially by epithelial lymphocytes and dendritic cells but also by dILC2s, is thought to mediate adhesion and migration of T cells in inflammatory skin reactions. We therefore examined the role of CD103^{+} dILC2 in murine contact hypersensitivity (CHS) models.

We found that CD103^{−/−}/B6J mice display a significantly higher total dILC2 count compared to WT mice under physiological conditions. Interestingly, CHS models resulted in similar inflammatory phenotypes in WT and CD103^{−/−} mice, and lead to concordant up- or downregulation in total leukocyte count. However, while total dILC2 count in WT mice was regulated identically compared to all leukocytes, total dILC2 numbers in CD103^{−/−} mice were left completely unchanged. Our functional studies indicate that alterations in total dILC2 numbers were mediated by a combination of proliferation and migration involving integrin aE (CD103). In vitro experiments evaluated the impact of CD103 on various leukocyte subsets and especially dILC2. Blocking experiments with a CD103-antibody (M290) were performed in vivo as well as adoptive transfers of WT and CD103^{−/−} leukocytes into Rag1^{−/−} mice.

Our data suggest an important role of integrin aE (CD103) in dILC2 physiology specifically during development of CHS, but not during irritant contact dermatitis. Lack of CD103 seems to affect proliferation and migration of dILC2 in allergic contact dermatitis. A better understanding of the role of integrin aE (CD103) in dILC2 physiology might pave the way for therapeutic interventions in eczematous skin diseases.

P140 | ERAP1 regulates the risk for psoriasis through affecting immunogenicity of melanocytes and the psoriatic autoantigen ADAMTS-like protein 5

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Psoriasis is an HLA-C*06:02-associated CD8^{+} T-cell mediated autoimmune disease, where the HLA-C*06:02 molecule promotes an

autoimmune response against melanocytes through presentation of a self-peptide from ADAMTS-like protein 5 (ADAMTSL5). The genetic predisposition of psoriasis involves epistasis between HLA-C*06:02 and variants of the gene encoding endoplasmic reticulum aminopeptidase I (ERAP1). ERAP1 is a polymorphic gene: Of the different genetic ERAP1 haplotypes (Hap), Hap2 increases the risk for psoriasis in genetic interaction with HLA-C*06:02, while Hap10 is protective. ERAP1 trims precursor peptides to the appropriate size for loading onto HLA-class I molecules and thus is thought to shape the immunopeptidome presented to CD8^{+} T cells.

We investigated the role of the two ERAP1 haplotypes, Hap 2 and Hap 10, using a T-cell receptor (TCR) activation assay. This assay employed a TCR hybridoma expressing a pathogenic ADAMTSL5-specific psoriatic Vα3S1/Vβ13S1 TCR. We generated ERAP1 deficient cells from HLA-C*06:02-positive melanoma cell line (MCL) using the CRISPR/Cas9 system. ERAP1 knockout in MCL significantly decreased HLA-C expression and ligation activity of the Vα3S1/Vβ13S1 TCR. Reconstitution ERAP1^{−/−} MCL with ERAP1 increased both HLA-C expression and immunogenicity for the Vα3S1/Vβ13S1 TCR hybridoma, with a greater effect of the psoriasis ERAP1 risk variant, Hap2, than the protective variant, Hap10. By using the defined autoantigen, ADAMTSL5, we show that ERAP1 Hap 2 and Hap 10 differentially affected ADAMTSL5 antigenicity by differential trimming of peptide precursors to the appropriate length for HLA binding. The psoriasis risk Hap2 efficiently generated the causative ADAMTSL5 self-peptide from NH2-extended precursor peptides, while the protective Hap10 reduced the availability of autoantigenic ADAMTSL5 peptides for TCR stimulation.

Overall our data explain that epistasis between HLA-C*06:02 and ERAP1 variants affects psoriasis risk through differential trimming of autoantigenic self-peptides. This alters the immunogenicity of melanocytes as target cells of the psoriatic autoimmune response by either increasing (Hap2) or decreasing (Hap10) their immunologic visibility for CD8^{+} T cells.

P141 | Phenotypic and functional characterization of the cutaneous immune response on contact with sodium lauryl sulfate (SLS)

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Sodium lauryl sulphate (SLS) is an anionic surfactant which is often used in toothpastes and detergents as an emulsifier. Due to its irritating properties, the addition of SLS to toothpastes has been a matter of debate in recent years. In contact dermatitis, SLS is regularly used as an irritant control in patch testing. About 22% of all patch-tested individuals show a positive reaction to SLS, and this cohort has a significantly higher risk to display frequent reactions to contact allergens. The reason for a positive reaction in almost one quarter of patients tested remains to be elucidated.

The pattern of the SLS-induced cutaneous immune responses was investigated in murine experiments. Repetitive topical treatment with SLS was compared with two three commonly used contact allergens (oxazolone, DNCB, DNFB) and the well-established irritant croton oil. SLS induced significant concentration-dependent skin inflammation. Based on the individual reactions, the current dynamics were recorded with ear thickness measurements and photography. To analyze the cutaneous immune response, cellular infiltrates in the affected skin as well as the draining lymph nodes were analyzed by immunohistochemistry, flow cytometry, and qPCR with regards to T-cell subsets, innate lymphoid cells and major cytokines involved. In order to dissect effects of systemic application of SLS on the SLS-induced cutaneous immune response, we injected SLS intraperitoneally at different times with or without topical treatment with SLS, contact allergens, as well as croton oil.

Our data permit an insight into the immunological response induced by SLS in comparison with allergic and irritative contact dermatitis. Systemic effects of SLS were evaluated for their impact on local skin inflammation induced in murine models of allergic and irritative contact dermatitis.

P142 (OP04/06) | Gold compound induces increased Treg frequencies in vitro and TGFβ expression in vivo

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Aryl hydrocarbon receptor (AHR) binds aromatic hydrocarbons and non-halogenated polycyclic aromatic hydrocarbons and mediates biological responses in a ligand-dependent way. The receptor functions as a transcription factor upon ligand activation and is inducing expression of various factors. Previous studies have shown that activated AHR regulates the induction of Tregs and Th17 cells in a ligand-dependent manner. A newly generated gold metal compound 3 (MC3) binds to the AHR and induces increased gene expression of cytochrome P450 enzymes (CYP1A1) in hepatocytes. Binding is 100 fold stronger compared to the ligand 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). TCDD was shown to induce foxp3 expression similar to CYP1A1 expression.

To investigate whether MC3 induces Treg conversion in vitro, primary murine CD4^{+} T cells were isolated from WT mice and treated over night with MC3 followed by stimulation with CD3/CD28. Treatment with MC3 showed an upregulation of CYP1A1 and TGFβ1, TGFβ2 and TGFβ3 in comparison with mock solution using real-time PCR.

Furthermore, MC3 treatment resulted in 2-3 fold higher frequencies of Tregs in comparison with mock-treated CD4^{+} T cells as detected by FACS analysis. In addition, Treg frequency was dependent on MC3 concentration; furthermore, MC3-induced foxp3 expression could be prevented by the AHR inhibitor resveratrol or a TGFβ inhibitor in vitro.

Since TGFβ induces anergy in activated T cells, we investigated the effect of MC3 on TGFβ expression in an autoimmune-related skin disease. Scurfy mice lack Treg control and show activation of autoreactive T cells which leads to T-cell mediated inflammation of several organs including the skin. Scurfy mice show severe skin disease (erosions, scabs and blister formation). After treating scurfy mice with 0.2 μM MC3 every 3 days for 21-24 days, we found reduced activation markers (CD69, CD25) on CD4^{+} pre-gated T cells in comparison with mock-treated mice. Furthermore, RT-PCR data show that MC3 treated mice (WT and scurfy) have higher TGFβ1 expression in liver, spleen and thymus in comparison with mock-treated mice. Similar results could be shown by TGFβ1 in-situ staining of thymus and liver. In summary, the gold compound MC3 functions as a new AHR ligand which induces increased TGFβ1 expression and thereby might lead to Treg induction. Furthermore MC3 seems to have an immune suppressive effect on T cells in an autoimmune model.

P143 | A new approach towards the development of 3D skin models for psoriasis

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We developed a functional full thickness 3D skin model consisting of keratinocytes and fibroblasts derived from skin biopsies of healthy individuals. The skin model was developed using a cell coating technique. This is a rapid manufacturing technology for fabrication of 3D cellular constructs by coating cell surface using layer-by-layer assembled nanofilms of extracellular matrices. In our models the thickness of the dermal equivalent could be easily controlled from approximately 5-100 μm by altering the seeded cell number. Comprising keratinocytes as the major epithelial cell population the model exhibited a fully differentiated epidermal equivalent after lifting to the air-liquid interface. A key feature of the model is its suitability for long term cell culture (>28 days). Moreover, the model is scaffold free and without the use of protein sources from animals.

Using this technique we could for the first time develop a 3D psoriatic skin model by mimicking the clinical condition for the treatment of psoriatic patients. To trigger a psoriatic phenotype we first stimulated our model system with IL-17A (50 ng/mL) for 5 days. On the following 14 days the model was additionally treated with the anti-IL-17A monoclonal antibody secukinumab (6 μg/mL). Models that were treated with IL-17A or secukinumab alone were used as controls.

While IL-17A treatment resulted in a psoriatic phenotype with a disturbed epidermal differentiation the addition of secukinumab inverted these abnormalities as demonstrated by histological examination. Moreover, RT-PCR analysis revealed that in comparison with IL-17A-induced effects the additional treatment of secukinumab forced an upregulation of keratinocyte differentiation markers such as FLG, KRT1, KRT10, DSC1, DSG1 and DSG4 and a downregulation of antimicrobial peptides such as DEFB4B and DEFB4A (HBD-2). Inhibiting IL-17A with secukinumab also downregulated different chemokines and proinflammatory cytokines (CCL20, CCL8, CXCL1, CXCL5, CXCL6, CXCL17, IL-1β, IL-6, IL-24, IL-33 and IL-36 (α, β and γ)). Here, secukinumab was able to reverse IL-17A induced effects on gene expression level.

Interestingly, despite the detected inhibiting effects of secukinumab on IL-17A-mediated gene regulations we detected an increased expression of IGFL2 and IL-18. It is tempting to speculate that this could be attributed either to the treatment protocol or the models require longer treatment periods with secukinumab to reveal a downregulation of these genes associated with inflammation.

In conclusion, using a cell coating technique we developed organotypic 3D skin models enabling long term in vitro cultivation. This feature was exploited to investigate the effects of IL-17A and its antibody secukinumab in psoriasis, which could be successfully accomplished due to the long term stability of the models. The present study not only shows the functionality of the developed skin model but also its versatility.

P144 (OP04/03) | Targeting heat shock protein 70 (Hsp70) in melanoma with functionalized theranostic superparamagnetic iron oxide nanoparticles

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Introduction: Nanotechnologies based on superparamagnetic iron oxide nanoparticles (SPIONs) have emerged as a promising tool for the theranostics of advanced and metastatic melanoma. Decoration of the nanoparticle surface with specific moieties that have specific anti-tumor immunotherapeutic activity could provide a theranostic potential for the nanocomplexes. Targeting Hsp70 that is presented on the cell membrane of tumor cells but on corresponding normal cells could provide the specificity of melanoma treatment. Combination of functionalized particles with

blocking of immune checkpoint inhibitors could further enhance the therapeutic benefit.

Material and Method: Developed nanoparticles conjugated with granzyme B (GrB-SPIONs) were in vitro assessed (employing confocal and electron microscopies, flow cytometry) for specific targeting of the membrane-bound Hsp70 in B16 melanoma cells. Intratumoral administration of GrB-SPIONs as a monotherapy or in combination with anti-CTLA-4 monoclonal antibodies and adoptive T-cell transfer was performed in the orthotopic model of B16 melanoma in C57/Bl6 mice. IHC sections were performed to assess the intratumoral distribution of nanoparticles as well as the caspase-3 expression in the melanoma following the treatment.

Results and Discussion: Internalized GrB-SPIONs in Hsp70-positive B16 melanoma cells induced apoptotic death in dose- and time-dependent manner (as shown by flow cytometry). Monotherapy with intratumorally injected GrB-SPIONs in B16 animal model resulted in delayed tumor progression (MRI volumometry). Biodistribution analysis employing highly sensitive magnetic resonance imaging (7T) confirmed the retention of particles in the tumor (that was further proved by histological studies). Subsequent therapy with anti-CTLA-4 antibodies and adoptive T-cell transfer improved the efficacy of GrB-SPIONs nanocomplexes. Inhibitory immune checkpoint blockade significantly enhanced the apoptosis in tumor cells as was demonstrated by the caspase-3 IHC analysis.

Conclusion: These findings reveal that GrB-SPIONs can specifically target Hsp70-positive melanoma cells. Combinatorial regimen of targeted nanocomplexes and immune checkpoint inhibitors has a high therapeutic potency that could be translated into clinical trials.

P145 | CRISPitope: a versatile approach to study how the target antigen choice influences antigen-specific T-cell therapy

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Background: The success of immune checkpoint blockade demonstrates that mobilization of the endogenous T-cell repertoire can effectively restrain tumor growth. However, not all patients benefit and other strategies like adoptive T-cell therapy (ACT) are needed to enforce anti-tumor immunity. Autologous CD8^{+} T cells can be engineered to express T-cell receptors (TCR) directed against specific tumor antigens. CD8^{+} T cells recognize antigen-derived short peptides (epitopes) presented by major histocompatibility complex (MHC) class I molecules, which allows for specific tumor cell killing. Hence, the choice of the target antigen is critical for ACT. Antigen expression level and peptide binding affinity to MHC class I

molecules are well-studied determinants of ACT efficacy in experimental models. However, other target antigen criteria like essentiality for tumor cell growth are insufficiently understood even though there is an unmet need, as personalized cancer immunotherapies are evolving rapidly.

Methods: We established CRISPR-assisted insertion of epitopes in order to render different endogenous gene products targetable by the same TCR-transgenic T cells trough fusions with a defined T-cell epitope. By this strategy we can directly compare how the target antigen choice influences therapeutic efficacy and resistance mechanisms.

Results: In a proof of principle study we applied CRISPitope to our established transplantable mouse melanoma models and we compared ACT targeting a melanosomal gene versus an oncogenic driver, which is critical for tumor cell growth. We found comparable therapeutic efficacies, but profound differences in resistance mechanisms. Indeed, the type of resistance mechanism defined the immune contexture of relapse melanomas with important implications for the efficacy of anti-PD-L1 salvage therapies.

Conclusions: CRISPitope represents a powerful and versatile experimental strategy that supports clinical efforts in antigen-specific personalized cancer immunotherapies to define target antigen selection criteria. The strength of CRISPitope is that it allows for addressing the relevance of the target antigen biology.

P146 | Tofacitinib downregulates antiviral immune defense in keratinocytes and reduces T cell activation

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Introduction: Tofacitinib is a novel Janus kinase (JAK) inhibitor approved for the treatment of rheumatoid and psoriatic arthritis. In clinical trials investigating the therapeutical effects of tofacitinib, the most common adverse events observed were nasopharyngitis and upper respiratory tract infections. JAKs are found downstream of the type II cytokine receptor family. A number of cytokines produced by TH17 cells stimulate their target cells via the type II cytokine receptors and these receptors use the JAK pathway for signal transduction. These TH17-associated cytokines are leading to the secretion of antiviral and antimicrobial peptides (AMP) by keratinocytes or synoviocytes. Blockage of the JAK pathway might therefore result in a diminished secretion of antimicrobial and antiviral peptides. This reduced capability of antimicrobial defense might result in a higher susceptibility to bacterial infections in patients treated with JAK inhibitors.

Material and Methods: We treated primary human keratinocytes with tofacitinib and subsequently added various cytokines and

bacterial surface proteins to the culture. The response of the keratinocytes was then evaluated using RT-qPCR. In order to test the activation of T cells, we pre-incubated PBMCs with tofacitinib and stimulated them with lipopolysaccharide (LPS) or Varicella zoster envelope glycoprotein (VZV gE). The activation of T cells was then investigated based on the expression of the activation marker CD69 on the cell surface via flow cytometry.

Results: We found that gene expression of antimicrobial peptides (e.g. S100A7 and LL37) in keratinocytes is not affected by JAK pathway inhibition using tofacitinib. But on the other hand, gene expression of all tested antiviral peptides such as MX1 or ISG15 is markedly reduced in a dose-dependent manner even without any costimulatory cytokine or bacterial component added. Additionally, we found that JAK inhibition leads to reduced activation of T cells after stimulation with bacterial LPS or viral VZV gE.

Conclusion: The antiviral immunity in keratinocytes is strongly inhibited in presence of tofacitinib in vitro, while the antimicrobial immunity does not seem to be affected. In T cells, the overall activation process seems to be influenced by tofacitinib. These findings are in accordance with our hypothesis that tofacitinib has an impact on antiviral immunity such as patients treated with tofacitinib often show adverse events like upper respiratory tract infections, and zoster.

P147 | Immunomodulation of melanoma by inhibition of stearoyl-CoA desaturase

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Recently, melanoma patients with preexisting antitumor immunity benefited greatly from new immunotherapies that block immune inhibitory checkpoints. To make these promising immunotherapies also available for patients with melanomas lacking T-cell infiltration, the challenge now is to turn immune cell-poor tumors into immune cell-inflamed tumors. A possible target to induce inflammatory signaling is the metabolic enzyme stearoyl-CoA desaturase (SCD) which is regulated by the lineage addiction oncogene MITF. The inhibition of the MITF-SCD signaling axis has been shown to induce inflammatory signaling in human melanoma cells in vitro.

Here, we analyzed the effect of specific SCD inhibitors on murine MITF-dependent melanoma cell lines and CD8^{+} T cells in vitro and on transplanted melanomas in vivo. SCD inhibition reduced melanoma cell proliferation and induced the expression of pro-inflammatory cytokines in vitro and in vivo. However, CD8^{+} T cell activation or proliferation was not impaired by SCD inhibitor treatment.

Taken together, our results reveal a great potential of SCD inhibitors as an adjuvant therapy for checkpoint blockade immunotherapy in experimental mouse melanoma models and suggests that concomitant SCD inhibitor treatment could offer therapeutic benefit in melanoma patients who did not respond to immune-checkpoint inhibitor treatment until now.

P148 | Effects of environmental signaling in patients with STAT1/STAT3-dependent primary immunodeficiencies

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Background: Patients with primary immunodeficiencies caused by STAT1/STAT3- imbalances frequently suffer from chronic microbial infections caused by a compromised Th17 immunity. By interfering with Th17 immunity and regulation of interleukin (IL)-22 and IL-17, the environmental sensor aryl hydrocarbon receptor (AHR) has immune-modulating functions and is possibly beneficial for Th17 immunity in STAT-imbalanced patients. Hence, we assessed healthy subjects and STAT1/STAT3-imbalanced patients for Th17 immunity and epithelial defense mechanisms upon stimulation of the AHR pathway.

Methods: Human peripheral blood mononuclear cells (PBMCs) of healthy subjects and STAT1/STAT3-imbalanced patients activated with anti-CD3/CD28 in combination with AHR agonists were assessed for the expression of AHR pathway related targets and Th17 cytokines. Epithelial response of human primary keratinocytes to AHR activation in combination with STAT1/STAT3 activating cytokines was analyzed regarding the production of antimicrobial peptides and pro-inflammatory mediators.

Results: AHR activation increased the Th17 cytokine IL-22 and AHR pathway related genes in PBMCs of healthy subjects and STAT1/STAT3-imbalanced patients. Despite induction, IL-22 concentration in patients remained below the level found in healthy subjects. AHR activation increased the production of antimicrobial peptides whereas pro-inflammatory chemokines were reduced in healthy keratinocytes.

Conclusion: The observed differences after environmentally induced signaling pathway activation between healthy subjects and STAT1/STAT3-imbalanced patients in the regulation of Th17 immunity and associated epithelial response direct to possible beneficial effects of AHR activation on an impaired epithelial defense.

P149 | Type I IFN responsiveness of melanoma is cell state dependent and can be both harnessed and suppressed to enhance oncolytic virotherapy efficacy

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Oncolytic virotherapy is a new promising approach to treat malignant melanoma. Tumor cells are often permissive for viral infection and oncolysis due to their active metabolism and decreased responsiveness to type I interferons (IFN-I). However, also IFN-I responsive tumors may be suitable targets for oncolytic virotherapy, as local IFN-I responses have often been associated with anti-tumor immunity. The underlying mechanisms accounting for the differences in IFN-I responsiveness of melanoma are poorly understood. The aim of this work was to analyze the responsiveness of a collection of human and mouse melanoma cell lines to IFNI utilizing an oncolytic Semliki Forest virus expressing EGFP (SFV-VA7-EGFP). 16 human melanoma cell lines with a spectrum of phenotypes ranging from very melanocytic (MITF-high) to poorly differentiated (MITF-low) were screened for their IFN-I responsiveness by treatment with varying concentrations of IFN-I followed by infection. The infection kinetics were monitored with fluorescence and bright field microscopy over 72 hours, after which the net result of cell proliferation was quantified using crystal violet staining. Following IFN-I pretreatment, healthy primary melanocytes were readily protected from infection, whereas all melanoma cell lines had, to varying degree, lowered antiviral type I IFN responsiveness. Melanoma cell lines, which had retained partial responsiveness to IFN-I displayed a basal IFN-I signature in a bioinformatic analysis. Interestingly, the one quarter of the melanoma cell lines with poorest IFN-I responsiveness were all melanocytic (MITF-high), suggesting a potential link between the differentiation status and the responsiveness to type I IFNs. Supporting the hypothesis, MITF overexpression utilizing a tet-ON system in MITF-low Mamel65 human melanoma cell line suppressed their type I IFN responsiveness allowing productive SFV-VA7-EGFP infection and oncolysis. RNA-seq and ATAC-seq massively parallel sequencing approaches in combination with available MITF ChIP-seq data revealed that MITF inhibits interferon stimulated gene expression and binds directly to several IFN-I regulatory areas. To test the hypothesis that both suppressing and harnessing the type I IFN responses may be utilized to benefit oncolytic virotherapy, we treated HCmel12 mouse melanomas with SFV-VA7-EGFP in combination with antibodies targeting either the type I IFN receptor or an immunosuppressive PD1 receptor on T cells. Both approaches were found to enhance oncolytic virotherapy efficacy and type I IFN-receptor deficient melanomas could be eradicated.

P150 | Environmental cues modulate neutrophil extracellular trap (NET) formation

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Neutrophils are the most abundant phagocytes and contribute to both innate and adaptive immunity. They are able to perform a new type of cell death called neutrophil extracellular trap (NET) formation (NETosis). During NETosis, neutrophils decondense and finally release their chromatin, decorated with cytosolic and granular proteins. These chromatin meshworks have been linked to chronic inflammatory diseases such as systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), vasculitis, diabetes, atherosclerosis and to cancer. For this reason, it is fundamental to understand the biological and biophysical conditions which may support or hinder NET formation.

Substrate elasticity ("stiffness") varies enormously between different tissues, ranging from 50 Pa in blood to 250 kPa in atherosclerotic plaques. As NETs have been shown to form in the context of the development of atherosclerosis and in some solid tumor tissue, we were interested in finding out whether substrate elasticity can influence and modulate the propensity of neutrophils to form NETs

To this end, freshly isolated human neutrophils were seeded on polyacrylamide gels with different substrate elasticity (1 kPa, 2 kPa, 4 kPa, 8 kPa, 16 kPa, 20 kPa, 30 kPa, 60 kPa, 128 kPa) coated either with collagen I or fibrinogen. The cells were stimulated to perform NETosis either with phorbol 12-myristate 13-acetate (PMA) or lipopolysaccharide (LPS), both classical inducers of NET formation. We found that under PMA stimulation NETosis did not depend on substrate elasticity. However, for stimulation by LPS there was a clear stiffness-dependent increase in NETosis rate for stiffer gels. Interestingly, there was also a correlation between neutrophil adhesion and spreading area to gels of different stiffness and NETosis rate.

Thus, we show here for the first time that NETosis is differentially regulated by mechanical and biophysical cues such as substrate elasticity. These novel finding may provide new insights into the pathophysiology of diseases such as atherosclerosis in which there is an increment in arterial stiffness or in Alzheimer's disease in which the brain stiffness is decreased.

P151 | Compensation of IL-6 through an increase of NLRP3 and IL-1 in BMDCs and keratinocytes of IL-6$^{-/-}$ mice

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Important inflammatory skin diseases such as psoriasis, lichen planus or atopic dermatitis are characterized by a hyperplasia of the epidermis. In these diseases, proliferation is controlled and only rarely leads to cancer development which can be supported by an inflammatory microenvironment. One key factor which controls and regulates proliferation is the activating transcription factor 3 (ATF3). ATF3 is a dual function protein as it suppresses pro-inflammatory IL-6 and IL-8, but also acts as a pro-oncogenic factor by the suppression of p53. To dissect the bi-modal role of ATF3 and its suppression of IL-6 we investigated the cytokine expression and secretion of BMDCs as well as keratinocytes out of WT, ATF3$^{-/-}$ and IL-6$^{-/-}$ mice. In BMDCs we could show an increase of IL-1β secretion and NLRP3 mRNA in all mice after treatment with different TLR ligands like R837, CPG and LPS, which was further significantly enhanced in IL-6$^{-/-}$ BMDCs. IL-6 mRNA was increased after treatment with TLR ligands, most prominently in ATF3$^{-/-}$ BMDCs compared to WT cells. We then investigated cytokine expression and secretion in keratinocytes out of WT, ATF3$^{-/-}$ and IL-6$^{-/-}$ mice upon R837 exposure. After TLR7 activation, IL-1, NLRP3 and IL-6 were increased, however no difference between WT and the knockout keratinocytes was observed. Therefore we decided to use UV as inflammasome activator. After UV treatment, keratinocytes responded with an increase of IL-1 and NLRP3, most pronounced in ATF3$^{-/-}$ and IL-6$^{-/-}$ keratinocytes.

Taken together, our results demonstrate that keratinocytes from ATF3$^{-/-}$ and IL-6$^{-/-}$ mice produce increased amounts of IL-1α, IL-1β and NLRP3 upon inflammasome activation by UV, while the absence of IL-6 is in dendritic cells compensated by an increased IL-1β secretion, partially due to increased NLRP3 mRNA-transcription upon TLR stimulation.

P152 | Selective targeting of JAK3 with new small molecular compounds

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The Janus kinase (JAK)/signal transducer and activator of transcription (STAT) signaling pathway plays an important role in inflammation and autoimmunity. Many cytokines critically involved in the pathogenesis of inflammatory skin diseases signal through the JAK/STAT pathway. JAKs like JAK1, JAK2, JAK3 and TYK2 associate with the cytokine receptors and are responsible for the activation of STAT transcription factors, which transmit the extracellular signals after phosphorylation and dimerization into the nucleus. The inhibition of this pathway can affect many cellular functions like proliferation, differentiation and survival. Inhibition of Janus kinases makes them popular targets for the development of new drugs. In mammals, JAK1, JAK2 and TYK2 are expressed ubiquitously, while the expression of JAK3 is limited to hematopoietic and lymphoid tissues. A specific JAK3 inhibitor should have limited but precise effects on immune cells and would not affect other cell types.

To study the effects of selective JAK inhibitors on cytokine signaling, we measured the dose-dependent inhibition of STAT phosphorylation in human CD4$^+$ T cells and compared new compounds with the clinically established inhibitor tofacitinib. T cells were stimulated with different cytokines that use either combinations of JAK3 and JAK1 or combinations of JAK1, JAK2 and/or TYK2. JAK3 selectivity was demonstrated for two new compounds, which inhibited STAT activation downstream of IL-2 and IL-4 receptor signaling. When stimulating T cells with certain ɣc receptor chain using cytokines, JAK3 inhibition by new compounds was achieved at lower concentrations than with tofacitinib. Since tofacitinib has been shown previously to inhibit not only JAK3 but also JAK1 and JAK2 activation, we next studied the impact of the compounds on JAK/STAT signaling activated by cytokines like IL-6 or IFN-α. As reported, tofacitinib inhibited IL-6 and IFN-α signaling in a dose-dependent manner. This has been tested by studying the activation of STAT3 and STAT1. In sharp contrast, the new compounds tested neither affected IL-6 nor IFN-α signaling in T cells, confirming their selectivity towards JAK3 in functional assays. The new generation of selective JAK3 inhibitors could provide new opportunities in clinical settings.

P153 | Near-infrared photoimmunotherapy as a new strategy to investigate inflammatory skin diseases

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Psoriasis is a common chronic inflammatory skin disease. The clinical picture results from hyperproliferative epidermal keratinocytes, a dense leukocyte infiltrate in the skin, and dermal hypervascularization. The local immune response of psoriasis is dominated by innate immune cells producing TNF-α, IL-23 and IL-12, thereby stimulating an IL-17-, IL-22- and IFN-α-dominated immune response mainly mediated by Th17/Th22/Th1 and Tc17 cells. In contrast to psoriasis, during acute cutaneous graft-versus-host disease (GvHD) alloreactive T cells are programmed to pathogenic effector Th1 and Tc1 cells, producing TNF-α and IFN-α leading to apoptosis of basal keratinocytes.

In this study, we aim to unravel the regulatory impact of inflammatory cells during the development and maintenance of inflammatory skin diseases. For this purpose we aimed for the ablation of different leukocyte subsets. For this purpose, we established a new depletion strategy in immunodeficient NSG mice using a psoriatic skin xenograft model as well as a healthy skin xenograft model exposed to allogenic leukocytes. The latter model resembles signs of human cutaneous GvHD defined by skin rejection.

In a first set of experiments, we coupled a near-infrared reactive photosensitizer via NHS-ester to a monoclonal antibody targeting CD4^{+} T cells. The efficiency of this mAb could be confirmed by flow cytometry. As published by others, when activated with the appropriate wavelength and energy, this photosensitizer causes a rapid selective cell disruption of target cells, with an insignificant amount of off-target effects.

With this method we could demonstrate in vitro cell specific depletion of CD4^{+} T cells within seconds of irradiation. After intradermal injection and target cell depletion in vivo we were able to prevent healthy skin rejection as seen in human cutaneous GvHD by depleting infiltrating allogeneic CD4^{+} T cells from the skin graft through irradiation. Additionally, after systemic application we could detect antibody penetration into psoriatic skin grafts which led to CD4^{+} T cell ablation from the inflamed tissue when exposed to near-infrared light thereby diminishing the amount of IL-17 producing cells.

We have previously demonstrated in a saporin-based depletion method the specific depletion of CD8^{+} T cells. With our current results, we are able to strengthen the importance of CD4^{+} T cells in Th1 and Th17 mediated skin diseases. By combining immunotherapy with near-infrared light therapy we present a new method for the cellular investigation of inflammatory skin diseases.

P154 (OP01/04) | Antigen-specific tolerance induction in a mouse model of pemphigus vulgaris

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Pemphigus vulgaris (PV) is an autoimmune blistering disease caused by autoantibodies (auto-ab) mainly against the desmosomal cadherins desmoglein (Dsg) 3 and Dsg1 leading to painful blisters and erosions of the skin and mucous membranes. So far, only unspecific treatment options such as systemic corticosteroids combined with adjuvant immunosuppressives or second-line therapies like immunoadsorption by high-dose immunoglobulins and recently B-cell depletion are being applied. The blatant need for antigen-specific therapies in PV targeting specifically autoreactive Dsg3-specific T and B cells prompted us to investigate a novel approach to induce T cell tolerance in an HLA-transgenic preclinical mouse model.

Our group has established a human leukocyte antigen (HLA)-transgenic mouse model of PV; the mice are transgenic for the PV-associated HLA-DRB1*04:02-DQ8 haplotype, the human CD4 coreceptor and they lack functional endogenous mouse major histocompatibility complex (MHC) class II (I-Aß^{−/−}). Immunization with recombinant human Dsg3 protein leads to the formation of Dsg3-specific T and B cells. Applying a set of previously identified immunodominant Dsg3 CD4^{+} T cell epitopes linked to different carriers (splenocytes or nanoparticles), the aim of this study was to modify the Dsg3-reactive immune response and to potentially induce Dsg3-specific T cell tolerance in this mouse model. HLA transgenic mice are immunized on day 0 and 14 with recombinant human Dsg3- protein in aluminum hydroxide and cellular and humoral immune responses to Dsg3 are characterized over a period of 28 days. In a preventive protocol, a set of immunodominant Dsg3 CD4^{+} T cell epitopes that are linked to different carriers are injected intravenously (i.v.) prior to the first Dsg3 immunization and the Dsg3 boost, respectively.

Here we could show a profound reduction of circulating Dsg3-specific IgG antibody titers in mice receiving a set of immunodominant Dsg3 T cell epitopes linked to splenocytes in a concentration dependent manner. Compared to control animals, anti-Dsg3-IgG titers were reduced by 30%-90%, respectively, in mice that were pretreated with Dsg3-epitopes bound to splenocytes. Moreover, Dsg3-specific T cell proliferation as well as secretion of IFN-γ by splenic T cells was significantly reduced in mice receiving Dsg3-peptide loaded splenocytes. Dsg3- peptide application alone did neither affect Dsg3-specific antibody formation nor T-cell proliferation. In addition to using cellular carriers for immunodominant Dsg3 T cell epitopes we applied nanoparticles as a more defined and novel method to induce Dsg3-specific T cell tolerance in this animal model. In a preventive setting, we could show that i.v. application of a set of immunodominant CD4^{+} T cell epitopes bound to nanoparticles significantly reduces the antibody formation 28 days after i.p. Dsg3-immunization by 74% and 80%, respectively, compared to control. We have set up a broad array of flow cytometry parameters to investigate in detail the distribution of specific B cell subpopulations such as transitional state and Dsg3-specific B cells. A non-significant reduction of total CD4^{+} as well as of T regulatory CD4^{+}CD25^{high}FoxP3^{+} cells in the spleen on day 28 after Dsg3-immunization points to effects of Dsg3-nanoparticle treatment on both the CD4^{+} T as well as on the B cell level. These immunological effects will be investigated in more detail in further PoC studies.

To summarize, for the first time we have successfully induced a Dsg3-specific tolerance in an HLA-transgenic mouse model of PV by using both Dsg3 T cell epitope-loaded splenocytes and nanoparticles in a preventive experimental setting. Both experimental approaches efficiently prevented th.

P155 | Detection of low-frequent desmoglein 3-specific T and B cells in an HLA transgenic mouse model of pemphigus vulgaris

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Pemphigus vulgaris (PV) represents a severe and difficult-to-treat blistering autoimmune disease of the skin and mucous membranes, despite recent therapeutic developments. IgG autoantibodies (auto-ab) mainly against desmoglein 3 (Dsg3) induce loss of keratinocyte adhesion and lead to the clinical presentation of flaccid blisters and widespread erosions in PV patients.

As in other autoimmune disorders, mouse models have been very important in revealing crucial steps in the pathogenesis of PV. Our group has established a human leukocyte antigen (HLA)-transgenic mouse model of PV; the mice are transgenic for the PV-associated HLA-DRB1*04:02-DQ8 haplotype, the human CD4 co-receptor and they lack functional endogenous mouse major histocompatibility complex (MHC) class II (I-Aß$^{-/-}$).

A single intraperitoneal immunization with recombinant human Dsg3 induces low titers of Dsg3-reactive IgG, while a second boost after 14 days increases antibody formation for several months. For identification and characterization of low-frequent Dsg3-specific CD4$^+$ T cells, we used novel HLA class II Dextramers®, which consist of a dextran polymer backbone carrying several HLA-DR0402-molecules and fluorochromes. In this study we used a set of HLA-DR0402-Dextramers® loaded with four immunodominant CD4$^+$ T cells epitopes of the human Dsg3-protein. Dsg3-reactive B cell receptors were detected by applying fluorochrome labeled recombinant Dsg3-protein in flow cytometry analysis. We found that the CD4$^+$ T cell compartment is primarily affected after the initial immunization, a significant increase up to approx. 3.5% of Dsg3$^+$CD4$^+$CD19$^-$ cells was found in the spleen 3 days after Dsg3-immunization. The second boost did not affect those Dsg3-specific CD4$^-$ T cells; however, a small population (approx. 0.2-0.4% of Dsg3$^+$CD4$^+$CD19$^-$ T cells) remained in the spleen compared to controls. Even though the total CD4 T cell compartment is increased after each immunization, T-regulatory cells (Tregs, defined as CD4$^+$CD25^{high}FoxP3$^+$ cells) are temporarily decreased in the spleen, suggesting a putative shift of CD4$^+$ T cells from the spleen into other lymphoid organs.

The kinetics within the B cell compartment differs from changes in the set of Dsg3- reactive CD4$^+$ T cells, as a maximum of total CD3$^-$CD45$^+$CD19$^+$ B cells in the spleen can be found 7-15 days after initial Dsg3-immunization. Conversely, Dsg3-specific B cells occur shortly after Dsg3-immunization (day 3) and are nearly undetectable already at day 7 in the spleen. Initially, the bone marrow as a primary lymphoid organ gives rise to transitional B cells (subgroups T1, T2, T3) after Dsg3- immunization. Interestingly, only the T3 subpopulation (defined as CD3$^-$CD45$^+$CD19$^+$CD93$^+$CD23$^+$IgM$^-$) which is known to recognize chronically present self-antigens increases in the

spleen after each Dsg3-immunization. A peak of Dsg3-specific T3 cells (2% of T3) can be detected only 7 days after initial immunization followed by a steady decline. B cell subpopulation T1 is decreased after initial immunization (reduced to 50% compared to control), and T2 B cells and the respective Dsg3-specific subpopulation remain stable. Three weeks after initial immunization, Dsg3-specific B cells can be detected in the bone marrow shown by Dsg3-specific B-cell ELISPOT analysis.

To summarize, in this study we have further characterized the complex Dsg3-specific T and B cell interactions in the HLA-transgenic mouse model of PV. The usage of highly sensitive techniques such as novel Dsg3-specific Dextramers® and fluorochrome labeled Dsg3-protein enabled us to identify the kinetics of very low frequent Dsg3-specific T and B cells in different lymphoid organs.

P156 | Activation of the lymphocyte function associated antigen 1 (LFA-1) is critical for the stable adhesion and migration of slan+ monocytes recruited via CD16 by intracapillary immune complexes

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Intracapillary immune complex (IC) deposition and accumulation of monocytes are hallmarks of lupus nephritis. A cell population directly recruited from the blood flow by IC are 6-sulfo LacNAc (slan+) cells. These cells express CD16 for the interaction with IC and have functional features of dendritic cells. They are now called slan+ monocytes (slanMo), which is in line with their monocytic progenitor and the most recent nomenclature of mononuclear phagocytes. We previously reported on the selective accumulation of slanMo for the early focal form of lupus nephritis (class III), in which immobilized ICs induced slanMo recruitment from the microcirculation via interaction with Fcγ receptor IIIA (CD16).

To illustrate the activation status of the integrin LFA-1, a monoclonal antibody specifically recognizing the activated α-subunit cation binding domain (mAb 24) was used. Furthermore, a perfusion assay-based approach was applied to investigate whether the integrin LFA-1 is required for the ICs-mediated recruitment of slanMo. Monolayers of dermal microvascular endothelial cells were preincubated with an endothelial cell-specific antibody to mimic deposited ICs. The arrest functions of purified slanMo were measured under physiological flow conditions. Moreover, a neuroprobe chemotaxis chamber was used to address the role of LFA-1 during the process of cell adhesion and migration.

We observed that LFA-1 on slanMo became activated upon IC-engagement via FcγRIII. By time-lapse video microscopy and blocking strategies with monoclonal antibodies and LFA-1 inhibiting small molecules such as lovastatin, we could demonstrate that rolling is

induced by the engagement of immobilized antibodies with CD16 and that firm adhesion as well as subsequent slanMo migration required activation of LFA-1.

Collectively, our results show that LFA-1 is required for the recruitment of slanMo to ICs deposited at to the vasculature. Understanding these critical nuances of recruiting inflammatory cells allows to specifically immunomodulate certain IC-mediated inflammatory and/or autoimmune diseases. Our results also underscore the rationale of previous immunomodulatory therapeutic strategies of using the LFA-1 specific mAb efalizumab as well as current strategies that employ the LFA-1 inhibiting small molecule lovastatin.

P157 | 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 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.

P158 | Identification of the mutation c.9886A>G, p.Lys330Glu in the plasminogen gene in a family from Northern Germany with hereditary angioedema

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Hereditary angioedema (HAE) is a life-threatening disease characterized by recurrent episodes of subcutaneous and mucosal swellings and abdominal cramping. Corticosteroids and antihistamines, which are usually beneficial in histamine-induced acquired angioedema, are not effective in HAE. Therefore, diagnosing HAE correctly is crucial for affected patients. We report a family from Northern Germany with six individuals suffering from recurrent swellings, indicating HAE. We evaluated clinical data, family history, and laboratory parameters. Furthermore, we performed genetic analyses of the genes for C1 esterase inhibitor (C1-INH; SERPING1), coagulation factor XII (F12) and plasminogen (PLG). In all affected members of the family, the symptoms manifested in adulthood, with swellings of the face, tongue and larynx, including a fatal case of a 19 year-old female individual. The mutation was also found in a yet unaffected 12 year-old member of the family. The frequency of the attacks was variable, ranging between once per year to once a month. Laboratory tests, including C1-INH concentration and function, and genetic diagnostics of SERPING1 and F12 were unremarkable. However, in three affected and the one yet unaffected member of the family, we were then able to identify the mutation PLG:c.988A>G, p.Lys330Glu mutation, which has recently been described in several families with HAE. This missense mutation leads to the amino acid exchange p.Lys330Glu in the kringle 3 domain of plasminogen. As reported in few other patients with PLG defects, icatibant proved to be very effective in controlling acute attacks, indicating an involvement of bradykinin in the pathogenesis of HAE associated with PLG mutations.

P159 | Restoration of anti-tumor T cell responses by restoration of skin dendritic cell numbers in a spontaneous melanoma mouse model

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One major characteristic of skin-related cancers is the drastic loss of skin-resident dendritic cell populations. In the tg(Grm1)EPv mouse model, ectopic expression of the metabotropic glutamate receptor-1 in melanocytes leads to a highly proliferative and anti-apoptotic phenotype, resulting in melanoma formation within the dermis. The slow progression of these tumors allows for the in depth analysis of the immune infiltrate in growing tumors. We here show that total DCs are gradually lost as the tumor progresses. Mainly the dermal CD11b^{+} DCs are affected, whereas epidermal Langerhans cells remain unchanged. We hypothesized that extrinsic cell death of the DCs may be induced within the growing tumor. Indeed, the tumor tissue upregulated the expression of Fas ligand (FasL) that can induce extrinsic apoptosis in immature DCs that express Fas (CD95). Fas-mediated apoptosis depends on the activation of caspase-8 and mice in which the autoproteolytic cleavage site D387 is mutated to alanine (casp8D387A) show strong resistance to Fas-mediated death. We compared the apoptosis induction in the different DC subsets in wild-type C57BL/6 and in casp8D387A mice upon intradermal administration of an agonistic anti-CD95 antibody. We found that indeed CD11b^{+} DCs in WT mice are susceptible to apoptosis via CD95. In order to increase the number of intra-tumoral DCs, we treated tumor-bearing tg(Grm1)EPv mice with Flt3L, an important growth factor for DCs. This treatment restored the numbers of CD11b^{+} DC-subset to the level of tumor-free mice. At the same time, T cells from both the tumor and the tumor-draining lymph node were able to produce more cytotoxic cytokines. Our data thus indicate that the loss of DCs in melanoma is mediated by apoptosis within the tumor; rescue of skin DCs may be a promising strategy in order to enhance the efficacy of existing immunotherapeutic strategies against skin-related cancer.

P160 | Identification and validation of novel immunoregulatory agents for the treatment of chronic inflammatory diseases

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There is still a high unmet medical need for better treatment options for patients with chronic inflammatory skin diseases. Many current therapies rely on systemic immunosuppression, which are linked to an increased risk of long term side effects such as infections and cancer. The use of strategies that strengthen already existing immune-control mechanisms such as regulatory T cells (Treg) is a novel and promising alternative treatment approach.

To identify Treg-promoting agents, we have developed a high throughput flow cytometry-based screening assay to monitor the expression of Foxp3 (master transcription factor of Tregs) in primary immune cells from Treg reporter mice. A library consisting of 40.000 small synthetic compounds (FMP Berlin) was screened, and compounds were selected as hits when Foxp3 expression was induced, proliferation and viability remained intact, and druggability was indicated according to the Lipinski rule of five. Finally, one validated hit compound and nine structural analogs were selected for further validation studies in primary human T cells and monocytes.

Dose response studies (40 nM to 20 μM) with human primary CD4 T cells showed upregulation of Foxp3 expression by all selected compounds even at the lowest concentration. In addition, compound-treated T cells and monocytes showed markedly decreased expression of the proinflammatory cytokines IL-17, IL-22 (T cells), IL-6, and TNF-α (monocytes).

Our data suggest that the compounds identified may be used to induce or strengthen inhibitory immunological pathways. As of now, no agents with a similar mode of action are available. Further development of this approach may lead to better treatment options for patients with autoimmune or other chronic inflammatory immunological skin disorders. Therefore, the further development of these compounds might lead to the development of drug candidates that display unique action profiles targeting chronic inflammatory diseases.

P161 | Foxo4 and AHR control a stress-induced immune-mediated host protection via secretion of IL-22

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Epithelial barrier integrity is of outermost importance to protect the human body from external harm. To ensure this protection, a close interaction of epithelial cells with the innate and adaptive immune system in barrier organs is needed to allow immediate and most appropriate defense mechanisms against harmful pathogens, but also to initiate repair mechanisms following inflammation induced tissue damage. One of the key cytokines involved in tissue regeneration is interleukin (IL)-22. During inflammation, cells capable of IL-22 production are recruited into barrier organs and activated to restore inflammation mediated barrier damage. The aryl hydrocarbon receptor (AHR) has been described to regulate IL-22 expression in T cells. Previous results from our group indicated that another transcription factor belonging to the Forkhead box O (FOXO) family, namely FOXO4, is expressed in human T cells. The FOXO family consists of four members—FOXO1, FOXO3, FOXO4 and FOXO6—with each member being expressed in different tissues and fulfilling distinct functions in cell-cycle regulation, tumor suppression, apoptosis, metabolism, senescence and ageing. By sensing environmental changes including oxidative stress, growth factor composition and inflammatory components, FOXO transcription factors can efficiently translate these stress signals into appropriate cellular reaction patterns.

FOXO4 has been mainly described as tumor suppressor and regulator of senescence and longevity. In line with these functions, FOXO4 mRNA is expressed in various tissues at low level and downregulated in most tumors. FOXO4 mRNA and protein have been detected in mouse CD4^{+} T cells and our group could assign FOXO4 expression to human T cells of the Th22 subset. Despite these first hints, a transcriptional contribution of FOXO4 to T cell phenotype and function has not been performed so far. To answer this question, we used a lentiviral transduction system to overexpress and vice versa downregulate FOXO4 in CD4^{+} effector T cells as well as in naïve T cells that were subsequently cultured under Th22 polarizing conditions.

Here, we now show that oxidative stress and pro-inflammatory cytokines stabilize FOXO4 expression in human T cells allowing cooperation with AHR to regulate IL-22 in effector cells and differentiating naïve T cells.

P162 (OP03/02) | Slan+ monocytes produce Interleukin-8 to recruit NK cells and to induce a p21-dependent growth arrest in melanoma cells

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In most instances leucocytes orchestrate effective anti-tumor responses to eliminate malignant cells before primary tumors or metastasis can arise. It was recently reported that murine patrolling monocytes initially detect newly formed metastasis and reduce metastatic load by recruiting NK cells. slan+ monocytes (slanMo) represent a subset of human CD14^{dim}CD16^{+} monocytes (homolog of patrolling monocytes in mice) and were previously identified in melanoma metastasis. slanMo are capable of initiating anti-tumor responses based on the secretion of proinflammatory cytokines after TLR ligand stimulation. A positive feed-back loop between slanMo and NK cells is based on the IFN-γ production by NK cells that amplifies IL-12 production by slanMo, which in turn perpetuates cytokine production by NK cells and slanMo leading to high levels of TNF-α and IFN-γ in the microenvironment. Here, we addressed the question whether slanMo actively recruit NK cells for starting an innate immune response that could control growth and induce senescence of tumor cells in malignant melanoma.

We initially validated the presence of both slanMo and NK cells in lymph node metastasis of melanoma patients. Since NK cells numbers in most tissues under homeostatic conditions are low, we next focused on the recruitment of NK cells by slanMo. We therefore established an in vitro migration assay to determine the slanMo-dependent local recruitment of NK cells. By using supernatants from TLR-activated slanMo, we could show a strong and specific NK cell migration towards slanMo conditioned medium comparable to recombinant CXCL-12 as the positive control. Neutralization of IL-8 in the slanMo conditioned medium reduced specific migration by 70%. Coculturing of TLR-activated slanMo with NK cells induced high levels of proinflammatory cytokines. Melanoma cell lines cultured with slanMo/NK cell-supernatants demonstrated a strongly reduced proliferation rate, increased senescence-associated beta-Galactosidase staining, and a senescence phenotype characterized by strong p21 (CDKN1A) upregulation. This phenotype could be abolished by combined TNF-α and IFN-γ neutralization, highlighting the function of these cytokines.

Our data suggests that slanMo are present in melanoma metastasis and can recruit NK cells in an IL-8 dependent mechanism to induce a proinflammatory immune microenvironment that inhibits the growth of melanoma cells.

P163 | Laser-assisted skin immunisation to target dendritic cells in human skin

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Skin dendritic cells (DC) are antigen presenting immune cells which induce immune responses against cutaneous infection and tumours. Due to their localisation in the skin, they are also able to recognise cancer cells developing in the skin and to start an immune response against tumours. The immunotherapeutic approach

called "epicutaneous immunisation" aims at loading DC subtypes with tumour antigens in situ. To improve this vaccination modality we loaded skin DC with fusion antibodies directed against DC surface molecules, such as the lectin receptors DEC-205 (CD205) and Langerin (CD207) that are essential for antigen incorporation. We hypothesised that laser poration of the skin may substantially improve epicutaneous immunisation. An infrared laser (P.L.E.A.S.E.® Laser System, Pantec Biosolutions, Liechtenstein) creates defined and adjustable micropores in the skin by excitation of water molecules. These micropores should allow macromolecules to diffuse into the skin, and therefore enable and facilitate the transcutaneous application of molecules with high molecular weight, like (fusion) antibodies. Human skin samples were laser-treated ex vivo to determine the optimal parameters for delivery of antibodies into epidermis and dermis. DC targeting by antibodies against Langerin and DEC-205 was evaluated. We were able to induce pores of definable depths. Laser-induced thermal cell damage seemed negligible since no increased apoptotic signals were found in the surroundings of the pores. Both Langerhans cells and dermal DC could be targeted via laser-"holed" skin by anti-DEC-205 and anti-Langerin antibodies, respectively. However, the DC targeting efficiency after conventional intradermal injection was higher as compared to laser treatment. Ongoing work is attempting to figure out conditions for better targeting and, eventually, investigate the benefit of co-applied adjuvants and the immune-stimulatory capacity of antigen-targeted DC in laser treated skin.

P164 (OP04/01) | Long-term effects on follicular and non-follicular T cell subsets in B cell-depleted patients with pemphigus

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Rituximab (Rtx) is a monoclonal antibody that selectively binds CD20, a transmembrane protein expressed on the surface of B lymphocytes. This binding leads to long-term depletion of circulating B cells, i.e. from pre-B cells to mature B cells, for at least one year. The efficacy of B cell-depleting therapy has been proven in different autoimmune diseases, like pemphigus, systemic lupus erythematosus or rheumatoid arthritis. Although the efficacy of Rtx treatment results from depletion of B cells producing autoantibodies, it has been shown that Rtx also affects the T cell compartment by interfering with the cross-talk between T and B cells. Furthermore, several in vitro and in vivo studies demonstrated the essential role of T helper (Th) cells in the generation of autoantibodies and T follicular helper (Tfh) cells in providing B cell help, respectively. However, data on the impact of Rtx on distinct $CD4^+CXCR5^+$ Tfh cell subpopulations in patients with pemphigus are lacking.

To get further insights in the long-term effects of Rtx treatment on circulating T cell subsets (Th cells, Tfh cells and regulatory T cells), we analyzed peripheral whole blood of pemphigus patients (P. vulgaris, n = 6; P. foliaceus, n = 3) treated with Rtx in comparison with healthy controls (HC; n = 10). The frequencies of T cell subsets in pemphigus patients were examined at baseline and followed longitudinally up to 12 months after Rtx administration by flow cytometric analysis. Eight different $CD4^+$ T cell subpopulations were characterized based on their individual expression of surface markers (i.e. Th1 cells: $CXCR5^-CXCR3^+CCR6^-$, Th2 cells: $CXCR5^-CXCR3^+CCR6^+$, Th17 cells: $CXCR5^-CXCR3^-CCR6^+$, Treg cells: $CXCR5^-CD25^+CD127^{low/-}$, Tfh 1 cells: $CXCR5^+CXCR3^+CCR6^-$, Tfh2 cells: $CXCR5^+CXCR3^+CCR6^+$, Tfh17 cells: $CXCR5^+CXCR3^-CCR6^+$, and Tfr cells: $CXCR5^+CD25^+CD127^{low/-}$).

Rtx-induced peripheral B cell depletion led to a substantial decline of serum autoantibodies associated with clinical improvement in all patients. In contrast to HC, at baseline the frequencies of Th and Tfh cells were elevated in patients with pemphigus. While B cell numbers showed a dramatic decrease within the first week, the frequencies of both Th and Tfh cell subsets started to decline at later time-points gradually adapting to levels seen in HC at the end of the observation period.

Our study shows that Rtx affects not only B and Th cell numbers but also results in normalization of increased Tfh cell subsets in patients suffering from pemphigus, thus pointing to a decisive role of Tfh cells in autoimmune diseases, probably by interfering with the interaction of autoreactive B and T cells.

P165 | Expression of the checkpoint receptors PD-1 and Tim-3 is increased in autoimmune blistering diseases

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Background: Bullous pemphigoid (BP) and pemphigus vulgaris (PV) are characterized by blisters and erosions in response to autoantibodies against specific structural proteins of the skin. Checkpoint receptors, such as programmed death-1 (PD-1), T cell immunoglobulin mucin domain-3 (Tim-3) and lymphocyte activation gene-3 (Lag-3), play a crucial role in maintaining immune homeostasis by modulating immunogenic tolerance. Blocking these checkpoint pathways is highly efficient in the treatment of tumors that co-opt checkpoint receptors as a major mechanism of immune escape. Recent studies have demonstrated that checkpoint receptors are also involved in the pathogenesis of various autoimmune diseases.

Methods: To assess the role of inhibitory checkpoint receptors in BP and PV, we analyzed skin sections of patients with BP, PV and control

subjects for expression of PD-1, Tim-3 and Lag-3 using immunohistochemistry. Next, we investigated expression of checkpoint receptors on different immune cells by double-stainings. Furthermore, we measured serum levels of PD-1, Tim-3 and Lag-3 in BP, PV and controls using ELISA.

Results: We observed a significantly increased expression of PD-1 and Tim-3, but not Lag-3, in the skin of patients with BP and PV compared to controls. Expression of PD-1 was particularly upregulated on CD8-positive T cells, whereas expression of Tim-3 was enhanced on CD8-positive T cells as well as on CD68-positive macrophages. In contrast, we did not observe upregulation of PD-1 and Tim-3 on CD4-positive T cells, CD20-positive B cells and ELA2-positive neutrophils. Serum levels of PD-1, Tim-3 and Lag-3 were comparable between BP, PV and controls, with the exception of decreased Tim-3 levels in PV compared to BP.

Conclusions: Collectively, we report on enhanced expression of the co-inhibitory checkpoint receptors PD-1 and Tim-3, but not Lag-3, in the skin of patients with BP and PV. These findings suggest an involvement of checkpoint receptors in the pathogenesis of autoimmune blistering diseases and provide a rationale for exploiting these pathways as potential targets in the treatment of BP and PV.

INFECTIOUS DISEASES

P166 | Fast and reliable diagnostics of fungal skin infections

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Up to 10% of all adults in Western countries have fungal infection of the nails. This percentage increases to 20% in adults who are 60 years or older. Physical exam alone is an unreliable method of diagnosing fungal nails as only 50% of cases of abnormal nail appearance are caused by fungi. Differential diagnosis includes psoriasis, lichen planus, contact dermatitis, trauma, nail bed tumor, eczema, yellow nail syndrome and others. Therefore, laboratory testing is indicated. Nail samples will be used for direct microscopic analysis, which has a low specificity and sensitivity. Culturing is necessary for differentiation between different types of fungi, which is important for therapy and further prophylaxis. Unfortunately, it takes weeks to get a result with high specificity and sensitivity and the differentiation between species requires good knowledge in cultural growing patterns and microscopy. To improve reliability and time we established a PCR-based molecular diagnosis of fungal nail infections. Using the EUROArray Dermatomycosis test system 50 different dermatophytes can be specifically detected, including 23 dermatophytes as well as 6 yeasts and molds. Even pathogens which are difficult to identify from culture or mixed infections or, most

importantly, already treated dermatomycoses can be diagnosed by the EUROArray Dermatomycosis test system. We compared the sensitivity and specificity of more than 300 samples by "expert" molecular diagnosis (in the EUROIMMUN laboratory), "user" molecular diagnosis, fungal culture and direct microscopy and found the EUROArray Dermatomycosis to be a prompt, safe and unambiguous test. It provides the basis for a timely identification of the pathogen and also the source of infection. In this way, an efficient therapy targeted to this specific pathogen can be initiated within 2-3 days after the suspected diagnosis.

P167 | Is there a correlation between *Trichophyton benhamiae* genotype and virulence?

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Introduction: *Trichophyton benhamiae* isolates are currently stratified into one of two mono-phyletic clades referred to as white and yellow colony types according to ITS sequencing [1]. The yellow type has been reported to exhibit higher collagenase, elastase and proteins/peptide degrading activities, while the white type seemed to depend on sugars [2]. The classification into two groups is convenient; however, fungal morphology and pigmentation are not always consistent within one group. Hence, it seems likely that members of one group are not clonal. Thus, the aim of this study was to scrutinize if members of one type are truly clonal and if confound, whether previously reported differences of *T. benhamiae* virulence are more severe or reversed upon re-classification.

Methods: Genetic variability of 11 *T. benhamiae* strains (5 white, 5 yellow, the DSM6916 strain) was determined by ITS sequencing, PCR of mating type specific fragments and RAPD-PCR [3]. Analysis of enzyme activity and growth requirements was performed as previously described [2].

Results: Tested white types belonged to 2 groups (CBS112371 and CBS280.83) with opposite mating types. Yellow types appeared to be clonal according to ITS sequences, but RAPD patterns differ between yellow isolates and DSM6916. Enzyme testing demonstrated higher activities of collagenase, elastase and amino acid hydrolases for yellow strains with comparable activities of the two white types. Previously shown differences in carbohydrate hydrolases were less distinct. No difference in growth requirements was observed on vitamin supplemented Trichophyton agars.

Conclusion: Although the phenotypical difference indicates a larger genetic variability of *T. benhamiae* beyond yellow and white colony types, no impact on virulence in form of enzyme activities and growth requirements were observed.

References:

[1] Symoens et al. 2013, J Med Microbiol, 62:377-385.

[2] Hesse et al. 2017, Mycoses, 60: 4-5.

[3] Seyfarth et al. 2011, Mycoses 54:e870-e876.

P168 | African four-toed hedgehogs or European hedgehogs can transmit *T. erinacei* infections

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Introduction: African four-toed hedgehogs (Atelerix albiventris) are common pets in Asian countries like Japan. But, the number of these animals kept as pets has grown in Germany as well. Therefore, infection with *T. erinacei* is an increased risk for their owners. In addition, European hedgehogs (Erinaceus europaeus) cared for in animal shelters can be a source for *T. erinacei* infections.

Cases: Two cases of infections with *T. erinacei* strains were documented 2017 in at the Department of Dermatology at the University Hospital Jena. One infected person lived together with a pet holder of African four-toed hedgehogs. The other infected person helped wild European hedgehogs to survive the winter period.

Results: ITS-Regions of both strains were identical with the ITS-region of the *T. erinacei* type strain CBS 511.73. RAPD-PCR analyses [1] showed similar patterns within all *T. erinacei* strains and the close relationship to African *T. benhamiae* strains as well as *T. verrucosum*. The European hedgehog derived strain demonstrated a strong orange pigmentation of the mycelium whereas the other strain exhibited a white phenotype. On Takashio agar [2] *T. erinacei* strains present a periodic growth pattern which is a specific morphological criteria and never seen with cultures of *T. benhamiae* strains or *T. verrucosum* on this medium.

Conclusion: *T. erinacei* strains of both ITS-types are able to be transmitted from European hedgehogs causing skin infection of humans. Wounding of the hands by Hedgehog quills leads to a high risk for fungal infection with *T. erinacei*.

References:

[1] Seyfarth et al. 2011, Mycoses 54:e870-e876.

[2] Takashio M (1970) Mykosen 15:11-17.

P169 | Cutaneous over-expression of RANKL leads to suppression of anti-microbial immune responses

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Cutaneous infections are controlled by the skin immune system eliciting innate as well as adaptive anti-microbial immune responses. Different studies, including our work, revealed that critical involvement of the tumor necrosis factor (TNF)/TNF receptor superfamily are regulating the cutaneous immunity. However, to complete their life cycle, pathogenic microorganisms need to suppress host anti-microbial immune responses. In particular CD4^{+}Foxp3^{+} regulatory T cells (Treg) and MDSCs play a crucial role in the inhibition of cellular immunity. The molecular mechanisms determining the activation, expansion or migratory behaviour of Treg and MDSCs during cutaneous infections are still poorly understood. Upon intradermal infection with *S. aureus* in the back skin, K14-RANKL tg mice exhibited an increased skin lesion size as well as bacterial load compared to WT controls. This effect was mediated by increased abundance of Treg in K14-RANKL tg mice. To address the in-vivo relevance of Treg for the suppression of anti-bacterial immunity in *S. aureus*, K14-RANKL tg mice were bred with DEREG mutants, expressing the diphtheria toxin receptor (DTR) under control of the Foxp3 promoter. Subsequently, Treg were depleted in K14-RANKL tg x DEREG double mutants by intraperitoneal injection of diphtheria toxin (DT) before and after *S. aureus* infection. This results in the accumulation of G-MDSCs (granulocytic Ly6G^{high} Ly6C^{low}) and downregulation of M-MDSC (monocytic Ly6C^{+}). Together, this data indicates that RANK-RANKL signaling increases Treg frequencies that lead to suppression of host anti-microbial immune responses while Treg deficiency results in an increase of G-MDSCs and the suppression of M-MDSC populations.

P170 | DNA release upon treatment of *Enterococcus faecalis* biofilms with cold atmospheric plasma

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During the last 10 years, treatment of infectious diseases such as wound infections of the skin and the mucous membranes has become increasingly more complicated and ineffective due to the emergence of drug-resistant bacteria. One major pathogen which often causes such infections is the Gram-positive coccus *Enterococcus faecalis*. *E. faecalis* combines many virulence factors like the ability to form biofilms as well as intrinsic resistances to several classes of antibiotics. Therefore, novel treatment modalities that are capable of killing pathogens in biofilms with less or even no risk of inducing resistance are desperately needed. In this light, cold atmospheric plasma (CAP), which has been shown to effectively inactivate microorganisms, independent from their resistance level, may be a promising alternative for application in the fields of dentistry and dermatology. CAPs are partly ionised gases, which operate at low temperature and are composed of electrons, ions, excited atoms and molecules, reactive oxygen and nitrogen molecules. In this study, the effect of CAP was investigated against *E. faecalis*, growing on

agar plates or as a biofilm. CAP reduced the CFU by more than 5 log10. The inactivation of biofilms (24 hours) of *E. faecalis* resulted in a reduction of CFU of 3 log10 for a CAP treatment duration of 5 minutes thereby revealing equal results as for the treatment with 0.2% CHX for 5 minutes. 2% CHX reduced the CFU by more than 5 log10. UV-C also resulted in a reduction of CFU over 3 log10. DNA release was measured 5 minutes and 20 minutes after the CAP treatment. No DNA release could be measured compared to the positive control (enzymatic digestion with lysozyme and proteinase K). Therefore, the release of DNA through damage of the bacterial cell wall/membrane seems to not be the primary mechanism of inactivation of *E. faecalis* by CAP. Overall, depending on distinct treatment time, CAP showed an antimicrobial efficacy as good as CHX or UVC.

P171 | Assessment of in vivo ASC speck formation as a putative biomarker in human inflammatory diseases: first results on sepsis patients.

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Background: IL-1β is an immunoregulatory cytokine relevant in acute and chronic inflammation. Critical for mature IL-1β production is the assembly of apoptosis-associated speck-like proteins containing a caspase recruitment domain (ASC). In humans little is known about in vivo inflammasome activation and generation of mature IL-1β. A flow cytometry based detection of ASC speck formation allowed us to identify blood leukocytes that had undergone inflammasome activation in vivo. We here report on our results studying sepsis patients for 7 consecutive days. Materials and methods: Blood and serum samples were collected from 27 patients with sepsis in intensive care unit (ICU) of Heidelberg university hospital from day 1 to day 7 constantly. To illuminate the kinetics of ASC specks in sepsis, surface marker and intracellular staining were performed on fresh whole blood samples to determine level of ASC specks in monocytes from day 1 to day 7. In addition, extracellular ASC specks were measured by ELISA as well. Furthermore, evaluation of dynamic change of cytokines was undertaken using BioLegend LegendPlex Assay to investigate the downstream inflammation induced by ASC specks.

Results: The level of ASC specks increased dramatically in patients on day 6 and day 7 compared with healthy donor controls. Linear regression analysis displayed LDH positively correlated with ASC specks on day 6 and day 7 as well indicating the association of ASC specks and organ injury. Moreover, Kaplan-Meier survival analysis showed patients with higher ASC specks on day 6 had favorable outcome suggesting ASC specks as potential predictors of sepsis prognosis. Furthermore, higher level of IL-18, the cytokine induced by ASC specks, was observed on day 6 and day 7 compared with healthy controls. We measured the released ASC specks in the serum of patients and healthy control by ELISA. Extracellular ASC specks only can be detectable in few patients.

Conclusions: Our study for the first time showed the kinetics of ASC specks and trigger of cytokine release by them in sepsis patients. Patients with higher level of ASC specks had favorable outcome suggesting ASC specks as predictors for sepsis. Next we investigate the role of ASC speck formation as a sign of systemic inflammation in chronic inflammatory skin diseases such as psoriasis and atopic dermatitis.

P172 | Presence of neutrophils in the skin enhances *S. aureus* colonization

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Introduction: Our skin is constantly exposed to a large number of pathogens while at the same time undergoing selective colonization by harmless commensal microorganisms such as *S. epidermidis*. Changes in the composition of the microbiome can promote colonization by the pathogen *Staphylococcus aureus* and can deteriorate barrier defects for instance in atopic dermatitis patients. Keratinocytes, as the most abundant cell type in the epidermis, are able to orchestrate specific immune responses in the skin in response to colonizing commensals as well as to pathogens. However, the mechanism how keratinocytes can discriminate commensals from pathogens is barely understood.

Objectives: In this work we investigate the role of different types of immune cells in *S. aureus* skin colonization and how skin commensals can modulate the recruitment of immune cells to the skin.

Materials and Methods: Using an in vitro co-culture model with primary human keratinocytes and PBMCs or neutrophils as well as by using an in vivo epicutaneous mouse skin infection model we analyzed the recruitment of specific immune cells subsets to the skin in response to commensals or pathogens as well as the effect of the recruited immune cells on *S. aureus* skin colonization.

Results: We show that skin inflammation induced by tape stripping induces a rapid recruitment of neutrophils which correlates with enhanced skin colonization with *S. aureus*. Consequently, depletion of neutrophils in vivo resulted in reduced *S. aureus* colonization. Moreover, in vitro co-culture of keratinocytes with neutrophils but not PBMCs led to enhanced *S. aureus* colonization. And finally, we show that *S. epidermidis* prevents excessive neutrophil recruitment to the skin.

Conclusion: In healthy skin *S. epidermidis*, as part of the skin microbiota, prevents excessive recruitment of neutrophils to the skin and by this protects the skin from *S. aureus* colonization. Further studies will provide deeper insight into the mechanism how neutrophils contribute to enhanced *S. aureus* skin colonization.

PHARMACOLOGY

P173 | Identification of promising drug candidates for development of novel topical anti-inflammatory medicinal products applying a straightforward preclinical efficacy assessment strategy

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Skin is not only biggest the barrier organ of our body, but it also has an important neuro-immuno-endocrine function. Chronic inflammatory skin diseases represent a global health problem. A quest for new anti-inflammatory locally applied, locally acting, non-immunosuppressive drug candidates with improved side-effect profile is thus of great importance. Here, we describe a strategy for research and development of anti-inflammatory drugs for topical as well as systemic application. The compound of interest (NME, repurposing or repositioning of known APIs) should target general immunomodulatory and not specific disease-bound pathways and should exhibit particular physico-chemical characteristics, e.g. molecular size (<500 g/mol), lipophilicity, and topological polar surface area. Moreover, pI and selectivity of the target are also of importance. After optimization of physico-chemical characteristics, compounds of interest undergo a core set of in vitro experiments on both human and murine epithelial and immune cells, e.g. T cells, keratinocytes including cytotoxicity assessment to ensure that findings in mice are not species-specific. Once the anti-inflammatory activity of the compound has been proven in vitro, it is tested in a set of in vivo "unspecific" acute and chronic models of dermatitis (mouse models of arachidonic acid-induced ear edema and oxazolone-induced delayed-type hypersensitivity). If in those models the compound shows anti-inflammatory action, it can be tested in models of other skin diseases, e.g. in mouse models of imiquimod-induced psoriasis-like inflammation and barrier disruption (tape striping or SDS patch). A broad, general anti-inflammatory activity is shown by testing the compound in models with different pathophysiology like the murine model of DSS-induced colitis or vasculitis (local Shwartzman reaction). Successful candidates may be selected for preclinical development. In the course of preclinical development the candidate compound and its topical formulation are tested in more sophisticated animal models such as the ovalbumin-induced dermatitis model in mice or the canine model of atopic dermatitis. Application of this strategy resulted in the nomination of two clinical and two preclinical candidates.

P174 | Influence of emulsions of different pH on skin barrier integrity

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The pH of the stratum corneum is slightly acidic (mean 4.9) and tightly regulated. The acidic pH was considered to present an antimicrobial barrier; later on, it was shown that the pH influences skin barrier function, lipid synthesis, epidermal differentiation and desquamation. The objective of the present study was to investigate whether topical application of emulsions with different pH values influences skin barrier function. The effect of the oil in water (O/W) emulsions adjusted to pH 4 in comparison with pH 5.8 adjusted formulation and the untreated control on the integrity of the skin barrier, mechanical stability, SC morphology and inflammation was examined. Twenty-four healthy volunteers aged 18-75 years were treated randomized on the lower arms for 30 days; TEWL, SC hydration and pH were documented. As a marker for the mechanical stability the number of tape-strips to achieve a threefold increase in TEWL was determined. Erythema induced by tapestripping was evaluated by clinical (visual) grading. The size of corneocytes adhered to D-Squame tape-strips was examined microscopically. After 30 days of treatment TEWL was slightly reduced by both formulations. SC hydration was significantly increased to the same level by both preparations. pH was significantly reduced by the pH 4 emulsion. The number of tape-strips to disrupt the skin barrier to a threefold increase in TEWL was significantly increased by both emulsion, but slightly more by the pH 4 emulsion. Erythema after tape-stripping was considerably lower at the pH 4 as compared to the pH 5.8 site. Corneocyte size was significantly increased after treatment with the pH 4 emulsion compared to the pH 5.8 emulsion and untreated. In summary, treatment with a pH 4 emulsion positively influenced SC hydration, pH, stability against mechanical irritation and inflammation as well as corneocyte size. The results could be potentially important for the formulation of topical products for effective acidification in several conditions which showed an increased pH, like eczema, dry and aged skin.

P175 (OP02/03) | Topical inflammasome inhibition—a novel treatment strategy for contact dermatitis

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Introduction: Contact dermatitis (CD) is a T cell-mediated common inflammatory skin disease, which presents with papules, vesicles and scaling at sites of skin contact with allergens or irritants. Recently, evidence suggests that inflammasome activation contributes to the skin inflammation induced by allergens and irritants. Topical steroids are the standard treatment for CD. However, their use has been associated with considerable side effects. Treatment strategies that target the inflammasome are not available so far.

Methods: We performed a high content screen with >60.000 small molecules (including SELLECK, WDI, LOPAC libraries, compounds from academia) using ASC speck formation as readout for inflammasome activation. The most active hit compounds were further validated in human peripheral blood mononuclear cells (PBMCs) for effects on interleukin (IL)-1β secretion. One selected hit compound, a carbamate derivative, was tested in a placebo-controlled skin patch test model of CD in 25 human healthy volunteers. Here, topical application of the carbamate derivative, mometasone 0.1% or vehicle (DAC Basiscreme) was followed by application of sodium dodecyl sulfate (SDS) for 24 hours each. Eczema induction was quantified by mexameter and laser speckle imaging. Epidermal protein was collected by corneocyte sampling of lesional skin and inflammasome-mediated cytokines were measured by ELISA.

Results: The carbamate derivative induced a dose-dependent inhibition of ASC speck formation and IL-1β release from PBMCs. In vivo, pretreatment with the carbamate derivative for 24 hours, but not with vehicle or mometasone 0.1%, inhibited SDS-induced eczema. This was demonstrated by significantly lower erythema and total flux values assessed by mexameter and laser speckle imaging, respectively, for the carbamate derivate compared to vehicle or mometasone 0.1% ($P \leq 0.000$). Also, corneocyte IL-18 levels were significantly reduced after application of the compound compared to vehicle ($P \leq 0.000$).

Conclusion: Our results demonstrate that the carbamate derivative identified by our high throughput screening is a strong and dose-dependent inflammasome inhibitor in cellular assays in vitro. The inhibitory capacity was confirmed by its preventive effects on SDS-induced eczema in vivo. Future studies should aim at investigating the efficacy of this compound in patients with CD.

P176 | Common determinants of nanocarrier penetration and drug release: practical implications for dermatotherapy

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A surplus value of certain nanocarrier types for cutaneous drug delivery has repetitively been suggested, but the field lacks comprehensive studies, which dissect common determinants of penetration and drug release.

Herein, comparison of core-multi-shell-nanocarrier, thermoresponsive nanogels, ethyl cellulose carriers and nanocrystals revealed improved drug penetration in deeper layers of ex vivo human skin. Drug release largely occurred during the stratum corneum (SC) passage. Changes in particle stiffness affected penetration depth, but whether the observed disturbance of SC hydration and lipid organization was a general penetration enhancement effect or specifically attributed to the nanocarriers remains unclear. Interestingly, a surplus value became especially apparent when concentrations lower than clinical standard doses were applied, but the successful exposure of the skin to higher local drug concentrations occasionally came at the price of irritation, as demonstrated for dexamethasone nanocrystals and for tacrolimus when applied on barrier-disrupted skin.

High-resolution microscopy and en bloc two-photon-microscopy helped monitor penetration pathways of drugs and nanocarriers across SC and confirmed translocation of low amounts into the viable epidermis predominantly at skin folds and hair follicles. Cellular uptake was increased in impaired skin barrier function and proinflammatory environment studied in full-thickness skin and keratinocyte-dendritic cell co-cultures.

Based on those results, we conclude that the investigated nanocarriers enabled modified penetration kinetics and may have dose-sparing effects. Practically, they provide an option for hydrophilic formulations of lipophilic drugs. Physical stratum corneum damage had greater impact on penetration than (bio-)chemical stimuli, but given that chronic skin diseases are typically associated with barrier abnormalities, cytokine increase and immune cell infiltrates, nanocarriers are likely to reach viable cells. This indicates potential for cell targeting, but future chemical compositions also need to consider toxicological and allergological implications.

PHOTOBIOLOGY

P177 | UV radiation on scalp skin surface induces scalp hair follicle damage ex vivo, but can be alleviated by topical treatment with caffeine

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Although the effect of ultraviolet radiation (UVR) on human skin has been extensively studied, very little is known about how UVR irradiating the skin surface impacts on human hair follicle (HF), and

whether any harmful effects can be reduced by cosmetics or nutraceuticals. Therefore, the goal of this study was to investigate the effect of solar spectrum UVR hitting the human skin surface on HF biology, and whether a widely used cosmetic ingredient, caffeine, interferes with this.

Human scalp skin with terminal HFs was irradiated transepidermally ex vivo using either 10 J/cm² UVA + 20 mJ/cm² UVB (low dose) or 50 J/cm² UVA + 50 mJ/cm² UVB (high dose). Subsequently, the skin was organ-cultured under serum-free conditions for 1 or 3 days. 0.1% caffeine (5.15 mM) was topically applied ex vivo for 3 days prior to UV exposure with 40 J/cm² UVA + 40 mJ/cm² UVB over 3 days after UVR. UVR effects on various skin and HF toxicity and vitality readout parameters were measured in defined skin and HF compartments. In agreement with previous results, transepidermal UVR exerted skin cytotoxicity and epidermal damage. Treatment with high and/or low UVA + UVB doses also induced oxidative DNA damage and cytotoxicity in human HFs. Furthermore, these treatments decreased proliferation and promoted apoptosis of HF outer root sheath (ORS) and hair matrix (HM) keratinocytes, induced catagen development, differentially modulated the expression of known HF growth factors, and stimulated perifollicular mast cell degranulation. UVR-mediated HF damage was more severe after irradiation with high UVR dose and also affected deeper HF compartments. Topical application of 0.1% caffeine did not induce skin or HF cytotoxicity and increased the expression of IGF-1 in the proximal HF ORS. However, it also promoted keratinocyte apoptosis in selected HF compartments. Moreover, caffeine conferred protection towards UVR-mediated HF cytotoxicity and dystrophy, keratinocyte apoptosis, and negative modulation of the catagen-promoting growth factor.

Our study provides the first evidence that transepidermal UVR negatively affects important human HF functions and suggest inclusion of agents that can act as HF photoprotectants, such as caffeine, into sun-protective cosmeceutical and nutraceutical formulations as a prophylactic strategy to protect HFs from UVR-mediated damage.

P178 | Biological effects of a conventional UVA1-light source and a LED-based UVA1 device prototype in the treatment of morphea in vitro and in vivo

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Ultraviolet A1 (UVA1)-phototherapy is a well-established treatment for several skin diseases such as scleroderma (morphea). Recent developments in improving the commonly used UVA1-light sources brought attention to a new Light emitting diodes (LED)-technology bringing remarkable advantages benefitting the handling and clinical routine.

In this study two UVA1-light sources (LED- and metal halide-lamp) with different UVA1-spectres (360-400 nm and 340-400 nm) were examined referring to their effectiveness in treating scleroderma. Experiments were done using human keratinocytes and fibroblasts and a bleomycin-induced scleroderma mouse model.

UVA1 significantly affects the collagen metabolism and has an impact on inflammatory cytokines as well as on growth factors, which play an emphasized role in sclerosis-conditional symptoms. Collagen type I (Coll-1), which is excessively increased in sclerotic skin conditions, was strongly reduced by UVA1-phototherapy, whereas simultaneously Matrix Metalloproteinase-1 (MMP-1), responsible for the collagen degradation, was significantly enhanced after UVA1-exposure. Furthermore, pro-fibrotic and inflammatory marker, dominating the clinical picture of sclerosis, including the inflammatory cytokines (IL-1α, IL-6, IL-8 and TNFα) and pro-fibrotic growth factors (TGFβ-1, TGFβ-2), were analyzed and showed marked changes following UVA1-radiation.

In summary, both UVA1 devices revealed beneficial effects of UVA1 light towards scleroderma and activate identical biological mechanisms in vitro and in vivo such as reduction of inflammatory cytokines, downregulation of collagen expression and enhancement of collagen degradation.

Referring to the comparable results, the new UVA1-therapy using LED-lights has the potential to improve the present UVA1-phototherapy in lowering costs and making the therapy session more comfortable for the patients by shorten the treatment duration and eliminating unpleasant heath development. Thus, the new UVA1-device presents a promising new therapy method for scleroderma patients.

P179 | UVA and blue light induces formation of neutrophil extracellular traps (NETs)

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Neutrophil Extracellular Traps (NETs) are networks consisting of decondensed chromatin decorated with antimicrobial substances, which are produced by neutrophil granulocytes. These networks are released as a response to various activating stimuli like bacteria and fungi, lipopolysaccharides (LPS) or chemicals such as phorbol-12-myristate-13-acetate (PMA), which engage different intracellular pathways to initiate NET formation. In most scenarios, NET release leads to the cell's death and can be clearly differentiated from apoptosis and necrosis in a morphological and molecular way. Beside the appearance of NETs as an important defense mechanism of the innate immune system, the dysregulation of NETosis appears to be involved in the pathology of diseases like psoriasis, systemic lupus

erythematosus, thrombosis or cancer. Deciphering how NET formation is induced and how it can be inhibited may therefore greatly contribute to our understanding of these diseases.

Until today, the influence of sunlight (particularly the skin-penetrating part of the spectrum) on the induction of NETosis has not been conclusively investigated. Light of the UV-VIS spectrum is well known to induce ROS-mediated skin damage and is associated with multiple inflammatory diseases such as systemic lupus erythematosus, solar urticaria or polymorphous light eruption. Therefore, we investigated whether NETs are released in response to defined light doses and wavelengths. Neutrophils from healthy donors were illuminated in vitro by LED light (375 nm (UVA), 470 nm (blue light) and 565 nm (yellow light)) and NET-rates were determined. Our results show that the number of cells undergoing NETosis depends on light doses and wavelength with a maximum in the UVA/blue light region. This light-induced release of NETs is mediated by granular enzymes like neutrophil elastase (NE) or myeloperoxidase (MPO) which are well described within the context of NETosis. Furthermore, light-induced NETosis is dependent on the generation of reactive oxygen species (ROS) derived from extracellular light-sensitive components such as riboflavin, HEPES and tryptophan, which are present in standard culture conditions as well as in the human skin.

These findings suggest that NETs can function as an important link between the impact of light on neutrophil granulocytes and the inflammatory reaction of the skin. Since the pathogenesis of diseases associated with a high light sensitivity of the skin remains largely enigmatic, this connection could be a starting point to develop a deeper understanding of these diseases and contribute to the development of new pharmacological strategies.

P180 | Ultraviolet (UV)-A irradiation induces long lasting changes in metabolism in non-malignant cells of normal human skin

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Ultraviolet (UV) radiation has a plethora of effects on human tissues. In the UV spectrum, wavelengths above 320 nm fall into the UVA-range and for these it has been shown that they induce reactive oxygen species (ROS), DNA mutations and are capable to induce melanoma in mice. In addition to this it was recently shown that UVA irradiation and UVA-induced ROS also increase glucose metabolism of melanoma cells. UVA irradiation causes a persistent increase in glucose consumption, accompanied by increased lactic acid production. But it has not been investigated if normal non-malignant cells of the skin also change their glucose metabolism upon UVA irradiation.

We treated primary human fibroblasts and murine fibroblasts and primary murine and human keratinocytes with repetitive UVA irradiation with physiological doses for 4 days. After irradiation we measured glucose consumption and lactate production. Interestingly fibroblasts and keratinocytes showed increased glucose consumption and lactate production upon UVA irradiation.

These findings indicate that UVA induced glucose consumption and lactate production is a general phenomenon in normal skin. As lactic acid is an immune modulator it could be that UVA induced production of lactate contributes to UVA induced immune suppression.

PRURITUS

P181 | Pruritus in inflammatory skin diseases—a brain issue?

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Pruritus, a desire to scratch, is a burdensome symptom accompanying many inflammatory skin diseases. It can be localized or generalized, it can occur as acute or chronic and can be classified according to its origin as cutaneous (pruritoceptive), neuropathic, neurogenic and psychogenic. Recently, clinical trials with orally available substances acting as antagonist on NK1 receptor (serlopitant) or peripherally active kappa-opioid receptor (KOR) agonist (asimadoline) failed to meet their primary efficacy endpoints as potential antipruritic drugs in patients with atopic dermatitis.

We hypothesized that failure of those compounds as an antipruritic agents is due to lack of activity in the central nervous system (CNS). In addition, a recent study by Takahashi in a mouse model of psoriasis demonstrated that topical or intraperitoneal administration of the μ-opioid receptor (MOR) antagonist naloxone and oral administration of ICI-199,441 (centrally acting KOR agonist) reduced pruritus, while asimadoline did not show any beneficial effect. We compared effects of nalfurafine (centrally acting KOR agonist), asimadoline and WOL071-007 (KOR agonist with CNS activity) in an arachidonic acid-induced ear inflammation mouse model. Application of arachidonic acid on mouse ears induces a fast inflammatory and a robust scratching response which allows for screening of compounds. Arachidonic acid-induced scratching was significantly decreased in nalfurafine treated mice at very low doses (0.03-0.3 mg/kg, p.o.). Effects on scratching were only seen for very high doses of WOL071-007 (30 and 100 mg/kg, p.o.) and asimadoline (100 mg/kg, p.o.). The differences can be attributed to the compounds pharmacokinetics (oral bioavailability, CNS penetration) and their potency at KOR. Of note, muscle relaxation and behavioral depression were observed at all doses showing antipruritic activity. Therefore, it is hard to separate the desired antipruritic effects from CNS-mediated side effects. Interestingly, in the same animal model, anti-inflammatory activity (as shown by reduced ear swelling) was independent from CNS activity.

Asimadoline and WOL071-007 dose dependently inhibited ear swelling (1-10 mg/kg, s.c.). For asimadoline no CNS effects were observed whereas treatment with WOL071-007 (3 and 10 mg/kg, s.c.) was associated with behavioral depression. Following topical application (2×0.1-2×3 mg) ear swelling was dose-dependently reduced by both compounds with no CNS effects observed. An optimized structural analog of WOL071-007 restricted to the periphery as shown in a PK study did not exhibit any CNS effects whereas its anti-inflammatory activity was maintained. Furthermore, a recent study in a murine model of AD using topical nalfurafine supports findings that anti-inflammatory effects are mediated peripherally, whereas the initial strong antipruritic activity was accompanied by a reduction in general activity.

Centrally acting drugs targeting KOR exhibit well documented adverse drug reactions like dysphoria, sedation, diuresis, and sleep disturbance. These adverse drug reactions limit their potential use not only to the most severe forms of pruritus, but also limit its use in children. However, the topical use of these compounds or analogs selective for the periphery as anti-inflammatory drugs to treat inflammatory epidermal skin diseases and thus pruritus as a symptom of these diseases is highly interesting as therapeutic strategy.

P182 | Sensitization of sensory, cutaneous nerve fibers in chronic pruritus

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Chronic pruritus (CP) is a symptom of many different diseases and the neuroimmunological cutaneous mechanisms underlying CP remain unclear. It is assumed that cutaneous nerve fibers undergo peripheral neuronal sensitization due to inflammatory mediators.

Within this study, we investigated the cutaneous neuroanatomy, the function of small and large nerve fibers and sensitization of different C-fibers (histamine sensitive C fibers: CMi fibers; cowhage-, mechano- and heat-sensitive C fibers: CMH fibers). 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). All patients and 40 sex- and age-matched healthy controls (HC) were stimulated with active cowhage spicules and inactivated spicules loaded with histamine, capsaicin or NaCl (neg. control). Pruritus induction was monitored for up to 30 minutes and intensity and duration were used to calculate areas under the curve (AUC). Function of nerve fibers was analyzed by quantitative sensory testing (QST) according to the protocol by the German Research Network for Neuropathic Pain containing 13 different thermal and mechanical tests. Finally, biopsies were obtained for assessment of cutaneous neuroanatomy by PGP9.5 staining.

In PN patients, stimulation with cowhage led to a significantly higher pruritus intensity as measured by the AUC when compared to HC (P = 0.02), while no differences were found between the remaining patient groups and matched controls (P > 0.1) or across patient groups (P > 0.1). Furthermore, cowhage induced a higher AUC pruritus intensity compared to histamine (AD: P = 0.02; BRP: P = 0.003; PN: P = 0.002) and capsaicin (AD: P = 0.002; BRP: P < 0.001; PN: P = 0.005) in all patient groups, but not in HC (cowhage vs. histamine: P > 0.1; cowhage vs. capsaicin: P > 0.05). AUC pruritus intensities induced by histamine and capsaicin did not differ (P > 0.1) in any group. The density of intraepidermal sensory PGP 9.5-positive nerve fibers (IENF) was significantly reduced in all three CP groups as compared to HC. But, only minor effects on nerve fiber function were shown by QST. Compared to HC only a reduced vibration detection threshold could be shown in AD patients (P = 0.026; Aß-fiber "loss of function"). BRP patients showed only an increased warm detection threshold (WDT: P = 0.018; C-fiber "loss of function") and no differences to HC could be shown in PN patients. Furthermore for WDT a correlation with decreased IENF could be shown in the CP patients. In sum, we found evidence for peripheral neuronal sensitization with an enhanced CMH fiber (but not CMi fiber) response upon experimental cowhage stimulation independent of the CP origin. The reduced IENF was seen in all CP patients but had only minor effects on the general cutaneous nerve fiber function.

P183 | Chronic pruritus in skin diseases—more common and more severe than we thought

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Many dermatological disorders are associated with pruritus. While in some diseases pruritus is a hallmark symptom, i.e. urticaria, atopic dermatitis or lichen planus, the prevalence and intensity of pruritus in other diseases and the impact of pruritus on the general well-being and quality of life is less well known. In recent years, there have been various reports on the prevalence and burden of itch in some selected skin diseases; a detailed characterization of the presence, intensity, localization, quality and impact of pruritus in different skin diseases was, as of yet, missing. We have therefore contacted more than 1,300 unselected, consecutive in- and outpatients with active dermatological disorders and have collected data from 880 patients with 19 different dermatological diagnoses (including chronic spontaneous urticaria [n = 143], psoriasis [n = 138], atopic dermatitis [n = 129], chronic prurigo [n = 75], cutaneous T and B cell lymphoma [n = 68, 26], mastocytosis [n = 54], and bullous pemphigoid [n = 15]). Using validated questionnaires we have collected information on the presence, intensity, quality and localization of pruritus, and have analyzed the impact

of pruritus on the quality of life, the effects on sleep and activity and work productivity and the impact of pruritus on suicidal ideation.

Our data indicate that chronic pruritus is more common and more severe than previously anticipated in most skin conditions and that pruritus in these diseases is often associated with an impairment of quality of life, sleep, and work productivity, and that pruritus is linked to depression and suicidal ideations. Body maps representing the localization of pruritus additionally show distinct bodily distribution patterns of pruritus, which, together with information on quality of pruritus and scratching behavior, indicate disease specific pruritus characteristics in different dermatoses. Consequently, the results can lead to a better understanding of the pathophysiology of itch in these diseases, help in the development of better treatment options and can lead to a better management of our patients.

P184 | Medical needs related to pruritus in Germany—a 2-year Google search engine analysis

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Background: Pruritus is one of the most frequent interdisciplinary medical complaints. Due to the very subjective character of the sensation and due to the fact that affected persons often do not visit a doctor, it is hard to determine the prevalence of chronic and acute pruritus in the general population.

Objectives: The aim of this study was to estimate the medical need related to pruritus and the most common localisations of the symptom in the German general population by analysing the pruritus-related Google search volume.

Materials and Methods: Using the Google AdWords Keyword-Planner we identified relevant keywords for the subject "pruritus" and analysed the corresponding search volume. The keywords were categorized in order to explore the most common localisations and the context in which pruritus is an issue in the German population. The assessment period was January 2015 to December 2016.

Results: In total we identified 701 relevant keywords for the subject "Juckreiz" (German lay word for pruritus), resulting in 7 531 890 pruritus related Google searches during the 2-year assessment period. The most common search terms were the German lay term for atopic eczema ("Neurodermitis", 23.7%), the German lay term for psoriasis ("Schuppenflechte", 17.8%) and "psoriasis" (13.0%). The German lay term for pruritus ("Juckreiz") was only the sixth most searched term (3.0%). 15.7% of search terms contained closer information about the localisation. Of those, the most commonly searched localisation was pruritus on the whole body (24.2%), followed by anal pruritus (17.6%) and pruritus on the legs (14.4%). Most searches (72.0%) included influencing factors for pruritus, and among those especially skin diseases (87%) and skin conditions (10.2%). Analysis of the

temporal course showed a higher monthly search volume during winter.

Conclusion: With its unconventional methodology of a Google search engine analysis this study allows a rough estimation of the medical need of pruritus in the German general population. Especially the identification of pruritus in the anal area as an unmet medical need proves that this methodology can contribute to a better understanding of major medical complaints in the general population.

P185 | Dichotomy of autoreactive peripheral blood T helper (Th) 1/Th2 cell responses in elderly patients with pruritic disorders and bullous pemphigoid

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Introduction: Chronic pruritic disorders of the elderly may be considered as a pre-clinical stage of bullous pemphigoid (BP) since a subset of these patients has IgG autoantibodies against BP180 and/or BP230. Pruritus is increasingly considered as a cardinal symptom of BP despite its clinical heterogeneity including bullous, urticarial, eczematous, erythematous and no lesions at all. In BP, pruritus has been linked to the presence of IgG autoantibodies which are also, but rarely, found in elderly individuals with pruritic dermatoses.

Objectives: In a prospective study, we sought to characterize peripheral blood T cell responses against BP180 in BP and elderly patients with pruritic disorders in relation to IgG autoantibodies and clinical parameters of disease severity and pruritus.

Material & Methods: A total of 12 elderly patients with pruritic disorders, 32 patients with bullous and 6 patients with non-bullous BP and 13 age-matched healthy controls (HC) were studied. Peripheral blood mononuclear cells (PBMC) were isolated and co-cultured in vitro with BP180 antigen (NH2- or COOH-terminal ectodomains), and desmoglein (Dsg) 1 or Dsg3 as control antigen. Subsequently, the frequency of BP180-specific T cells producing IFN-γ (Th1), interleukin (IL)-5 (Th2) and IL-17A (Th17) was determined by ELISpot assay in the patients and HC.

Results: T cell recognition of the NH2- and COOH-terminus of BP180 was observed in both, elderly patients with pruritic disorders and BP patients. However, BP180-specific T cells in the elderly patients were mainly Th1 cells whereas BP patients showed a mixed Th2- and Th17 cell response against BP180 which was correlated with anti-BP180 IgG in sera of these patients. In contrast, low anti-BP180 serum IgG concentrations were observed in a subset (3/12) of elderly patients with pruritus. Of note, T cell recognition against BP180 correlated with the extent of pruritus in both patient groups. Moreover, topical treatment with glucocorticoids led to a strong decrease of peripheral blood BP180-specific T cells and the severity of pruritus suggesting a pathogenic role of autoreactive T cells in pruritus development.

Elderly patients with pruritic disorders as well as age-matched HC further showed a distinct Th1 cell response against Dsg1, but not against Dsg3, which was not observed in the BP patients.

Conclusion: We here demonstrate T cell recognition of BP180 in elderly patients with pruritic disorders and BP, although with a different signature (Th1 versus Th2/Th17 cells). The presence of all the autoreactive T cell subsets correlates with pruritus independent from the presence of anti-BP180 serum IgG autoantibodies. Our findings further suggest that targeting of autoreactive T cells via topical application of glucocorticoids has a major impact on pruritus control.

TUMOR BIOLOGY

P186 | p53 is involved in basal and IFN-gamma induced PD-L1 expression in melanoma

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The introduction of monoclonal antibodies inhibiting the immune checkpoint programmed cell death protein 1 (PD-1) has significantly improved treatment of advanced melanoma. Notably, a high expression of programmed death-ligand 1 (PD-L1) has been associated with a better clinical outcome in melanoma patients receiving anti-PD-1 immunotherapy. Since in non-small cell lung cancer (NSCLC) PD-L1 is negatively regulated by p53, we were interested to analyze the potential role of p53 in regulating PD-L1 in melanoma cells.

In cell culture experiments, we addressed the impact of p53 expression levels on basal and interferon (IFN)-gamma (200 IU/mL for 48 hours) induced PD-L1 expression. To this end, we modulated p53 expression in melanoma cell lines by shRNA-mediated inducible p53 knockdown or knockout using CRISPR/Cas9 technology. The effect of p53 knockdown on the IFN-gamma/JAK1-JAK2/STAT1-signaling pathway was determined by immunoblots. Since these analyses revealed diminished JAK2 after p53 knockdown, we determined the effect on cells that ectopically expressed JAK2. We also analyzed publicly available expression data (the cancer genome atlas (TCGA) for skin cutaneous melanoma (SKCM)) on correlations for PD-L1 expression.

IFN-gamma treatment led to a strong up-regulation of PD-L1 in melanoma cells. Knock-down of p53 by inducible shRNA expression clearly blocked PD-L1 induction by IFN-gamma. Notably, lack of consistent PD-L1 alterations after CRISPR/Cas9-mediated truncation of p53 as well as diminished IFN-gamma-induced PD-L1 expression after p53 knockdown in TP53 mutant cell lines suggested that PD-L1 depends on p53 protein rather than on TP53 transcriptional activity. In accordance, TCGA data analyses revealed no correlation between expression of the p53 target gene EDA2R and PD-L1 mRNA, but a positive correlation between p53 and PDL1 expression at the protein level. Mechanistically, p53 knockdown consistently resulted in a decrease of JAK2 in melanoma cell lines, and reconstitution of JAK2 by ectopic expression clearly enhanced IFN-gamma-induced PD-L1 expression after p53 knockdown.

Our study provides evidence that p53 plays a role in the regulation of PD-L1 expression in melanoma cells. Importantly, p53 knockdown led to a decrease of JAK2 and thus interfered with the IFN-gamma signaling pathway. Further studies have to investigate whether p53 is involved in adaptive immune resistance, as in vivo data have already demonstrated that decreased IFN-gamma responsiveness is associated with a diminished response to PD-1-directed immunotherapies.

P187 | 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.

P188 | Novel targeted treatment strategies for genetic melanoma subgroups

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Background & Objectives: New therapy concepts, such as immunotherapy as well as targeted therapy with BRAF and MEK inhibitors, have significantly improved the overall survival of melanoma patients. However, about 20% of patients do not respond to initial targeted therapy and at the same time, most of the tumors develop resistance through long-term therapy. For particular melanoma subgroups like the NRAS mutated tumors it is known, that they are associated with aggressive disease, but there is no approved targeted therapy for this subset. In clinical trials, the MEK inhibitor (MEKi) binimetinib displayed modest antitumor activity, making combinations a requisite. In a previous study, the BRAF inhibitor (BRAFi) vemurafenib was shown to induce endoplasmic reticulum (ER) stress that together with inhibition of the RAF-MEK-ERK (MAPK) pathway amplified its pro-apoptotic activity in BRAF-mutant melanoma. The present study investigated whether this effect might extent to NRAS-mutant melanoma, in which MAPK activation would be expected.

Other clinical studies in breast cancer show that PI3K inhibitors have antitumor activity. This raises the question of whether these inhibitors are also a therapeutic option for melanoma and whether a combination of them with MEK inhibitors could further restrict growth and prevent possible development of resistance in different melanoma subgroups.

Material & Methods: Melanoma cells of different genetic subtypes, as well as tissue slice cultures of patient tumors are treated with BRAF and PI3K inhibitors alone and in combination with MEK inhibitors. In addition to the investigation of cell cytotoxicity and cell cycle remainder, the altered signal transmission is detected. Furthermore, the patient cells are sequenced in order to identify mutations that promote a positive therapeutic response.

Results: BRAFi increased pERK, but also significantly increased growth inhibition and apoptosis induced by the MEKi in monolayer and patient-derived tissue slice cultures of NRAS-mutant melanoma. BRAFi such as encorafenib induced ER stress via upregulation of the ER stress-related factors ATF4, CHOP and NUPR1 and the pro-apoptotic protein PUMA. MEKi such as binimetinib induced the expression of the pro-apoptotic protein BIM and activation of the mitochondrial pathway of apoptosis.

While the pan-PI3K inhibitor BKM120 is cytotoxic in almost all cell lines and patient cells, the PI3Kα-selective inhibitor BYL719 does not have an antitumour effect. However, the combination of the PI3K inhibitors with the MEK inhibitor already shows a significantly stronger cytotoxic effect at lower concentrations compared to monotherapies.

Conclusion: The data presented herein strongly encourage the clinical use of MEKi in combination with ER stress inducing BRAFi as a strategy to treat rapidly progressing NRAS-mutant melanoma; the combination of PI3K inhibitors with MEK inhibitors could be a new therapeutic option for BRAF wild-type melanomas.

P189 | Lactate exchange promotes oxidative stress resistance and melanoma metastasis

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The vast majority of cancer deaths, including from melanoma, are caused by distant metastasis. Metastasis is an inefficient process that requires cancer cells to undergo poorly understood metabolic changes. It was recently discovered that lung cancer cells consume lactate, and that increased lactate consumption is correlated with worse outcomes; however, it is unclear whether lactate consumption promotes cancer progression or how. Here we show that lactate exchange promotes melanoma metastasis by reducing levels of reactive oxygen species and oxidative stress. Efficiently and inefficiently metastasizing patient-derived xenografts did not exhibit any difference in the uptake or use of isotopically labelled glutamine or glucose in vivo; however, efficient metastasizers took up more lactate and used it to fuel the TCA cycle. Efficient metastasizers expressed higher levels of the lactate transporter Monocarboxylate Transporter 1 (MCT1) and pharmacological inhibition of MCT1 reduced lactate uptake in vivo. MCT1 inhibition had a limited effect on the growth of primary subcutaneous tumors, but substantially reduced the frequency of circulating melanoma cells in the blood and metastatic disease burden. MCT1 inhibition significantly reduced flux through the pentose phosphate pathway and increased ROS levels. Treatment with the anti-oxidant N-acetylcysteine rescued these effects on ROS levels and metastasis. Metastasizing melanoma cells are particularly dependent upon MCT1-mediated lactate exchange to manage oxidative stress.

P190 | The transmembrane proteins LRIG1 and LRIG2 differentially affect skin carcinogenesis

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Skin cancer is dominated by two entities: melanoma and keratinocyte carcinomas. Incidence rates for melanoma are increasing every year; keratinocyte carcinomas are by far the most common type of all cancers in humans. The three transmembrane proteins of the leucine-rich repeats and immunoglobulin-like domains family (LRIG 1-3) attracted attention due to their potential as prognostic markers in different cancer types. LRIG proteins are regulators of receptor tyrosine kinases (RTKs), which are involved in essential cellular processes such as proliferation, differentiation or cell death, and are often dysregulated in tumors. While tumor suppressive functions of both LRIG1 and LRIG3 were reported in malignant glioma, LRIG2 seems to have a pro-oncogenic role. However, the molecular mechanisms and their impact on tumorigenesis in the skin are unknown.

To investigate the function of LRIG1 and LRIG2 in the skin during development, homeostasis and tumorigenesis, we generated two skin-specific transgenic (TG) mouse lines overexpressing LRIG1 or LRIG2 using the Tet-Off system with the keratin-5-promoter. In contrast to LRIG2 TG mice, which showed no obvious abnormalities, newborn LRIG1 TG mice showed a severe phenotype when LRIG1 expression was induced during pregnancy. LRIG1 TG mice were born with open eyes and abnormal whiskers and died within one week. Therefore, to assess later effects of LRIG1 over-expression, we delayed LRIG1 over-expression in the skin until birth. This resulted in mice developing alopecia at 3 months of age, showing hyperplastic epidermis, enlarged hair follicles and sebaceous glands as well as an altered epidermal differentiation. These results suggest that LRIG1 and LRIG2 have different functions in skin homeostasis and physiology.

Next, to analyze the function of LRIG1 and LRIG2 during tumorigenesis, we subjected the generated TG lines to a multi-stage chemical carcinogenesis protocol with DMBA (7,12-Dimethylbenz(a)anthracene) and TPA (12-O-Tetradecanoylphorbol-13-acetate). As expected, first papillomas arose after four to five weeks. However, while the papilloma incidence was not altered in LRIG1 or in LRIG2 TG mice compared to the respective controls (Ctrl), LRIG1 and LRIG2 manipulation changed the course of tumor progression: while LRIG1 TG mice showed no difference in papilloma burden or size compared to Ctrls, LRIG2 TG mice revealed increased tumor progression along with an early onset of squamous cell carcinomas (SCC). In contrast, LRIG1 TG mice developed melanocytic nevi nine weeks after tumor initiation covering the whole back at the end of the experiment. Histologically, melanin accumulation was detected in the dermis as well as in the basal layer of the epidermis and lymph nodes.

The present data indicate different, but not opposite roles of LRIG1 and LRIG2 in the skin. Both proteins seem to support tumor progression, however, towards different types of tumors. Whereas LRIG1 may be an important player in the development of melanocytic lesions, LRIG2 seems to be involved in SCC progression.

P191 (OP02/02) | Hgf overexpression reveals spontaneous conditional activation of the BrafV600E allele for the development of melanoma in mice

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Introduction: BrafV600E is the most frequent oncogenic driver mutation in human melanoma. In order to uncover the mechanisms of disease progression, metastasis and therapy resistance we require matching experimental tools. Most Braf-driven mouse melanoma models however do not readily metastasize, making them unsuitable for understanding melanoma dissemination. Additionally, Braf-driven mouse melanomas frequently develop amelanotic phenotypes not commonly seen in primary human melanoma. In our previous work we showed that overexpression of the hepatocyte growth factor (Hgf) cooperates with the oncogenic Cdk4R24C mutation to drive pigmented, spontaneously metastasizing melanomas.
Methods: In order to obtain a metastasizing BrafV600E-driven mouse melanoma model, we crossed mice carrying an Hgf transgene (Mt::Hgf) with our previously described mice carrying knock-in alleles for Cdk4R24C and a conditionally activatable BrafV600E mutation along with a transgene for tamoxifen inducible cre-recombinase expression under the control of a melanocyte specific promotor (Mt::Hgf-BrafLSL-V600ECdk4R24C/R24C-Tyr::CreERT2, or short Hgf-Braf-Cdk4 mice). Spontaneous tumor development was observed in Hgf-Braf-Cdk4 mice. Additionally, 6-8 week old Hgf- Braf-Cdk4 mice were treated epicutaneously with 33 µg 4-hydroxy-Tamoxifen (4OHT) on three consecutive days.
Results: All Hgf-Braf-Cdk4 mice spontaneously developed fast growing, nodular melanomas within the first year with both amelanotic, immune cell rich as well as pigmented, immune cell poor phenotypes. Surprisingly, we detected a spontaneous activation of the conditional Braf-allele within all amelanotic and 21% of pigmented tumors. Hgf overexpression and activation of the Braf mutation cooperatively promoted the development of skin melanomas. Again, we detected both amelanotic as well as pigmented melanomas. Importantly, Hgf overexpression also drives the metastatic spread of Braf mutant melanomas to the lungs.
Discussion: In our work we show that overexpression of Hgf cooperates with the conditional activation of BrafV600E for the development of primary mouse melanomas in the skin and for spontaneous metastases to the lungs. Interestingly, we detected spontaneous activation of the conditional Braf mutated allele in Hgf-Braf-Cdk4 mice, a finding we have not seen in our previous work with Braf-Cdk4 mice. We speculate that Hgf overrides the Braf induced oncogenic senescence and drives the growth and the metastatic spread of melanoma. The detection of two distinct tumor phenotypes with a genotype-phenotype correlation of Braf mutations in all amelanotic tumors raises the question, if these tumors originate from different cellular origins or whether the tumor microenvironment sculpts the

transcriptional program of the developing melanoma cell, directing it towards either melanocytic or neural-crest-precursor-like cell fates.

P192 | Inhibition of the neural crest transcription factor SOX10 leads to cell cycle arrest and apoptosis in uveal melanoma cells

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Background: 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. The transcription factor SOX10 plays a key role in the development of melanocytes and other neural crest derived cell types and is also expressed in melanoma. It was previously shown that SOX10 inhibition reduced the invasion capacity, decreased proliferation and induced cell death in cutaneous melanoma (CM) cells. Recently, SOX10 expression was also described in UM primary tumors, but its functional role is still unclear.

Methods and Results: We determined the constitutive gene and protein expression of SOX10 in UM cell lines 92.1, Mel270, OMM1.5 and Mel285, four CM cell lines and human melanocytes (HM) by qPCR and Western blot analysis. High SOX10 expression was found in all CM cells and HM (as described previously by Graf et al. 2014) as well as in three of the tested UM cell lines. We further examined the effects of SOX10 downregulation by RNA-mediated silencing in all SOX10 high-expressing UM cell lines. Cell viability was massively decreased already 24 hours after siRNA transfection in UM cells but not in 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. Annexin V/PI staining and FACS analysis showed that RNAi-mediated SOX10 inhibition also led to a strong induction of apoptosis. Cleavage of caspases 9 and 3, but not of caspase 8, indicated that SOX10 inhibition led to cell death by activating the intrinsic apoptosis pathway. Furthermore, higher levels of the DNA damage marker γ-H2A.X were found after SOX10 inhibition.

We also analyzed the impact of ectopic SOX10 expression in low-expressing Mel285 UM cells. Cell viability was examined in CellTiter Blue assays and anchorage-independent growth in soft agar assays but no differences between SOX10-overexpressing and control cells were observed. After embedding in collagen, no differences regarding the invasive capacity were observed, suggesting that SOX10 overexpression does not alter the invasive capacity, cell viability and proliferation of low-expressing cells.

Summary: 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. Thus, SOX10 may be a crucial factor for UM cell survival and may offer novel therapeutic options for the treatment of UM.

P193 | A comprehensive view of splice variants and gene fusions in melanoma

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Differential gene expression between melanoma and its precursors has been extensively studied, but differential expression of transcriptomic isoforms due to gene splicing or of gene fusions are largely understudied in melanoma and other solid tumors. Here we used a data set of an own large-scale RNA-seq analysis of benign melanocytic nevi and primary melanomas to provide a comprehensive view of transcriptomic isoforms due to RNA splicing and gene fusions in melanoma. Overall, gene expression between benign nevi and different types of primary melanomas (inflammatory versus non-inflammatory lesions) showed a large number of differentially expressed transcriptomic isoforms (splice variants). The major gene ontology categories included metabolic pathways, RNA degradation, carbon metabolism, adherens junctions and ribosome pathway genes. Among differentially expressed splice variants were histone genes such as H2AFJ, signalling phosphatases such as PTPN16 (Protein Tyrosine Phosphatase, Non-Receptor Type 18), putative oncogenes such as MTA3 (Metastasis Associated 1 Family, Member 3) and TNF receptor superfamily members such as TNFRSF14 (Tumor Necrosis Factor Receptor Superfamily, Member 14), which is known to be involved in the regulation of inflammatory and inhibitory T-cell immune responses. Recurrently occurring gene fusions included ITPKC, ENOX1, and PPP1R12C. These latter variants were validated in independent melanoma sample sets and were present in a significant number of samples (up to 25%). Functional analysis using an inducible gene expression system showed that the ITPKC fusion protein indeed impacted on in vitro melanoma cell migration. Taken together, we provide a comprehensive view of new melanoma transcriptomic isoforms and gene fusions with a potential role in melanoma development which significantly expands our understanding of this complex tumor.

P194 | Elucidating the mechanism of action of domatinostat (4SC-202) in cutaneous T cell lymphoma cells

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Targeting epigenetic modifiers is effective in cutaneous T-cell lymphoma (CTCL). Here we compared the mode of action of romidepsin (FK228), an established class I histone deacetylase inhibitor (HDACi), and domatinostat (4SC-202), a novel inhibitor of class I HDACs, which has been reported to also target the lysine-specific histone demethylase 1A (LSD1). While both agents potently induced cell death in six different CTCL cell lines, only in case of 4SC-202 death was preceded by an accumulation of cells in the G2/M phase of the cell cycle. Surprisingly, apoptosis and accumulation of cells with double DNA content occurred already at 4SC-202 concentrations hardly affecting histone acetylation and methylation and provoking significantly less changes in gene expression compared to biologically equivalent doses of FK228. Indeed, we provide evidence that the 4SC-202-induced G2/M arrest in CTCL cells is independent of de novo transcription. Furthermore, neither enforced expression of HDAC1 nor knockdown or knockout of LSD1 affected the 4SC-202-induced effects. Together, these results demonstrate that 4SC-202 effectively inhibits growth of CTCL cells, but that activity beyond LSD1-inhibition, modulation of histone modifications and consecutive alteration of gene expression may contribute to the anti-cancer cell activity of 4SC-202. Indeed, time-lapse microscopy using adherent model cells revealed that 4SC-202 could affect mitotic spindle formation. Accordingly, results obtained from an in vitro tubulin polymerization assay suggest that 4SC-202 can directly inhibit microtubule formation.

P195 | Post-transcriptional regulation of Argonaute2 in malignant melanoma

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MicroRNA (miRNA)-mediated gene silencing regulates many cellular processes such as proliferation, invasion and angiogenesis. Based on this it is obvious, that a dysregulation of the miRNA expression pattern is involved in the development of numerous types of cancer. Interestingly, in melanoma a major share of miRNAs is upregulated compared to normal human epidermal melanocytes (NHEM). This stands in contrast to the miRNA expression patterns in many other types of cancer.

MiRNAs are part of the RNA induced silencing complex (RISC) mediating translational repression and degradation of complementary target mRNAs. We found that in malignant melanoma the expression of Argonaute proteins (Ago1-4), which are the key protein components of the RISC, is reduced compared to other cancer cell lines. Especially the expression of Ago2, which is the most abundant Ago protein in human cells, was significantly lower. We quantified the cellular Ago protein levels using the newly developed AGO-APP, a specific affinity purification method followed by mass spectrometry analysis. We could further confirm an Ago2 downregulation via

Western blot and immunofluorescence compared to NHEMs, suggesting an involvement of the Ago2 reduction in melanoma development. Interestingly, the mRNA levels of Ago2 analyzed via qPCR showed no significant differences between melanoma cell lines and NHEMs as well as compared to other cancer cell lines. We conclude that the Ago2 expression has to be reduced by posttranscriptional regulation during melanoma development. On a functional level the re-expression of Ago2 diminished tumorigenic properties of melanoma cells, for example migration. However, even after ectopically expression the Ago2 protein level only increased weakly compared to other cell types. This finding strongly supports a post-transcriptional regulation of Ago2 based on regions in the coding sequence (CDS). To identify the specific region in the Ago2-CDS responsible for regulation, we recombinantly expressed the four functional Ago2 domains separately in different melanoma cell lines. The N-, PAZ- and MID-domain resulted in a higher protein expression than the PIWI-domain. These results provide evidence for a posttranscriptional regulation specifically of the Ago2 PIWI-domain. A reporter system with a luciferase-Ago2-MID + PIWI fusion construct revealed downregulation of the luciferase activity further confirming an association of the AGO2 regulation with the PIWI-part of the AGO2 mRNA.

Our data indicate that not only the miRNA expression pattern itself, but also the regulation of the miRNA processing enzyme AGO2 has severe impact on cellular function and can contribute to tumor progression. The decreased AGO2 protein expression and the elevated expression of plenty of miRNAs in malignant melanoma lead to the conclusion that miRNAs compete for AGO2 protein binding and only the most efficiently expressed miRNAs result in effective gene regulation. Influencing the molecular mechanisms driving the AGO2 downregulation in malignant melanoma would therefore offer an advantage for repressed tumor suppressive miRNAs. This provides a promising tool minimizing the tumorigenic properties triggered by the AGO2 dysregulation in melanoma.

P196 | Secreted YB-1—a novel tumour marker and functional player in melanoma progression

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Secreted factors play an important role in intercellular communication and are therefore not only indispensable for the regulation of various physiological processes but can also decisively advance the development and progression of tumours. In the context of inflammatory disease, the oncogenic transcription and translation factor Y-box binding protein 1 (YB-1) can be actively secreted promoting cell proliferation and migration. Based on an increased intracellular YB-1 expression during melanoma progression, secretion of YB-1

by melanoma cells and its functional effects as well as its potential usefulness as a melanoma marker were to be analysed in this study. Intriguingly, we can show that in contrast to benign cells of the skin such as melanocytes, fibroblasts or keratinocytes, melanoma cells can actively secrete YB-1. YB-1 secretion seems to correlate with the stage of melanoma progression and depends on a calcium- and ATP-dependent non-classical secretory pathway leading to the occurrence of YB-1 in the extracellular space as a free protein. Along with an elevated YB-1 secretion of melanoma cells in the metastatic growth phase, extracellular YB-1 exerts a stimulating effect on melanoma cell migration, invasion as well as tumorigenicity.

These data suggest that extracellular YB-1 secreted by melanoma cells plays a functional role in melanoma cell biology stimulating metastasis and may serve as a novel tumour marker in malignant melanoma.

P197 | Use of single-cell transcriptome analyses to reveal new mechanisms of melanoma progression and treatment resistance

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Cellular heterogeneity and clonal selection processes in many tumors are major contributors to early recurrences and resistance to targeted and checkpoint inhibitor treatment. Cellular heterogeneity may now be analysed in more detail by single-cell sequencing technologies. Here we performed a single-cell transcriptome analysis of thousands of melanoma cells and confirmed the molecular heterogeneity of different melanoma short-term cultures and melanoma cell lines. A recently described bioinformatics tool for the analysis of developmental processes in evolutionary biology (analysis of pseudotime dynamics) was used to provide a more detailed analysis of developmental paths of melanoma cells to highly aggressive cells. Pseudotime progression towards higher tumor aggressiveness appeared to be largely driven by cell cycle genes. Furthermore, a pseudotime-dependent, bipolar expression of microphthalmia-associated transcription factor (MITF) and AXL receptor tyrosine kinase (AXL) signatures was identified leading to different transcriptomic states in highly aggressive cells. Also, epigenetic modifications of promoter regions changed during the development of more aggressive single-cell phenotypes suggesting an epigenetic reprogramming during melanoma progression. Finally, a specific metabolic reprogramming occurs during pseudotime progression. In a subsequent study on treatment resistance to BRAF inhibitor treatment, a melanoma subclone with a hypoxic tumor signature was eradicated by BRAF inhibitor treatment while others sustained treatment and obviously contributed to treatment resistance. Taken together, single-cell transcriptomics identified different clonal subpopulations which might be of relevance for future treatment decisions. Pseudotime analysis allows for a more detailed analysis of gene signatures changing during melanoma progression.

P198 | Inhibition of RSK family members can effectively target malignant melanoma cells with MAPK pathway hyperactivation

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The MAPK signalling pathway is frequently hyperactivated in malignant melanoma and plays a central role in tumour cell proliferation and survival. Accordingly, its inhibition has proved to be an efficient treatment option in melanomas harbouring BRAF mutations. However, there is still a considerable need for effective targeted therapies for other melanoma subgroups with constitutive MAPK activation, such as RAS and NF-1 mutated tumours, as well as for therapeutic options targeting MAPK pathway inhibitor resistant BRAF mutated melanomas, which commonly exhibit a striking reactivation of this pathway. The p90 ribosomal S6 kinases (RSKs) are central effectors of MAPK signalling regulating cell cycle progression and survival.

Indeed, we can show an increased RSK activity going along with a MAPK pathway hyperactivation in BRAF mutated melanoma cells. Interestingly, RSK inhibition can effectively target those cells, particularly in the case of MAPK pathway inhibitor resistance. In line with an enhanced activity of the MAPK pathway based on activating RAS mutations or loss-of-function of the tumour suppressor NF-1, the antitumoural activity of RSK inhibitors appears to extend to melanoma cells of these genetic subgroups.

Overall, these data indicate a potential general usefulness of the p90 ribosomal S6 kinase family members as prospective targets in malignant melanoma with hyperactivated MAPK signalling pathway.

P199 (OP06/02) | Increased permeability of tumor blood vessels is triggered by membrane attack complex activated neutrophils

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The coagulation and complement systems are evolutionarily related enzymatic cascades and it is known that both systems interact with each other. Although growing body of evidence suggest that tumor mediated hypercoagulation supports neutrophil recruitment and metastasis, the impact of complement effectors on melanoma metastasis formation are poorly understood. Increased activation of the complement system has been measured in various malignancies. Previous studies indicated that the complement activates endothelial cells (ECs) and neutrophils. However, in the context of tumor progression, knowledge on the crosstalk between the vascular endothelium, the complement system and the neutrophil associated innate immunity is still scarce. Here, we report the systemic complement activation in patients suffering from malignant melanoma. Using mouse and human tumor tissue samples, we showed that complement effectors such as C3b and C5a fragments deposit around tumor blood vessel walls. However the complement cascade terminated by the formation of the membrane attack complex (MAC) not on the endothelium but on perivascular neutrophils. In vitro experiments with human ECs and neutrophils could confirm this complement mediated crosstalk. Further in vitro experiments demonstrated that MAC positive neutrophils release reactive oxygen species (ROS) and neutrophil extracellular traps (NETs). In close proximity to the endothelium, complement activated neutrophils were able to increase the vascular permeability allowing the transmigration of melanoma cells. Interference with the deposition of complement factors on the EC surface through the low-molecular weight heparin tinzaparin prevented MACs formation and thus ROS and NETs release from neutrophils. Moreover, tinzaparin treatment stabilized the vascular permeability and might contribute to a reduced metastasis as previously published. In summary, we discovered a triangular communication between the complement, neutrophils and the vascular endothelium mediating NETosis, endothelial dysfunction and subsequently melanoma cells extravasation. Therefore, targeting complement activation envisions a new therapeutic strategy for malignant melanoma.

P200 (OP03/04) | The myelin protein PMP2 is regulated by SOX10 and drives melanoma cell invasion

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Background: The transcription factor sex determining region Y-box 10 (SOX10) plays a key role in the development of melanocytes and glial cells from neural crest precursors. SOX10 is expressed in melanoma and involved in a variety of phenotypes from early tumor initiation to metastasis. However, specific mediators which impart its oncogenic properties remain widely unknown.

Methods and Results: To identify novel target genes of SOX10, we performed RNA sequencing after ectopic expression of SOX10 in the human melanoma cell line 1205Lu. Nine genes were differentially regulated and were further evaluated. Among these, peripheral myelin protein 2 (PMP2) was consistently upregulated in several melanoma cell lines after ectopic SOX10 expression. Gene expression analysis in a panel of melanoma cells revealed that basal PMP2 expression was highly variable. Inhibition of SOX10 by siRNAs led to reduced PMP2 mRNA and protein levels in all tested cell lines. In silico analysis of the PMP2 promoter region revealed three highly conserved SOX10 binding sites p1-p3, which were subsequently analyzed in chromatin immunoprecipitation (ChIP) and electrophoretic mobility shift assays (EMSA). SOX10 bound to the proximal PMP2 promoter at all predicted SOX10 binding sites, but most prominently to the most proximal site p1. To further elucidate the functional relevance of these sites, reporter constructs bearing the sites p1, p2, and p3 and a control site with a disrupted SOX10 binding motif (p1mut) were created and luciferase reporter assays were conducted in cells with high basal SOX10 expression and after ectopic SOX10 expression. The highest promoter activity was observed for constructs harboring p1 and p2, whereas no activity was detected for p1mut, indicating that there is a relevant transcriptional regulation of PMP2 by SOX10 at p1. Moreover, we identified early growth response 2 (EGR2), a common co-factor of SOX10 in myelin gene regulation, as a co-regulator of PMP2 expression in melanoma cells. We further analyzed the contribution of PMP2 expression to melanoma malignancy in cell lines that stably overexpressed PMP2 wild-type or defective PMP2 mutant proteins (L27D and Mut3). In 2D and 3D invasion assays, the invasive capacity was strongly increased with wild-type PMP2, but significantly diminished in cells expressing L27D and Mut3, suggesting that only fully functional wild-type PMP2 was able to mediate the pro-invasive effects. Moreover, knockdown of PMP2 by siRNAs led to decreased invasion in transwell assays. Ectopic PMP2 expression in SOX10-depleted cells partially rescued the invasive phenotype, implying that PMP2 contributed to the SOX10-mediated pro-invasive phenotype.

Summary: In this study, we have identified PMP2 as a novel target gene of SOX10 in melanoma, which is originally known for its function in the myelin sheath of the peripheral nervous system. Functionally, PMP2 contributed to the SOX10-mediated invasive phenotype. Our data imply that a subset of melanoma cells can hijack properties of glial cells to gain a more invasive phenotype and that myelin-associated proteins may play a hitherto unappreciated role in melanoma progression.

P201 | Inhibition of DNA repair related protein RAD51 as a therapeutic strategy for the treatment of metastatic melanoma

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Melanoma is a cancer type with a high mutational load. Therefore, maintenance of genomic stability is required for melanoma progression which is often conferred by overexpression of DNA repair related genes. High expression of DNA repair genes is associated with a higher metastatic potential of melanoma cells.

We found by database analysis that especially a high RAD51 expression among the different DNA repair related genes, significantly correlates with a worse survival of melanoma patients. In addition, we confirmed an enhanced RAD51 expression in metastatic melanoma cell lines compared to the expression level in melanocytes or primary melanoma cell lines, which supports the role of RAD51 as potential therapeutic target. We further treated melanoma cells with novel molecular RAD51 inhibitors and observed DNA damage accumulation and apoptosis induction in treatment-naïve as well as in melanoma cells resistant to the approved MAPK inhibitors (MAPKi). Furthermore, the combined inhibition of RAD51 and MAPK signaling reduced melanoma cell viability and induced apoptosis in a synergistic manner. Such synergistic viability decline could even be observed in melanoma cell lines with acquired resistance to MAPKi.

We propose that DNA damage repair proteins such as RAD51 may be promising targets for melanoma therapy and could enhance the effect of MAPKi therapy and thereby prevent MAPKi resistance.

P202 | PPAR-alpha suppresses lymphangiogenesis via VEGFR-1 and Neuropilin-2

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Lymphedema, cancer or chronic inflammatory diseases, are associated with abnormal lymphatic vessel formation. Hence, influencing lymphangiogenesis is a promising target. PPAR alpha agonists are known to be effective anti-angiogenic and anti-tumorigenic agents and there is no evidence whether this property can be extended to an anti-lymphangiogenic action. To prove this assumption, we performed proliferation and functional assays with primary human dermal lymphendothelial cells (DLEC). We could demonstrate that fenofibrate suppresses DLEC proliferation, formation of capillary-like structures and migration. There was significant apoptosis induced by PPARα agonists in DLECs and cell cycle inhibition could be ruled out by FACS analysis. Since signaling via important lymphendothelial growth factor receptor (VEGFR1-3, neuropilin-1,-2,

VE-Cadherin) pathways is critical for lymphangiogenic responses, we explored whether fenofibrate acted by diminishing the expression. Here, we could demonstrate a significant concentration dependent suppression of VEGFR1 and Neuropilin-2 protein expression. In contrast, the other receptors were not or only slightly (highest treatment concentration) affected by PPARα agonist treatment. PPAR alpha knockdown experiments demonstrated the abrogation of fenofibrate effects proving the PPAR dependent mechanism. Via neuropilin-2 knockdown experiments we analyzed whether neuropilin-2, known as an important regulator of lymphangiogenesis might be involved in the regulation of the other important VEGF-receptors. Interestingly, neuropilin-2 knockdown resulted in reduced lymphendothelial proliferation and was associated with reduced VEGFR expression. Hence, VEGFR-1 and neuropilin-2 expression are critical molecular targets of PPARα and might be responsible for its anti-lymphangiogenic effects.

P203 | Dimethyl fumarate influences endothelial PDL-1 and PDL-2 expression in part via Stat-3 and IRF-1

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Tumor associated vessels play multiple roles during tumor progression including metastasis and immune responses. Therefore, the expression and regulation of programmed death ligand (PDL)-1 and PDL-2 in endothelial cells in the proximity of the tumor is an important prerequisite of tumor immune response. Recently, it could be demonstrated that stimulated endothelial cells activate Tregs via the PD-1/PDL signaling pathway and therefore display an effective immunosuppressive action. Up to now there is only sparse information concerning the expression of both ligands in endothelial cells. Dimethyl fumarate (DMF) is employed successfully as a drug for the treatment of inflammatory skin diseases, e.g. psoriasis, and lately also for immunomodulatory therapy of the autoimmune disease multiple sclerosis. Furthermore, recent studies have proven that DMF has a marked antiproliferative impact on diverse cancer entities in both in vitro and in vivo trials, e.g. on malignant melanoma or head and neck cancer. We analyzed the basal and IFN-γ-induced expression of PDL-1 and PDL-2 in primary human vascular endothelial cells (HUVEC) and the influence of DMF.

In FACS analysis the cell surface expression of PDL-1 and PDL-2 was significantly induced by IFN-γ and diminished by DMF treatment. To analyze the underlying mechanisms of regulation we performed Western-Blot and quantitative mRNA analysis. Here we could demonstrate a different regulation of PDL-1 and PDL-2. Whereas whole cell protein and mRNA expression of PDL-1 was not changed, PDL-2 expression was regulated on both levels. We further analyzed whether DMF inhibits the JAK/Stat pathway regulating PDL

expression. Here, we could demonstrate that DMF inhibits Stat-3 phosphorylation and nuclear translocation which was accompanied by increased p53 expression known to inhibit Stat-3 activity. In addition, the nuclear translocation of the important transcription factor IRF-1 was inhibited by DMF treatment. These results were in line with the reduced PDL-2 luciferase promoter activity. 3′UTR promoter regulations could be ruled out.

In summary, DMF suppresses PDL-1 and PDL-2 surface expression on activated endothelial cells. Concerning PDL-1 this might be conveyed by increased PDL-1 surface turnover whereas PDL-2 expression is regulated on the transcriptional level. Therefore, DMF might increase immunoreactivity by suppression of tumor vascular PDL-1/-2 expression.

P204 | miR-129-5p: a potential therapeutic approach to overcome BRAFi resistance

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Melanoma is the most lethal form of skin cancer with increasing incidence.

Background: Almost 60% of melanomas harbor a BRAF mutation, most frequently the V600E substitution, for which therapeutic inhibitors 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 microRNA (miRNA) alterations. MiRNAs are small noncoding RNAs (20-27nt) with posttranscriptional regulatory functions, via suppressing protein translation or inducing mRNA degradation by binding to the 3′ untranslated region (3′UTR) of target mRNAs.

Aim: To find out if microRNAs (miRs) are a potential target to modify BRAFi resistance.

Results: Evaluation of next generation sequencing analysis (GSE94423) reveal that miR-129-5p expression is induced after BRAFV600E pathway inhibition in treatment sensitive but not in the resistant A375 melanoma cell line. This data were validated with three additional BRAFV600E associated cell lines (WM35, WM902B, WM9) and their corresponding resistant clones (WM35R, WM902BR, WM9R) by qRT-PCR. Thus only BRAF mutation associated cell lines, but not BRAFi resistant ones show raised miR-129-5p expression by BRAF inhibition. A putative target of miR-129-5p is SOX4, a SRY-related HMG-box transcription factor. In malignant melanoma tissues and the most melanoma cell lines SOX4 is overexpressed. SOX4 on the other hand transcriptionally regulates EZH2, a H3K27 histone methyl transferase, which itself regulate miR-129-5p expression in a negative feedback loop. We were able to show that the expression growth of miR-129-5p by BRAF inhibition leads to a

decrease of SOX4 on protein level and lower EZH2 mRNA expression in BRAFV600E sensitive cell lines, while it is unaffected in the corresponding resistant clones.

Conclusion: miR-129-5p may be an important factor to overcome resistance mechanisms in BRAF-associated melanoma.

P205 | Tumor derived exosomes alter macrophage function by delivery of microRNAs

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Metastatic melanoma is an aggressive tumor with still unfavorable limited prognosis.

Background: Melanoma cells secrete exosomes, which are small (50-200 nm depending on their origin) cell derived membrane nanovesicles loaded with proteins, DNA, mRNA and noncoding RNAs as microRNAs (miRNAs). Melanoma derived exosomes interact with several tumor infiltrating immune cells like T cells (TILs), myeloid derived suppressor cells (MDSCs) or tumor associated macrophages (TAMs) Their uptake mediates the recipient cell function and phenotype, thereby influencing tumor growth, metastasis, drug resistance and immune escape mechanisms. Macrophages represent tumor infiltrating immunomodulatory cells and are mainly divided into subpopulations such as tumor suppressive M1 and protumorigenic M2.

Aim: To better understand the mechanisms of immunomodulatory activities of melanoma we have analysed the effects of melanoma-derived exosomes within the tumor microenvironment, especially on macrophages.

Methods: Therefore, we established the exosome isolation via ultracentrifugation or total exosome isolation reagent (ThermoFisher Scientific). For the quantitative and quality control isolated exosomes were analyzed by nanoparticle tracking analysis (NTA) and western blot for tetraspanins CD63 and CD81 or calnexin. Uptake of SYTO stained exosomes into M1 polarized macrophages was demonstrated by fluorescence microscopy and flow cytometry.

Results: In vitro the treatment of M1 polarized THP-1 derived macrophages with melanoma exosomes induce a proinflammatory and proangiogenic phenotype, in a time and concentration dependent manner. By qRT-PCR analyses we revealed that melanoma exosomes induce the expression of immunomodulatory genes like IL-6, TNFα, IL-1b, CD80 as well as angiogenesis factors IL-8 and VEGFA. To better understand the molecular mechanisms we analyzed the miRNA cargo of melanoma and normal melanocyte secreted exosomes by next generation sequencing (NGS). Here we found that miR-125b-5p was enriched in melanoma derived exosomes, which could be validated by qRT-PCRs. Also miR-125b-5p was accumulated in M1 polarized Macrophages after treatment with melanoma exosomes.

Conclusion: Melanoma derived exosomes deliver miRNAs into macrophages, thereby affecting the macrophage phenotype in a way, which could support tumor immune evasion and finally tumor progression.

P206 | 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% 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.

P207 | Tasquinimod inhibits tumor growth in a humanized mouse melanoma model

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Tumors hijack the patient's immune system by exploiting its immune suppression mediating mechanisms to sustain tumor growth, avoid rejection and enable metastasis formation. Repolarizing tumor associated macrophages into immunostimulatory M1 macrophages is a promising strategy to flip the switch in the tumor microenvironment from immune suppression towards an immune reaction against the tumor.

In order to achieve repolarization of macrophages it is necessary to have (i) efficient nano carriers to transport small molecules or siRNA into macrophages and (ii) to subsequently release the functional cargo in an adequate amount. We developed destabilized liposomes being sensitive to low pH levels and physiological temperatures. The chemotherapeutic Doxorubicin was used as model substance to quantify drug release because of its fluorescence and DNA binding.

To analyze cargo release we perform in vitro cultures of human monocyte-derived macrophages and human melanoma cells. Doxorubicin release inside the cell is quantified by using flow cytometry and confocal microscopy. siRNA-mediated transfection is detected via qPCR.

In vivo studies are carried out in a subcutaneous melanoma model of human melanoma cell lines in humanized NOD/SCID mice transgenic for HLA A2.1. 2 out of 3 tested melanoma cell lines gave rise to xenograft tumors. Spleen and tumor showed a distinct composition of human immune cell infiltration in immunohistochemistry staining proving the model to be valid for our purpose.

For therapeutic use, liposomes containing tasquinimod or siRNA will be used as cargo. Tasquinimod, a quinoline-3-carboxamide derivate of roquinimex, has been shown to influence macrophages in the tumor microenvironment and blood vessel formation in several studies. By using the free substance as therapeutic agent we were able to inhibit tumor growth in our melanoma model in the humanized mice. This effect was limited to humanized mice, as in the control group of mice without human immune cells tumor growth was unaltered. This indicates that tasquinimod rather influences the tumor microenvironment than blood vessel formation. Furthermore, we validated destabilized liposome species as suitable in vivo drug carrier.

P208 | Senolysis is needed for the clearance of senescent tumor cells

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Cellular senescence, known as an intrinsic growth control mechanism that prevents the transformation of pre-malignant lesions into overt malignancy, plays an important role in tissue development, homeostasis and cancer control. Besides endogenous stress signals, exogenously delivered Th1-cytokines (IFNγ & TNF) can initiate senescence in a variety of human and murine cancer cells. However, these senescent cancer cells remain a potential harm due to their senescence associated secretory phenotype (SASP). The SASP promoted by cytokine induced senescence (CIS) in murine RIP1-Tag2 beta cancer cells was highly pro-inflammatory and contained chemoattractants. Its components CCL2 and CCL5 not only attracted bone marrow derived macrophages (bmMQ) and polarized them

into a pro-tumorigenic M2 phenotype but also promoted proliferation of non-senescent beta cancer cells. Therefore, we analyzed the mechanisms required for the clearance of senescent cancer cells. Surprisingly, we found that senescent cancer cells were resistant to phagocytosis, despite the production of chemoattractants and the expression of phosphatidylserine. However, senescent cancer cells were specifically more sensitive to apoptosis then non-senescent cancer cells. Following senescence with secondary apoptosis allowed phagocytosis by bmMQ. In consequence, senolysis is required for the clearance of senescent tumor cells. These findings will allow to answer the urgent question whether senescent cancer cells cause harm and should be deleted or whether they are protective by inducing tumor specific immune responses.

P209 | Preclinical development of salvage virotherapy to target immune-escaping melanomas with diminished Jak1 expression

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Most primary melanomas are detected early and can be cured by surgical resection. However, a subset of patients develops an incurable, metastatic disease. Recently, the treatment of metastatic melanoma has been revolutionized by the introduction of immunotherapies such as checkpoint blockade and adoptive T cell transfer. Long-lasting clinical responses can be achieved nevertheless only in a small subgroup of patients, and most patients show either primary or secondary treatment resistance. The experimental development of novel salvage therapies to target the resistant cell populations remains one of the major challenges in the field. Primary resistant tumors are often devoid of T cell infiltrates while secondary resistance may develop through acquisition of mutations in genes such as Jak1 that render tumors unresponsive to interferons and incapable of upregulating MHC molecules. Interestingly, recent studies have demonstrated that oncolytic virotherapy can counteract primary resistance both in the animal models and in the clinics, whereas their role as salvage therapy for interferon unresponsive melanomas has not been investigated so far. In the current work we utilized CRISPR/Cas9 genome engineering system to knockout Jak1 in HGF-CDK4 mouse derived transplantable HCmel12 melanomas and hypothesized that tumors derived from this cell line would mimic immune-escaping human melanoma. Moreover, we hypothesized that because Jak1 is a crucial component of the cell autonomous antiviral immunity, the escaping tumors would constitute good targets for salvage virotherapy. To test these hypotheses experimentally we first generated and functionally validated several monoclonal HCmel12-Jak1-KO cell lines and proceed to retrovirally transduce them with either mCherry or tagBFP transgenes to allow further

experiments with WT/Jak1-KO tumor cell mixtures. We carried out in vitro experiments with highly pure mouse CD8^{+} T cells carrying transgenic receptor against melanocytic antigen gp100 and could show that Jak1-KO HCmel12 melanoma cells were positively selected from WT/Jak1- KO cell mixtures upon immunotherapy. We then took advantage of the oncolytic alphavirus SFV VA7-eGFP as an experimental tool and demonstrated that the virus infects and lyses specifically Jak1-KO mouse melanomas both in vitro and in vivo. Taken together, these data suggest that oncolytic virotherapy may represent an attractive salvage therapy approach for immune escaping melanomas.

P210 | Endothelial Notch signaling controls hepatic metastasis by adhesive, but not angiocrine mechanisms

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The interaction of tumor cells with organ-specific endothelial cells (EC) is an important step during metastatic progression. In recent reports, Notch signaling in organ-specific niches has been implicated in mediating opposing effects on organotropic metastasis to the lungs and the liver, respectively. As EC specificity and mechanistic details have not yet been comprehensively analyzed, the role of endothelial Notch activation during liver metastasis was scrutinized here. To target hepatic EC (HEC), a novel EC subtype-specific Cre driver mouse was generated. Clec4g-Cretg/wt mice were crossed to Rosa26N1ICD IRES-GFP to enhance Notch signaling in HEC (NICDOE-HEC). Hepatic metastasis of malignant melanoma and colorectal carcinoma was significantly reduced in NICDOE-HEC. NICDOE-HEC mice revealed reduced liver growth and impaired metabolic zonation due to suppression of hepatic angiocrine Wnt signaling. Hepatic metastasis, however, was not controlled by angiocrine Wnt signaling as deficiency of the Wnt cargo receptor Wls in HEC of WlsHEC-KO mice did not affect hepatic metastasis. In contrast, the hepatic microvasculature in NICDOE-HEC revealed a special form of sinusoidal capillarization with effacement of endothelial zonation functionally paralleled by reduced tumor cell adhesion in vivo. Notably, expression of endothelial adhesion molecule ICAM1 by HEC was significantly reduced. Similarly, tumor cell adhesion to HEC was significantly inhibited by anti-ICAM1 antibody treatment of wild-type mice confirming that Notch controls

hepatic metastasis via modulation of HEC adhesion molecules. As endothelial Notch activation in the lung has been shown to promote lung metastasis, tumor therapy will require approaches that target Notch in an organ-, cell type- and context-specific manner.

P211 | Raising MAPK pathway inhibition to a new level using ERK inhibitor combinations

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The clinical availability of small molecule inhibitors specifically targeting BRAF mutated at V600 and its downstream target MEK marked a significant breakthrough in the therapy of BRAF mutant melanoma. Despite a vast anti-tumour activity and improved patient survival, rapidly emerging resistance to these inhibitors, however, 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 ultimate kinases and consequently 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 (GDC-0994) in the treatment of BRAF mutant melanoma cells.

To this end, melanoma cell lines 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. Intriguingly, long-term treatment with the ERK inhibitor could considerably reduce melanoma cell growth, which seemed to be independent of the sensitivity to BRAF or MEK inhibitors. Moreover, cell cycle analyses and cell viability assays revealed a distinct benefit of adding ERK1/2 inhibitor to BRAF and/or MEK inhibitors to effectively target the melanoma cells with BRAF mutation.

These data suggest that combinatorial treatment regimens including ERK1/2 inhibitors might be an attractive therapeutic strategy in BRAF mutated melanoma cells.

P212 | Glucocorticoid-induced suppression of UVB-mediated apoptosis in melanocytes

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The human 11ß-Hydroxysteroid-Dehydrogenase (HSD11B1) acts as a catalyst for the conversion of the inactive glucocorticoid cortisol into the active glucocorticoid cortisol. Tumours from melanoma patients harbour HSD11B1-mutations in the Glycine residues at positions G41, G45 and G47. These amino acids are conserved across mammals and located within the NADPH-binding side of the protein. Point mutations in this region have an

UV-Signature and occur in tumours with high mutation counts. We cloned and characterized these key-mutations by qRT, WB, ELISA, FACS and microscopy and found that they reduce the stability and completely abolish the activity of the murine and human enzyme in vitro. Subsequently we irradiated glucocorticoid-treated melanocytes with UVB to determine the importance of glucocorticoid-signaling during UV-stress. The glucocorticoids cortisol and dexamethasone increase expression of the anti-apoptotic gene BCL2L1 and decrease expression of the pro-apoptotic genes BAX, PMAIP1 and BAK1. Glucocorticoid-mediated suppression of apoptosis was shown by growth kinetics and stainings for Annexin V, Propidium Iodide, Caspase 3 and Caspase 7 activation. We conclude that Glucocorticoid-signaling plays a role in the context of UV-mediated carcinogenesis and plan to characterise the clinically relevant HSD11B1-mutations in more detail.

P213 | Sulforaphane (SFN)—a dietary supplement for treatment of metastatic Merkel cell carcinoma?

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There is great interest in dietary supplements for treatment of cancer. Isothiocyanates like sulforaphane (SFN), which can be found in cruciferous vegetables like broccoli, are known to have anti-cancer effects. SFN inhibits proliferation and induces apoptosis in several tumor cell lines, such as melanoma cells.

In the present study we are investigating the effects of SFN on the Merkel cell carcinoma cell lines MCC-13 and MKL-1. Effects of SFN on proliferation and viability were analyzed by BrdU and MTS assays. Potential changes in cell cycle were examined using flow cytometric analysis (FACS). Apoptotic effects were evaluated by annexin V binding capacity with FACS, cell death detection ELISA and Caspase-Glo® 3/7 assays. Furthermore we investigated potential effects of SFN on the expression of pro- and anti-apoptotic proteins by western blot analysis.

Treatment with SFN led to a dose- and time-dependent reduction of cell viability and proliferation of the treated cell lines. Since induction of apoptosis is described for SFN, we investigated whether the seen effects on viability and proliferation could be due to induction of apoptosis. We were able to detect an increase in the sub-G0/G1 phase in cell cycle and an increase in annexin V binding capacity via FACS, formation of cytoplasmic histone associated DNA fragments and cleavage of poly (ADP-ribose) polymerase (PARP).

Our first results demonstrate antiproliferative and pro-apoptotic effects of SFN in the Merkel cell carcinoma cell lines MCC-13 and MKL-1. We assume that SFN may provide a novel therapeutic option in Merkel cell carcinoma. Further investigations are necessary

to identify the specific mechanisms resulting in the observed changes.

P214 | Intraperitoneal immunotherapy of melanoma ascites induces cancer cell senescence

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Cellular senescence is an important endogenous barrier against cancer development. Preclinical data reveal that besides endogenous signals, exogenous, cytokine-delivered signals can induce senescence in murine cancers in vivo and human cancers in vitro. This raises the question whether immunotherapy of human cancers exclusively relies on killing or whether cytokine-induced senescence contributes to efficient immunotherapy of human cancer. Here we show that intra-peritoneal injection of interferon-α (IFN-α) induces extensive senescence in melanoma cells during salvage treatment of life-threatening, malignant ascites. Following IFN-α application, melanoma cells started to strongly express senescence-associated β-galactosidase (SA-β-gal), and the cell cycle inhibitors p16Ink4a or nuclear phospho-p21. Simultaneously, the melanoma cells became permanently growth-arrested, also in the absence of the cytokines, and cleared from the peritoneum. To validate the therapeutic potential of IFN-α in vivo we transferred the treatment protocol in a murine model of peritoneal carcinosis in NOD-SCID mice. During four cycles of IFN-α, the tumor burden was efficiently controlled by arresting cancer cell proliferation. We could demonstrate that IFN-α treatment is a functional pro-senescence immunotherapy. Therefore, besides killing through cytolysis or apoptosis, cytokine-induced senescence critically contributes to therapeutic cancer immune control.

P215 | RAB27A promotes melanoma cell invasion and metastasis via regulation of pro-invasive exosomes

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Despite recent advances in targeted and immune-based therapies, advanced stage melanoma remains a clinical challenge with a poor prognosis. Understanding the genes and cellular processes that drive progression and metastasis is critical for identifying new therapeutic strategies.

Here, we found that the GTPase RAB27A was overexpressed in a subset of melanomas, which correlated with poor patient survival. Loss of RAB27A expression in melanoma cell lines inhibited 3D

spheroid invasion and cell motility in vitro, and spontaneous metastasis in vivo. The reduced invasion phenotype was rescued by RAB27A-replete exosomes, indicating that exosomes drive RAB27A-mediated invasion. Furthermore, while RAB27A loss did not alter the number of exosomes secreted, it did change exosome size and altered the composition and abundance of exosomal proteins, suggesting RAB27A promotes the biogenesis of a distinct proinvasive exosome population.

These findings support RAB27A as a key cancer regulator, as well as a potential prognostic marker and therapeutic target in melanoma.

P216 | Enforcing cellular stress promotes apoptotic and immunogenic responses in melanoma

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Neo-antigens derived from apoptotic tumour cells are sensed by the immune system and drive effective anti-tumour immunity. This immunogenic cell death (ICD) can be elicited in vivo following bortezomib (26S proteasome inhibitor) treatment in some cancers. Here, we test the dynamics of bortezomib-induced ER stress and apoptosis in melanoma and whether this promotes ICD. We first confirmed that a clinically relevant dose of bortezomib induced hallmarks of ICD in vitro. Necessary markers of ICD, including cell-surface expression of calreticulin, HSP70 and HSP90, were upregulated in human and murine melanoma cells following bortezomib treatment. The secretion of HMGB1 and ATP by apoptotic melanoma cells was also detected, further suggesting ICD initiation. We confirmed bortezomib-induced ER stress by real-time imaging of an ER stress biosensor in melanoma cells. We next asked whether bortezomib-treated melanoma cells could elicit anti-tumour immunity in vivo. Mice were primed subcutaneously with bortezomib-treated melanoma cells undergoing ER stress and apoptosis. Mice were then challenged a week later on the opposing flank with matched, live melanoma cells and tumour growth was monitored. We observed a delay in tumour onset and decreased tumour growth in mice that were vaccinated compared to controls. Together, these data demonstrate that melanoma cells undergo ICD following bortezomib treatment and this cellular stress can be co-opted to enable effective anti-tumour immunity. Next we will use this vaccination method to further determine the effects on the innate immune system. ICD induction could be a therapeutic method to further potentiate patient responses to immunotherapy.

P217 | Microphthalmia-associated transcription factor regulates dynamic heterogeneity and structural integrity in melanoma

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Differential tumor cell behavior caused by environmental conditions, termed dynamic heterogeneity, is a prime source for drug resistance. We utilize real-time cell cycle imaging (FUCCI) to study melanoma heterogeneity. As distinct proliferative and invasive capabilities reflect variable drug sensitivities, identifying these different responses is crucial to design effective therapies. Mouse xenograft tumors generated from cell lines with high microphthalmia-associated transcription factor (MITF) level displayed a homogeneous distribution of cycling cells throughout. In contrast, tumors generated from cell lines with low MITF levels were composed of clusters of cycling cells and clusters of G1-arrested cells. The proliferating areas were in close proximity to blood vessels, presumably characterized by oxygen/nutrient availability. Melanoma spheroids recapitulated the in vivo cycling behavior, considering that here oxygen and nutrients are supplied by diffusion. MITF was undetectable within the hypoxic G1-arrested spheroid core, indicating hypoxia-induced MITF downregulation. Furthermore, modulation of MITF expression impacted spheroid morphology, with overexpression giving rise to flatter structures whereas knockdown to smaller aggregates with unaffected sphericity. The loss of morphological integrity caused by increased MITF expression did not reduce spheroids' inner hypoxic level, dismissing the hypothesis that these compromised structured could be more permeable to oxygen resulting in decreased hypoxia induced G1-arrest. Proteomic analysis revealed that modulation of MITF level induced differences in cell-cell and cell-ECM adhesion. In addition, inhibition of the Rho/ROCK signalling pathway, known to control cell contractility, partially mimicked the morphology and cell cycle effects of MITF overexpression. We conclude that MITF protects from cell cycle arrest induced by oxygen deprivation. We hypothesise that high MITF levels prevent cell cycle arrest by reducing the cell-intrinsic propensity to arrest in response to low oxygen via a mechanism involving cell-cell/ECM crosstalk.

P218 (OP05/05) | Uncovering microtubule-driven mechanisms of melanoma invasion

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Despite the significant progress in targeted therapies for melanoma, advanced stage metastatic melanoma is still currently incurable. This highlights the urgent need for novel therapeutics that specifically act to inhibit metastasis—known as "migrastatics". Further, suitable migrastatic targets can only be uncovered by studying and elucidating the mechanisms and biology that drive metastatic melanoma. It is widely understood that the cell motility underlying invasion is driven by the orchestration of various elements of the cell cytoskeleton, identifying these proteins as key targets. Additionally, the modality that cancer cells employ to undergo invasion is highly dependent on the bio-chemical composition of the micro-environment, which underscores why metastatic disease is regarded as a heterogeneous disease state. This adaptability of cancer cell motility arises through a cellular process of "mechanosensing", whereby the bio-chemical properties of the extracellular matrix can influence the spatiotemporal regulation of cell adhesion-cytoskeletal crosstalk to influence cell migration. Here, we report that the protein Cytoskeletal Linker ASsociated Protein or CLASP, that has functions to tether and stabilise microtubules, are highly expressed in a panel of melanoma cell lines. Further, melanoma cells utilise 2 unique paralogs of CLASP for differing functions to drive 3D invasion. Using fluorescent tagging and quantitative live cell imaging approaches, we report that the selective depletion of CLASP paralogs in 1205Lu melanoma cells abrogates invasion by interfering with crucial microtubule-dependent functions during 3D-invasion. Further pan-depletion of CLASPs within 1205Lu melanoma cells results in 3D migration-stasis and reduced cell viability when cells are exposed to conditions of 3D confinement. These findings suggest that the microtubule associated protein, CLASP, functions in melanoma cells to facilitate cellular processes of both invasion and survival.

P219 | miRNA nanoparticles for melanoma treatment in a murine melanoma model

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The current concept of oncogene addiction refers to the fact that malignant tumors may depend on the activity of one or more oncogenes or oncogenic pathways. It has recently been extended towards microRNAs (miRNAs), i.e., small non-coding RNAs that play important roles in various cellular processes by interfering with the expression of genes, including oncogenes and tumor suppressor genes. In a recent analysis of miRNA expression in benign melanocytic nevi, primary melanomas and melanoma metastases, miR-638 and miR-150 were shown to be differentially expressed, with increasing levels of expression during tumor progression. Subsequent functional analyses showed that

melanoma cell lines treated with antimiRs, small oligonucleotides directed against both miRNAs, inhibited melanoma cell proliferation in vitro. However, no effect was seen in colony formation. In a xenograft mouse model using immunodeficient mice, subcutaneous and intravenous injection of human melanoma cells led to local and distant tumor formation (lung colonization), respectively. We then used specific antimiRs directed against miR-638 and miR-150 formulated in polymeric, polyethylenimine-based nanoparticles to treat these mice. Local tumor growth as well as systemic dissemination of melanoma cells could be significantly reduced by this treatment. In order to further validate these findings, the expression of miRNA target genes SOX2 and MDM4 was analysed by immunofluorescence analyses in local tumors. The miR-150 target MDM4 showed enhanced expression in antimiR-treated, regressing lesions, further arguing for a specific effect of this treatment modality. The role of MDM4 in melanoma may be complex, since it exerts pro-tumorigenic effects while also reversing MDM2-targeted degradation of p53. Taken together, the present study demonstrates antimiRs complexed with nanoparticles as innovative treatment approach in melanoma, which might substantially enrich the current therapeutic repertoire of melanoma therapy.

P220 (OP02/05) | T-helper-1-cell-induced senescence stabilizes the differentiation and physiological function of neuroendocrine tumors

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Cellular senescence, or permanent cell cycle arrest, is linked to a number of physiological and patho-physiological conditions such as aging, age-related diseases, embryogenesis, tissue homeostasis and tumor development. T helper 1 (TH1) cell-mediated immunity has been shown to control neuroendocrine beta-cell cancers of RIP-Tag2 mice by induction of tumor dormancy. Further detailed analyses revealed that this TH1-mediated, therapeutic and life-prolonging effect is based on cytokine-induced senescence. Thus, and in accordance with the histological data from other cancers, e.g. melanoma, cytokine-induced senescence can be considered as an anti-tumoral barrier in RIP-Tag2 mice. However, senescent cells also display apoptosis resistance and a senescence-associated secretory phenotype, and thus might be harmful and foster tumor cell dedifferentiation. Here, we asked whether senescence induction in beta-cancer cells rather promotes or protects from dedifferentiation. We show that TH1 cell-based immunity not only controlled the growth but also the genetic stability of those cancers. The immune cells also stabilized the expression of three important beta-cell differentiation markers synaptophysin, insulin and glucose transporter-2 (Glut2) in a tumor necrosis factor (TNF) receptor-1-dependent manner.

Further experiments showed that TH1 cells preserved the beta-cell function of the cancer cells, i.e. the glucose-dependent activation of their mitochondria and the glucose-dependent release of insulin. The preservation of the beta-cell function was also TNF receptor-1-dependent. In addition, treatment of beta-cancer cells with the TH1 cell cytokines interferon-gamma and TNF mimicked the immune cell-mediated effects on beta-cell differentiation. Although the cytokine-treated cells displayed clear morphological and functional hallmarks of senescent cancer cells, the cells highly expressed the differentiation markers insulin and Glut2. Taken together, our data reveal that immune cell-mediated senescence surveillance of cancers protects against loss of differentiation in specialized, hormone-producing tumors rather than driving the cells into epithelial mesenchymal transition.

P221 | Generation of in vitro cellular dormancy models

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Many years after removal of the primary tumor and treatment with adjuvant therapy melanoma patients can face a severe recurrence of the cancer. The appearing metastasis at different organ sites most likely arise from tumor cells disseminated at an early stage to distinct niches such as the bone marrow. They are assumed to survive adjuvant therapy, being protected by their microenvironment, e.g. by cancer associated fibroblasts and their extracellular matrix or osteoblasts. The mechanism of the survival of the disseminated tumor cells (DTC) is attributed to an induced reversible cell cycle arrest which results in a so called cellular dormancy.

Although cellular dormancy in tumor cells is already known since the late 1940s, in vitro models to investigate the cellular mechanisms of dormancy in melanoma remain to be established.

Therefore, we develop cell culture systems to examine the influence of the tumor niche and tumor-intrinsic signaling pathways on the initiation, maintenance and reactivation of dormant melanoma cells based on methods already used in prostate and breast cancer.

Our cell culture systems include the induction of cellular dormancy in melanoma cells by different stress signals, the cultivation of dormant melanoma cells on different extracellular matrices, the co-culture of dormant and proliferating melanoma cells and the co-culture with bone marrow stromal cells.

Cellular dormancy is confirmed and measured by simple cell counting, clonogenic assays and cell cycle analysis or immunostaining (Ki-67). The in vitro dormancy model for melanoma cells should help to understand the mechanism of dormancy initiation, which factors influence the dormant state and possible therapies for the inhibition of reactivation of dormant melanoma cells.

P222 | Does cGAS/STING signaling in melanoma patients correlate with clinical response?

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Talimogene laherparepvec (T-VEC) is a herpes simplex virus 1 (HSV-1) strain, which was genetically modified through the functional deletion of 2 genes (ICP34.5 and ICP 47) and the insertion of a coding sequence for the human granulocyte-macrophage colony-stimulating factor (hGM-CSF). These modifications grant selective replication of the virus in tumor cells as well as a systemic antitumor immune response, mediated through GM CSF production. A phase III study published in 2016 reported an objective response rate (ORR) of 26% in the cohort of patients treated with T-VEC (melanoma stage IIIB-IVM1a). The study also showed that 64% of the injected and 34% of the non-injected lesions had a response, defined by a tumor area decrease of 50% or more.

It was recently suggested that the susceptibility of melanoma cells to oncolytic viruses is dependent on the expression of the genes cGAS (cyclic GMP-AMP synthase) and STING (stimulator of interferon genes), which are located upstream of type I interferon (IFN-1) induction.

To investigate the correlation between cGAS, STING and melanoma, we initially analyzed the expression of both genes through immunohistochemistry in tissue microarrays (TMAs) composed from patient tissue samples: primary melanomas, melanoma metastasis, nevi and healthy skin. We then analyzed the expression of cGAS and STING through immunohistochemistry of tissue samples additionally in patients treated with T-VEC. The results obtained will enable us to determine the frequency of a defective signaling pathway at the level of cGAS and STING as well as to explore the relation between STING/cGAS expression and patient clinical response.

P223 | Pharmacologically active high-dose vitamin C blocks melanoma cell energy metabolism and efficiently inhibits tumor growth in vitro and in vivo

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In recent years, we and others have discovered that high-dose vitamin C paradoxically acts as a prooxidant and causes the formation of a large amount of hydrogen peroxide in an oxygen pressure-dependent manner, especially in extracellular space. This formation of reactive oxygen species (ROS) could not be compensated by tumor cells, especially melanoma cells, but was rather well tolerated by benign cells such as fibroblasts. Therefore, ROS formation by high-dose vitamin C could be an attractive approach to treat therapy-refractory melanomas such as melanoma metastases with primary resistance to immunotherapy or tumors with secondary resistance to targeted therapies such as BRAF plus MEK inhibitor combinations. Vitamin C is mainly transported into the cancer cell by the facilitative glucose transporter GLUT1 in its oxidized form and by the ATP-dependent sodium transporters SVCT1/2 in its reduced form. Therefore, we have speculated whether there are additional intracellular effects of vitamin C that support the cytotoxicity of vitamin C in high concentrations. Besides the rapid formation of hydrogen peroxide, we have measured a rapid degradation of cellular ATP and a rapid decrease of reduced glutathione (GSH) and NADPH. The decrease in ATP levels coincided with the end of glycolysis in vitamin C-treated melanoma cells. Energy metabolism blockade and redox homeostasis disorder were observed in both NRAS- and BRAF-mutated cell lines. Melanoma cell lines that are resistant to BRAF + MEK inhibitors were similarly sensitive to the induction of cell death after treatment with pharmaceutically active amounts of vitamin C.

To investigate the effect of high-dose vitamin C on standard melanoma therapies, we treated three different BL6 mouse models, based on subcutaneously injected B16F10, D4M.3A (BRAFV600E) or 1274 (HgfxCdk4R24C) melanoma cells, with intraperitoneal injections of vitamin C (1-2 g/kg body weight). This resulted in short-term ascorbate serum levels in the pharmacologically active range of 1-10 mM and significantly improved the therapeutic effect of the corresponding combined standard melanoma therapy, which was either surgery, immune checkpoint blockade with anti-PD1 or BRAFV600E inhibition.

Therefore, we conclude that intravenous high-dose vitamin C therapy may be beneficial for melanoma patients by interfering with the tumor's energy metabolism and can be safely combined with standard melanoma therapies without interference.

P224 | Prognostic relevance of interactions of H2A deubiquitinase MYSM1 with BRAF/CREB- and WNT-MITF/c-MET modules in human melanoma

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In melanoma and other tumors, altered expression and activities of histone-modifying enzymes and transcription factors collaborate with genetic alterations to determine tumor cell plasticity at different steps of tumorigenesis including transformation, tumor progression, metastasis and drug resistance. The histone H2A deubiquitinase 2ADUB/MYSM1 has recently been shown to have critical functions

in skin pigmentation and melanoma growth. Based on our data from Mysm1-deficient mouse models and human melanoma we here analyzed signaling pathways and transcriptional modules regulated by MYSM1 at different stages of melanoma development in greater detail using human melanoma samples and melanoma cells lines.

In accordance with our in vitro data, high MYSM1 expression in melanoma patients correlated with an overall poor prognosis and reduced overall survival rates. Expression of MYSM1 in melanoma cell lines partially depended on RAS-MEK-ERK and AKT-pathway activity. During transformation and tumor progression of melanoma cells, MYSM1 regulated the overall abundance of H2A-K119ubi generating an overall growth permissive transcriptional environment. According to ChIP and colocalization data, MYSM1 specifically interacted with the c-MET promoter region in close vicinity to PAX3 in A375 melanoma cells enhancing the HFG/c-MET axis, tumor cell invasiveness, and potentially drug resistance to BRAFi. In addition, MYSM1 interactions with other growth and survival promoting transcriptional regulators including CREB, FOSL1, ß-Catenin and MITF as detected by co-localization and ChIP experiments may guide tumor subset fate at different stages of melanoma pathogenesis. Current inhibitor and knock-down experiments aim at dissecting the differential roles of MYSM1 interactions in different transcriptional cascades and cell populations in melanoma. Based on our present in vitro and patient data, we propose that 2A-DUB/MYSM1 might be a valuable prognostic marker in human melanoma and potentially promising target for combinational therapy.

P225 | Dissecting the impact of MEKi on BRAF wild-type melanoma cells

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Targeted therapy has greatly improved patient survival in metastatic melanoma but the development of drug resistance limits long-term efficacy. The small molecule inhibitor Cobimetinib targets MEK. MEK is part of the MAPK pathway, a signaling pathway which is important for cell proliferation, survival and migration. In BRAF mutated melanoma the combination of BRAF inhibitor and MEK inhibitor is more effective than BRAF inhibition alone. However, the impact of MEK inhibition on tumors without a BRAF mutation is partly unknown. Here we use HCmel12 a cell line derived from HGF/CDK4 mice.

First, we confirmed the activity of the MAPK pathway by analyzing pERK levels by phospho-specific antibody flow cytometry. We noticed that phosphorylated ERK was detected in each cell but that the expression differed between cells and between experiments. When cells were treated for two hours with Cobimetinib, phosphorylation of ERK was effectively inhibited in all cells.

Next, we treated HCmel12 cells for 72 hours with Cobimetinib and noticed that Cobimetinib strongly impaired cell proliferation.

In vivo there was only a marginal effect of systemic Cobimetinib on tumor growth. Surprisingly, we observed a strongly enhanced number of lung metastasis in MEKi treated mice compared to vehicle treated mice. We hypothesize that Cobimetinib stimulates migration of tumor cells and that the enhanced migration from the primary tumor site is the main reason for elevated number of lung metastasis after Cobimetinib treatment.

To test this hypothesis, we performed transwell migration assays with a fluorescent version of HCmel12. Fluorescent protein expressing cells were treated for 72 hours, counted and plated in Boyden chamber transwell inserts. After 20 hours migrated cells were fixed and counted using fluorescence microscopy. Indeed we observed that cobimetinib treated HCmel12 cells migrated significantly more than vehicle treated cells.

We hypothesised that MAPK inhibition could stimulate PI3K/AKT signaling, thereby enhancing cell migration. Levels of phosphorylated AKT were indeed elevated after 72 hours Cobimetinib treatment, compared to vehicle treated HCmel12 cells. To inhibit this pathway during migration assays we pretreated cells for 72 hours with Cobimetinib. When either AKT (wortmannin inhibitor) or MET (Capmatinib) were coinhibited in the last two hours of this pretreatment, migration was severely reduced.

Together these data indicate that Cobimetinib treatment inhibits cell proliferation, but that tumors can escape from MEK inhibition through overactivation of the PI3K/AKT pathway, resulting in enhanced migration. We are currently repeating similar assays with a variety of BRAF wild-type murine and human melanoma cells.

MISCELLANEOUS

P226 | Antibacterial efficacy of a nanosecond-driven cold plasma device against common wound pathogens in vitro

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The control of bacterial super-colonization in wounds has recently become an immense challenge owing to the extensively increasing antibiotic resistance exhibited by various bacterial species. This has called for the urgent investigation of novel alternative antibacterial and disinfection approaches. Cold atmospheric pressure plasma (CP) has achieved considerable medical developments especially as a novel antimicrobial procedure for the treatment of superficial skin infections and chronic wounds. In this paper, the antibacterial efficacy of cold plasma generated from a prototype nanosecond-driven

dielectric barrier discharge (DBD) plasma device was tested in vitro using six, antibiotic-sensitive and antibiotic-resistant, bacterial species that represent common wound pathogens.

Three gram-positive and three gram-negative bacterial cultures were inoculated onto the surface of blood agar and exposed to cold plasma for 30, 60, 90, 120, and 180 seconds. The diameters of inhibition zones obtained after treatment were measured and the corresponding number of inactivated bacterial colonies (CFUs) in each inhibition area was calculated using the viable plate count method. The log10 reduction factor (log RF) was then calculated from the logarithmic values of CFUs of treated and untreated (control) plates. Our results show that the growth of all six bacterial species tested was effectively inhibited by cold plasma at all exposure times compared to untreated controls. Already after 30 seconds a reduction in bacterial count of around 2-5 log steps was achieved ($P < 0.05$). Observing the log RF values at different plasma exposure times revealed that bacterial inhibition was time-dependent. Increasing CP exposure from 30 seconds to 120 seconds significantly enhanced the killing of all strains which is manifested by the increase in log RF from around 2-5 magnitudes to around 5-11, respectively. Further plasma exposure (180 seconds) did not significantly augment bacterial reduction compared to plates treated for 120 seconds. However, significantly larger inhibition zones were observed in three of the tested species ($P < 0.05$).

It was also observed that different bacterial strains exhibited variable sensitiveness to CP. Methicillin-resistant *Staphylococcus aureus* (MRSA) demonstrated the most susceptibility (log RF = 5.16-10.7) while Methicillin-sensitive *Staphylococcus aureus* (MSSA) was the least susceptible (log RF = 1.79-5.34).

In conclusion, this study points out the promising potential of cold plasma generated from our prototype DBD device in inhibiting common wound bacteria in vitro. Our results provide a detailed database of the susceptibility of different species to cold plasma which would allow a more successful utilization of the device for skin decontamination and wound treatment in vivo. This is of great importance when alternatives to conventional antimicrobial methods are warranted.

P227 | Hyaluronan/collagen hydrogels containing sulfated hyaluronan increase efficiency of HB-EGF during wound healing

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Functional biomaterials that are able to bind, stabilize and release bioactive proteins in a defined manner are required for the sustained delivery of such to the desired place of action stimulating wound healing in health-compromised patients. Glycosaminoglycans (GAG) represent a very promising group of constituents since they may be functionally engineered and are well tolerated by the recipient tissues due to their relative immunological inertness.

Ligands of the EGF-receptor activate keratinocytes and dermal fibroblasts and, thus, contribute to skin wound healing. HB-EGF bound to GAG in biomaterials (e.g. hydrogels) might serve as a reservoir that induces prolonged activation of the EGF receptor and recover disturbed wound healing.

Based on previous findings we aimed to investigate the capacity of hyaluronan (HA) and its chemically sulfated derivatives (sHA) to bind and release HB-EGF from HA/collagen-based hydrogels. The development of a molecular model of HBEGF followed by docking and molecular dynamics-based analysis allowed us to identify residues at the heparin-binding domain of the protein being essential for the recognition of GAG derivatives. Furthermore, molecular modeling and surface plasmon resonance (SPR) analyses demonstrated that sulfation of HA increases binding strength to HB-EGF thus giving a rational basis for the development of sHA1-containing hydrogels. In line with computational and SPR results, gels with sHA displayed a retarded HB-EGF release compared to pure HA/collagen gels. Collagen hydrogels containing HA or its mixture with sHA were shown to bind and release bioactive HB-EGF over at least 72 hours, which induced keratinocyte migration, EGFR-signaling and HGF expression in dermal fibroblasts. Importantly, hydrogels containing sHA strongly increased the effectivity of HB-EGF in inducing epithelial tip growth in epithelial wounds shown in a porcine skin organ culture model. These data suggest that hydrogels containing HA and sHA can be engineered for smart and effective wound dressings.

P228 | Significantly reduced diversity and altered composition of the skin microbiome in peeling skin disease

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Inflammatory peeling skin disease (PSD, OMIM 270300), also referred to peeling skin syndrome type B, is an unusual and rare autosomal-recessive ichthyosiform erythroderma. Affected individuals present a severe cutaneous inflammation with patchy peeling of the entire skin, associated with pruritus, recurrent *S. aureus* infections, allergic manifestations and elevated serum IgE levels. PSD is caused by a homozygous nonsense mutation in the gene encoding corneodesmosin

(CDSN). The mutation leads to a complete loss of CDSN, a glycoprotein expressed primarily in the upper layers of the epidermis and hair follicles. CDSN contributes to keratinocyte cohesion and represents a protease target during epidermal desquamation. The absence of CDSN induces a separation of the epidermis between the stratum corneum and the stratum granulosum, which leads to skin barrier defects, thus facilitating allergen penetration and contributing to atopy manifestation in PSD. Therefore, similar to Netherton syndrome and ichthyosis vulgaris, PSD has been proposed as a novel human model disorder for atopic dermatitis (AD). Hence, we speculated that the skin microbiota in these erythematous-desquamative lesions should be similar to the microbiome signature found in AD, and aimed to investigate the taxonomic composition of the cutaneous microbiota in lesional and perilesional skin from PSD patients. To answer the question, we performed, for the first time in this disease, Metagenomic Sequencing to characterize the skin microbiome of lesional and perilesional site from individuals affected by PSD. After the collection of samples from these skin areas of individuals with PSD with a swab soaked in PBS, we established the isolation of sufficient amounts of microbial DNA from swabs. Then we amplified the variable regions V3-V4 of the 16S rRNA genes in a PCR reaction. Subsequently, barcoded libraries were prepared and subjected to high throughput next generation sequencing using the Illumina MiSeq®. The subject affected by PSD showed a decreased diversity and genera richness. We observed a distinct bacteria configuration that was clearly different from that of healthy subject. The most prevalent phyla detected in all subjects included *Actinobacteria*, *Firmicutes*, *Proteobacteria* and *Bacteroidetes*. In particular, PSD patients showed a decreased abundance of the phyla *Proteobacteria* and *Actinobacteria*. In parallel, the abundance of *Firmicutes* was significantly increased compared to corresponding skin areas from healthy donors or individuals with other skin diseases such as psoriasis. In addition, at the class and order level, the abundance of *Bacilli*, as well as *Bacillales* was also significantly increased in PSD patient; whereas, the abundance of *Actinobacteria*, as well as *Actinomycetales* was reduced. At the family and genus level, the relative abundance of *Staphylococcaceae*, as well as *Staphylococcus* was increased in PSD patients. In accordance with this, the abundance of *S. aureus* was higher in PSD group, which interestingly and in contrast to subjects with AD, could also be observed in non-lesional skin samples. Overall, within PSD subjects, we did not observed any significant difference in the skin microbiota composition between non-lesional and lesional skin. This data support the hypothesis that PSD microbial configuration resembles the one seen in AD and is very different from the one found in healthy subject. Our results support the role of PSD as a model disorder for AD and generate the hypothesis that interventions targeting the microbiome could perhaps provide a therapeutic benefit for PSD patients as already published in AD.

P229 | The PPARg modulator N-Acetyl-GED-0507-34-Levo (NAGED) is more effective in reversing experimentally induced EMT than the PPARg agonist Pioglitazone

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Lichen Planopilaris (LPP) is one of the most common primary cicatricial alopecia. LPP patients show multifocal, coalescing areas of hair loss in the scalp with mild to moderate perifollicular erythema and scarring. Lesional hair follicles (HFs) show collapse of immune privilege in the bulge, the epithelial HF stem cell niche (eHFSCs), with dense perifollicular inflammation localized exactly in and around the bulge. Bulge epithelial stem cells (eSCs) undergo epithelial-mesenchymal transition, which leads to irreversible destruction of this central HF cell population with scarring appearing at the HF location. Unfortunately, therapies of substantial clinical benefit for LPP and other scarring alopecia remain to be developed.

Previous reports revealed that reduced PPARγ signaling is involved in LPP susceptibility but that lesional HFs are still capable of responding to PPARγ modulation. Indeed, PPARγ agonists are currently being tested for the treatment of LPP and other scarring alopecias, in particular the orally administrable PPARγ agonist Pioglitazone. Few case studies have reported favorable results for LPP patients treated with Pioglitazone but therapy was discontinued in some cases due to severe side effects.

We have previously shown that N-Acetyl-GED-0507-34-Levo (NAGED), a topically applicable PPARγ modulator with a favorable toxicology profile, deserves to be further explored as a novel therapeutic for LPP and other scarring alopecias. NAGED not only reduced inflammation in and around the bulge of LPP lesional affected HFs ex vivo but was also capable of protecting and partially rescuing bulge eSCs from experimentally induced EMT ex vivo, and reversing established EMT in affected HFs ex vivo.

Therefore, in the current study we aimed to compare the effectiveness of NAGED and Pioglitazone in reversing experimentally induced-EMT in human HF bulge epithelial stem cells ex vivo.

For this purpose, full lengths HFs were microdissected from occipital scalp skin from 3 independent donors. 48 hours after inducing EMT, using a previous published EMT-triggering cocktail, the HFs were treated for 3 days ex vivo with either 0.1 mM NAGED or 0.1 mM Pioglitazone.

As previously shown, EMT induction in HF bulge eSCs after treatment with the EMT-inducing cocktail was confirmed by significant reduction of E-cadherin protein expression and a significant increase in vimentin and SLUG positive cells within the HF bulge.

The application of 0.1 mM NAGED 48 hours after EMT induction significantly reduced the number of Vimentin and SLUG positive cells

in the bulge compared to HFs treated only with the EMT-triggering cocktail. By contrast, the number of vimentin or SLUG positive cells in HFs treated with 0.1 mM Pioglitazone did not change compared to HFs treated only with the EMT-triggering cocktail, and was significantly increased compared to HFs treated with the EMT-inducing cocktail and 0.1 mM NAGED.

Neither NAGED nor Pioglitazone could completely reverse decreased expression of E-cadherin or K15^{+} cells in the bulge of HFs treated with the EMT-inducing cocktail in these donors.

Our data demonstrate that NAGED is more efficient than Pioglitazone in reversing experimentally induced EMT ex vivo by negatively regulating early EMT. Given that NAGED, in contrast to Pioglitazone, is topically applicable our results highlight NAGED as an alternative, more efficient and favorable treatment for LPP and other scarring alopecia that deserves to be clinically assessed.

P230 | Tofacitinib impacts the polarization of human macrophages and the differentiation of human dendritic cells

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Introduction: Psoriasis is a common chronic inflammatory skin disease characterized by the appearance of red scaly plaques. Tofacitinib is a small-molecule Janus kinase (JAK) inhibitor. JAK proteins function in pairs and are responsible for signalling of cytokines involved in several immune processes. Each pair of JAK proteins has specificity for a different set of cytokines. Tofacitinib is a potent inhibitor of JAK1/3 and JAK1/2 signalling, while also inhibiting JAK2/2 and JAK2/TYK 2 signalling with reduced potency.

Aim: to examine the in vitro effects of tofacitinib on human macrophage and dendritic cell functions.

Material and Methods: Monocytes were isolated from peripheral blood mononuclear cells of 9 healthy donors. Monocytes were differentiated into immature DC (iDC) in presence of IL-4 and GM-CSF, or into M0 macrophages upon M-CSF stimulation. M0 macrophages were further polarized into inflammatory M1 or regulatory M2 macrophages via LPS and IFNγ or IL-4 stimulation, respectively. LPS was used as a general trigger of activation. Tofacitinib was added at a non-toxic dose of 10μM. The mRNA levels of cytokines and surface markers were profiled by qPCR.

Results: We first tested the effects of tofacitinib on the function of macrophages. M0 cells differentiated in presence of tofacitinib showed a reduced ability to undergo further polarization into M2 (but not M1) subtype, as observed by a decreased expression of CD200R molecule. In addition, Tofacitinib had a general inhibitory effect when added during the polarization of M0 macrophages, as it decreased the expression of the M1-specific molecule CD80 and the M2-specific molecule CD200R. Increased mRNA levels of IL-12 were observed in M2 macrophages polarized in presence of tofacitinib. Finally, when tofacitinib was added to fully polarized M0, M1 and M2 macrophages, it counteracted the inflammatory effect of LPS on IL-12 mRNA. We also assessed the effects of tofacitinib on the function of DC. Tofacitinib prevented the expression of CD209 and CD80 on iDC. Strikingly increased level of IL-12 was observed when monocytes were differentiated into DC in presence of tofacitinib. However, tofacitinib decreased the response of fully polarized DC to LPS, as observed by a reduced expression of IL-12 mRNA.

Conclusions: Tofacitinib impacts the polarization and differentiation of macrophages, in particular of the M2 subtype. In addition, it prevents DCs differentiation and decreases their ability to respond to LPS.

P231 | Chronic inflammation drives the mechano-transduction cascade in the epidermis

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Chronic skin inflammation leads to significant alteration of tissue stroma with respect to ECM deposition and remodelling, thus changing tissue mechanical properties and ultimately the activity and function of epithelial stem and progenitor cells. Components of the mechanotransduction cascade respond to mechanical stimuli and induce activation of cell signalling pathways leading to metaplasia of epidermis as well as functional alterations such as expression of immunomodulatory cytokines. Epithelial dysfunction induced by altered mechanical properties may contribute to the chronicity of psoriasis, rendering components of the mechanotransduction cascade as putative therapeutic targets.

Using immunohistochemistry on biopsy material from patients, we provide evidence for the activation of the mechanotransduction cascade in response to deposition of extra-cellular matrix (ECM) materials—namely tenascin C and fibronectin—in chronic inflammatory conditions such as psoriasis. In-vivo experiments in mice were performed, using imiquimod (IMQ) or IL-17E injections to induce a psoriasis-like phenotype also documented substantial deposition of tenascin C and fibronectin in the dermis, which resulted in the activation of the mechanotransduction cascade through b-integrin signalling. Further immuno-fluorescence experiments showed phosphorylation of focal adhesion kinase through activated b-integrin, resulting in subsequent activation and nuclear translocation of ROCK2 and YAP1. Absence of YAP1 in cytoplasm spares ß-catenin from proteasome degradation, resulting in hyper-proliferative and migratory signals in epidermal keratinocytes in IMQ treated mice. Upon ablation of the inflammatory stimulus in the IMQ mouse model via injection of anti-IL-17E antibody, we observed reduced tenascin C deposition in the dermis and reduced translocation of ROCK2 into the nucleus; this effect may result from the anti-inflammatory effect of the treatment with the anti IL-17E antibody.

In summary, we provide evidence for the activation of the mechanotransduction cascade in psoriasis through deposition of tenascin C and fibronectin, resulting in b-integrin activation, as well as subsequent activation and nuclear translocation of ROCK2 and YAP1. Future investigations in the mechanotransduction pathway may allow identification of novel potential therapeutic targets for treating chronic cutaneous inflammation.

P232 | Assessment of ammonia molecules leaving the skin surface by diffusion along with calcium ions rinsed from the skin surface in order to assess their relation in the upper stratum corneum layer

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In several investigations an inverse relation between ammonia molecules diffusing out of the skin and pH of the skin surface was found. While this cannot be explained easily by the Henderson-Hasselbalch equation favoring ammonia diffusion with increasing pH values, pH dependent enzymatic activity in particular transglutaminase activity producing ammonia allows to explain such inverse relations. As calcium ions are a key factor of transglutaminase activity quantification of calcium levels of the skin surface in vivo might reveal further insights into the relation of ammonia molecules diffusion out of the skin with the pH of the skin surface. Therefore the aim of the present study is to assess ammonia molecules diffusing out of the skin along with calcium ions extractable from the skin surface as well as the pH of the skin surface. Overall 16 volunteers (8 m/8 w) were included after written informed consent. Site of investigation was the median volar portion of the non-dominant forearm. Ammonia was assessed using a diffusion test based on trapping of ammonia with water. By maintaining a gap of air between skin surface and water trapping the ammonia it was assured that only ammonia molecules and not ammonium ions were collected. The following quantification of ammonia was performed using a photometric test. Collection of calcium ions was performed by rinsing the skin surface using gradient grade water. The quantification of calcium ions was performed using a photometric test. The pH of the skin surface was measured potentiometrically using a glass electrode. For the statistical evaluation of the relation of the different parameters correlation coefficients were calculated and curve fitting procedures were applied.

For ammonia a median amount of 1.52 ng/cm² min leaving the skin surface could be measured and a highly statistically significant inverse relation between ammonia and skin surface pH, which was in the physiological range could again be found ($r = -0,659$, $P = 0.007$). For the calcium ions, a median amount of 0.31 μg/cm² was found. With respect to the relation between calcium ions and ammonia molecules a very slight increasing linear relation between ammonia and calcium was found ($r = 0,320$; $P = 0,245$). The increasing relation became stronger and reached almost statistical significance when applying non-linear curve fitting procedures ($P = 0.051$). Furthermore, a tendency to an inverse relation between calcium ions and skin surface pH ($r = -0.450$; $P = 0.093$) was found.

The results obtained indicate besides confirming the inverse relationship between ammonia and pH of the skin surface a non-linear positive relation between ammonia molecules leaving the skin surface by diffusion and calcium ions. However, the relation appears not uniform enough to conclude that there is a relationship exclusively due to enzymatic activity. Interestingly, the results indicate also an inverse relation between skin surface pH and calcium levels suggesting that there is another mechanism influencing at the same time both ammonia and calcium ions. From the literature it is known that calcium binding to proteins is pH dependent and decreases with decreasing pH values which might lead to higher free calcium levels and higher extractability of calcium ions in lower pH values. As ammonia is in equilibrium with ammonium ions which are probably also bound pH dependently to proteins, this might also apply for ammonia. Further studies and data from a higher number of volunteers are required to try to separate which of the effects might be due to enzymatic activity and which might be due to general pH dependent protein binding.

P233 | Modulation of melanin biosynthesis by cold plasma derived reactive species

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The biosynthesis of melanin in the human skin gives rise to our skin colour and is an important protector against UV induced cellular damage. Formation and transport of melanin pigments is a well-orchestrated process in which various enzymes are involved. The activity of enzymes in the melanin formation is dependent on multiple factors; among them are reactive oxygen species. Cold atmospheric plasma provides reactive nitrogen and oxygen species in a tunable manner that can be applied to liquids, biological materials or tissue. Therefore it was tempting to investigate a modulating role of cold atmospheric plasma on melanogenesis.

To experimentally approach melanin synthesis, melanoma cell lines with different endogenous melanin content were selected. Cold atmospheric plasma was generated by the kINPen MED® (neoplas tools GmbH). This device belongs to plasma jets and it received a certificate as a medical device class II in 2013. Initially the presence and distribution of melanosomes was investigated by immunofluorescence staining with the melanosomal marker NKi/beteb. The expression of the key transcriptional regulator MiTF was investigated by qPCR before and after plasma exposure. Melanin content after plasma stimulation was determined.

In all tested cell lines, the melanin content increased significantly after incubation with plasma treated cell culture medium. Because

of poor solubility of eumelanin in alkali solution this method may lack the changes in eumelanin content but detect only the increased amount of pheomelanin. To include the eumelanin in the analytic approach, alkaline hydrogen peroxide oxidation was performed and degraded products were separated by HPLC.

In addition, the impact of cold atmospheric plasma on morphology and viability was minor. The pattern of melanosomal distribution remained similar. In the least pigmented SK-Mel-28 cells MiTF expression doubled after plasma treatment. In the other tested cell lines, plasma exposure revealed only minor effects on the expression of MiTF in our experimental setup.

Modulation of melanin by cold plasma can be a useful tool. However, further investigations are required to elucidate the underlying signaling cascade.

P234 | Measurement properties of patient-reported outcome measures (PROMs) for women with Genitourinary Syndrome of Menopause: a systematic review

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Background: Genitourinary Syndrome of Menopause (GSM) is a chronic and usually progressive skin disease which affects up to 50% of postmenopausal women. Symptoms, such as vaginal dryness, itching and burning have negative impacts on the women's sexual activity and often come along with urinary problems. Furthermore, these consequences influence the women's quality of life (QoL). Patient-reported outcome measures can be used to measure the impact of GSM.

Objectives: We aimed to identify all existing PROMs that were developed and/or validated for measuring patient-reported outcomes in women with GSM or vulvovaginal symptoms during menopause and assess the quality of these PROMs in a transparent and structured way.

Methods: We performed a systematic literature search in MEDLINE, EMBASE, Web of Science and smaller data bases, and hand-searched reference lists of included studies. Only studies in English, German, French or Italian aiming at the evaluation of measurement properties, the development of a PROM, or the evaluation of the interpretability of the PROMs of interest were eligible. The methodological quality of eligible studies was evaluated with the COnsensus-based Standards for the selection of health Measurement INstruments (COSMIN) risk of bias checklist. Quality criteria for good measurement properties were applied and the quality of evidence was graded using a GRADE approach. Information on interpretability and feasibility was extracted as well. PROMs were then categorized into

three categories. PROMs of category A had evidence for sufficient content validity and at least low quality evidence for sufficient internal consistency, PROMs of category C had high-quality evidence for an insufficient measurement property, and PROMs of category B could not be categorized in A or C.

Results: Eight studies, two of which were found by reference list screening, were included. These studies reported on four PROMs. All of the included PROMs showed sufficient content validity. Two of the PROMs, the Vaginal Symptoms Questionnaire (VSQ) and the Day-to-Day Impact of Vaginal Aging (DIVA) showed moderate-to-high quality of evidence for sufficient structural validity and internal consistency, and were categorized as A. They can be therefore recommended for future use. The UGAQoL still has the opportunity to be recommended for use, but further validation is needed. The overall rating was often indeterminate since structural validity or important reliability parameters were not reported. The Urogenital symptom scale cannot be recommended for use since there was high quality of evidence for insufficient structural validity and internal consistency.

Conclusion: Currently, two PROMs for women with GSM or vulvovaginal symptoms can be recommended. Nevertheless, those PROMs do not cover the urinary component of GSM. Future validation research should try to confirm and extend the measurement properties of those PROMs to strengthen this recommendation. PROSPERO registration CRD42018092384.

P235 | Evaluation of responsiveness and estimation of smallest detectable change (SDC) and minimal important change (MIC) scores for the Childhood Atopic Dermatitis Impact Scale

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Background: The Childhood Atopic Dermatitis Impact Scale (CADIS) is an instrument to measure quality of life (QoL) in young children affected by atopic dermatitis (AD) and their parents. It consists of five domains, "Symptoms", "Activity Limitations and Behaviour", "Family and Social Function", "Sleep", and "Emotions".

Objectives: We aimed to evaluate the responsiveness (sensitivity to change in those whose condition had changed), smallest detectable change (SDC) and minimal important change (MIC) for the CADIS total score and each domain separately.

Methods: Parents and primary caregivers of 300 young children completed the CADIS and a global rating of their child's skin condition at baseline and four-week follow-up. Kruskal-Wallis-tests, Wilcoxon-tests and effect sizes were used to assess responsiveness. The SDC

can be seen as a change beyond measurement error. Anchor-based, distribution-based, and an integration of both methods were used to estimate the MIC. An anchor is an external criterion to which the score change is linked. In our study, the global rating of the child's skin served as an anchor.

Results: 270 families provided data at baseline and 228 at follow-up. Mean CADIS total score was 54.87 (30.68) at baseline and 41.14 (28.21) at follow-up. The CADIS total change score and the domain score for "Symptoms" had a correlation greater than 0.5 (p < 0.001) with the skin change score and served as an anchor. The patients were grouped according to the anchor. Children whose parents noted an improvement of the skin showed lower CADIS scores at follow-up (P < 0.001). For the SDC, we obtained a score change of 11.52 points on the total score and a score change of 4.40 on the domain "Symptoms". A MIC value of 14.61 on the total score and 5.05 on the domain "Symptoms" passed the SDC cut-off. For the remaining domains, appropriate MIC values could not be found.

Conclusion: The CADIS is highly sensitive to change towards improvement of QoL. A change of 14.61 on the total score and 5.05 on the domain "Symptoms" likely represents clinically important changes. For the remaining domains, more appropriate anchors should be chosen in future research.

P236 | Whole exome sequencing reveals characteristic mutations in patients with epidermolysis bullosa acquisita

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Epidermolysis bullosa acquisita (EBA) is an acquired autoimmune disease that is characterized by severe lesions, blisters and erosions of the skin and/or mucous membranes. The development of lesions is associated with the presence of IgG autoantibodies that bind to different extracellular structures of the epidermis, basal lamina, and dermal anchor fibrils.

EBA is difficult to treat; therefore, there is a high medical need to study its etiology and to identify new therapeutic targets.

EBA develops in middle age of life and therefore have multiple environmental and genetic causes. To elucidate a putative genetic predisposition towards EBA and to find molecular pathways affecting the disease we determined the mutations of 10 patients using Whole exome sequencing. In total, we identified 802 exonic mutations that are predicted to be both rare and deleterious. Interestingly, most patients have mutations in collagen (type 7A1, 4A1 and COL4A2-AS2) together with variants in laminin $\alpha5$, $\beta3$ and $\beta2$. To predict the mutation effect on cell signaling we projected the mutations on the protein interaction network and extracted affected subnetworks through network propagation methods that diffuse the effect of

mutated nodes onto adjacent proteins. A functional enrichment of these affected subnetworks showed a clear predisposition towards integrin signaling, cell adhesion or matrix formation.

Potentially, these mutations have significance influence on the tertiary structure of the folded protein(s). Therefore, these mutations can affect the stability of basal lamina, and dermal anchor fibrils, and thus contribute to this disease.

P237 | What happens to psoriasis patients under a controlled therapy in a real-life setting?

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Introduction: Today, psoriasis can be treated well, our knowledge increased and we are realizing, that there are more complex interactions between the symptoms of the skin disease and comorbidities which are only insufficiently understood. In a rural dermatology practice with a therapeutic focus on systemic therapies for psoriasis vulgaris, patients with moderate-to-severe psoriasis have been studied. In this local patient-centered care research project we analyzed data from disease burden (PASI, PSA), BMI, DLQI, and comorbidities from over 90 patients suffering from psoriasis vulgaris. The goal was to evaluate in a real life setting the disease burden, comorbidities in patients with good therapeutic response.

Material and Methods: All patients (n = 91, 47 f, 44 m) were treated with system therapeutics and biologics. The patient group was subdivided in control of disease, relapse or naïve patients. Most patients showed a very good and stable response to the systemic therapy (PASI improvement). Psoriasis patient data were measured and evaluated with the help of standardized questionnaires like DLQI, and PASI.

Results: Data were analyzed from 91 well-adjusted patients, only 13 patients (PASI > 10, 6 female/7 male) had still a moderate-to-severe psoriasis. Eighteen patients (12 female/6 male) had a DLQI over 10, only 8 patients (5 female/3 male) had a moderate-to-severe psoriasis, 6 patients (5 female/1 male) mentioned comorbidities and 12 of these patients (7 female/5 male) showed a BMI over 25. In the whole study group 13 patients (7 female/6 male) had a BMI over 25. Thirty-six patients (24 female/12 male) mentioned different comorbidities and 55 (22 female/33 male) did not give any information about comorbidities.

Discussion and Conclusion: Interesting is that DLQI seem not correlate with disease burden alone, BMI and comorbidities and other factors may play also a role. Comorbidities seem to be common but should be specified better in psoriasis patients. Our results show some new insights, but there are still many open questions. To get a better understanding of patient care and satisfaction, a focused study should be analyzed with more detail and a larger patient group, more standardized questionnaires for better therapy guidance, comorbidities, disease control and satisfaction.

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