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ABSTRACT

ALLERGY

P001 | *Staphylococcus aureus* Fibronectin-binding protein 1 induces a specific type-2 immune response in atopic dermatitis

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Atopic dermatitis (AD), one of the most common inflammatory skin diseases worldwide, is frequently accompanied by *Staphylococcus aureus* colonization and secondary infections. The question, if allergic sensitizations against microbial antigens can act as trigger factors of AD, is under debate. In earlier studies, we reported measureable levels of IgE that specifically bind *S. aureus* Fibronectinbinding protein-1 (FBP1) in a subgroup of patients suffering from AD. The objective of this study was to detect, enumerate and phenotypically characterize FBP1-specific T cells.

In order to identify immunodominant T-cell epitopes, proliferation testing of patientderived FBP1-specific T cell lines after stimulation with single 15mer peptides was performed. Major histocompatibility complex class II-tetramers carrying newly identified immunodominant epitopes were generated and successfully bound T helper cells in 8 out of 8 HLA-matched, IgE-sensitized AD patients. MHC-tetramer sorted T helper cells secreted predominantly the type 2 cytokines IL-13 and IL-4. IL-17, a hallmark cytokine for the response to extracellular pathogens, however, was only detectable in low amounts.

In conclusion, *S. aureus* FBP1 induces specific pro-inflammatory T helper cell responses in sensitized AD patients via immunodominant epitopes and thereby contributes to increased Th2 levels that can drive the allergic inflammation.

P002 | Investigation of adverse reactions in tattooed skin through histological and chemical analysis

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As the number of tattooed people increased in recent years, so did the adverse reactions in tattooed skin. Tattoo colorants contain numerous, partly unidentified substances, which have the potential to provoke adverse skin reaction like allergy or granulomatous reactions. Identification of the initiating substance is often difficult or even impossible.

Ten patients with typical adverse reactions in tattooed skin were enrolled in the project. Skin punch biopsies were taken and the paraffin-embedded specimens were analysed by various histological and immunohistochemical stainings. In addition, chemical analyses of tattoo inks provided by two patients and of pigments found in punch biopsies of six patients were possible using matrix-assisted laser desorption/ ionization mass spectrometry (MALDI-MS), pyrolysis gas chromatography mass spectrometry (Py-GCMS), coupled plasma mass spectrometry (ICP-MS) and X-ray fluorescence spectroscopy (XRF). Blood samples of patients were screened for angiotensin converting enzyme (ACE) and soluble interleukin-2 receptor (sIL-2R).

In histological analysis, variable skin reactions such as granulomatous reaction, foreign body reaction, contact dermatitis, Schamberg's disease or pseudolymphoma were observed. The dermal cellular infiltration was dominated by CD3+ T lymphocytes (in 90%) and bcl-2 expression was positive in 80%. Most adverse skin reactions occurred in combination with red tattoo ink (n = 7), followed by white ink (n = 3). The red tattooed skin areas predominantly contained pigment Red 170, but also pigment Red 210, pigment Orange 13, pigment Blue 15, pigment Red 22, polystyrene, propylene glycol, dipropylene glycol and 2-(2-ethoxyethoxy) ethanol. The white ink showed inorganic elements such as rutile titanium dioxide, iron, nickel, manganese, chromium, cadmium and copper. None of the patients showed increased levels of ACE and sIL-2R with regard to sarcoidosis. Half of the study participants showed partial or complete remission after treatment with topical steroids, intralesional steroids or topical tacrolimus.

The combination of the presented methods might now be a first approach towards the identification of substances that trigger adverse reactions in tattoos. This identification process has the potential

to push forward the prohibition of toxic substances in tattoo inks, which in turn is likely to reduce the number of adverse skin reactions in future.

P003 | Prevention of allergen-dependent airway and intestinal inflammation in PBMC-engrafted humanized mice by short chain fatty acids or the probiotic formulation BactoFlor® 10/20

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Probiotic bacteria and their metabolites, particularly short chain fatty acids (SCFA), have been shown to prevent or ameliorate allergic inflammation in OVA- or house dust mice-driven allergy mouse models. The aim of this study was to investigate the role of SCFA and the probiotic formulation BactoFlor® 10/20 in a recently developed humanized mouse model of allergen-induced IgE-dependent lung and gut inflammation. Therefore, immunodeficient NSG mice were injected intraperitoneally with human PBMC from highly sensitized aeroallergen allergic donors together with the respective allergen or PBS as control. Mice were treated with 200 mM sodium butyrate, acetate or propionate in drinking water or with 1 × 108 cfu of BactoFlor® 10/20 (containing 3 Bifidobacteria, 5 Lactobacilli, Lactococcus lactis and Enteroccocus faecium) every other day per gavage. Three weeks later, inflammation of the gut and lung was monitored by video mini-endoscopy evaluating translucency, granularity, fibrin production, vascularity, and stool, or by invasive body plethysmography and histology after rectal or intranasal allergen challenge, respectively. Compared to control mice, allergen-specific human IgE production in mouse sera, being only detectable in allergen-treated groups, was strongly reduced in mice receiving SCFA or BactoFlor® 10/20. Consequently, allergen-induced IgE-dependent intestinal inflammation, airway hyperreactivity and mucus-producing goblet cells were significantly inhibited in these mice. Importantly, numbers of FoxP3+ cells were slightly increased in the colon, lung and spleen. Furthermore, butyrate but not acetate and propionate concentration in stool was enhanced in BactoFlor® 10/20-treated mice. These results confirm the importance of microbiota-related metabolites for tolerance induction and possible therapeutic intervention of allergic diseases.

P004 | Anti-allergic effect of a vitamin E derivate semi-synthesized from garcinoic acid

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Introduction: The African bitternut Garcinia kola is used in traditional medicine to treat infections and inflammatory diseases. Several studies showed that the long-chain metabolite of vitamin E (α-13-carboxychromanol or α-13'-COOH) can be semisynthesised from the seed. Recent studies demonstrated that α-13'-COOH inhibits 5-lipoxygenase, a key enzyme for pro-inflammatory eicosanoid biosynthesis, such as leukotrienes (LT). The quantification of sulfido-leukotrienes (sLT), released from blood leukocytes (basophils, mast cells), are used in routine allergy diagnostics to detect specific IgE reactions (allergy type 1). Since α-13'-COOH inhibits the sLT biosynthesis, we hypothesized that this compound might reduce cellular allergic reactions.

Methods: The anti-allergic effect of α-13'-COOH was investigated using the cellular antigen stimulation test (CAST). In brief, leukocytes were isolated from blood samples, reconstituted in an IL-3-containing buffer solution and stimulated with the unspecific cell activator N-formylmethionyl-leucyl-phenylalanine (fMLP) or the complement factor C5a with or without pre-incubation with α-13'-COOH. Moreover, leukocytes from non-allergic donors and those with a confirmed elevated specific IgE level against the house dust mite allergen d1 (HDM) were first pre-incubated with α-13'-COOH and then stimulated with HDM. Secretion of sLT (LTC4, LTD4, and LTE4) was then measured by enzyme linked immunosorbent assay (ELISA). The effect on basophil activation was studied further by the detection of cluster of differentiation (CD) 63+ surface marker expression using flow cytometry. Cytotoxicity was determined by photometric measurement of released lactate dehydrogenase and cell viability by fluorometric quantification of ATP.

Results: Analyses in leukocytes did not show significant effects on cell viability and cytotoxicity after incubation for 60 or 120 min with 0.5 µM and 5 µM α-13'-COOH compared to control. Moreover, neither 0.5 µM nor 5 µM α-13'-COOH modulated CD63+ expression on the cell surface of leukocytes. Blood cells were not activated by the complement factor C5a with or without addition of α-13'-COOH. Production of sLT was not reduced by stimulation of blood cells with fMLP and α-13'-COOH when given simultaneously. However, significant reduction of sLT release was observed ($p < 0.05$) when cells

were pre-incubated with α-13'-COOH for 60 min and then stimulated with fMLP or HDM for further 60 min.

Conclusion: Our results demonstrate that the vitamin E metabolite α-13'-COOH is able to reduce specific and unspecific cellular allergic reactions significantly. However, the effect was only achieved when cells where incubated with α-13'-COOH prior to stimulation with an allergen or unspecific cell activator. For dermatological therapeutic applications of α-13'-COOH further research is needed.

P005 | Anti CCL17/22 aptamers as possible therapeutic agents to suppress inflammation in contact hypersensitivity

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Around 20% of the general population have a contact allergy towards common environmental allergens. In allergic contact dermatitis (ACD), immune cell migration is regulated by chemokine secretion and chemokine sensing, which is a key process required for the manifestation of ACD symptoms. We are aiming to understand the role of the CCL17/CCL22-CCR4 axis in immune cell migration leading to ACD. Since treatment options for ACD are limited, we are aiming to understand if targeting the CCL17/CCL22- CCR4 axis might represent a possible therapeutical approach. Aptamers are short, folded RNA or DNA oligonucleotides that can bind target proteins specifically, thereby facilitating different functions. Aptamers combine beneficial properties of small molecules and monoclonal antibodies, making them promising therapeutic agents.

The chemokines CCL17 and CCL22 are so far the only known ligands for the chemokine receptor CCR4. CCL17 is predominantly expressed by dendritic cells and is associated with the recruitment of cells in inflammatory allergic diseases such as asthma, atopic dermatitis and allergic rhinitis. CCL22 on the other hand is rather involved in regulatory processes, preferably recruiting regulatory T cells over conventional T cells. Despite their different functions, we observed similar effects of CCL17- and CCL22- deficiency in contact hypersensitivity (CHS), the mouse model for ACD.

In CCL17-deficient mice, the CHS reaction was suppressed, resulting in a decreased ear swelling as well as reduced immune cell infiltration to the ear skin. This suggested that CCL17 could be a promising pharmacological target. In support of this notion, CHS symptoms were ameliorated after injection of anti-murine CCL17 RNA aptamers. The reduced inflammation was explained by reduced migration of activated T-cells when CCL17 was functionally blocked. Together with our collaborators, we now also developed an anti-human CCL17 aptamer and plan to test the effectiveness of this reagent in human allergic reactions.

Furthermore, we generated eight different murine CCL22 candidate aptamers to investigate whether CCL22 also represents a potential therapeutic target for CHS. Here, we already identified functionally binding aptamer candidates that were able to inhibit T cell migration in in vitro transwell assays. Additional tests of these functional aptamers in in vitro assays, as well as in vivo, using the CHS mouse model, are required to further delineate the migratory mechanisms of immune cells and to define suitable targets for pharmacological treatment of contact allergies.

P006 | Mast cell activation syndrome is more often suspected than diagnosed—A prospective real-life study

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Background: Mast cell activation syndrome (MCAS) is increasingly used to describe non-specific symptom patterns claimed to be caused by inappropriate activation of mast cells.

Objective: To characterize patients referred with suspected MCAS and investigate the applicability of the current consensus diagnostic criteria.

Methods: 100 patients were prospectively examined at three visits within 12 weeks. Patients listed their top-5 symptoms and answered the Hospital Anxiety and Depression Scale (HADS). Changes in blood tryptase levels linked to episodic exacerbation were assessed. Response to mast cell-mediator targeted treatment was assessed by patient reported outcome measures (PROMs) for symptom activity, quality of life, and symptom control.

Results: On average, females (80%) were 45 years and males (20%) 37 years old. In half of the patients, suspicion of MCAS was based on self-diagnosis. 87 different symptoms were reported; mostly fatigue (n = 57), muscle and joint pain/weakness (n = 49), abdominal pain (n = 43), pruritus (n = 34) and diarrhea (n = 27). Depression and anxiety disorders (n = 23 each) were the most frequent reported comorbidities. 39% reported continuous, 61% continuous and intermittent, none exclusively intermittent symptoms. Tryptase increased by 20% + 2 ng/ml in two patients during episodic exacerbation. Most patients showed non-response in the PROMs; pathological values of HADS (n = 65) were related to high disease impact and poor symptom control.

Discussion: Especially middle age woman diagnose themselves with MCAS after internet research. In most cases, MCAS cannot be confirmed based on current consensus criteria. Known psychiatric comorbidities, abnormal HADS values and poor symptom control indicate other causes than MCAS in this patient population.

P007 | Prevalence and relevance of IgE-autoantigen immunocomplexes in chronic spontaneous urticaria

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Background: IgE autoantibodies to thyroid peroxidase (TPO) and interleukin 24 (IL-24) are held to contribute to the pathogenesis of chronic spontaneous urticaria (CSU) in a subpopulation of patients. As of now, it is unclear if these IgE autoantibodies can engage with their autoallergens in the blood to form mast cell-activating autoIgE-antigen immunocomplexes (autoICs).

Aim: To investigate the prevalence and relevance of autoICs in CSU.

Methods: Levels of free, complexed, and total IgE-anti-TPO and IgE-anti- IL-24 were quantified in the serum of 332 CSU patients by capture ELISA and assessed for correlations with clinical and laboratory parameters. We used the betahexosaminidase release assay to investigate autoICs for human skin mast cell-activating effects.

Results: More than half of CSU patients (56%, 187/332) exhibited elevated levels of IgE-anti-IL-24, IgE-anti-TPO, or both, and 20% and 8% of patients had IgE/TPO and IgE/IL-24 autoICs, respectively. Levels of free IgE-anti-TPO correlated with levels of IgE/TPO autoICs, this was also the case for IgE-anti-IL-24. IgE/IL-24 autoICs were weakly linked to disease activity (r = 0.172, p = 0.007) and blood basophil counts (r = -0.218, p < 0.001). Sera of patients with high levels of IgE/ IL-24 autoICs induced dose-dependent mast cell activation without the addition of IL-24.

Conclusions: About 50% of CSU patients have autoreactive IgE, and these IgE autoantibodies can form mast cell-activating immunecomplexes with their autoallergens. Further studies are needed to confirm the clinical relevance of these autoICs including their impact on treatment responses.

P008 | Characterization of itch-associated mediators in mycosis fungoides

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Patients with Mycosis fungoides (MF), the most frequent variant of primary cutaneous T-cell lymphomas (CTCL), often suffer from severe pruritus that greatly impacts their quality of life and that is exceptionally difficult to treat. As of yet, little is known about the characteristics of pruritus in different stages and subforms of cutaneous lymphomas and the underlying mechanism of pruritus in CTCL is largely unclear. Here, we analyzed potential mediators of itch in the blood of MF patients and correlated these with itch intensity.

We have collected data and blood from 53 MF patients seen at the Department of Dermatology, Charite—Universitaetsmedizin Berlin. Among them, 47 MF patients (17 female and 30 male) aged from 44 to 91 years (mean age: 67 years) have detailed clinical information, including itch severity and tumor stage. Plasma (n = 57) and serum samples (n = 33) from matched healthy individuals were included as controls. Itch severity was assessed using the VAS (visual analogue scale, 0–10) and plasma or serum level of IL-33, ST2, Thymic stromal lymphopoietin (TSLP), Gastrin-releasing peptide (GRP), IL-31 and substance P were measured by ELISA and serum tryptase level was measured by ImmunoCAP.

As compared to healthy controls, MF patients exhibited increased levels of IL-31, tryptase, substance P, GRP, IL-33, ST2, and TSLP in the serum/plasma. Differences between MF patients with and without itch were found for IL-31, substance P and GRP (all p < 0.05) and a correlation with itch intensity was identified for IL-31 (r = 0.32, p = 0.04), tryptase (r = 0.5, p = 0.02), GRP (r = 0.33, p = 0.03).

IL-31 is considered an important mediator involved in the induction and maintenance of chronic pruritus in various diseases, i.e., atopic dermatitis, chronic prurigo, scabies and stasis dermatosis. Our data indicate that IL-31 could be of equal importance in pruritus experienced by MF patients. Apart from T cells, mast cells are a potential source of IL-31, and our data furthermore indicate that mast cells could be involved in pruritus in MF. The alarmins TSLP and IL-33 and its soluble receptor ST2 as well as the neuropeptide substance P all can activate mast cells and induce degranulation. Activation of mast cells results in the release of tryptase, another mediator that was found to correlate with itch in MF patients. GRP, also found to be elevated in MF and to correlate with itch intensity, is considered to be an itch-specific neurotransmitter and most likely acts downstream of the activation of sensory nerves in the periphery.

Successful therapy of pruritus is an essential goal in the treatment of MF patients, which, however, is currently hardly or not at all achieved. Our data indicate that drugs targeting IL-31, mast cells and/or substances activating mast cells could present promising treatment options in pruritus associated with CTCL.

Keywords: Mycosis Fungoides, Itch, pruritus, IL-33, ST2, Thymic stromal lymphopoietin, Gastrin-releasing peptide, IL-31, Tryptase, Substance P, Mast cell

P009 | Serologic approach to allergy diagnostics in hymenoptera allergy considering ratios of specific IgE, IgG, IgG4 and total IgE levels

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Allergic reactions to hymenoptera venoms belong to the most common causes of anaphylactic shock. Despite different types of cutaneous and serologic test methods in currently there is no strategy to correlate the test results with the severity of the allergic reaction. Advances in molecular allergy diagnostics did not result in relevant progress in this issue. In other immediate type allergic reactions, for example food allergies, specific IgE levels are thought to be related to more severe reactions on one hand. On the other hand they have to be interpreted in relation to total IgE levels, with less importance of specific IgE in case of high total IgE levels. In contrast specific IgG and IgG4 levels are negatively correlated with immediate type allergic reactions with an increase of values during successful specific immune therapy.

Therefore we investigated the correlation of severity of allergic reactions with different calculated values of serologically gained parameters: IgE, IgG, IgG4, the ratios of specific IgE to total IgE, specific IgG or IgG4, and the ratio of specific IgE to the calculated product of total IgE and IgG4. The severity of allergic reactions was assessed by a categorising anamnestic data into one of 2 categories: either non-severe reactions (local and systemic) or severe systemic reactions.

Categorised this way, the severity of the sting reaction did not show a correlation to the serological markers or their mentioned ratios. In general it cannot be deduced that, as with other allergies of the immediate type a high IgE level leads to a severe reaction. Furthermore, even high IgG levels do not protect, as suggested in some studies, against a severe reaction but may rather indicate immunological contact with the allergen. In addition, it can be concluded that it is probably not beneficial to consider ratios of specific IgE and IgG or IgG4 values as the values considered individually. Thus, our results suggest that there must be even more processes and components involved in allergic reactions of the immediate type, which have not been researched yet.

P010 | MRGPRX2 is highly expressed in skin lesions of patients with indolent systemic mastocytosis and shows a distinct pattern

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Objective: Mas-related G-protein coupled receptor X2 (MRGPRX2) is known to activate mast cells and held to be involved in the pathophysiology of several mast cell-driven skin disorders. Cell-surface and intracellular patterns of MRGPRX2 expression have been described, with the latter described as linked to receptor internalization due to mast cell activation. Recently, adult patients with cutaneous mastocytosis were reported to exhibit skin expression of intracellular MRGPRX2. As of yet, little is known about the relevance of MRGPRX2 and its expression pattern in patients with mastocytosis.

Methods: We evaluated lesional and non-lesional skin biopsies of 22 patients (33–65 years) with indolent systemic mastocytosis and 8 healthy controls (23–68 years) for expression of MRGPRX2 by immunohistochemistry. Additionally, we stained lesional skin biopsies for cortistatin, a known MRGPRX2 agonist. Colocalisation of MRGPRX2 and the mast cell marker tryptase was assessed by immunofluorescence. Clinical and demographic data, laboratory parameters, including serum tryptase and KIT D816V mutation burden, and mastocytosis activity score (MAS) were assessed.

Results: The number of MRGPRX2-positive cells was significantly higher in lesional skin (median [IQR]: 22.3 [2.5–64.8] cells/mm2, n = 22) as compared to non-lesional skin (5.2 [1.5–13.5] cells/mm2, p = 0.001, n = 21) and skin of healthy controls (2.9 [1.2–5.2] cells/mm2, p = 0.03, n = 8). In lesional skin, the number of mast cells correlated with the number of MRGPRX2-positive cells (r = 0.784; p < 0.0001, n = 22) and cortistatin-positive cells (r = 0.865; p < 0.0001; n = 12). Double staining for MRGPRX2 and tryptase identified most MRGPRX2-positive cells as mast cells. Membrane expression of MRGPRX2 was only seen in mastocytosis skin lesions, whereas intracellular expression was detected across all samples.

Conclusions: Skin lesions of patients with mastocytosis show high numbers of MRGPRX2-positive cells and a distinct pattern of MRGPRX2 expression. Further studies should evaluate the mechanisms and relevance of MRGPRX2 expression in mastocytosis.

P011 | A novel beads-based protocol to obtain high purity total IgE from human serum

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Background: Purified total Immunoglobulin E (IgE) from human sera is of great value for allergy research. The most frequently applied method for IgE purification is affinity chromatography. However, limited access to these facilities and requirement of specific knowledge precludes widespread use of this technique. Protocols are scarce and commercial purification kits for human IgE are unavailable. Therefore, we established a magnetic beads-based protocol for human IgE purification.

Methods: Allergic sensitization of 17 adults was confirmed by skin prick testing using the 14 most common aeroallergens and food allergens. Of each subject, a volume of 500 µl serum was applied to a protein G spin-column for depletion of polyclonal IgG. To capture total serum IgE, anti-human IgE antibodies pre-coupled to magnetic beads were added to these IgG-depleted sera. Next, glycine was used to elute total serum IgE. Finally, the eluates were incubated with Protein G-coupled beads to remove the capture antibodies. IgE-purity was analyzed by Western Blot and total serum IgE or purified IgE concentrations were measured using enzyme-linked immunosorbent assay. The functionality of the IgE purified by this approach is currently investigated by sensitization of peripheral blood-derived human mast cells followed by activation with anti-IgE antibodies or the relevant allergen. Mast cell degranulation will be measured by analysis of mediator release and CD63 upregulation.

Results: The quantity of total IgE purified with the help of our new protocol corresponded with total serum IgE levels. Concentrations up to 300 ng of pure IgE were obtained from 500 µl of serum. Minor fractions of IgE remained attached to the beads, confirming an effective elution. Total polyclonal IgG was purified from serum fractions with minimal amounts of IgG remaining in the purified IgE fractions, confirming high IgEpurity. Evaluation of the IgE-functionality is still in process.

Conclusion: Our novel method represents a fast, easy and highly effective protocol for the purification of human serum IgE, which may contribute to allergy research.

P012 | Use of two tools for the assessment of the methodological quality of SRs on early compared to later introduction of allergenic complementary foods and fluids for early childhood allergy prevention

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Background: Recent systematic reviews (SRs) have summarised primary research testing the hypothesis that early introduction of specific allergenic complementary foods and liquids (CFL) to infants may lead to a lower incidence of one or more allergic outcomes. Generally, the quality of SRs varies. The aim of this study was to examine the methodological quality of SRs on the effects of earlier versus later introduction of CFL on allergy incidence in infants and children.

Methods: A comprehensive search of PubMed, Medline (Ovid), and Web of Science Core Collection was conducted for SRs published since 2000. Suitability for inclusion was determined by two or more reviewers. SRs were eligible for inclusion if they included at least one RCT of earlier versus later introduction of any CFL. Outcomes of primary interest included food allergy (FA), eczema, asthma and allergic rhinitis (AR). The Measurement Tool to Assess Systematic Reviews (AMSTAR-2) and the risk of bias in systematic reviews tool (ROBIS) were used to assess the methodological quality of the included SRs. Assessment was done by two reviewers.

Results: Eleven SRs were identified for inclusion. Methodological quality based on AMSTAR-2 revealed that only one SR was deemed to be of high quality. One was of moderate quality, four of low quality and five of critically low quality. Assessment by ROBIS yielded different results: Two SRs were judged at overall low risk of bias while the remainder was judged at overall high risk of bias.

Discussion: This systematic overview highlights that many SRs, which include RCTs on the timing of CFL for ECAP, already exist. However, the individual methodological quality as assessed by AMSTAR-2 across these SRs varied widely. There was less variation when a risk of bias assessment tool for SRs was used. Both assessments need to be considered together as the results of the methodological assessment varied as a function of the tool used.

Conclusion: Reliance on only one methodological quality assessment tool may be insufficient.

P013 | Assessment of histamine in skin interstitial fluid (ISF) extracted by hollow microneedles in ex vivo skin models of allergy and chronic urticaria

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The pathomechanisms of chronic inflammatory skin diseases such as urticaria, psoriasis, or atopic dermatitis are complex and challenging to investigate. Histomorphometric analyses of skin biopsies combined with the analysis of blood, serum and skin microdialysis (SMD) samples are gold-standard tests in skin research. However, these tests are either invasive, or samples are difficult to obtain and with the risk of infection. Moreover, locally produced tissue biomarkers are often diluted in the circulation below detection thresholds of routinely used assays. To improve diagnostic options and facilitate biomarker discovery we have developed a dermal interstitial fluid (ISF) extraction method using sharp hollow microneedle chips (hMNs).

Microneedle chips with 37 hollow microneedles of 420 µm length were manufactured from monocrystalline silicon wafers and applied to human abdominal ex vivo skin for ISF extraction. On average, a volume of 12.6 µl could be extracted with hMNs by application of -70 kpa subpressure (skin soaked in PBS for 2 h). The recovery of biomolecules such as histamine or immunoglobulins in the ISF was enhanced when extracted with hMNs compared to sampling by SMD. In addition, we could demonstrate that this method can be applied in ex vivo skin-serum transfer models for allergy and inducible urticaria, e.g. cold urticaria (ColdU) and symptomatic dermographism (SD). Mast cell degranulation, as indicated by an increase of histamine in the ISF, could be detected in response to injection of serum of an allergic patient followed by injection of the respective allergen, whereas this was not the case when serum of a non- allergic donor was injected. Similarly, injection of SD or ColdU serum followed by application of the respective trigger, i.e. cooling and rewarming or scratching of the skin, induced a detectable increase of histamine in the ISF.

In summary, we have developed a novel, accessible, efficient, and minimally invasive tool for sampling ISF and used it successfully in ex vivo models of allergy and inducible urticaria to detect MC degranulation. Thus, analysis of ISF extracted by hollow microneedles is a promising approach for research and diagnosis of various skin diseases.

P014 | Microbial dysbiosis in a mouse model of atopic dermatitis mimics shifts in human microbiome and correlates with the key pro-inflammatory cytokines IL-4, IL-33 and TSLP

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Skin dysbiosis is a characteristic hallmark of atopic dermatitis (AD) that strongly influences the severity of the disease. Nevertheless, frequently used AD models have not been characterized regarding the changes of their skin microbiome compositions. Here we analyze the skin microbiome of two frequently used murine AD models for assessing their applicability in translational research. AD was induced by topical application of calcipotriol, or oxazolone. Following comparable elicitation of AD-like dermatitis, skin microbial communities were analyzed using 16S rRNA gene sequencing. We detected critical differences in the microbiota composition of diseased skin. In contrast to calcipotriol, application of oxazolone induced significant changes of the cutaneous microbiota with a drastic drop of bacterial richness. Furthermore, an expansion of Staphylococci, particularly S. xylosus was observed in the oxazolone group, also displaying positive correlations with AD key markers including pH, TEWL, IL-4, TSLP and IL-33. In this article we show that (i) the model of choice to investigate AD needs to be characterized for the cutaneous microbiota if applicable and (ii) the oxazolone-mediated mixed Th1-Th2 immune response triggers microbiota-induced alterations which share similarities to dysbiosis in human AD and represents therefore a suitable model for translational research on AD if alterations of the microbiome are in the focus of the investigation.

P015 | A prospective case-control study with double exposure to ozone and grass pollen in the allergen exposure chamber: Rhino-conjunctival symptoms and skin physiology are altered in patients with allergic rhinoconjunctivitis

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Introduction: Due to the dramatic changes in climatic conditions, an increase in allergic symptoms as well as an increase in the number

of pollen allergy patients has been reported. In addition, an increase in clinical symptoms can be observed with ozone exposure. There seems to be a connection between allergies and a negative influence on epithelial barrier functions. The aim of the study was to quantify the effect of ozone and pollen in a controlled pollen chamber exposure. We tested the hypothesis whether ozone induces an increase in mucosal and skin symptoms under pollen exposure in grass pollen allergic patients.

Methods: The prospective case-control study included 8 grass pollen allergic patients and 8 non-allergic subjects. Exposure was performed in the pollen chamber with grass pollen as well as ozone. We compared double exposure (DE) of shorter and longer duration. The subjects' general well-being, bronchial and nasal symptoms were documented. Skin physiological parameters were determined by non-invasive assessment of stratum corneum moisture, skin redness, surface pH and basal epidermal barrier function.

Results: We could show that DE leads to a significant induction of nasal secretion in pollen allergic subjects compared to healthy subjects, which was more pronounced with longer exposure duration. The general well-being was significantly worsened under DE compared to pollen or ozone alone, with a negative influence of DE duration. No relevant bronchial symptoms were recorded in both groups. With regard to skin physiology, DE leads to a deterioration of epidermal barrier function and stratum corneum hydration, an increase in pH values and an increase in skin redness.

Conclusion: The nasal mucosa is negatively affected by DE with ozone and grass pollen. Epidermal barrier damage could be demonstrated in allergy patients with a dose (time)-response relationship. The surface pH can be regarded as a possible modulation mechanism.

P016 | Epidermal barrier homeostasis is mast cell dependent

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Introduction: Many chronic inflammatory skin diseases, such as atopic dermatitis and psoriasis are associated with impaired epidermal barrier function and changes in skin pH. A disturbed epidermal barrier can promote skin inflammation, as potentially pathogenic microbes, chemical substances, and allergens can reach the dermis more easily. As of now, the effects of mast cells, critical effector cells in skin inflammation, on epidermal barrier functions remain ill characterized.

Aim: To assess the effects of mast cells, in two different mouse models of mast cell deficiency, on skin pH, transepidermal water loss (TEWL) and epidermal recovery after standardized barrier disruption.

Methods: Two mast cell-deficient mouse models, a c-Kit-dependent model B6.Cg-KitW-sh/ HNihrJaeBsmJ (W-sh) and a c-Kit-independent model C57BL6-Cpa-Cre;Mcl-Ifl/fl (Hello Kitty), with their wild-type (WT) littermates as controls, were studied. After shaving the flanks and recording the baseline parameters (TEWL, stratum corneum hydration, epidermal pH), tape stripping followed using DSquames tapes. TEWL was monitored at defined time intervals (1, 6, 24 h), to assess the barrier recovery in percentage.

Results: We could not detect any difference between baseline TEWL and stratum corneum hydration in mast cell deficient mice compared to their WT littermates. Hello Kitty mice showed significantly (p = 0.001) higher epidermal pH (7.01 0.23) compared to their WT littermates (6.75 0.21), whereas we could not detect any significant difference in W-sh mice and their WT littermates. Barrier recovery was significantly delayed in both mast cell-deficient mouse models across the first 24 hrs following tape stripping (p < 0.0001). The largest difference in barrier recovery was visible after 1 h (immediate repair signal), when W-sh and Hello Kitty mice showed 28.8 11.9 % and 25.6 15.5% recovery, respectively, as compared to 70.6 16.5% and 54.3 18.2 % in their respective WT littermates.

Conclusion: Our findings indicate that epidermal barrier recovery, in mice, is skin mast cell dependent, at least in part. Skin mast cells also appear to be involved in epidermal pH regulation. Further investigations are needed to determine the involved mechanisms and to assess the relevance of these mast cell effects on epidermal biology in the human system.

Cellular Biology

P017 | KDM5B's role in creating radiation synthetic vulnerabilities and its effects on sequential therapy combinations

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Cutaneous melanoma is the most aggressive form of skin cancer, and survival rates dramatically drop following distant organ metastases. Moreover, it is not fully understood which molecular mechanisms of adaptation are responsible for the observed melanoma cells escape from initial therapeutic hits such as targeted (MAPKi) or radiation-induced killing, nor how to target these surviving cells. We have previously described the existence of a small subpopulation of slow-cycling cells that survives multiple available drugs and re-populates the tumor. This multidrug- resistance and universal self-renewal capacity seems to be dependent on the upregulation of the histone H3K4 demethylase KDM5B. We have also recently shown an important role of KDM5B in melanoma repopulation after combined targeted plus radiation therapy (RT) and that the degree of therapy resistance may be even dependent on the temporal treatment sequence chosen. However, the molecular mechanisms and dynamics

downstream of KDM5B that actually mediate cell survival and radiotolerance and -resistance are not known. In this study, we attempted to define the role of KDM5B in mediating tolerance and acquired radio-resistance induced by chronic cycling hypoxia and to identify protein targets that may drive radiation resistance through an RPPA screen. We show that cells undergoing initial repeated cycles of hypoxia and intermittent reoxygenation become more tolerant to RT and have an increased endogenous expression level of KDM5B. We also demonstrate that prolonged cycles of hypoxia have the opposite effects, where the cells have a decreased expression level of KDM5B and a proliferative phenotype. We plan to test different sequences of therapy application of MAPKi plus radiation in our established preclinical 2D/3D to assess the risk of radio-resistance development. These results highlight the importance of characterizing possible vulnerabilities in the context of KDM5B to unravel the involved cell survival pathways.

P018 | Assessment of senescence markers after ex vivo treatment of human skin biopsies with senolytics

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Cellular senescence is a physiological mechanism in which the cell stops proliferating due to endogenous or exogenous stress, cell damage, telomere dysfunction, or oncogene induction. This proliferation-arrest may protect the organism from neoplastic transformation. While there are positive aspects of cellular senescence, the accumulation of senescent cells is also associated with a variety of age-related diseases, such as idiopathic lung fibrosis, osteoarthritis, diabetes, arteriosclerosis, or cancer metastasis. Senolytics such as quercetin (usually in combination with dasatinib) have the ability to selectively kill senescent cells and measurably reduce senescence, measured by, e.g., CDKN2A (p16INK4A) or TP53BP1. The aim of this study is to assess the effect of an intervention with senolytics on senescent cells in human skin biopsies ex vivo. Therefore, two punch skin biopsies each were collected from a total of 52 healthy volunteers. One of the biopsies was treated ex vivo with senolytics and the other biopsy served as a control and was treated with the vehicle only. Preliminary results from two independent pairs of treated and untreated skin tissue samples showed that gene expression of CDKN2A and TP53BP1 was reduced after treatment with quercetin and dasatinib. This was true for RNA isolated from tissue sections as well as for RNA isolated from exosomes from the medium. Further validation on a cellular level was performed using immunohistochemistry. All 52 healthy volunteers participate in an interventional study assessing the effect of consuming foods rich in geroprotectors, including polyphenols with potential senolytic effects. Comparison of results from the nutritional intervention with ex vivo treatment of skin tissues will provide valuable insights on the effects of this intervention on senescence biomarkers in blood and skin. Furthermore, our preliminary results suggest that a topical intervention to eliminate senescent cells in the skin may be feasible and, hence, may have positive effects on skin aging as well as skin cancer risk.

P019 | NADPH oxidase inhibition rescues keratinocytes from elevated oxidative stress in a 2D atopic dermatitis and psoriasis model

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Emerging evidence suggests oxidative stress plays a role in the pathophysiology of both atopic dermatitis (AD) and psoriasis (PSO). We established in vitro models of AD and PSO skin, and characterized these models in regard to their oxidative stress state. Both AD and PSO model keratinocytes exhibited elevated reactive oxygen species (ROS) levels and accumulated more DNA damage than control cells after oxidative stress induced by 250 µmol/L H2O2. Elevated ROS levels and DNA damage accumulation could be inhibited by the NADPH oxidase (NOX) inhibitor diphenyleneiodonium (DPI). Further, immunofluorescence analysis revealed the presence of both NOX1 and NOX4 in keratinocytes. By inhibiting NOX1, stress-related signalling cascades and elevated ROS levels could be abrogated, and survival of AD and PSO cells improved. Taken together, this study reveals that inhibition of NOX inhibition could abrogate elevated oxidative stress in a 2D model of AD and PSO.

P020 | Hidradenitis suppurativa-like reconstructed epidermal equivalents

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Hidradenitis suppurativa (HS), also known as Acne inversa, is a chronic inflammatory skin disease of the sebaceous gland unit. Clinical symptoms comprise deep-seated, painful nodules, abscesses to fistula tracts, and sinus tract formation. Since the exact pathophysiology of this auto-inflammatory disease is not yet fully understood, no causal therapies for HS are available, making HS one of the most studied skin diseases. The HS research includes in vitro, ex vivo as well as clinical studies. The in vitro experiments mostly use primary cells from the blood or skin of HS patients. Alternatively, cell lines or healthy primary cells are treated with various cytokines

associated with HS to create an inflammatory environment. For ex vivo studies, punch biopsies, so-called explants, are taken from lesional and non-lesional HS tissues and cultured in a transwell system. This system allows research that reflects the in vivo situation most closely and thus screening for biomarkers in the laboratory. In addition, there is a debate whether established animal models for other diseases are also suitable as animal models for HS. A research model that has already been used several times in atopic dermatitis and psoriasis are reconstructed human skin equivalents. To our knowledge, no skin equivalent has yet been developed for HS, which would also be a good addition to the already established models for studying biomarkers or topical drugs.

Therefore our study aimed to determine whether primary keratinocytes derived from lesional HS skin can form reconstructed HS epidermis equivalents. In this context, we examined the expression of epidermal differentiation markers in lesional HS skin compared to the reconstructed HS epidermis equivalents to assess whether they are suitable as models for HS research. Our first observations in reconstructed HS epidermis equivalents suggest a similar expression pattern of differentiation markers as in lesional HS skin.

P021 | Function of TRPV4 ion channel during hypotonic stress response and epidermal barrier formation

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The barrier function of the human epidermis is constantly challenged by various environmental stressors such as pathogens, UV radiation as well as osmotic stress. Hypotonic stress leads to cell swelling that is counteracted by efflux of osmolytes and influx of Ca2+. Since Ca2+ is also a major regulator of epidermal differentiation, it is of interest to further elucidate the possible link between Ca2+ uptake, cell volume regulation and epidermal development.

Since the Ca2+-permeable TRPV4 (transient receptor potential vanilloid 4) ion channel was shown to contribute to epidermal barrier integrity and cell volume regulation in other tissues, we directly tested the importance of TRPV4 by creating genetic TRPV4 knockout in human HaCaT keratinocytes using the CRISPR-Cas9 genome editing technology. Interestingly, we found an ambiguous role of TRPV4 during cell volume regulation: when Ca2+ influx and regulatory volume decrease was triggered by osmotic stress, TRPV4 was dispensable. On the other hand, direct stimulation of TRPV4 by a specific small-molecule activator triggered Ca2+ influx and isotonic cell shrinkage. Surprisingly, this triggered the downstream activation of Ca2+- activated (CaCCs) and not volume-regulated anion channels (VRACs).

To address the contribution of TRPV4 to epidermal development, we analyzed Ca2+- induced differentiation of HaCaT keratinocytes. We found that the expression of TRPV4 increases during 2D in vitro differentiation and that absence of TRPV4 results in increased expression of keratin 10 and involucrin at later stages of differentiation. However, knockout of TRPV4 did not lead to morphological changes or expression of differentiation markers in 3D reconstituted epidermal equivalents. Nonetheless, in TRPV4 knockout epidermal models the epidermal barrier was compromised as penetration of Lucifer yellow could be detected. Whether this detrimental effect is accompanied with impaired tight junction formation is currently under investigation.

Altogether, we provide evidence for a role of the TRPV4 ion channel in epidermal development and barrier function; probably by controlling Ca2+ influx and cell volume changes of keratinocytes. Our study also suggests that hypotonic cell swelling and isotonic cell shrinkage lead to activation of two distinct pathways but involving Ca2+ as a major regulator and two distinct classes of effector chloride channel classes. How these distinct pathways are activated during epidermal development will be the scope of future studies, in particular with respect to conditions of disturbed differentiation in inflammatory skin disorders.

P022 | Pitfalls in the application of dispase-based keratinocyte dissociation assay for in vitro analysis of Pemphigus

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Pemphigus vulgaris (PV) is a chronic life impacting autoimmune blistering disease characterized by the production of anti-Desmogleins 3 (Dsg3) and 1 (Dsg1) antibodies with loss of cell-cell adhesion in keratinocytes (acantholysis) and blister formation in the skin and mucous membranes.

The dispase-based keratinocyte dissociation assay (DDA) is the approach of choice to examine the pathogenic effect of antibodies and additional co-stimuli on cell cohesion in vitro. Despite the common use of this assay, there is a high variability of experimental conditions resulting in inconsistent and hardly repetitive experimental results.

We have here identified and discussed the typical pitfalls in the application of DDA which are as follows: generation of a monolayer with optimal density; appropriate culturing conditions in order to prevent the generation of an unbreakable or too fragile monolayer; application of mechanical stress in a standardized manner and performing of consistent data processing.

We describe a detailed protocol for performing a successful and reliable DDA with the respective optimal conditions for three different types of human keratinocytes. Therefore, we have directly compared and detected the best working conditions for application of the DDA with primary keratinocytes, HaCaT spontaneously

immortalised keratinocyte cell line and for the first time the recently characterized spontaneously immortalised HaSKpw keratinocyte cell line.

Taken together, our study provides detailed protocols which will allow to guarantee intra- and inter-experimental comparability of DDA.

P023 | Cellular and cutaneous biocompatibility of anti-inflammatory compounds

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Introduction: Inflammatory skin diseases and reactions affect a high number of the world's population and thus, are commonly seen in daily dermatology. The class of glucocorticoids with broad-spectrum anti-inflammatory actions represents the first choice of treatment but is often associated with adverse effects, e.g. inhibition of wound healing. Hence, new therapeutic approaches allowing a patient-specific treatment and the characterization of new drugs with specific antiinflammatory properties, but less side effects are of interest. Celastrol (CS), a natural root ingredient of Tripterygium wilfordii and the vitamin E metabolite α-13'- carboxychromanol (α-13'-COOH) represent such promising compounds since they have already been described to exert anti-inflammatory effects. The goal of this study is to provide biocompatibility analyses in two-dimensional epidermal cells and three-dimensional skin models as the initial step of compound suitability testing for dermatological applications.

Methods: HaCaT and primary keratinocytes were treated with CS or α-13'-COOH followed by cell viability and cytotoxicity studies considering endogenous ATP levels and lactate dehydrogenase (LDH) release. Effects on wound healing were analysed using a scratch assay. After the two-dimensional tests, compounds were applied topically to three-dimensional skin equivalents and cytotoxicity was measured via LDH release in undernatants of the skin models. Inflammatory responses were investigated via interleukin (IL)-6 and IL-8 ELISA. Dexamethasone (Dexa) was used as anti-inflammatory drug control. Morphological treatment effects on skin models were histologically evaluated by hematoxylin-eosin staining and immunohistochemistry of structural proteins and differentiation markers.

Results: The compounds were tolerated in a concentration-dependent manner in two-dimensional cell monolayers. CS concentrations <1 μM and α-13'-COOH concentrations <5 μM did not potently affect cell viability or exerted cytotoxic effects. The immortalized HaCaT keratinocytes showed higher tolerance than primary cells. Wound healing was not negatively affected with lower compound concentrations. The vitamin E metabolite even slightly accelerated scratch closure of primary keratinocytes. This improvement was also observed in a HaCaT scratch wound model where cells were stimulated with wound milieu typical cytokines (IL-1β, TNF-α). Compounds were further well tolerated in three-dimensional skin models; even 1 μM CS did not increase LDH release. Unlike Dexa, our test compounds did not affect IL-6 and IL-8 release by the skin models. Morphological changes or abnormalities in protein expression of the skin models were not observed.

Conclusions: CS as well as α-13'-COOH can be considered biocompatible and therefore represent promising candidates for dermatological applications. Compared to the known wound healing delay caused by glucocorticoids, the vitamin E metabolite might be an advantageous alternative. Cutaneous systems were shown to tolerate higher drug concentrations. Hence, these three-dimensional test systems can mimic in vivo-like conditions and thus, are more suitable for drug testing than susceptible monolayer cell cultures.

P024 | A standardized 3D-skin wound model for antimicrobial testing

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Aim: Chronic wounds are a growing socio-economic problem. Impaired wound healing is often caused by the colonization and infection with different microorganism. Accordingly, various antimicrobial agents have been suggested for treatment of chronic wounds. The antimicrobial activity of these products is commonly tested using in vitro suspension tests. As such tests do not feature the conditions found in chronic wounds, more realistic test models are urgently needed. 3D models provide a lifelike situation for antimicrobial testing. Here, a human 3D-skin wound model for the evaluation of antimicrobial substances is introduced. This wound model demonstrates physiological wound healing capacity within 9 days and can be infected with different bacteria.

Methods: Skin models were injured using a biopsy punch to create a standardized wound and infected with *Staphylococcus aureus* or *Pseudomonas aeruginosa*. Skin cell damage was determined by the release of lactate dehydrogenase (LDH) in the supernatant of the skin models up to 48 h after infection. The secretion of proinflammatory cytokines was determined by Enzyme-linked-Immunosorbent-Assay (ELISA). The expression of pro-inflammatory cytokines and was investigated using quantitative real-time PCR. Morphological changes were examined after hematoxylin & eosin staining of histological sections.

Results: The infection of the wound model with *P. aeruginosa* led to a stronger tissue damage compared to *S. aureus*, which is characterized by tissue dissociation and cell damage. Furthermore, an

increased expression and secretion of proinflammatory cytokines was observed during infection for both bacteria.

Conclusions: It could be shown that a standardized wound model was established, which responds to an infection by an inflammatory reaction hampering wound healing. This 3D-skin wound model provides a far more realistic basis for the future testing of antimicrobial products, where antimicrobial efficacy under more realistic conditions as well as effects on wound healing progression can be evaluated simultaneously.

P025 | Immortalized keratinocyte cell lines as in vitro models for inflammatory skin diseases

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Chronic inflammatory skin diseases such as psoriasis vulgaris or atopic dermatitis (AD) are common dermatoses and are associated with a high physical and psychological burden for patients. Despite progress in the elucidation of the pathogenesis and the development of new therapeutic strategies, there is still no adequate therapy available for many patients. Thus, to investigate therapeutic targets and to pre-clinically evaluate novel topical treatments, in vitro models are required, that resemble the pathophysiological situation in the epidermis most accurately. However, most of these models rely on primary human keratinocytes (NHEKs), that are limited by availability, have a short life-span and display donor-specific variations. To overcome these issues we validated immortalized cell lines that were generated from NHEKs by the expression of SV40 large T antigen and hTERT(NHEK-SV/ TERT, Evercyte GmbH) or HPV E6/E7 (NEHK-E6/E7, BRAIN Biotech AG) for their use in inflammatory in vitro epidermal models.

Immortalized keratinocytes showed a prolonged life-span for up to over 50 passages and increased proliferation rates when compared to NHEKs, but were still sensitive to contact inhibition. While NHEK-E6/E7 showed a differentiation behavior comparable to primary cells, NHEK-SV/TERT required complete removal of proliferative signals and displayed a delayed onset of differentiation. Both cell lines reconstituted a stratified epidermis, which was thinner than in models generated from NHEK or HaCaT cells. Nevertheless, the epidermal barrier was fully functional and superior to HaCaT models as Lucifer yellow was not able to penetrate into the dermis. In addition, transepidermal resistance (TEER) values and expression of tight junction markers were similar to NHEKs. Analysis of differentiation markers like keratin10, Involucrin, Filaggrin showed a degree of differentiation, which was similar to primary keratinocytes. However, both immortalized cell lines showed increased numbers of Ki-67 positive nuclei in suprabasal layers indicative of their remaining proliferative capacity.

As inflammatory skin diseases are characterized by dysregulation of cytokines of the Th1 (psoriasis) or Th2 (AD) spectrum, epidermal models were treated with either a Th1 cocktail consisting of IL-22 and TNF-alpha or a Th2 mix comprising IL-4 and IL-13. Similar to NHEKs, the immortalized cell lines responded to the Th1 mix with a psoriasis-like phenotype, characterized by acanthosis and reduced expression of keratins. Similarly, the Th2 treated immortalized cell models displayed signs of spongiosis and reduced TEER values. Currently additional inflammatory markers such as TSLP, HBD-2 or pro-inflammatory cytokines are being investigated to further assess the degree of equivalency to the human disease. Interestingly, first data indicate that immortalized cell lines seem to be more sensitive to cytokine treatment, resulting in a more fragile epidermal morphology.

In addition to these induced models, we investigated the use of the CRISPR/Cas 9 technology to generate genetically induced inflammatory models. We were able to isolate cell clones from the NEHK-SV/TERT cell line with stable gene editing in disease relevant genes such as those of the STAT3 or Akt/mTOR pathway, which are currently being investigated for their disease-like features.

In summary, immortalized keratinocyte cell lines are suitable substitutes for primary cells and have the potential to be transformed into inflammatory disease models through genetic engineering or treatment with pro-inflammatory cytokines in order to investigate molecular pathomechanisms and to test novel therapeutics during preclinical evaluation.

P026 | Functional characterization of ELOVL7, an enzyme strongly expressed in sebaceous glands

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Long-chain fatty acid derivatives are major components of skin lipids and play a critical role in skin barrier function. Elongation of very long-chain fatty acids (ELOVL) enzymes are crucial factors for the generation of skin lipids by catalysing the elongation of fatty acids to both saturated and unsaturated very long chain fatty acids (chain length ≥22 carbons). So far seven members of this family (ELOVL 1–7) have been described, each exhibiting characteristic substrate specificity and tissue distribution. ELOVL7 is the latest addition to this family of enzymes and its physiological functions are largely unexplored.

Pivotal roles in skin development and its barrier function have been discovered for several members of the ELOVL family using genetically modified mice. ELOVL1 and ELOVL4 knockout mice are perinatal lethal, probably due to skin barrier defects and concomitant excessive water loss. Absence of ELOVL3 causes defects in hair coat formation and water repulsion. In humans, mutations in ELOVLs

have also been described in various syndromes with mutations in ELOVL1 and ELOVL4 leading to debilitating skin pathologies.

Here, we established a knockout mouse model for studying the function of ELOVL7 in the skin. In this model, the Lacz gene is expressed under the endogenous Elovl7 promoter and disrupts the expression of ELOVL7. Therefore, we can use this model to analyse ELOVL7 tissue distribution as well as to generate knockout mice (knockout first strategy). In addition, exons 4–6 are floxed, thus facilitating conditional inactivation of Elovl7.

Analysis of Elovl7 heterozygous mice revealed lacZ-staining in several tissues including salivary glands, brain and testis. Most interestingly there is prominent ELOVL7 expression in the sebaceous glands, indicating an important function of ELOVL7 in this skin appendage. The analysis of knockout first animals revealed that ELOVL7 knockout mice are viable, grow normally, showed no macroscopic or histological phenotype. Future studies will reveal whether the loss of ELOVL7 affects the function of the sebaceous gland and consequently skin barrier.

P027 | Differential induction of antimicrobial peptides and proinflammatory mediators in keratinocytes exposed to bacteria of the skin microbiota

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To protect the human body from its infectious environment the skin barrier is indispensable. To maintain the functionality of this barrier, balanced interaction between keratinocytes and the skin microbiota is essential. An imbalance is associated with various skin diseases. Some of the commensal bacteria of the skin microbiota are already known to induce specific antimicrobial peptides (AMPs) and proinflammatory cytokines in keratinocytes and thereby enhance or inhibit the inflammatory response. For example, *Staphylococcus epidermidis* is reported to induce human beta-defensin (hBD)-2, -3 and RNase 7. However, the effects of a variety of commensal bacteria and their potential interaction with each other as a microbiome system are less known.

Therefore, this study aimed to get a better insight into the commensal bacteria of skin microbiota regarding their ability to induce AMPs (e.g. hBD-2, -3 and RNase 7) and proinflammatory cytokines on their own and combined as a simulated microbiota variety.

To achieve this aim we produced supernatants of seven commensal bacteria strains isolated from healthy skin microbiota including Staphylococci, Micrococcus luteus, Corynebacterium singular and Roseomonas mucosa. Primary keratinocytes were stimulated with supernatants of the strains alone and in combination to simulate the microbiota. Real-time PCR analysis revealed differential AMP induction by the distinct bacteria strains. Alongside a moderate NFkappaB induction, the expression of IL-17C and other proinflammatory cytokines were increased by certain strains as well. Interestingly, the combination of the different strains to simulate the microbiota induced a high level of AMP response, which could indicate an additive effect between the single strains and needs further investigation.

These results may offer beneficial knowledge to re-establish a healthy skin microbiota and could provide important information to treat inflammatory skin diseases by combining the positive effects of different strains of commensal bacteria.

P028 | A plant extract mixture rich in flavonoids inhibits induction of inflammatory factors and restores down-regulation of differentiation markers in an atopic dermatitis in vitro model

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Atopic dermatitis (AD) is a common chronic inflammatory skin disease. AD is characterized by a complex interaction between skin barrier dysfunction and a dysregulated immune system, partly triggered by genetic and environmental factors. The resulting inflammation and skin barrier defects cause dry and itchy skin strongly lowering the quality of life of AD patients. Treatment with corticosteroids and systemic immunosuppression can ease the symptoms but may cause severe side effects.

In search of local treatments with fewer side effects, plant extracts rich in flavonoids get into focus. Flavonoids are known for their antioxidant character and show health benefits in different diseases. Thus, possible beneficial effects of a mixture of plant extracts rich in flavonoids on AD were examined in this study.

Therefore, differentiated normal human keratinocytes were stimulated with cytokines Interleukin-4 (IL-4), Interleukin-13 (IL-13), Interleukin-22 (IL-22) and tumor necrosis factor-alpha (TNF-alpha) or the RNA analog poly (I:C) instead of IL-22 and TNFalpha to mimic an AD-like inflammatory environment. Cells were incubated with the plant extract for 20 h. Effects on gene expression were determined by quantitative real-time PCR.

Stimulation of the keratinocytes with the AD-relevant cytokine mix was able to mimic an atopic dermatitis-like inflammation and barrier disruption in vitro. This was shown by a significant downregulation of AD-relevant skin barrier factors like loricrin, filaggrin and involucrin and upregulation of carbonic anhydrase 2 (CA2) as well as inflammatory markers such as IL-1beta, IL-17C, thymic stromal lymphopoietin (TSLP) and TNF-alpha.

Stimulation with the plant extract mixture restored the downregulation of the barrier factors loricrin, filaggrin and involucrin to normal levels or higher. In addition, the induction of inflammatory markers was significantly downregulated by the plant extract.

Together, a flavonoid-rich plant extract mixture restored the AD-related reduction of barrier factors and inhibited the AD-associated expression of inflammatory markers in an in vitro AD model. These results suggest that such flavonoid-rich plant extracts may be promising candidates for novel treatment options of atopic dermatitis.

P029 | *Staphylococcus aureus* activates the aryl hydrocarbon receptor in human keratinocytes

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The aryl hydrocarbon receptor (AhR) is a ligand-activated transcription factor mediating xenobiotic metabolism, skin barrier integrity and the innate immune response in the skin. There is increasing evidence that the AhR regulates innate immune defense as a pattern recognition factor by sensing bacterial metabolites and signal molecules. *Staphylococcus* (*S.*) *aureus* is a major skin pathogen and has been shown to induce proinflammatory factors in keratinocytes. The molecular mechanisms underlying the *S. aureus*-keratinocytes interaction are yet not fully understood. We hypothesized that *S. aureus* may activate the AhR in keratinocytes.

To follow this hypothesis we stimulated human primary keratinocytes with living *S. aureus* and investigated the activation of the AhR responsive genes CYP1A1 and CYP1B1 by real-time PCR. This revealed a strong induction of CYP1A1 and CYP1B1 which was blocked by a specific AhR inhibitor. Whole transcriptome sequencing of primary keratinocytes stimulated with *S. aureus* in the presence or absence of an AHR inhibitor also identified CYP1A1 and CYP1B1 as major AhR-regulated genes in *S. aureus*-stimulated keratinocytes. The *S. aureus*-mediated induction of CYP1A1 and CYP1B1 was also inhibited by siRNA-mediated downregulation of the AhR in keratinocytes. In concordance with these data, an AhR luciferase gene reporter plasmid transfected in keratinocytes was activated by stimulation with *S. aureus*. Moreover, the *S. aureus*-induced expression of the cytokines IL-24, IL-6 and IL-1beta in keratinocytes was partially inhibited by an AhR inhibitor and upon transfection of AhR-specific siRNA. Culture supernatants of *S. aureus* had similar effects and stimulation of keratinocytes with size-filtrated *S. aureus* culture supernatants revealed that the secreted AhR-activating factor(s) has a molecular weight <2 kDa. Screening of 45 clinical *S. aureus* isolates revealed that culture supernatants of 18 strains induced CYP1A1 gene expression more than two-fold and 12 supernatants induced a more than five-fold expression indicating that *S. aureus* in general has the capacity to activate the AhR. Finally, *S. aureus* induced CYP1A1 and CYP1B1 also in a 3D skin equivalent. The *S. aureus*-mediated induction of cytokines such as IL-24 and IL-1beta in 3D skin equivalents was inhibited in the presence of an AhR inhibitor.

Taken together, our data highlight a crucial role of the AhR in keratinocytes to mediate sensing of *S. aureus* and indicate an important role of the AhR in innate cutaneous defense against *S. aureus*.

P030 (OP04/05) | LRRC8A: A new player in epidermal physiology?

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Cell volume regulation is a crucial mechanism during cell proliferation and differentiation. A proper balance between these two processes is necessary for barrier formation within the epidermis and dysregulation often occurs in inflammatory conditions. We recently showed, that the volume-regulated anion channel (VRAC) LRRC8 and its essential subunit LRRC8A is necessary for cell volume regulation upon hypotonic stress in keratinocytes. Therefore, we were interested whether deregulated cell volume control through loss of LRRC8A could contribute to inflammatory skin diseases such as psoriasis or atopic dermatitis.

Immunohistochemical staining showed that LRRC8A is mainly expressed in the basal layer of healthy skin. RNA-Sequencing and Western Blotting of keratinocyte subpopulations displayed that LRRC8A is strongly expressed in transient amplifying cells. This was supported by the finding that in vitro differentiated keratinocytes displayed a bell-shaped expression pattern of LRRC8A, with a peak shortly after the onset and a decrease during further differentiation. Interestingly, reduced LRRC8A expression was detected in lesional psoriatic skin. In addition, siRNA-mediated knockdown led to reduced expression of differentiation markers like involucrin and cytokeratin 10 after Calcium-switch, thus, LRRC8A seems to be important during differentiation initiation.

For a better understanding of the underlying processes, we established a LRRC8Aknockout in the immortalized keratinocyte cell line NHEK/SV-TERT using CRISPR/ Cas9. The knockout was not only validated on DNA and protein levels, but further verified by reduced regulatory volume decrease in a hypo-osmotic environment and complete disappearance of VRAC-mediated ionic currents using manual patch clamping. The knockout cells showed slowed proliferation and disturbed differentiation in 2D assays arguing that LRRC8 indeed plays a role at the switch between proliferation and differentiation. Interestingly, the knockout cell line was not able to establish a stratified epidermis in in vitro 3D reconstructed epidermal models. Rescue of LRRC8A by transient overexpression will verify that LRRC8A-absence is indeed causal for these effects.

Our results suggest that LRRC8A is an important mediator in keratinocytes switching from proliferation to differentiation as abolished protein expression deteriorates both processes. These effects could be even more pronounced in an inflammatory environment. In summary, our study highlights the emerging role of ion channels not only in cell volume regulation, but also its link to epidermal development.

Further work will focus on underlying molecular mechanisms via which LRRC8A effects epidermal (patho-)physiology.

P031 | High-resolution expansion microscopy of neutrophil granulocytes and NETs to visualize nuclear and cellular morphology

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Neutrophil granulocytes are short-lived immune cells which play a significant role in the innate immune system. They are the most abundant immune cell type in the blood as well as part of the first line of defence of the body against pathogens. With the discovery of neutrophil extracellular traps (NETs) in 2004, neutrophil granulocytes became a focus in biomedical research, as NETs appear to be involved in a plethora of malignant, cardiovascular, autoimmune and chronic inflammatory diseases. Understanding neutrophil function and morphology could yield novel therapeutic approaches in the above-mentioned illnesses. Moreover, neutrophils possess unique properties that enable them to quickly migrate to the site of inflammation. In particular, nuclear morphology and biomechanics appear to be crucial to many immune defence functions.

In spite of the obvious importance of neutrophil cellular morphology and nuclear composition, studying nuclear functions and morphology remains difficult as they require high-resolution imaging up to super-resolution, which is technically challenging and costly. Here, we have developed an efficient method to analyse single-cell organelles such as the nucleus by expansion microscopy, achieving an 4–5 higher resolution. Expansion microscopy is based on linking labelled epitopes to a polymer, which is then expanded in a swelling process. Combining this method with conventional fluorescence microscopy or confocal microscopy yields very specific information about cellular structures at higher resolution.

We used this method to image typical neutrophil markers such as myeloperoxidase and to study the distribution of lamins within the nuclear envelope of neutrophils. Furthermore, we characterized neutrophil chromatin composition in unstimulated cells and in neutrophils undergoing NETosis and analysed heterogeneity of microdomains within the nucleus.

We also combined this approach with super-resolution methods (STED) to achieve an even higher magnification.

In conclusion, expansion microscopy is an useful tool to image neutrophils but also other immune cells with ultra-high resolution to reveal novel aspects of their biology.

P032 | Aberrant destabilization of the tuberous sclerosis complex (TSC) controls mTORC1 activity in psoriasis

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We previously found that aberrant activation of the Akt/mTORC1 cascade significantly contributes to the pathogenesis of psoriasis. In healthy skin mTORC1 signaling is only active in the basal layer and regulates proliferation while preventing differentiation. When cells leave the proliferative compartment, mTORC1 signaling is switched off which promotes differentiation. However, under inflammatory conditions this switch is hijacked by inflammatory cytokines, which prevents proper differentiation. Beyond this model, it is currently unknown how mTORC1 activity is regulated to promote these effects on keratinocyte differentiation. It is known from other tissues that mTORC1 is regulated through various pathways via the tuberous sclerosis complex (TSC). This complex, consisting of TSC1, TSC2 and TBC1D7 can be phosphorylated by different kinases such as Akt, Rsk1 or Erk, which cause its dissociation from the lysosome. Thus, TSC2, the catalytic subunit of TSC2, can no longer act as a GTPase activating protein for Rheb, which remains GTP-loaded and is able to activate mTORC1 on the lysosome.

To elucidate the regulation of mTORC1 in inflammatory skin diseases, we investigated whether pro-inflammatory cytokines that are known to activate these kinases are in turn mediating inactivation of TSC2. TNF-a as well as IL-1b induced robust phosphorylation of TSC2 especially on Ser939. Blocking the PI3-K/Akt or the MAPK pathway with kinase inhibitors not only reduced TSCSer939 phosphorylation, but also blocked mTORC1 activity. Surprisingly, we could not detect increased TSC2 phosphorylation in psoriasis patients. Instead, we found that TSC2 is strongly downregulated in lesional psoriatic skin compared to non-lesional skin of the same patients or healthy skin, which is in line with previously reported RNAseq data. We also found that in vitro chronic exposure to inflammatory cytokines seems to destabilize the TSC complex and induces degradation. Thus, we hypothesize that downregulation of TSC2 contributes to hyperactivation of mTORC signaling in psoriasis. To further study this mechanism we generated TSC2 knock-out keratinocytes using CRISPR/Cas9 genome editing. This cell lines displays enhanced mTORC1 activity, thus resembling a psoriasis-like phenotype. We are currently investigating whether this cell lines displays psoriasis-like features and thus will help to elucidate whether the TSC complex could represent the epidermal switch that integrates different extracellular cues and crucially controls mTORC1 activity to allow proper keratinocyte maturation.

P033 | Short chain dehydrogenase/reductase family 16C member 5 (SDR16C5) is a differentiation-dependent regulator of retinoid metabolism in human keratinocytes

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The metabolism of retinoids influences the homeostasis of the skin and represents an important target of therapies in dermatology. Short chain dehydrogenase/reductase family 16C member 5 (SDR16C5) is a member of the SDR enzyme family that converts retinol into retinaldehyde and retinaldehyde into retinoic acid. The aim of this study was to determine the expression pattern of SDR16C5 in human skin and skin appendages. Human primary epidermal keratinocytes were induced to differentiate in vitro. SDR16C5 mRNA and protein levels were determined by quantitative RTPCR and western blot analysis. The expression pattern of SDR16C5 in human skin was investigated by immunohistochemistry. Gene expression analysis in human organs showed predominant expression of SDR16C5 in the skin. SDR16C5 mRNA and protein strongly increased in abundance during terminal differentiation of keratinocytes in monolayer culture and in organotypic skin models. In human skin, SDR16C5 was detected in the granular layer of the epidermis. In addition, SDR16C5 was expressed in sebaceous glands. The results of this study suggest that SDR16C5 is a keratinocyte differentiation-dependent regulator of cutaneous retinoid metabolism.

P034 | Epithelial transglutaminase activities in biomedical model systems

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The cornification of epidermal keratinocytes depends on protein cross-linking by transglutaminases. The evolution of cornification and the control of epidermal transglutaminases are only incompletely understood. The aim of the present study was to determine the distribution of transglutaminase activity in model systems for keratinocyte differentiation. We established an in situ fluorescence labeling assay that reveals calcium-dependent transglutaminase activity using a fluorescent substrate. Transglutaminase activity was prominent in the granular layer, but absent in basal and early differentiated keratinocytes of human epidermis. A similar pattern of transglutaminase activity was detected in the skin of the mouse and in the epidermis of non-mammalian model species, chicken and Xenopus frogs. The enzymatic activity of transglutaminases was also conserved in the esophagus of mouse, chicken and frogs. In vitro, transglutaminase activity could be modeled in human keratinocytes undergoing differentiation in confluent monolayer culture and in three-dimensional skin models. Together, these results suggest that epithelial transglutamination is evolutionarily conserved among mammals and non-mammalian tetrapods and keratinocyte cultures are suitable models for the study of mechanisms that regulate epidermal transglutamination.

P035 (OP06/03) | Using natural immunity to control cellular senescence

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Cellular senescence is referred to as permanent state of cell cycle arrest where the cell becomes completely unresponsive to cell cycle promoting stimuli. This permanent cessation of cell division can be triggered by several endogenous and exogenous factors, such as DNA damage, chromosomal abnormalities, redox imbalance, mitochondrial dysfunction, proteostasis, ER stress, activation of checkpoint proteins etc. Senescent cells through secretion of several soluble factors, collectively known as senescence associated secretory phenotype (SASP) propagate the senescence in the neighboring cells and in this way can spread the senescence throughout the tissues. Accumulation of senescent cells and release of SASP are known to be associated with several age-associated disorders. Immune system naturally participates in the elimination of senescence cells. During aging due to probable impairment of immune function, the elimination of senescent cells is compromised, resulting in accumulation of senescent cells and SASP pileup. Removal of senescent cells and/or inhibition of SASP has been shown to improve or delay many age-associated disorders in experimental animals. Natural antibodies, which are germline encoded immunoglobulins generated in the body without prior antigen exposure are interesting candidates to tackle SASP release from senescent cells. Among these natural antibodies, IgM, a soluble pentameric polyreactive antibody released by B1 B cells is one of the most important one because of their role in protection against a wide range of infection and cellular abnormalities. We observed that natural IgM inhibited generation and release of reactive oxygen species (ROS) in replicative senescent human dermal fibroblasts (HDF). In addition, IgM also inhibited ROS production in primary and chronic lymphoid leukemic B cells. IgM significantly inhibited expression/synthesis and release of several SASP factors from senescent HDF as revealed from transcriptome analyses and antibody arrays. Furthermore, IgM also binds and neutralizes important SASP factors, such as IL1α, IL1β, IL6, TGFb, TNFβ, GROα, etc., as revealed from co-IP assays. IgM also binds and neutralizes IGFBP3 and IGFBP6, which are known to bind and reduce bioavailability and therefore activity of IGF1. IgM treatment also increases the release of IGF1 from senescent human dermal fibroblasts. In this way, during senescent condition IgM can increase the action of

IGF1, which is reported to be reduced during aging and considered as one of the reasons for higher degeneration during aging process. Therefore, natural antibodies including IgM can be in future used as an emerging opportunity to intervene as well as new therapeutics in controlling cellular senescence and SASP in ageing and ageing-associated diseases.

P036 | Sodium butyrate accelerates healing of diabetic wounds through resetting the epigenetic machinery of macrophages

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Non-healing wound disorders including diabetic ulcers, chronic venous leg ulcers and pressure ulcers are still remains uncured and pose a stern challenge to health care system. Persistent accumulation of dysfunctional macrophages have been identified as a major underlying cause for chronic wounds, however molecular details regulating these macrophages are still lacking. Here, we report that histone H3K27 acetylation, an epigenetic mark regulating macrophage transcriptome, suppressed under inflammatory diabetic microenvironment. In a series of in vitro and in vivo experiments we found that diabetic microenvironment markedly suppressed the acetylation of histone by activating histone deacetylase-dependent pathways. Furthermore, we observed a shift in transcription control from STAT1 to JUN in palmitate-exposed stimulated macrophages. Of note, histone deacetylase inhibitor sodium butyrate markedly enhanced the healing of diabetic skin wounds via restoring histone H3K27 acetylation-dependent pathways in wound associated-macrophages and reinstalled the STAT1 signaling. In addition, histone deacetylase inhibition preserved morphological features as well as improved the phagocytic and migratory activity of macrophages under inflammatory diabetic microenvironment. Our study reveals a novel pathogenic mechanism controlling perturbed macrophages, which can be therapeutically exploited to cure chronic wounds dominated by unrestrained macrophage.

P037 | The adaptive response of old ABCB5+ MSCs is changed upon exposure to LPS

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Previously, our laboratory uncovered that by contrast to fibroblasts, mesenchymal stem cells (MSCs) are endowed with the unique capacity to raise an adaptive response to environmental cues. This allows MSCs to control their direct neighborhood and endogenous tissue niche. Upon exposure of MSCs with infection mimicking lipopolysaccharide (LPS), a wall component of gram negative bacteria, MSCs completely shift their transcriptome with the release of neutrophil activating chemokines. The LPS induced transcriptomic shift resulted in a significant increase in neutrophil expulsed DNA traps (NETs) and proteolytic enzymes. This adaptive response guarantees the defense from bacterial attack. Wound healing decreases with age and the propensity for infection significantly increases in elderly individuals. Therefore, I set out to address the question whether MSCs from young healthy donors (<30 years) as opposed to MSCs from old healthy donors (>65 years) may change their adaptive response upon LPS exposure towards a reduced microbicidal response. Cultured ABCB5+ MSCs from young and old donors treated with LPS revealed differences in the time kinetic of NF-B translocation from the cytoplasm to the nucleus where it transactivates target genes. By contrast to ABCB5+ MSCs from young donors, ABCB5+ MSCs from old donors depicted a significantly delayed backregulation of nuclear NF-B translocation. Furthermore, significant differences in the expression level of p-p65, indicative of NFB activation with an impressive increase in p-p65 in ABCB5+ MSCs from elderly adults was observed when compared to ABCB5+ MSCs derived from young individuals. This correlates with a higher and longer persisting expression of different NF-B target genes and their corresponding proteins like IL-1 and IL-6 in MSCs of elderly individuals as opposed to young individuals. Notably, ABCB5+ MSCs from young donors point out higher expression levels of IL-8 as well as the anti-inflammatory cytokine IL-10 compared to ABCB5+ MSCs from old donors. Apart from this, PARP, an enzyme contributing to DNA repair, is over the time highly expressed in ABCB5+ MSCs from young donors compared to a low expression level in ABCB5+ MSCs from old donors.

Interestingly, LPS primed ABCB5+ MSCs from young donors can activate neutrophils by producing NETs to a higher level in comparison to ABCB5+ MSCs from old donors. NE activity indicative for microbicidal NET formation from co-cultures of LPS primed ABCB5+ MSC from young donors with PMA stimulated neutrophils is significantly higher and occurred LPS -concentration-dependently compared to moderate NE activity from PMA stimulated neutrophils by LPS primed ABCB5+ MSCs from old donors. Remarkably, ABCB5+ MSCs from young donors express higher levels of GCP-2, a chemoattractant for neutrophils, which is crucial for participation to the immune response after a bacterial infection.

These data imply, that ABCB5+ MSCs from old individuals cannot at the same extent raise an adaptive response towards infectious cues of their microenvironment. This finding may preclude MSCs from old individuals to refine current MSC-based therapies employing LPS primed MSCs for the treatment of infected wounds in clinical routine.

P038 | Disulfiram enhance the apoptotic activity of MEK inhibitor trametinib by inducing endoplasmic reticulum stress in NF1 loss-of-function Melanoma

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Despite current advances in therapy and diagnostics, melanoma is a difficult to treat, aggressive skin cancer with a high mortality rate. The third most common subgroup in the genomic landscape of melanoma includes melanomas with aberrations in the NF1 gene (12–14%). Most mutations are loss-of-function (LoF) events. Since NF1 is a GTPase-activating protein known to down-regulate RAS activity through its intrinsic GTPase activity, NF1-LoF mutations are an alternative way to activate the canonical MAPK signaling pathway. Consequently, activation is critical for the RAS/ MAPK signaling pathway in melanoma. An effective targeted therapy is currently only available for BRAFV600 mutant melanomas, indicating the clinical need for novel treatment options, especially for metastatic NF1 LoF melanomas.

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.

Initially, 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 NF1 LoF melanoma cells.

We initially performed cell viability assays to investigate the efficacy of CuET on NF1 LoF melanoma cells. Actually, Cu2+ as monotherapy had no influence on cell viability. In contrast, treatment with CuET, in particular, the combination with 10 nM of trametinib, showed a significant and complete inhibition of cell growth in NF1 LoF cells with a minimal effective dose of 125 nM of CuET. In addition, CuET/trametinib persistently inhibited melanoma cell growth for 12 days after treating the cells for 3 days in a colony formation assay. Interestingly, the inhibitory effects achieved by CuET in NF1-LoF melanoma 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/trametinib we could measure a dramatic increase of intracellular Cu2+ in the melanoma cells, demonstrating that ET/trametinib mediates the uptake of Cu2+. The additional Cu2+ was localized at the nucleus of treated melanoma cells. CuET/ trametinib in addition induced high expression of ER stress-related genes in a dose-dependent manner in NF1 LoF melanoma cells. Furthermore, the inhibitory effect was fully dependent on CuET/trametinib ER production since adding the potent and specific inhibitor of ER chaperone BiP/GRP78/HSPA5 totally abolished the CuET/ trametinib mediated cytotoxicity. To confirm this mechanism of action, the high levels of ER produced by CuET/trametinib were observed measured by the upregulation of the ER stress-related factors as ATF4, CHOP, and NUPR1 (P8) and the proapoptotic proteins from the BCL-2 family (BIM and BAX). A second mode of action of CuET/ trametinib could be the activation of cJun/JNK pathway. Indeed, we were able to detect a rapid cytoplasmic induction of cJun in NF1 LoF melanoma cells treated with CuET/trametinib. Lastly,

We conclude from our results that the drug disulfiram induces apoptosis in melanoma cells in general, but especially NF1 LoF melanoma cells, and adds additional cytotoxicity to MEK inhibitors as trametinib to obtain an effective therapy for this group of melanomas.

Chemokines/Cytokines

P039 | Macrophage migration inhibitory factor (MIF) and its homolog D-dopachrome tautomerase (D-DT) are significant promotors of UVB- but not chemically induced non-melanoma skin cancer

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Non-melanoma skin cancer (NMSC) is the most common cancer in Caucasians worldwide, caused by chronic exposure to environmental factors such as ultraviolet (UV) radiation and chemicals. MIF is a pleiotropic cytokine that is described as a pro-tumorigenic factor in various tumour entities. D-DT was recently described as a homolog of MIF that shares many biological activities. The aim of the present study was to assess the pathophysiological role of MIF and D-DT in the development and progression of UVB- and chemically induced NMSC.

Investigating the effects of MIF and D-DT during the acute inflammatory phase, we irradiated WT, Mif-/-, D-dt-/- and Mif-/-/D-dt-/- SKH1-HrHr mice dorsally with a single UVB dose of 2240 J/m2. Here we found that the epidermal thickness was significantly increased in UVB-irradiated Mif-/- and D-dt-/- mice compared to WT controls. For long-term UVB irradiation studies mice were irradiated three times a week for 25 weeks. Mif-/-, D-dt-/- and Mif-/-/D-dt-/- mice developed significantly later and fewer tumors. For chemical skin carcinogenesis, mice were treated with benzo(alpha)pyrene twice a week for 23 weeks. In contrast to our long-term UVB experiments, we did not observe any differences in the timing of tumor onset and tumor incidence between all groups. In addition, we investigated the chemotactic effects of MIF and D-DT using an in vitro 3D skin model in which we included macrophages. When recombinant MIF and D-DT were applied to these models, we observed an accumulation

of the macrophages within the models. Here we saw that D-DT exhibits the same chemotactic effects on macrophages as MIF, but the additive effects of the two cytokines were very small.
In summary, our data indicate that MIF and its homolog D-DT play important roles in the development and progression of UVB- but not chemically induced NMSC.

P040 | Dissection of the effects of TH2 cytokines on a novel atopic dermatitis model using hair follicle-derived keratinocytes

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TH2 cytokine stimulation of healthy keratinocytes is an often-used model to study inflammatory processes in atopic dermatitis (AD). To consider potential intrinsic alterations of AD-derived keratinocytes and to avoid invasive surgical procedures we established the growth of hair follicle-derived keratinocytes from AD patients (FDK-AD). Stimulation of these FDK-AD with the AD-associated TH2 cytokines IL-4 and IL-13 revealed a higher upregulation of several AD-relevant factors (e.g. TSLP, CCL26, IL-33, carbonic anhydrase 2) as compared to stimulation of hair follicle-derived keratinocytes from healthy donors.
Staphylococcus (*S.*) *aureus* is highly abundant in the lesional skin of AD patients and a correlation of *S. aureus* abundance with the severity index of AD has been reported. However, less is known about the interplay of TH2 cytokines and *S. aureus* in the context of AD. To elucidate whether *S. aureus* pathogenicity is associated with TH2 cytokines, we stimulated the FDK-AD with *S. aureus* supernatants and IL-4/ IL-13. *S. aureus* supernatants alone upregulated expression levels of AD-relevant markers and down-regulated expression of keratinocyte differentiation markers. Stimulation of the FDK-AD with *S. aureus* in the presence of IL-4/IL-13 further amplified the expression of several AD-relevant markers. In concordance, the expression of differentiation markers (e.g. keratin 1 and 10) was further decreased in cells treated with *S. aureus* together with IL-4 and IL-13 as compared to treatment with *S. aureus* or IL-4/IL-13 separately. These data suggest that AD-associated TH2 cytokines amplify *S. aureus*-induced inflammation and barrier disruption of the skin.
To further evaluate if such FDK-AD are also useful to analyze AD-related inflammation in a differentiated epidermal model, we established generation of a 3D skin model using the FDK-AD. Stimulation of the 3D model with IL-4 and IL-13 revealed induction of several AD-relevant factors. In addition, enhanced appearance of AD-associated spongiosis was detected in the 3D model treated with the combination of IL-4/IL-13, although IL-13 alone also induced spongiosis.
Together, our results indicate that AD-keratinocytes derived from hair follicles function as a useful in vitro tool to study AD and open new perspectives to investigate AD-associated inflammatory scenarios mediated by an AD-relevant cytokine and microbial environment.

P041 | Unraveling the function of autocrine IL-9/IL-9Rα signals in atopic dermatitis

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αSkin-resident memory T (TRM) cells persist long-term in the skin to provide immune protection. However, their activation by allergens or autoantigens can cause chronic inflammatory skin disease. Atopic dermatitis (AD) and allergic contact dermatitis (ACD) are common T cell-driven diseases in which TRM cells play a key role and contribute to their chronicity. Current treatments suppress inflammation, but fail to cure patients long-term. Thus, there is a need for a better understanding of pathogenic TRM in T helper 2 cell (Th2)-driven skin disease, such as AD, as a prerequisite for the development of curative therapies.
Our research uncovered that skin tropic pathogenic Th2 cells, that infiltrate AD and ACD, are characterized by high expression of both interleukin 9 (IL-9) and its receptor, IL-9Rα. Although the cytokine IL-9 has been discovered more than three decades ago, an overarching role in humans remains elusive. Our finding hence suggests an unidentified function of autocrine IL-9 signals in human skin inflammation.
Since previous data showed that pathogenic Th2 cells are associated with the expression of the transcription factor PPAR-g, we hypothesized that PPAR-g controls IL-9Rα expression. Indeed, knocking-out or inhibiting PPAR-g in pathogenic Th2 cells downregulates the expression of IL-9Rα at the mRNA level as well as at the protein level.
To unravel the autocrine function of IL-9 on Th cells, we isolated human Th cells expressing high levels of IL-9Rα from skin and peripheral blood. Transcriptional profiling of these cells in presence and absence of recombinant IL-9 (rIL-9) revealed that approx. 650 genes are differentially expressed in response to rIL-9. From these genes, the majority shows induction of gene transcription upon rIL-9 stimulation. Interestingly, there is a considerable overlap with IL-2 signaling. Nonetheless, we observed that rIL-9 strongly induces genes specifically associated with the pathogenic Th2 phenotype, such as IL9, IL17RB, and HPDG.
To conclude, we discovered that PPAR-g—a transcription factor closely linked to the pathogenic Th2 phenotype—regulates IL-9Rα expression and that autocrine IL-9 signals promote pathogenic features of Th2 cells. This suggests that IL-9 plays an important role in cutaneous immunity and allergy, which might open up new avenues for therapeutic intervention.

P042 (OP06/05) | Targeting mast-cell plasticity re-shapes the tumor microenvironment of melanoma

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The incidence of malignant melanoma is steadily rising. Due to its metastatic potential the cancer has a poor prognosis and is responsible for 80% of patients with skin cancer dying. The treatment of metastatic melanoma was revolutionized by the development of new immunotherapies, however long-term treatment responses are still limited to few patients and complete remissions are rare due to resistance to therapy. Therefore, there is need for new therapeutic options for melanoma. Resistance to therapy in malignant melanoma is linked to an increase of phenotypic heterogeneity of melanoma cells to their response to pro-inflammatory signals from the tumor microenvironment (TME). For different tumor entities an infiltration of mast cells (MCs) into the TME has been reported, but their impact on tumor progression has been discussed controversially. Within the TME of melanoma, an accumulation of MCs has been found, especially in areas of regression. We have recently demonstrated that LPS-activated tumor-resident MCs initiate effective antimelanoma immune control. Since MCs are plastic in regard to their functions and can secrete various mediators, we are currently investigating how different stimuli activate MCs and how this affects the TME and the control of melanoma.

To decipher the role of MC plasticity in melanoma progression, we first isolated MC precursors from murine femur (BMMCs) and matured them in medium containing IL-3 and stem cell factor. Maturity of MCs was determined by flow cytometry, gating for CD117 (c-kit) and high-affinity IgE receptor (FcεRI) double positive cells. The cells were then stimulated with the alarmin IL-33 in combination with TLR ligands for 24 h. RNA Sequencing showed a strong enrichment in genes involved in cytokine-mediated signaling pathways in BMMC stimulated with IL-33. Through the stimulation with IL-33, MCs were activated to produce a wide range of cytokines that could be detected in the supernatant. Different combinations of stimuli acted antagonistically or synergistically in regard to the production of cytokines. Upon the highest concentration of detected cytokines in the supernatant were chemokines that play a crucial role in the recruitment of immune cells, such as CCL3 and CCL22. In vivo experiments were performed using the B16 melanoma model in mast-cell deficient Mcpt5-cre mice demonstrating that IL33R-TLR2/6 targets tumor-associated MCs to initiate anti-tumor immune defense, by mediating T cell recruitment, especially CD4+ T cells. Hence, we show that the plasticity of MCs can be manipulated to act pro-tumorigenic.

Clinical Research

P043 | How the molecular classifier can enhance the clinical and histopathological differentiation between psoriasis and eczema. Insights from the Heidelberg cohort study

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The clinical similarity between psoriasis and eczema may pose major diagnostic challenges even to experienced dermatologists. Even the dermatohistopathological analysis does not always contribute to the reliable differentiation of both diseases. This is partly because eczema may have psoriasiform characteristics and on the other hand psoriasis may appear eczematoid. The newly developed Molecular Classifier offers the possibility to determine the probability of the presence of psoriasis or eczema, thus providing new insights for the diagnostic process. Both diseases were found to have typical molecular signatures. CCL27 and NOS2 were identified as the most significant genes that are regulated differently in the respective diseases. Using these, a classifier could be trained that achieved accuracies averaging nearly 100% in test cohorts. In a cohort study of the department for occupational dermatology of the University Hospital Heidelberg, at least 250 occupational dermatology patients will be followed on over 3 years and the impact of a diagnosis by means of this Molecular Classifier on the course and severity of the skin disease, therapeutic measures, occupational retention and quality of life will be investigated.

By September 2021, 134 patients (men: 72, women: 52, age: min.: 20 years, max.: 70 years, median: 52 years) were included. As expected, there were high discrepancies between the clinical diagnosis by the dermatologist and the classifier. Both assessments were congruent in only 40% of the cases. Psoriasis was diagnosed by dermatologists in 19.4% (n = 26), eczema in 44.8% (n = 60) of the cases. The Molecular Classifier showed a clear tendency towards psoriasis in 18.7% (n = 25) of the cases, towards eczema in 68.7% (n = 92).

In 32% (n = 43) of all cases dermatologists could not make a clear diagnosis. However, by using the molecular classifier, a clear tendency to psoriasis or eczema could be established in 98% (n = 42) of these cases. To date additional histological examination was performed in 49 patients. In 71% (n = 35) of the cases, this provided a different diagnosis compared to the clinical diagnosis of the dermatologist. Dermatohistopathology and Molecular Classifier were congruent in 45% (n = 22) cases only. An overall congruence between all three instances of dermatologist, pathologist and Classifier was only observed in 6% (n = 8) of the cases and underlines the diversity and the problems of differentiation of the two disease patterns.

At this early stage of the study it is already evident that the Molecular Classifier can become a "game changer" in the diagnosis of psoriasis

and eczema, also in hand eczema. Clinically and/or pathologically unclear cases can receive a decisive impulse for the correct diagnosis and a targeted therapy through this method.

In the future, it will be of particular importance to study those cases in which the diagnosis of the dermatologist and the classifier differ. The follow-up surveys will investigate the extent to which the dermatologist's assessment changes, which disease-specific therapies are implemented and whether this leads to an improvement in the severity of the disease and an improvement in the quality of life.

P044 | IRAK4 drives pathogenic processes in inflammatory skin diseases

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In recent years innate immunity has moved into focus of therapeutically approaches due to its ability to drive downstream adaptive responses. Modulators of innate immune responses are expected to have high therapeutic potential across immunemediated inflammatory diseases (IMIDs). The kinase IRAK4 integrates signaling downstream of receptors acting at the interface between innate and adaptive immune responses (e.g. TLRs, IL-1R, IL-18R).

Here, we profiled a novel, selective IRAK4 inhibitor, GLPG2534, in an extensive series of in vitro, ex vivo, and in vivo models of inflammatory skin diseases (ISD). In vitro, IRAK4 inhibition resulted in substantial inhibition of TLR and IL-1 responses in primary keratinocytes, granulocytes, and T cells. Furthermore, disease activity in murine models of psoriasis and atopic dermatitis (AD) skin inflammation (Il-23-, Il-33-, imiquimod- and MC903-induced) was markedly dampened by IRAK4 inhibition. Finally, neutralizing IRAK4 reversed pathogenic molecular signatures in human lesional psoriasis and AD biopsies.

The variety of models highlighted IRAK4 as central mediator of psoriasis and atopic dermatitis by regulating cytokines (e.g. IL-17A, IL-22, IL-23, IL-4, IL-13, IL-5, TNF-α), chemoattractant chemokines (e.g. CXCL8, CXCL5, MCP1) and antimicrobial peptides (e.g. S100A7, DEFB4A). Overall, our data demonstrate the potential of IRAK4 inhibition as a therapeutic strategy in ISD.

P045 | Identifying predictors of high response levels in ixekizumab-treated patients with moderate-to-severe plaque psoriasis

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Introduction: The UNCOVER-1, -2, -3, and IXORA-S trials demonstrated ixekizumab's (IXE) efficacy in treating patients with moderate-to-severe plaque psoriasis (PsO), which has been confirmed by several network meta-analyses. Since patient baseline profiles may influence their response to treatment, it is important to assess whether these characteristics can also affect the response to IXE treatment. Additionally, understanding which patients are most likely to benefit from treatment can help provide patients with effective treatment more rapidly.

Objectives: The aim of this analysis was to evaluate whether patient baseline characteristics or early clinical responses could predict achievement of Psoriasis Area and Severity Index (PASI)90 or PASI100 responses in IXE-treated patients at Week (W) 12 and W52.

Methods: This post hoc analysis pooled 375 patients from the Phase 3, randomized clinical trials, UNCOVER-1, -2, -3, and IXORA-S, who received IXE as per label through W52. Specifically, patients received IXE 160 mg at W0, 80 mg every 2 weeks through W12, and 80 mg every 4 weeks thereafter. Patients were ≥18 years old with moderate-to-severe PsO defined as ≥10% Body Surface Area, a static Physician Global Assessment ≥3 and a PASI ≥12 in UNCOVER-1, -2 and -3, and a PASI ≥10 in IXORA-S.

Baseline characteristics and PASI75 responses at W2 and W4 were evaluated as potential predictors of PASI90 or PASI100 achievement at W12 and W52 using multivariate logistic regression models which were adjusted for all reported patient variables at baseline. Accuracy ranged between 0.68 and 0.78 for all models. Results are presented as odds ratio (OR) with 95% confidence intervals. Non-responder imputation was used for missing data.

Results: Higher baseline PASI and achievement of PASI75 at W2 or W4 were predictors of PASI90 responses at W12 and W52. In addition, achieving PASI75 at W4 was predictive of PASI100 responses at both timepoints, while reaching PASI75 at W2 was predictive of a PASI100 response at W12 only. Males were more likely to achieve PASI90 and PASI100 at W52.

Higher weight and presence of palmoplantar PsO at baseline were associated with reduced odds of achieving PASI90 or PASI100 at W12 and W52. Additionally, patients with prior biologic treatment were less likely to achieve PASI90 at W52 but not at W12. Concomitant psoriatic arthritis, presence of nail or scalp PsO, or

higher age at baseline were not predictive of PASI90 or PASI100 responses at W12 or W52.

Conclusions: Although most patients respond well to IXE, this analysis demonstrates that certain baseline characteristics (male, lower weight, higher PASI, absence of palmoplantar PsO, biologic naive) are associated with higher level responses to IXE over time. Partial response rates at W2 and W4 reliably predicted high clinical response rates at later time points.

P046 | Progressive microbiome dysregulation with predominance of *Staphylococcus* correlates with wound burden and disease activity in recessive dystrophic epidermolysis bullosa: A prospective case-control study

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Background: Recessive dystrophic epidermolysis bullosa (RDEB) is characterised by skin fragility, with blistering and wounds. Bacterial wound colonisation and infection, mainly caused by *Staphylococcus aureus*, are common, but the interplay of microbiome dysregulation and RDEB disease severity, systemic inflammation, and age remained unexplored.

Objectives: By analysing the microbiome of skin, wounds, oral mucosa, and stool in a well-characterised paediatric RDEB cohort, we aimed to uncover the natural history and dynamics of skin and mucosal microbiome and its impact on clinical and inflammatory laboratory parameters.

Methods: Microbiome swabs from wounded and unwounded skin, oral mucosa and stool samples of children and adolescents with RDEB were prospectively collected, subjected to 16S rRNA gene sequencing, and compared to age- and sex-matched healthy controls. Blood samples were analysed for parameters of inflammation. Taxonomy, alpha diversity, and abundances were correlated with clinical and laboratory items in statistical models.

Results: 28 children with RDEB, and 28 healthy age-matched controls were included. Skin microbiome showed significantly reduced alpha diversity compared to healthy controls and early, age-progressive predominance of *Staphylococcus* first in wounds, then in unwounded skin. Changes in oral mucosal and gut microbiome in RDEB were discrete with no significant differences in alpha diversity. Patients with severe disease showed reduced alpha diversity and higher abundances of *Staphylococcus* on wounded and unwounded skin compared to intermediate disease. *Staphylococcus* abundance correlated significantly with both acute and chronic wound burden. High CRP correlated with higher abundance of *Staphylococcus* on unwounded skin and of Bifidobacterium on both unwounded skin and wounds. No significant correlations were found with IL-6, INF-γ, TGF-β, or TNF-αlevels. The stepwise linear regression modelling for *Staphylococcus aureus* abundance on wounds included total wound burden, chronic wound burden, total EBDASI, CRP, leukocyte count, IL-6, TGF-β and TNF-α.

Conclusions: Children with RDEB experience early and progressive skin microbiome dysregulation starting on wounds and later extending to unwounded skin. The predominance of *Staphylococcus* significantly correlates with wound burden and disease activity, and to some extent with systemic inflammation. This study emphasises the need to target *Staphylococcus* early not only on wounds, but on the entire skin surface.

P047 | Urticarial vasculitis versus chronic spontaneous urticarial—Natural history, disease course and response to treatment—A UCARE study

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Background: Urticarial vasculitis (UV) is defined by urticarial lesions lasting >24 h with histopathologic criteria for leukocytoclastic vasculitis. The clinical spectrum varies from mild wheals to severe systemic disease. In contrast, chronic spontaneous urticaria (CSU) is characterized by short-lasting wheals often associated with severe pruritus.

Methods: In this prospective observational study, 109 UV patients and 127 CSU patients were recruited at 10 specialized urticaria centers of reference and excellence (UCAREs) and university hospitals from Brazil, China, Ecuador, Germany, Iran, Oman, Russia and Turkey and completed a questionnaire (on the natural history, clinical symptoms and localization, triggers, associated diseases and treatment effects).

Results: The majority of UV and CSU patients was female, middle-aged with elevated mean BMI levels (UV 25.7, CSU 25.6). Mean diagnostic delay was significantly higher in UV compared to CSU patients (UV 34.2 months; CSU 20.6 months). Wheals (UV 94%, CSU 95%) and pruritus (UV 72%, CSU 89%) were the most frequently reported initial symptoms. UV patients reported more often systemic symptoms both at disease onset (UV 45%, CSU 24%) and over time (UV 72%, CSU 52%). as well as a longer duration of wheals and angioedema than CSU patients. Of UV patients, 39% described wheals lasting ≤24 h, and in this subgroup, angioedema was more prevalent than in UV patients with wheals lasting >24 h. UV and CSU patients reported a similar anatomical distribution pattern of wheals. The most frequently prescribed drugs were antihistamines and oral corticosteroids in both groups. Patients reported higher efficacy for oral corticosteroids in UV and for omalizumab in CSU patients.

Conclusions: Our results suggest a considerable overlap but also distinct differences in clinical presentation and treatment responses between UV and CSU patients. Further studies are necessary to clarify whether UV and CSU are part of a disease continuum or separate entities.

P048 | Cold atmospheric plasma versus diclofenac 3% gel in patients with actinic keratoses

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The influence of the cutaneous microbiome (CM) on the development and progression of various cutaneous malignancies is part of active research[1, 2].

This study is looking at the effect of cold atmospheric plasma (CAP), a partially ionised gas compared to diclophenac-3% gel as a therapy for actinic keratoses (AK), as well as the change in the CM caused by the two therapies.

In the prospective ACTICAP study, 60 patients with AK in treatment area 1 were treated with diclophenac 3% gel twice a day and treatment area 2 with CAP (SteriPlas, Adtec®) twice a week for a total of 3 months.

Raterblinded evaluations of lesion count, AK-affected area and evaluation of local skin response score (LSRS)[3] were performed before, during and after the treatment, as well as cutaneous swabs for a microbiological analysis.

After 3 months of treatment with CAP, the lesion count was reduced by an average of 33%, whereas a reduction of only 21% was observed in the area treated with diclophenac ($p = 0.048$).

The AK affected area was reduced by 41% (CAP) vs. 27% (diclpophenac) ($p = 0.033$).

30% of adverse skin reactions (>2 point increase in LSRS) appeared after diclophenac treatment and only 8% ($p = 0.0046$) in CAP area.

Before treatment, the CM was characterised by highly significant differences in alpha and beta diversity associated with sample location. In contrast to the extremities, significantly more Propionibacteriacae were found on the capillitium and torso. *Staphylococcus* spp were distributed in nearly similar numbers in all regions.

At the end of both treatments an increase in alpha diversity, significant for diclophenac and insignificant for CAP was observed. The relative abundance of *Staphylococcus* spp. reduced after initiation of therapy for CAP and diclophenac.

Patients responding to CAP treatment, assessed by the size of the area of AK, were associated with a significant higher relative abundance of the genus Corynebacterium compared to non-responders.

The data from the study allow us to assume that both therapies are effective against actinic keratoses, with CAP offering the advantage of good tolerability, as no severe side effects occurred.

Staphylococcus spp. are already described as a possible malignancy factor and may lead to progression into Squamous cell carcinoma The role of genus Cornybacterium as a possible protective factor has not been discussed yet and needs additional exploration[1, 4].

It is already known that CAP is proapoptotic and tumour selective, but the immediate effect of CAP is part of active research[5].

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P049 | Association between atopic dermatitis and cardiovascular diseases: A large multicenter observational study (ProRaD)

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Background: There is contradictory evidence on the association between atopic dermatitis (AD) and cardiovascular comorbidities. The aim of this study was thus to further explore this connection and how it relates to the presence of atopic comorbidities.

Methods: 705 patients suffering from AD and 80 healthy individuals participating in the observational multicenter study ProRaD (Prospective Longitudinal Observational Research in Atopic Dermatitis) were included. EASI, BSA involvement, SCORAD and objective SCORAD were used to grade AD severity. The presence of atopic, cardiovascular and metabolic conditions was assessed by a dermatologist. Cardiovascular risk factors (age, sex, smoking habits, physical activity, body mass index) were asked in a standardized questionnaire.

Results: Our analysis did not show any overall association between AD and cardiovascular outcomes. AD patients with atopic comorbidities have a lower incidence of cardiovascular comorbidities in comparison to AD patients without atopic comorbidities (pure AD). Furthermore, the presence of severe or pure AD was found to be associated with cardiovascular diseases in a bivariate model. In patients with pure AD, there was even a statistically significant relation between the severity of AD (EASI and BSA) and the presence of cardiovascular comorbidities.

Conclusion: Our study does not suggest an overall association between AD and cardiovascular comorbidities but suggests a more complex relation between the two conditions: A higher BSA involvement may be indicative of a stronger inflammatory reaction in pure AD and represent a risk factor for CVD. Conversely, a more prominent type 2 response (clinically evidenced by atopic comorbidities) might exert a protective effect.

P050 | Nectin-1 determines the susceptibility of malignant melanoma to oncolytic herpes simplex virus in vitro and in vivo

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Talimogene laherparepvec (T-VEC), an oncolytic herpes simplex virus, is approved for intralesional injection of unresectable stage IIIB/IVM1a melanoma. However, it is still unclear which parameter(s) predict treatment response or failure. Our study aimed at characterizing surface receptors Nectin-1 and herpes virus entry mediator (HVEM) in addition to intracellular molecules cyclic GMP-AMP synthase (cGAS) and stimulator of interferon genes (STING) as potential biomarkers for oncolytic virus treatment. In 20 melanoma cell lines, oncolytic activity of T-VEC was correlated with the expression of Nectin-1 but not HVEM, as evaluated via flow cytometry and immunohistochemistry. Knockout using CRISPR/Cas9 technology confirmed the superior role of Nectin-1 over HVEM for entry and oncolytic activity of T-VEC. Neither cGAS nor STING as evaluated by Western Blot and immunohistochemistry correlated with T-VEC induced oncolysis. The role of these biomarkers was retrospectively analyzed for the response of 35 cutaneous melanoma metastases of 21 patients to intralesional T-VEC injection, with 21 (60.0%) of these lesions responding with complete (n = 16) or partial regression (n = 5). Nectin-1 expression in pretreatment biopsies significantly predicted treatment outcome, while the expression of HVEM, cGAS, and STING was not prognostic. Altogether, Nectin-1 served as biomarker for T-VEC induced melanoma regression in vitro and in vivo.

P051 | Addiction in acne inversa/hidradenitis suppurativa patients—Much more common than estimated

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Background: Acne Inversa/ Hidradenitis Suppurativa (AI/HS) is a chronic inflammatory skin disease presenting with deep seated painful nodules and abscesses, pus secreting sinus tracts and extensive

scarring. In conjunction with the involvement of the axillar, inguinal and perigenital region AI/HS represents a considerable psychosocial burden for those affected and massively restricts their quality of life. Although nicotine abuse is considered a comorbidity of AI/HS, other addictive disorders have barely been studied.

Objective: To investigate whether addictive disorders occur more frequently in patients with AI/HS in order to develop evidence-based recommendations for dermatologists in their clinical practice and to improve the care situation of patients.

Methods: Patients diagnosed with AI/HS were asked to complete a paper based self-reported anonymous questionnaire with a total of 94 items. The questionnaire contained validated screening instruments for a total of 7 addictive disorders (smoking, alcohol, drugs/medications, cannabis, food addiction, gambling, compulsive use of the internet/video games). In addition, screening for depression, as well as for the presence of an anxiety disorder was performed. Furthermore, the following were documented: Age, weight, height of the patients, BMI, disease severity according to Hurley and IHS4, pain scale from 1 to 10, Dermatologic Life Quality Index (DLQI), previous surgeries as well as the current systemic therapy. The data obtained was compared to the prevalence of these addictions in the german general population provided by the Deutsche Hauptstelle für Suchtfragen (DHS).

Results: Between January 2021 and September 2021, 100 consecutive patients (53 males, 47 females; mean age 39.2 years (SD 13.6 years), range 18–78 years) participated in the study. Of these, 68 patients showed evidence of the presence of an addictive disorder. An addictive disorder with the exception of smoking was identified in 38 patients. Twenty-eight patients reported having more than one addiction. 55% of patients were smokers, 17% had evidence of alcohol abuse, 18 % showed signs of food addiction, 3% were compulsive gamblers, 11% abused illicit drugs or prescription medications, 18% reported abusing cannabis in the past 12 months and 12% could be identified as cannabis addicts. Compared with the general population, these results are significantly higher for smoking ($p < 0.001$), alcohol abuse ($p < 0.001$), gambling ($p < 0.01$), cannabis addiction ($p < 0.001$) and the prevalence of cannabis use in the past 12 months ($p < 0.001$). Surprisingly, no correlation was found between disease severity (Hurley Stage or IHS4 Score) and the presence of addictive disorders.

Conclusion: Addictions are much more prevalent in patients with AI/HS compared with the general population. Addictive behavior can either be a cause of AI/HS as demonstrated for nicotine abuse or a consequence due to an assumed individual withdrawal from social activities. Using validated screening instruments as the questionnaires presented enables physicians to identify those being at risk or even suffering from addictions. Furthermore, this approach helps to pave the way for appropriate therapeutic options. This is of utmost importance for patients displaying only minor signs of AI/HS as disease severity does not show any correlation with development of an addictive disorder.

P052 | Profiling of autoreactive CD4+ T cells in pemphigus vulgaris patients

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Autoreactive CD4+ T cells against the desmosomal protein Desmoglein 3 (Dsg3) are critically involved in the pathogenesis of pemphigus vulgaris (PV) as they intimately interact with B cells and thereby promote autoantibody production. Here, we aimed to profile pathogenic CD4+ T cells of PV patients for evaluation of possible new treatment avenues. Peripheral blood mononuclear cells of healthy controls (HC, $n = 6$) as well as of acute and remittent PV patients ($n = 8$ and 15) were phenotypically analyzed by flow cytometry. Proliferation of CD4+ T cells in response to immunodominant T cell epitopes of Dsg3 was studied after 7 days of in vitro stimulation by CFSEdye dilution (HC $n = 5$, PV $n = 15$). Additionally, a broad range of T helper cytokines was simultaneously assessed in these supernatants by cytometric bead arrays to investigate key T helper subclass involved in PV pathogenesis.

In the studied PV patients we found increased frequencies of memory CD4+ T cells with a chronically activated and stimulatory capacity. Several pro-inflammatory markers as CXCR3 and CD161, which may contribute to T cell recruitment in inflamed tissue, were upregulated in PV. In vitro, autoreactive CD4+ T cells proliferated in response to Dsg3 peptide stimulation dependent on presence of PV associated HLA-DRB1*04:02 and -DQB1*05:03 alleles. Here, the assay revealed low frequencies of Dsg3-reactive CD4+ T cells within PBMCs. Moreover, PV patients showed a heterogeneous reactivity, mostly for peptides within the EC3 domain and to a lesser extent against EC2 and EC1 domains of Dsg3. Preliminary results point towards a crucial role of Th2 cells in PV by enhanced IL-5 secretion in response to Dsg3 protein stimulation. Extension of detected analytes in supernatants by cytometric bead arrays will reveal more details about the identity of Dsg3-reactive CD4+ T cells in PV patients.

Collectively, our data profile autoreactive CD4+ T cells in PV introducing new treatment targets for modulation of inflammation.

P053 (OP04/02) | Circulating tumor derived cell-free RNA (cfRNA) as a potential biomarker in melanoma

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Background: Melanoma is one of the most common cancers with steadily increasing incidence and poor prognosis in advanced stages.

New treatment options have expanded the therapeutic landscape and significantly improved both overall and progression-free survival. Early detection of progression and selection of the right therapeutic regimen appear to be critical in the treatment of melanoma patients. Therefore, the identification of novel biomarkers that allow not only diagnosis, but also monitoring of treatment response in real-time could significantly improve outcomes for melanoma patients. Plasma-derived tumor-specific cell-free nucleic acids such as circulating cell-free tumor-specific RNA (cfRNA) are increasingly utilized as non-invasive and real-time biomarker approach in many solid tumors.

Objectives: The aim of this study was to test proof-of-principle gene candidates which showed significantly higher expression in melanoma vs. benign nevi tissues (KPNA2 (Importin subunit alpha-2), DTL (Denticleless E3 Ubiquitin Protein Ligase Homolog), BACE2 (Beta-Secretase 2), and DTYMK (Deoxythymidylate Kinase)) as potential universal cfRNA biomarkers across various melanoma genotypes.

Methods: We analyzed 344 plasma samples from 100 melanoma patients. Samples were collected before therapy and at standard follow-up time points. Absolute cfRNA copies of KPNA2, DTL, BACE2 and DTMYK were quantified on digital droplet PCR (ddPCR). We validated the diagnostic utility of our cfRNA candidates, where plasma samples from healthy donors (HD; n = 18) and psoriasis (PS; n = 20) served as controls.

Results: cfRNA levels of KPNA2, DTL, BACE2 and DTMYK did not correlate with demographic characteristics such as age and sex, histological subtype, tumor mutational state, and therapy line. More importantly, they were significantly higher in plasma samples of melanoma patients as compared to HD and PS ($p < 0.05$, receiver operating characteristics are under the curve (ROC AUC) >79%). Elevated baseline cfRNA levels of KPNA2, DTL, BACE2 and DTMYK were significantly higher in patients with distant metastases and proportionally increased with higher stage ($p < 0.05$). We found significant association between cfRNA copies and elevated clinical tumor markers LDH and S100 ($p < 0.05$), however, the correlation was weak to moderate (rho < 0.54). Finally, we found that KPNA2, DTL, BACE2 and DTMYK copies significantly increased during therapy in non-responders, while decreased in responders. Furthermore, increasing levels in DTMYK copies predicted significantly shorter progression-free survival (hazard ratio 0.47, $p = 0.0209$).

Conclusion: Our study demonstrates the potential diagnostic and treatment monitoring role of cfRNA in melanoma.

P054 | In vivo visualization of tattoo particles and tattoo complications using multiphoton tomography and fluorescence lifetime imaging

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Aim: In the last decades, the prevalence of tattoos is rising continuously worldwide. Numerous reports on tattoo complications raise awareness of health problems related to tattoo components. These tattoo reactions comprise a broad spectrum, including allergies, chronic inflammation and carcinogenic skin lesions. To diagnose and monitor the course of tattoo-based skin changes sufficiently, high-resolution noninvasive imaging techniques are necessary. This first preliminary study introduces the multiphoton tomography with fluorescence lifetime imaging (MPT-FLIM) in the assessment of in vivo tattoo particles and tattoo complications in human skin.

Methods: Different aged black and colored tattoos in three volunteers were analyzed using the multiphoton tomographic system MPTflex (JenLab GmbH, Jena, Germany).

Results: After new tattoo ink is injected, a high amount of the particles is being transported and scaled off from the papillary layer to the epidermal layer, simultaneously with the keratinocytes. Existing tattoo pigments are located in the papillary layer between collagen bundles. Distribution of these particles may vary throughout the papillary dermis, depending on the pigments used. In MPTFLIM, tattoo pigments appear as different sized particles with clustered or diffused organization. By generating high-resolution optical biopsies, morphologic changes of living cells and extracellular matrix in tattoo complications can be analyzed. Alterations in autofluorescence pattern and molecule specific fluorescence lifetime enable investigations of tissue metabolism.

Conclusions: MPT-FLIM may be a qualified non-invasive method in clinical practice to analyze in vivo tattoo particles and the course of tattoo complications. Optical biopsies not only visualize morphological alterations but also enable the imaging of metabolic activities of the human skin. This diagnostic tool may add an essential benefit to plan following therapeutical procedures and may act as an alternative to the conventional skin biopsy.

P055 | Systemic treatment response and response-prediction using skin microbiome and clinical data from the TREATgermany registry

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The identification of diagnostic, prognostic and predictive biomarkers is an essential task in the development and implementation of treatment in atopic dermatitis (AD). Therefore, the availability of high-quality patient samples with associated clinical data is central to this task. Here, we aimed to investigate associations of AD patients' skin microbiome, clinical data and treatment response in the TREATgermany registry. Skin swabs of 151 patients were profiled with 16S rRNA amplicon sequencing before and after treatment. Defined by a decrease in EASI of 75% (EASI-75) to treatment, 51% of patients classify as responders. After 3 months of treatment, responder skin samples showed a significant increase in alpha diversity and a more pronounced change in beta diversity compared to non-responders. Using the before-treatment abundance data as input we trained a random forest algorithm to predict responder status. By the inclusion of additional clinical parameters, we were able to further enhance the accuracy of the prediction to 72%. In conclusion, to successfully predict treatment responder status, we suggest a combined and machine learning based approach using microbiome and clinical data.

P056 | Evaluation of a sodium hypochlorite-containing wound gel in the treatment of split-skin donor sites

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Background: The use of split thickness skin graft in the therapy of wounds of different aetiology is an established operation. Further this operation is among the most frequent operations in Germany in the fields of dermatologic surgery. Chronic wounds represent a burden for the health care system; in the context of demographic change, the prevalence of chronic ulcers will continue to increase; at the same time, split-thickness skin grafting may become more important in this development. The donor site of the split thickness skin is usually a self-limiting wound. Advanced patient age and comorbidities can delay the healing of split-skin donor sites. In addition, the resulting wound is often accompanied by significant pain. (Feldmann 1991). The optimal wound care of the split skin donor site is therefore a subject of ongoing research.
Objective: To evaluate a wound gel containing sodium hypochlorite in the treatment of split thickness skin donor sites.
Methods: Patients were randomized in two groups and wound care was organized with and without the gel according to randomisation. Epithelisation of the wound was documented using "mskin", a tool to communicate with the patient. Further a hyperspectral camera was used to follow the healing and a wound score was obtained. Pain of the donor site was evaluated using a numeric analogue scale (0–10). Follow up visits took place 1 day preoperative and postoperatively at day 1, 5, further 1 and 2 weeks after the demission from the hospital.
Results: Interim evaluation of 16 Patients (11m:5f; age 18–84; mean 67.3). Pain throughout the day one day postoperative was lower in the cohort who were treated with the wound gel (2.63 vs. 5 NAS), further the change of the wound dressing was less painful (2.75 vs. 6.25 NAS). The epithelisation was in favour in the cohort with wound gel (17.5 vs. 18.5 days). St02 as the oxygen saturation in the tissue obtained by hyperspectral imaging climaxed one day after the operation and decreased in the follow up without reaching the initial values. An increasing wound score was seen in the process of epithelisation.
Conclusions: Adherence of dressing material provokes a painful dressing change and can lead to fresh epithelium being loosened again. The use of an antibacterial wound gel can reduce pain. In the interim evaluation, the healing time in the area of the donor site is also shortened.
Literature
Feldman DL, Rogers A, Karpinski RH. A prospective trial comparing Biobrane, Duoderm and xeroform for skin graft donor sites. Surg Gynecol Obstet. 1991;173:1–5.

P057 | EARLY emollient: A randomised controlled pilot trial of daily emollient use in children with high risk for atopic eczema

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Background: Small pilot studies provided initial evidence that early emollient application directly after birth could prevent atopic

eczema (AE). However, results were inconclusive and there is further need of studies confirming these initial results.

Methods and study design: We performed a two-center, parallel group, assessor-blind, randomized open study of emollient use in neonates at high-risk for developing AE. 50 newborns were randomly assigned (1:1) within day 1 and 21 after birth to the emollient (daily application of Lipikar Baume AP+ for the first year) or control group. Skin examinations, skin physiology measurements and biosampling were performed. The primary clinical outcome was the cumulative incidence of AE at 2 years.

Results: Preliminary results showed no significant differences of skin physiological parameters between the two groups, but linear regression analysis showed a trend of longitudinal treatment over time: TEWL remained constant in the emollient group, whereas it increased in the control group. Skin hydration increased in both groups, but was more pronounced in the emollient group. Additionally, we measured inflammatory mediators from tape strips by using multiplex ELISA analysis. We identified important Th1/2 and Th17 cytokines to be significantly altered at onset of AE. The identified proinflammatory AE onset signature could be partially reverted by early emollient application. Finally, we identified elevated levels of IL-8, TNFb and VEGFa at birth as possible predictive biomarkers for AE development.

Conclusions: Preliminary results show a trend towards lower TEWL values and increased SC hydration over time by early emollient treatment. In addition, early emollient treatment reduces proinflammatory cytokine levels in children.

P058 | An adipose-derived stem cell-engineered patch represents a promising treatment for chronic wounds

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Mesenchymal stem cell-based therapies are emerging as promising approach to promote cutaneous regeneration of chronic wounds. We assessed here the regenerative potential of a patch concentrating mesenchymal stem cells extracted from the human fat, i.e. adipose-derived stem cells (ASC), in a gelatin matrix. ASC cultures were derived from the abdominal/skin fat of four ischemic patients. Principal component analysis on microarray data revealed that ASC grown within the patch upregulate the transcription of genes important for wound healing, including VEGF, CXCL12 and FGF7. Secretome analysis confirmed the observed upregulations at the protein level. ASC-patches subcutaneously transplanted in immunodeficient Nu/Nu nude were early invaded by new vessels. Of note, transplanted mice did not form any tumors. A marked vasculogenesis was similarly demonstrated in a preclinical chick chorioallantoic membrane model when compared to empty matrix or with a matrix containing human fibroblasts. Finally, ASC-patches prepared from Wistar rats were used to treat full-thickness skin wounds created on the foot of syngeneic rats whose paw was rendered ischemic by surgical resection of the femoral artery. The ASC patch allowed a faster wound closure when compared to an empty matrix. Together, we provide here evidence that an ASC-containing patch represents a promising approach for the treatment of chronic wounds thanks to its healing and vasculogenic properties.

P059 | Merkel cell Polyomavirus (MCVyP) as a biomarker of treatment response in patients with Merkel Cell Carcinomas

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Merkel Cell Carcinoma (MCC) is an increasing but scarce skin cancer. It primarily affect immunocompromised and elderly persons. Known risk factors are UV-light exposure, fair skin and the integration of the Merkel cell polyomavirus (MCpyV) genome into Merkel cells. Since the discovery of its oncogenic driver capacity in 2008, it became clear that the majority of patients are MCpyV-positive. This discovery makes MCpyV as an ideal surrogate marker for the tumor. Therefore, we asked if MCpyV DNA in the plasma of patients with MCC is useful as a dynamic biomarker for monitoring treatment response.

For our study, we identified 65 patients and 77 formalin fixed paraffin embedded (FFPE) tissue specimens with MCC that were eligible for further analysis. In a first analysis, we examined 21 patients and 27 tissue samples by quantitative PCR (qPCR) and immunohistochemical staining (IHC). In the analysis of the tumor samples ($N = 24$), all of them were clearly identified as MCC based on the morphology and by CK20 staining. Viral DNA was detected in 19 of the 21 patients. IHC staining confirmed this result in 17 of the 19 cases. In addition, we were able to collect plasma samples and isolate cell-free DNA (cfDNA) from 11 of the 21 patients before initiation and during therapy. Examination of the amount of cfDNA in plasma revealed a dichotomy of the cohort, with one-half of the patients showing an initial increase in cfDNA following therapy initiation and the other half showing a decrease.

Our preliminary results are in agreement with other studies demonstrating that qPCR is superior to IHC staining in regard of sensitivity for the detection of MCPyV. We are now examining the cfDNA for the presence of viral DNA in 11 of the 21 patients receiving immunotherapy. Longitudinally collected samples are now analyzed by

digital droplet PCR (ddPCR). The target sequence will be the LT4 and LT2 gene of MCPyV, respectively. Based on the results of the qPCR we expect that the plasma viral load will correlate with treatment response in patients with MCC treated with anti-PD-1 or PD-L1 therapy.

P060 | Successful treatment of Netherton syndrome with dupilumab implicates a role for Th2 signaling in this type of ichthyosis

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Background: Netherton syndrome (NS) is a rare syndromic ichthyosis, characterized by erythroderma, ichthyosis linearis circumflexa, hair shaft defects and atopic diathesis. It is caused by biallelic SPINK5-mutations resulting in impaired skin barrier, an activation of the KLK5-PAR2-TSLP axis and increased serum IgE levels.
Objective: Due to insufficient treatment options treatment of NS is challenging. Within the scope of compassionate use therapy we assessed whether dupilumab, an antibody targeting IL-4Ra is effective and safe in NS.
Methods: Three adults with genetically confirmed NS were treated with dupilumab every other week and closely monitored. The outcome was measured by assessing NASA, IASI-S, IASI-E, EASI and IGA scores, DLQI, TEWL and laboratory tests including serum IgE, serum LDH and blood eosinophils.
Results: Clinical improvement was observed as early as week 8 and all scores continuously improved with treatment duration. NASA scores were reduced significantly at weeks 8, 16, 24 and 32 by 43.4%, 47.7%, 59.0% and 70.0% respectively. Likewise, IASI-S, IASE-E, EASI and IGA scores declined. Furthermore, DLQI clearly improved with ongoing dupilumab treatment and IgE levels declined steadily. In contrast, LDH levels and eosinophil counts remained unchanged. Decrease of serum IgE clearly correlated with improvement of disease severity scores. Importantly, no serious adverse effects and no bacterial skin superinfections were reported. After 24 months duration of treatment, dupilumab showed no loss of effectiveness in all patients, i.e., NASA scores remained constant.
Conclusions: In summary, these data implicate a role of Th2 inflammation in NS and point at dupilumab as promising new long-term treatment option for NS.

P061 | Full-body blue light irradiation improves pruritus in atopic dermatitis—A randomized placebo-controlled clinical trial (AD-Blue)

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Introduction: Irradiation with visible blue light (400–495 nm) is a promising and safe new treatment option in chronic inflammatory skin diseases such as psoriasis and atopic dermatitis (AD). Previous clinical trials on visible blue light emitted from various devices and covering different wave lengths in treatment of AD did not fulfil the current standards for objective evaluation of its benefits in humans.
Patients and methods: 87 AD patients were enrolled into this international, placebo-controlled, double-blinded, three-armed, prospective, randomized trial to investigate the efficacy and safety of full-body blue light devices (wavelengths: 415 nm and 450 nm, respectively) compared to placebo irradiation for treatment of AD.
Results: At baseline, disease activity of AD patients (65.8% female, age: 33.1 ± 13.6 years) was mild to moderate (EASI: 6.9 ± 8.0; SCORAD: 37.3 ± 14.4) with moderately impaired health-related quality of life and pruritus (DLQI: 9.8 ± 5.6; Itch-VAS: 5.5 ± 2.1). Irradiation with 450 nm effected significant improvement of itch (Itch-VAS: -1.6 ± 2.3; $p = 0.023$ vs. placebo). PO-SCORAD decreased significantly in the 415 nm arm (-11.5 ± 18.4; $p = 0.028$ vs. placebo). For other outcome measures (EASI, SCORAD, IGA, DLQI), treatment with blue light was not significantly different from placebo. Transcriptome analyses of skin biopsies, cytokine levels and Elispot assays from blood revealed effects on IL-31 signaling by blue light irradiation. No safety concerns were observed.
Conclusions: Full-body blue light irradiation did not improve objective signs of AD significantly but effected significant and clinically important decrease of pruritus in AD patients at a favorable safety profile. Its application in other pruritic skin diseases remains to be investigated.
ClinicalTrials.gov: NCT03085303
International Registered Report Identifier (IRRID): DERR1-10.2196/11911

P062 | Sex-disaggregated population analysis in patients with hidradenitis suppurativa

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Hidradenitis suppurativa (HS) is a common chronic inflammatory skin disease with a high medical need, which affects both sexes. The aim of our study was to identify sex-specific risk factors, comorbidity, clinical manifestations, and treatments in HS patients. To this end, a prospective, non-interventional, cross-sectional, monocentric study with 500 HS patients was performed. All patients were examined by dermatologists. Demographic, anamnestic, clinical data, and blood parameters were evaluated.

Our data show no significant differences in age at HS onset and in disease duration between female and male patients. Furthermore, no differences regarding the family history for HS were found between sexes, suggesting equal contribution of genetic factors to the disease onset. Regarding further risk factors for HS, central obesity was more frequent in women while extensive cigarette smoking and acne vulgaris were more commonly found among male patients. Analyzing the clinical manifestation of HS, the groin was more frequently involved in women while the axillae were more often involved in men. Women showed a higher number of skin sites with inflammatory nodules, whereas fistulas were observed more frequently in men. However, the HS severity evaluated by means of the Sartorius score did not significantly differ between the sexes. In line, blood leukocyte counts were similar in female and male patients, suggesting a comparable extent of inflammation in both groups. Regarding comorbidity, lower HDL-levels and higher blood glucose levels were significantly more frequent in men. Female patients were found to suffer significantly more often from back pain, especially in the neck/shoulder region and lower back. Surprisingly, there was no difference in HS treatment applied to female versus male patients.

In conclusion, our study indicates significant differences in HS risk factors, comorbidity, and clinical manifestation between female and male patients. Thus, sex should be taken into account in HS patient care.

P063 | Shift of the microbial composition profile in atopic dermatitis patients upon dupilumab treatment

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Introduction: Patients with AD show a higher colonization rate with *S. aureus* in lesional, but also non-lesional skin than healthy individuals. Moreover, *S. aureus* colonization has been suggested to promote disease severity.

Dupilumab is a selective IL-4Ra blocker approved for the treatment of moderate to severe atopic dermatitis.

In this study, we aimed to investigate the impact of long term dupilumab treatment on skin microbiota known to be dysregulated in atopic dermatitis patients

Results: In a real-life cohort 77 atopic dermatitis patients were longitudinally followed. The mean SCORAD decreased from 60 to 15 after 2 years of therapy. Skin swabs from 7 AD patients (lesional and non-lesional skin) and 7 healthy individuals were sequenced using the Illumina platform (16S). The relative abundance of *S. aureus* was higher in atopic skin and most prominent in lesional skin of AD patients. On the other hand, we observed a decrease in the relative abundance of C. acnes in atopic skin. Importantly, the abundance of S. epidermidis was stable among all groups.

The ratio of *S. aureus* to C. acnes was determined by RT-qPCR (standardized interscapular skin swabs) during the course of dupilumab treatment. It gradually decreased in a period of 24 months and returned to values that resemble healthy skin.

Conclusion: The ratio of *S. aureus* to *S. epidermidis* may provide an objective measurement of therapy effectiveness in atopic dermatitis. Further studies are currently underway to elucidate the mechanistic basis for this observation.

Dermato-Endocrinology

P064 | The alpha7 nicotinic acetylcholine receptor—A protective factor against endothelial to mesenchymal transition (EMT) in fibrosis?

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Systemic sclerosis (SSc) is a complex autoimmune disease involving the connective tissue leading to excessive production of collagen and microvasular injuries resulting in fibrosis of skin and other organs. Activated myofibroblasts, a mesenchymal cell population, play a central role in tissue remodeling leading to a fibrotic state. Interestingly, vascular endothelial cells can differentiate to extracellular matrix producing myofibroblast-like cells via a process called endothelial to mesenchymal transition (EMT). Previously, we reported that partial and full pharmacological agonists of the alpha7 nicotinic

acetylcholine receptor (alpha7nAChR) have a promising protective potential in human dermal fibroblasts (HDF) and experimentally induced skin fibrosis. Further, we have identified the alpha7nAChR as an essential mediator of the molecular action of the antifibrotic effect of alpha7nAChR in HDF and skin. Here, we aim to investigate a novel facet of the pathogenesis of fibrosis addressing to effect of alpha7nAChR on EMT in human dermal microvascular endothelial cells (HDMEC). Our first preliminary data proved the expression of the alpha7nAChR in primary HDMEC at RNA level using semiquantitative RTPCR. Further, the presence of this receptor could be confirmed at protein level via Western immunoblotting in HDMEC. Immunofluorescence analysis with a specific anti-alpha7nAChR antibody disclosed expression and a cell membrane associated localisation of this receptor in these cells. Finally, we demonstrated that the alpha7nAChR is functional in HDMEC by measurements of calcium influx using specific alpha7nAChR agonists and blocking experiments with alpha-bungarotoxin an alpha7nAChR antagonist. This preliminary work represents the basis for intensive future investigations of endothelial cells e.g. HDMEC in fibrosis to clarify the role of the alpha7nAChR in EMT and support therapeutic exploitation of alpha7nAChR receptor agonists in fibrotic skin diseases.

P065 | Fibroblast function in vitro can be modulated by the melanocortin tripeptide derivatives KdPT and WOL074-029

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We previously reported on the impact of the melanocortin peptide alpha-melanocytestimulating hormone (alpha-MSH) on fibroblast function including experimentally induced skin fibrosis. Truncated peptides from the C-terminal domain of alpha-MSH as well as some derivatives have persevered immunomodulating effects but do not bind to the melanocortin-1 receptor (MC-1R). Thus, they do not elicit MC-1Rmediated pigmentation as an obligatory adverse effect of MC1R agonistic peptides. Among the melanocortin tripeptide derivatives is Lys-Pro-d-Thr (KdPT) which not only suppressed interleukin-IL-1beta-mediated expression of proinflammatory cytokines but also exhibited beneficial effects in animal models of inflammatory bowel disease as well as in patients with Crohn´s disease. Here we investigated as to whether KdPT and another more stable derivative, WOL074-029 are capable of suppressing fibroblast activation induced by the profibrotic cytokine transforming growth factor-beta1 (TGF-beta1). Both KdPT and WOL074-029 suppressed mRNA expression of collagen type 1, fibronectin I and alpha-smooth muscle actin in human dermal fibroblasts compared with TGF-beta1 alone as shown by real-time RT-PCR analysis. Expression of connective tissue growth factor was likewise suppressed. These effects were dose-independent and detectable in part at subnanomolar levels of both peptides. In accordance with these findings, secretion of collagen type I was significantly suppressed by KdPT and WOL074-029 using procollagen type I peptide ELISA. Mechanistically, both peptides did not interfere with canonical smad2/3 signalling as shown by Western immunoblotting and immunofluorescence analysis. Interestingly, expression of PepT2 but not PepT1 were found to be expressed by human dermal fibroblasts raising the possibility that KdPT and WOL074-029 elicit the observed effects by means of these tripeptide transporters. Future studies are in progress to further define the mode of action of KdPT and WOL074-029 in fibroblasts and the in vivo relevance of these findings.

Dermatopathology

P066 | Efficient homology-directed repair CRISPR/Cas9-based gene editing in junctional epidermolysis bullosa keratinocytes

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Homology-directed repair (HDR) CRISPR-Cas9 based gene editing can be used as a tool for precise mutation repair in the treatment of genetic disorders. Epidermolysis bullosa (JEB), a severe skin disorder manifested by skin blistering, is caused by mutations in the genes that encode dermal-epidermal junction proteins. A twobase-pair deletion mutation within exon 52 of the COL17A1 gene leads to junctional epidermolysis bullosa (JEB). In this study, we used HDR as a gene-editing approach to treat JEB keratinocytes either wild-type Cas9 or catalytically impaired Cas9 (Cas9n) ribonucleoproteins (RNP) together with symmetric or asymmetric single-stranded oligonucleotides (ssODN), that served as HDR templates. As analyzed by next-generation sequencing, in RNP-nucleofected keratinocytes we achieved 43% and 22% repair efficiency with wild-type Cas9 or Cas9n, and HDR oligonucleotides, respectively. High correction efficiencies were also shown at mRNA, protein and cellular level together with correct deposition of type XVII collagen within the basal membrane zone of 3D skin equivalents. These data could serve as a proof of concept for the potential treatment of JEB via traceless COL17A1 gene editing.

P067 | The C5a/C5a receptor axis as a potential target in bullous pemphigoid

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Bullous pemphigoid (BP), the most frequent autoimmune blistering disorder (AIBD), is characterized by autoantibodies against the hemidesmosomal proteins BP180 (type XVII collagen) and BP230. Complement activation at the dermal-epidermal junction is an immunopathological and diagnostic hallmark of BP. The anaphylatoxin C5a is a key mediator of the complement response, exerting its effector functions through binding to C5aR1 and/or C5aR2. C5aR1 has a proinflammatory role in diverse autoimmune diseases, though the function of C5aR2 is still enigmatic. Here, we aimed to assess the role of C5aRs and complement components in BP patients' skin and blood in more detail. To address this aim, perilesional skin biopsies of BP patients, representative for the early phase of autoantibody-mediated skin inflammation, were subjected to RNA sequencing and immunohistochemistry. Skin biopsies matched for biopsy sites, age, and sex in control patients with noninflammatory dermatoses. In addition, plasma levels of major soluble complement factors and complement modulating factors were measured in BP patients and controls by ELISA. To further investigate C5aR-mediated AIBD-related effector functions in vitro, naïve human leukocytes were activated with either Col17-specific IgG immune complexes or C5a and their functional responses were subsequently analyzed. Interestingly, transcriptome analysis showed upregulated expression of C5aRs and complement-associated genes in BP skin. Supporting these observations, double immunofluorescence staining revealed that C5aR1 in BP skin was predominantly expressed on T cells and macrophages. In contrast, C5aR2 was mainly expressed on mast cells and eosinophils. Evaluation of systemic complement activation by ELISA demonstrated significantly higher plasma levels of C3a and lectin pathway components in BP patients than in controls. Selective inhibition of neither C5aR1 nor C5aR2 affected release of reactive oxygen species (ROS) from immune complex-stimulated neutrophils. However, pretreatment of neutrophils with specific C5aR antagonists reduced their migration towards C5a. Collectively, this study improves our understanding of the complement network in BP and identifies C5aR1/R2 as potential targets in BP and, potentially, other complement-mediated AIBD.

P068 (OP02/05) | A new mouse model showing a psoriasis-like phenotype

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Psoriasis is an immune-mediated inflammatory skin disease that is associated with multiple medical conditions and affects more than 60 million people worldwide. Given the urgent need to improve current therapeutic strategies, establishing new mouse model of psoriasis reproducing key features of the human disease, would extensively contribute to the development of new medications. Keratin 79 (KRT79), expressed in the suprabasal cells of the infundibulum, has been poorly characterized. To establish a new driver mouse line expressing the tetracycline transactivator (tTA) under the control of the Krt79 promoter, exons 1 and 2 of the Krt79 gene were replaced by the cDNA of tTA. Unexpectedly, heterozygous mice showed induration, desquamation and erythema on skin of the mice, resembling a psoriatic phenotype. Preliminary data suggest that the phenotype is due to an unintentional loss-of-function mutation of an enhancer in intron 1, which is currently being characterized. Histologically, the epidermis was thickened with enlarged sebaceous glands and hair follicles. Likewise, cell proliferation markers such as Ki67 and proliferating cell nuclear antigens were highly expressed in the skin of the Krt79wt/tTA mice. Compared with wild type (WT) littermates, Krt79wt/tTA mice showed increased levels of sebum production and skin angiogenesis. Skin inflammation was demonstrated by enhanced level of interleukin (IL) 1A, IL1B, IL6 and increased number of mast cells in Krt79wt/tTA mice. Compared with WT littermates, an elevated white blood cell count, in particular neutrophils, monocytes, mast cells, and basophils, was detected in Krt79wt/tTA mice. Moreover, Krt79wt/tTA mice exhibited higher weight of some secondary lymphoid organs such as axillary lymph node and spleen. Immunofluorescence staining of mouse skin for KRT5, KRT10, and loricrin revealed an increased expression of all three differentiation markers in Krt79wt/tTA mice compared with WT littermates. Furthermore, expression of leucine-rich repeats and immunoglobulin-like domains 1 (LRIG1) was enhanced in the epidermis of Krt79wt/tTA mice pointing to an increase of epidermal stem cells. RNA-Seq analysis of the skin showed differential expression of many genes with known importance to the pathophysiology of psoriasis such as caspase recruitment domain family member 14 (Card14), tumor necrosis factor (Tnf), signal transducer and activator of transcription 3 (Stat3), and transforming growth factor (Tgf). Further pathway analysis depicted that IL17 and nuclear factor kappa B (NFKB) signaling pathway-associated genes are

differentially expressed in Krt79wt/tTA mice compared with WT littermates. Consistent with RNA-Seq results, an increased level of IL17A and IL17F proteins in skin as well as sera of the Krt79wt/tTA mice were identified. Collectively, we generated a new mouse model of psoriasis which recapitulates most phenotypic, histologic, and immune-pathogenic processes of psoriasis. It has a great potential to increase our understanding of the pathomechanisms involved in psoriasis and to investigate novel therapeutic agents.

P069 (OP02/04) | An unexpected role of Schwann cells in keloid formation

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Keloids are characterized by a constant expansion beyond the margin of the original skin wounds. The driving forces behind this itching, painful and movementrestricting scar type are, however, only poorly understood. To obtain novel insights into the pathology of keloids, we investigated in the present study keloids on a single cell resolution. Single cell RNA sequencing (scRNAseq) confirmed the characteristic accumulation of fibroblasts and endothelial cells, but also uncovered a yet undescribed population of Schwann cells (SC) in keloids. Interestingly, these SCs showed a completely changed phenotype when compared to normal skin and normal scars-derived SCs. Whereas myelinating and non-myelinating SCs constituted the SC population in healthy skin, the majority of keloidal SCs showed a repair-like phenotype. In addition, these keloidal SCs expressed a variety of factors involved in extracellular matrix formation, blood vessel development and wound healing. Comparison of our data set with data sets of three independent research groups corroborated our findings, and led to the identification of a set of fifteen genes which are characteristic for repair SCs in keloids. Immunofluorescence confirmed our scRNAseq data and showed that keloidal SCs were spread throughout the whole dermal layer in a scattered, disorganized way. Moreover, these SCs were not associated with axons. As repair SCs are known to interact with macrophages, we next investigated the macrophage populations present in keloids in more detail. We were able to identify several factors, including CCL2, MMP9, CCN3, IGFBP5, strongly deregulated in keloids. Interestingly, the reciprocal interplay of these factors has been reported to inhibit SC differentiation and affect macrophage polarization, both contributing to exacerbated matrix formation. Together, our findings indicate a strong contribution of SCs to keloid formation. Targeted therapy of keloidal SCs might therefore represent a new treatment option for keloids.

P070 (OP03/03) | Local accumulation of iron in the skin affects crosstalk and phenotypes of immune and tissue cells

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In this project, we focus on chronic venous insufficiency (CVI), a disease where patients present Lipodermatosclerosis and valve dysfunction leading to erythrocytes extravasation and iron accumulation in the lower dermis, conditions predisposing to leg ulcer formation. For decades, researchers studied CVI and chronic venous ulcer (CVU) and iron has been considered as an important pathological factor in this context, especially in relation to oxidative stress and ROS formation. However, a clear understanding on the pathogenic effects of iron on the tissue network and the crosstalk of resident immune and skin tissue cells in the course of CVI is still missing. At first, we collected skin samples of CVI/CVU patients and confirmed via Prussian blue staining a massive accumulation of iron in the dermis. Then, we generated a mouse model of local iron-overload via intradermal injection of iron dextran (FeDX) to mimic the accumulation of iron in the skin. H&E and Prussian blue staining proved that iron accumulates in the lower dermis of these mice similar to patient samples. Immunofluorescence staining showed in the iron-treated skin an expansion of F4/80+ macrophages confirmed by FACS analysis that also disclosed a shift in F4/80+ macrophage subtypes. In fact, in F4/80+ cells we found up-regulated CX3CR1+ and Ly6C+ (in F4/80+ med), and CCR2+ (in F4/80+ high) populations and TNFa induction while M2-like factors as CD301 and Relma were down-regulated. These data together with up-regulated gene expression of IL1ß and HMOX-1 in the skin cells indicate a shift in macrophages profile and a pro-inflammatory activation in the iron-overloaded skin. In order to better dissect the response of macrophages to iron-overload we mimicked erythrocyte accumulation in CVI-bothered skin in vitro by co-culture of primary human M2- like-differentiated macrophages with autologous erythrocytes. Gene expression microarray analysis revealed a shift in the gene signature towards pro-inflammatory activation states and an up-regulation of inflammatory pathways and iron-related genes. Hence, cytokine analysis confirmed a clear pro-inflammatory activation of erythrocyte-overload macrophages with an increased release of TNF, IL-6, IL-12, but decreased IL-10. Further immunohistochemistry and FACS analysis of the skin of our local iron-overload mouse model revealed an increased cellularity of the lower dermis, which we linked to an expansion of fibroblasts, in particular to a population of CD140a+ (PDGFRa)/Sca- cells. In addition, dermis of the iron-treated skin presented less collagen and down-regulated expression of main extracellular matrix (ECM) genes like Col1A1, Col3A1 and EDA-FN. To unravel the impact of iron-overloaded macrophages on the observed alterations of fibroblasts, we transferred conditional medium of erythrocyte-fed macrophages to human dermal fibroblast (HDF) culture, which enhanced the proliferation of the cells. At the same time, HDFs treated with

conditional medium of erythrocyte-fed macrophages presented a downregulation of typical genes related to ECM production and an induction of PDGFRa expression. In conclusion, our data suggest that local iron-overload cause a complex skin phenotype with a maintained inflammatory status, enhanced proliferation of immune and tissue cells, and induced a shift in resident macrophage and fibroblast subtypes. Lately, we are investigating the relation between PDGFRa fibroblast subset, macrophages overloaded with iron and adipocytes in the dermal white adipose tissue (dWAT) and the fibrotic changes in the skin architecture of these iron mice.

P071 | The translatome of junctional epidermolysis bullosa

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Epidermolysis bullosa (EB) is an umbrella term for a large group of genodermatoses with mild to severe phenotypes. The leading cause for junctional EB (JEB), one of the most severe manifestations of EB, are pathogenic mutations affecting the skin-anchoring protein complex laminin-332, a crucial component of the basement membrane bridging the epidermis und the dermis (1,2). At present, there is neither a cure nor a treatment for the most critical cases of this devastating disease. While the underlying genetic causes of JEB are usually well defined, other molecular aspects of the disease—such as epigenetic alterations or changes in proteostasis—are mainly unexplored (3).

Studies of the translatome are a growing research topic, providing not only important insights into the proteostasis of various organs but also revealing the coding potential of genomic regions that were previously assumed to be non-coding. Improved methods recently uncovered a new class of genes expressing functional microproteins that previously escaped detection. These microproteins where shown to play important roles in the context of development, physiology and human disease (4,5). So far, no translatome studies in JEB have been reported and the status of proteostasis as well as the contribution of microproteins and unconventional reading frames during disease progression remain unexplored.

Here, we describe the translatome of JEB using a CRISPR/Cas9 engineered, homozygous JEB keratinocyte model and Ribo-Seq-based ribosome profiling, a modern NGS technology. We adapted the Ribo-Seq protocol to measure ribosome protected footprint abundance and identified active translation at nucleotide resolution in annotated protein-coding as well as non-coding regions (lncRNA, uORFs and smORFs) (6,7). Our data highlight the impact of JEB-associated genetic mutations on perturbations of the keratinocyte translatome, compared to healthy controls, beyond the loss of functional lamin-332 expression. Ultimately, these differences could be important predictors of JEB disease progression that should be closely monitored during future therapeutic interventions.

Keywords: epidermolysis bullosa, microprotein, translation, crispr/cas9, ribosome profiling, translatomics

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P072 | Characterization of DIRAS1 in skin homeostasis and tumorigenesis

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Skin cancer is the most common type of cancer worldwide and incidence of melanoma and non-melanoma skin cancer has increased tremendously in recent years. The members of the GTP-binding protein Di-Ras (DIRAS1-3) family are RAS-related small G-proteins, but only little is known about its biological function besides its potential action as a tumor suppressor in glioblastoma, renal cell carcinoma, and squamous cell carcinomas (SCC). DIRAS1 is strongly expressed in the cortex, cerebellum, spinal cord, pituitary gland, adrenal gland, testis, and skin. Even though DIRAS1 is expressed in SCC, its function in cutaneous SCC (cSCC) is still completely unknown, but it is assumed that DIRAS1 could act as tumor suppressor in cSCC. The aim of this study is to investigate the biological function of DIRAS1 in the skin homeostasis and tumorigenesis in vivo and in vitro.

In order to determine the impact of DIRAS1 in vivo, we generated a transgenic mouse model and overexpressed Diras1 under the control of the ubiquitous chickenbeta- actin (CBA) promoter. We confirmed an overexpression of DIRAS1 in several organs of the transgenic animals (CBA-Diras1) compared to control mice using real-time quantitative PCR (RT-qPCR) and western blot analysis. Diras1 transgenic mice were viable, showed no macroscopic

phenotype, grow normally, bred in a Mendelian ratio, and the skin revealed no histological alterations. To investigate if DIRAS1 acts as a tumor suppressor in the skin we will perform with our transgenic mouse model an inducible 7,12-dimethylbenz(a)anthracene (DMBA) and 12-Otetra- decanoylphorbol-13-acetate (TPA) two-stage skin carcinogenesis and UV-light irradiation experiment.

Surprisingly, transgenic mice showed a higher relative and absolute lung weight compared to control littermates. Histopathological findings showed perivascular lymphocytic infiltrates in the lung and islets of Langerhans of the pancreas. Cell culture experiments will complete the results from our in vivo experiments. We want to investigate the impact of DIRAS1 in a human keratinocyte cell line (HaCaT), human cSCC cell line (A431), and human melanocyte cell line (A375), by generating CRISPR/Cas9 knockout cell lines for DIRAS1.

P073 | Psoriatic inflammation modulates mechanotransduction and cell adhesion in human epidermis

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Aberrant mechanotransduction and disrupted epithelial barrier function are associated with numerous human pathologies including inflammatory skin disorders. However, the involved molecular mechanisms are not well understood. We investigated the alterations in cell-cell adhesion, mechanosensitive effector molecules and mechanosignaling induced in human epidermis during inflammation using psoriasis as a model disease. Primary human keratinocytes and immortalized human keratinocytes N/TERT were grown in 2D and 3D cultures (epidermal equivalents) and stimulated with "M5" cytokine cocktail (IL-17A, IL-22, Oncostatin M, TNFa, IL-1a) to induce psoriatic phenotype in vitro.

The treated cells exhibited disrupted adherens junctions, decreased beta-catenin and E-cadherin expression and increased intercellular gaps. Same phenotype was observed in lesional psoriatic skin. Consistent with this, experiments on epidermal permeability showed a disruption of epidermal barrier in 2D and 3D cultures.

M5 cytokines enhanced intracellular contractile forces via phosphorylation of myosin light chain (MLC) and induced nuclear translocation of major mechanotransduction regulators YAP/TAZ, which are crucial for cell proliferation and differentiation. Inhibition of MLC kinases ROCK1 and ROCK2 with Y-27632 further disrupted adherens adhesions and surprisingly induced stronger nuclear YAP in M5-stimulated cells. Another inhibitor KD025 specific for ROCK2 has been shown to improve scores of psoriasis patients in clinical trials via changes in cytokine production; therefore we tested if its mechanism of action also involves effects on keratinocyte mechanotransduction and cell adhesion. KD025 restored normal cell junctions and inhibited nuclear translocation of YAP in M5-stimulated cells.

In conclusion, we show that a psoriasis-like cytokine milieu reduces cell-cell adhesion integrity that is directly implicated in the barrier function. Our data also indicate that YAP mechanosignaling in keratinocytes can be induced by inflammatory cytokines independently of MLC phosphorylation. ROCK2 inhibitor KD025 is able to suppress YAP signalling and restore cell junctions in keratinocytes, revealing a complementary mechanism by which it can improve debilitating effects of cutaneous inflammation.

P074 | Improvement of experimental bullous pemphigoid by targeting the IL-17A axis is not dependent on neutrophils and macrophages

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Bullous pemphigoid (BP), the most common autoimmune blistering skin disease, is mediated by autoantibodies targeting type XVII collagen (BP180). The binding of anti-BP180 IgG to the dermal-epidermal junction, complement activation, and the influx of inflammatory cells into the upper dermis are prerequisites for blister formation. Treatment of BP is still based on unspecific long-term immunosuppression/ immunomodulation. IL-17A has been identified as a key regulator in several chronic inflammatory diseases. Previously, we showed that IL-17A-deficient mice are largely protected by the pathogenic effect of anti-BP180 IgG. In addition, we identified CD3+ T cells as a major source of IL-17A in early skin lesions of BP patients. Here, we aimed at clarifying the contribution of the cells targeted by IL-17A using the well-established antibody transfer mouse model of BP. IL-17RA-/- and C57BL/6J wild-type (WT) mice were injected with affinity-purified rabbit anti-murine BP180 IgG. IL-17RA-/- mice were protected from the otherwise pathogenic effect of anti-BP180 IgG, confirming our previous findings. As expected, no difference in the intensity of IgG deposits by direct immunofluorescence was detected between the two groups, and in IL-17RA-/- mice, the inflammatory infiltrate in the upper dermis was less pronounced compared to WT animals. Since neutrophils and macrophages were previously considered important drivers of anti- BP180 IgG-mediated tissue destruction in this model, we applied this model in LysMCre+ IL-17RA-/- deficient of myeloid cells, particularly neutrophils and monocytes/ macrophages. LysM-Cre+IL-17RA- mice showed a similar severity of skin lesions as LysM-Cre-IL-17RA compared to control mice, suggesting that IL-17RA expression on neutrophils and macrophages is

not crucial for mediating the pathogenic effect of IL-17A in BP. No difference in the density and composition of the inflammatory cell infiltrate in the upper dermis was observed between the two groups. In future experiments, the pathogenic contribution of IL-17RA on T cells and keratinocytes may identify the main effector cell type(s) targeted by IL-17A. These data will be valuable to pave the way to more selectively target the IL-17A/IL-17RA axis in the fragile patient population of BP.

P075 | Specialized histological and histomorphometrical analytical methods for biocompatibility testing of biomaterials in (pre-) clinical studies

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Background and Methods: For the comprehensive analysis of complex tissue reactions, both preclinical in vivo analysis and clinical trials are essential in order to show overall compatibility of biomaterials or medical devices. Thereby, analysis regarding the cell types interacting with the inserted biomaterial, its integration or degradation behavior, vascularization patterns within the implant bed and immunological reactions are of special interest. In this context, the preferred test methods differ depending on the respective test material, its, composition, character and field of application. We provide a brief overview of the main workflows of the various processing and analysis methods used in preclinical and clinical biopsies as they are applied by both beginners and more experienced researchers in the field of biomaterials science.
Results: Beside the histological processing of a sample (embedding, cutting as well as histochemical and immunohistochemical staining), additional qualitative and quantitative analyses supplement the steps required in order to be able to adequately assess the biocompatibility of biomaterials. We present the currently most common variants of the above-mentioned procedures in their processes, preferred areas of application as well as their respective advantages/disadvantages and complemented these with advices for the application of individual analytical methods We were also able to draw attention to various potential sources of error within the respective test procedure and to point out strategies for error reduction.
Conclusion: In summary, it can be stated that all techniques presented in this work are suitable for the adequate and systematic assessment of tissue-specific reactions to biomaterials. Although there are a number of potential systemic sources of error in the processing of the various preparation and analysis steps, these methods have a sufficient suitability for obtaining well-founded test results and the quality of their informative value can be improved even further over time, as the examiner's experience increases.

P076 | Human skin organ culture is a model for spontaneous skin ageing

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Skin ageing is significantly influenced by exogenous risk factors such as solar UV, diet, smoking or alcohol consumption. Despite the growing understanding of the highly complex interactions between different cell types and signalling pathways in chronological (intrinsic) aging, the development of potent anti-ageing cosmetics failed due to the lack of clinically relevant testing models. Since the current in vitro approaches using cultured cells cannot recapitulate normal skin physiology and aging in its complexity, human ex vivo whole skin organ culture (hSOC) remains the most instructive model for pre-clinical testing. Here, we present intriguing results showing that biopsies from healthy subjects intrinsically age over a 3-day culture in a well-defined medium. We found decreased cell proliferation, reduction in mitochondrial activity as well as Collagen 17 and Sirtuin-1 expression, and elevated γH2A.X levels in epidermal keratinocytes. In the dermis, we observed decreased dermal hyaluronan and collagen I levels. Gene expression analysis revealed up-regulation of metalloproteinases, inflammatory cytokines IL6 and IL8, the chemokine CXCL10 as well as the cell cycle regulator/senescence marker CDKN1. Furthermore, media supplementation with caffeine and tretinoin, well-known anti-aging compounds, reduced the senescence-associated γH2A.X accumulation. In addition, caffeine increased dermal collagen I protein levels and prevented CXCL10, IL6, IL8, MMP2 and CDKN1 upregulation. In summary, our data suggest that skin biopsies spontaneously undergo aging/senescence in the hSOC system. We also present a proof-of-concept showing that the spontaneous hSOC ageing system can be used for assessing novel compounds for their anti-aging properties in a cost-effective, pre-clinical model.

P077 | Unraveling the role of extracellular matrix modifications in skin inflammation

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The extracellular matrix (ECM), which is composed of a mix of macromolecules in varying combinations, builds a complex meshwork of fibres and associated glycoproteins. Previously it was thought that the ECM mainly has structural, supportive functions. However, recent studies showed that regulation of matrix modifications, its biochemical properties and architecture are important factors to maintain tissue homeostasis. Collagen is the major ECM component

of the basement membrane and the dermal interstitial matrix. Enzymatic inter- and intramolecular crosslinking, which result in changes to the ECM geometry, structure and mechanical properties, can affect tissue-resident immune cell adhesion and migration. We hypothesized that inflammation-induced hypoxia can result in ECM modifications and thereby maintain chronic inflammatory skin diseases by influencing cell migration and tissue repair. Therefore, we would like to investigate whether the ECM is changed in inflammatory skin diseases, and if hypoxia may be a driving factor. We further want to explore molecules that modify the biochemical properties and architecture of the ECM, such as lysyl oxidase (LOX), one of the most prominent regulators of ECM topography. Here, we present a novel image analysis tool to investigate expression levels of HIF-1 and LOX in human healthy and psoriatic skin samples. We developed a semi-automated, customized analysis pipelines for cell and organelle segmentation and quantification in FiJi, machine learning-based programs and R to obtain localized intensity values. This pipeline will allow unbiased analysis of skin samples in batch mode and future findings will contribute to a better understanding of the role of hypoxia and matrix modifying molecules in skin tissue homeostasis and inflammation.

P078 | Characterization of type IIb autoimmune chronic spontaneous urticaria markers (CAPTUM)

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Introduction: Chronic spontaneous urticaria (CSU) is held to be autoimmune (aiCSU, also known as type IIb CSU) in a subpopulation of patients, i.e. associated with mast cell-targeted and -activating autoantibodies. Markers for aiCSU remain poorly characterized. The aims of our project are (i) to develop a comprehensive overview of the markers/features of aiCSU that have been published to date, (ii) to understand how these parameters are linked, and (iii) to identify gaps of knowledge. Here, we analysed the association of the best single tests for aiCSU, the basophil activation test (BAT) and basophil histamine release assay (BHRA), with clinical and laboratory characteristics of CSU.

Materials and method: We performed a systematic and comprehensive literature search of the electronic database MEDLINE/Pubmed. Evidence profiles were developed, and the relationship between the results of basophil tests and predefined clinical parameters of CSU was assessed by correlation analyses.

Results: In total, 1060 relevant CSU studies were identified and their full-texts were downloaded. Of them, more than 90 studies reported BAT and/or BHRA results and other CSU parameters, and 40 of them included at least 50 patients. The analyses of these 40 reports showed that positive BHRA or BAT results are significantly associated ($p \leq 0.05$) with at least one of the following characteristics: high disease activity ($n = 10$), low disease control, and longer duration, positive ASST ($n = 6$), increase after cyclosporine A treatment ($n = 1$), or other factors ($n = 12$). Of the 40 studies analysed, 22 and 18 performed the BHRA and BAT, respectively; only 2 performed both tests. Regarding the type of assays, there was also high variability in the technique and cut-off values used among the studies.

Discussion: Our results suggest that CSU is more severe in patients with a positive BAT/BHRA, a known marker of aiCSU. It is also clear the need to standardize the available techniques to unify diagnosis. The strength of this association and the supporting evidence will be analysed within the framework of the CAPTUM project.

Epidemiology

P079 | Somatization in skin diseases—Results from a population-based study

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Background: Psychodermatology has primarily focused on depression and anxiety, and studies investigating somatization in adults with skin diseases and allergy are rare. Somatization has been conceptualized in many different ways, but is generally considered as the tendency to experience psychological distress in the form of somatic symptoms and to seek medical help for these symptoms. Evidence indicates that the skin might be particularly vulnerable to the development of somatization, and both somatization and skin diseases have been linked to impaired health-related quality of life (HRQoL). Until now, epidemiological data regarding the relationship between somatization, skin diseases, allergy and HRQoL are limited. Using data from a general population sample of adults, the present study pursued two research aims: (i) To investigate the association of skin diseases and allergy with somatization, (ii) To analyze the association of somatization with physical and mental HRQoL in individuals with skin diseases and allergy.

Methods: Data from 3048 participants from the population-based Study of Health in Pomerania (SHIP) Trend were analyzed (aged 20 to 83 years; 51.3% female). All individuals participated in a standardized

dermatological examination. A history of atopic eczema, psoriasis, allergy, allergic asthma and hay fever was assessed in a standardized face-to-face dermatological interview. Using a self-administered questionnaire, we assessed somatic symptoms (24 items from the Zerssen Complaint list) and physical and mental HRQoL (Short Form 12 Health Survey). Linear and logistic regression analyses adjusted for age and sex were conducted.

Results: Our analyses revealed that allergy (Odds Ratio [OR] 1.04; 95% Confidence Interval [CI] 1.02, 1.07) and allergic asthma (OR 1.12; 95% CI 1.06, 1.18) were significantly related to a higher number of somatic symptoms. Psoriasis, hay fever and atopic eczema were not significantly associated with the number of somatic symptoms. Among our population, 938 (30.8%) individuals had allergy or allergic asthma, and reported on average four somatic symptoms. Further analyses in this subgroup showed that a higher number of somatic symptoms was related to diminished physical and mental HRQoL (OR -1.24; 95% CI -1.36, -1.12 and OR -0.95; 95% CI -1.09, -0.81, respectively).

Conclusions: The results of the present study have important implications for clinical practice. First, our data indicate that somatization is a significant comorbidity in particular in individuals with allergy and allergic asthma, and show that somatization is a determinant of impaired HRQoL in these individuals. Second, our findings suggest that somatic symptoms should be evaluated in individuals with allergic diseases. Thereby, our results may contribute to the improvement of screening practices, prevention, patient counseling, and treatment.

P080 | The association of childhood maltreatment with skin diseases and allergy in adult age: Results from a population-based cohort study

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Background: Since the seminal work of Felitti and collegues in the late 1990s—known as the Adverse Childhood Experiences (ACE) study—numerous studies have demonstrated the devastating consequences of childhood maltreatment (CM) for physical and mental health in adult age. Until now, little is known about the impact of CM on skin and allergic diseases, and particularly studies in the adult general population are limited. However, to improve patient care and to promote screening practices, prevention and treatment, precise data about CM prevalence in individuals with skin and allergic diseases are critical. Contributing to research in this field, the present study aimed to investigate the relationship between different types of CM with skin diseases and allergy in the adult general population.

Methods: Data from 3018 participants from the population-based Study of Health in Pomerania (SHIP) Trend were analyzed (aged 20 to 83 years; 51.5% female). All individuals underwent a standardized dermatological examination. Self-reports of atopic eczema, psoriasis, allergy, allergic asthma and hay fever was obtained from a standardized face-to-face dermatological interview. The self-administered Childhood Trauma Questionnaire (CTQ) was used to assess CM experiences (emotional abuse and neglect, physical abuse and neglect, sexual abuse). Logistic regression analyses adjusted for age and sex were conducted to investigate the association of CM types with skin diseases and allergy.

Results: The proportion of individuals who had experienced at least one type of CM was 51.9% (n = 467) in allergy, 52.4% (n = 176) in hay fever, 55.1% (n = 43) in atopic eczema, 60.2% (n = 142) in psoriasis and 61.5% (n = 72) in allergic asthma (total study population: 54.2%). We found that physical neglect was significantly associated with allergy (Odds Ratio [OR] 0.92; 95% Confidence Interval [CI] 0.85, 0.99) and hay fever (OR 0.95; 95% CI 0.90, 0.99).

Conclusions: Our data revealed substantial rates of CM experience in particular among participants with psoriasis and allergic asthma, emphasizing that CM is a significant public health problem. Giving support to previous studies demonstrating that CM is linked to allergy and higher hay fever prevalence in adult age, our results suggest that physical neglect might be involved in the etiology of allergy and hay fever. Overall, the present findings indicate that evaluating a history of CM might be appropriate in healthcare for individuals with dermatological and allergic diseases.

P081 | Effectiveness, safety and drug survival of dupilumab in patients with moderate to severe atopic dermatitis: an interim analysis of the TREATgermany registry

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Background: In clinical trials, safety and efficacy of therapies are tested in selected patient populations. Clinical registries like the atopic dermatitis (AD) multicentre registry TREATgermany provide the opportunity to collect longitudinal data on patients to generate evidence on the effectiveness and safety of therapies under real-life conditions, and provide information on many other aspects of patient management in clinical routine.

Methods: TREATgermany is a prospective observational registry that collects clinical and molecular data of atopic dermatitis patients receiving or eligible for systemic treatment. Patients are recruited in 39 study centers comprising both hospitals and private practices. Here, we describe results from patients included in the registry from June 2016 to April 2021 with a special focus on those who received a systemic therapy with the anti-IL4R antibody dupilumab.

Results: Between 06/2016 and 04/2021, 1095 patients (mean age 41.1 years, 42.7% female, mean oSCORAD 40.94 ± 16.08, mean EASI 16.32 ± 12.96 at baseline) were enrolled in the registry. Of these patients, 831 received one or more systemic treatment for AD. Over two-third (n = 675) had received treatment with dupilumab. 298 of these got their first prescription during the observation period of the registry and had at least one follow-up visit after 3 months. At the first follow up visit after initiation 75.46%, 51.28% and 23.08% had reached an EASI50, EASI75 and EASI90, respectively. After 6 months, 82.42%, 63.19% and 31.32% had reached an EASI50, EASI75 and EASI90. At month 12, response rates were 87.50%, 68.27% and 40.38%. Of the 298 patients treated with dupilumab, 26 (8.7%) discontinued treatment during the observation: 14 (53.8%) due to side effects, 6 (23.1%) due to ineffectiveness, 2 (7.7 %) due to patient's request and 5 (19.2%) due to other reasons (multiple reasons possible). In 34.56% of the dupilumab-treated patients a treatment-related adverse event (AE) was reported. 26.8% of the dupilumab-treated patients developed ocular side effects. 173 of the dupilumab patients were followed up at least 24 months. After 2 years 147 still continued with the treatment after 2 years, 26 discontinued. None of them discontinued in the first 6 months, 5 between month 6 and 12 and 21 between month 12 and 24. The drug survival rate was 97.1% after 12 months and 85.0% after 24 months of treatment with dupilumab. Other systemic therapies were prescribed less often, e.g. cyclosporine was initiated in 13.2% of the patients.

Conclusion: In conclusion, dupilumab appears to be a safe and effective long-term therapy for patients with moderate to severe AD under routine conditions, and has emerged as most commonly used systemic treatment in TREATGermany patients. Ocular side effects were more common than in trials, but rarely led to treatment discontinuation. Overall, drug survival rates for dupilumab were high.

P082 | Content validity of RECAP in Dutch, English and German to measure eczema control in young people with atopic eczema: cognitive interview study

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Background: Recap of atopic eczema (RECAP) is a patient-reported outcome measure (PROM) assessing eczema control. This instrument has been developed and validated in the UK. There is a self-reported and a proxy-reported version in English, Dutch and German. However, it is unclear whether the self-reported version shows adequate content validity when completed by young people in these languages.

Objectives: To assess the content validity (comprehensibility, relevance and comprehensiveness) of the English, German and Dutch versions of the self-reported RECAP in young people with atopic eczema and to identify the most appropriate age cut-off for self-completion.

Methods: We conducted 23 semi-structured cognitive interviews with young people from 8 to 16 years, using the "think-aloud" method. In Germany and the Netherlands, participants were recruited in dermatology clinics and in the UK through social media and existing mailing lists. Interviews were audio recorded, transcribed verbatim and analysed in the three languages, using a problem-focused coding manual. Transcripts were coded by two independent reviewers in each country. Themes were translated into English and compared across the three countries.

Results: Significant age-related comprehensibility issues with the last three items of the questionnaire occurred with young people aged 8 to 11 years, causing difficulties completing RECAP without help. However, older children had only minor problems and were able to complete the questionnaire by themselves. Due to language specific issues, some translational changes were made to the German version without altering the meaning of the questions. The self-reported version of RECAP has sufficient content validity for self-completion in young people aged 12 years and above. However, the German version with its translational adaptations is appropriate for young people from the age of 8 years.

Conclusions: The self-reported version of RECAP is appropriate for use from the age of 12 years. The proxy-version could be used in children younger than 12 years. Other measurement properties should be further investigated.

P083 | Hyperhidrosis Quality of Life Index (HidroQoL): Further validation by applying classical test theory and item response theory using data from a phase III b randomized controlled trial

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Background: The Hyperhidrosis Quality of Life Index (HidroQoL) is a well-developed and validated patient-reported outcome measure assessing the quality of life impacts in hyperhidrosis. Our aim was to assess the psychometric properties (especially structural validity) of the HidroQoL in patients with primary axillary hyperhidrosis in order to extend the already existing validity evidence.

Methods: Data from a phase III b randomized placebo-controlled clinical trial were used. Confirmatory factor analysis (CFA) was conducted to confirm the two a priori scales of the HidroQoL within the classical test theory (CTT). Furthermore, the assumptions of the Rasch model (model fit, monotonicity, unidimensionality, local independence) and Differential Item Functioning (DIF) were assessed using modern test theory.

Results: The sample included 529 patients with moderate to severe hyperhidrosis. The two-factor structure could be confirmed by CFA. Using item response theory (IRT), the item characteristic curves showed mainly optimally functioning response categories, indicating monotonicity. The overall fit to the Rasch model was adequate and unidimensionality for the HidroQoL overall scale could be confirmed. However, local independence was slightly violated in few cases. DIF analysis, controlling for age or gender, was critical for four and three items, respectively, however, this DIF could be explained.

Conclusion: Using CTT and additional IRT/Rasch analyses, this study provided further evidence for the structural validity of the HidroQoL questionnaire using data from a phase 3b trial. The findings underline the excellent measurement properties of the HidroQoL and are consistent with results of previous validation studies.

Genetics

P084 | Xeroderma pigmentosum: Genetic und functional analyses of eight new patients

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Xeroderma pigmentosum (XP) is a rare, autosomal recessive DNA repair defect syndrome with a worldwide prevelance of 1:1,000,000. XP can be subdivided into eight complementation groups: XP-A to XP-G, involved in nucleotide excision repair (NER), and XP variant (XPV), coding for the translesional DNA polymerase η. The NER is necessary for the repair of UV-induced DNA damage such as cyclobutane pyrimidine dimers (CPD) and (6–4) photoproducts (6,4-PP). Therefore, it is a natural protective mechanism against UV-induced skin cancer. XP patients suffer from premature skin aging, sun sensitivity and the development of skin cancer in early childhood.

The Clinic and Policlinic for Dermatology and Venerology in Rostock is a reference center in the European Reference Network for rare skin diseases (ERN skin) focusing on DNA repair defect syndromes such as XP, Cockayne syndrome and trichothiodystrophy. We offer diagnostic analyses such as sequencing of the potentially affected genes and functional testing of patients' cells via e.g. Host Cell Reactivation (HCR) Assay. At the moment, we are analyzing cells from eight new XP patients regarding their genetic background and the influence of the mutations on DNA repair capacity. Until now, we were able to assign one patient to the complementation group XP-C, two patients to XP-D and one patient to XPE. These analyses help to further understand the molecular causes and functional consequences of XP.

P085 | Quality of life and clinical characteristics of self-improving congenital ichthyosis within the disease spectrum of autosomal recessive congenital ichthyosis

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Autosomal recessive congenital ichthyosis (ARCI) is a heterogeneous group of ichthyoses presenting at birth. Self-improving congenital ichthyosis (SICI) is a subtype of ARCI and is diagnosed when skin condition improves remarkably (within years) after birth. So far, there is sparse data on SICI and quality of life (QoL) in this ARCI subtype.

This study aims to further delineate the clinical spectrum of SICI as a rather unique subtype of ARCI.

This prospective study included 78 patients (median age: 15 years) with ARCI who were subdivided in SICI (n = 18) and non-SICI patients (nSICI, n = 60) by their ARCI phenotype.

Quality of life (QoL) was assessed using the (Children's) Dermatology Life Quality Index.

The genetically confirmed SICI patients showed causative mutations in the following genes: ALOXE3 (8/16; 50.0%), ALOX12B (6/16; 37.5%), PNPLA1 (1/16; 6.3%) and CYP4F22 (1/16; 6.3%). Hypo-/anhidrosis and insufficient Vitamin D levels (<30 ng/ml) were often seen in SICI patients. Brachydactyly (a shortening of the 4th and 5th finger) was statistically more frequent in SICI (p = 0.023) than nSICI patients. A kink of the ear's helix was seen in half of the SICI patients and tends to occur more frequently in patients with ALOX12B mutations (p = 0.005). QoL was less impaired in patients under the age of 16, regardless of ARCI type.

We conclude that SICI is an underestimated, milder clinical variant of ARCI including distinct features such as brachydactyly and kinking of the ears. Clinical experts should be aware of these features when seeing neonates with a collodion membrane. SICI patients should be regularly checked for clinical parameters such as hypo-/anhidrosis or vitamin D levels and monitored for changes in quality of life.

P086 | MC4R mediated melanocortin signaling antagonizes central endogenous opioid signaling to cause high pain thresholds in genetic mouse models of red hair

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Humans and mice with natural red hair have altered pain sensation, increased thermal pain thresholds and an increased sensitivity to opioid analgesics. Here, we investigated the mechanisms responsible for higher nociceptive thresholds in various genetic mouse models, focusing on red-haired mice resulting from a loss of melanocortin 1 receptor (MC1R) function in non-albino and albino backgrounds. Furthermore, we used a number of mouse models that vary in the number of epidermal melanocytes (K14SCF mice and MITFmiwh mice) to demonstrate the role of epidermal melanocytes in the regulation of pain.

We found melanocyte-dependent but melanin-independent changes that underlie the increase in pain thresholds. We found that Pro-opiomelanocortin (POMC) transcription is regulated by MC1R in melanocytes and MC1R loss of function results in decreased POMC transcription and diminished systemic melanocyte stimulating hormone (MSH) levels in the plasma of red-haired (Mc1re/e) mice. The decrease in peripheral α-MSH de-represses central opioid signaling that is mediated by the opioid receptor OPRM1, which results in increased nociceptive thresholds. Using MC4R KO mice, we identified MC4R as the major MSH-responsive receptor in vivo that is responsible for opposing central OPRM1 signaling under physiologic conditions. Using bioinformatic analyses we have identified a number of brain regions that coexpress MC4R and OPRM1 that potentially contribute to the altered pain sensitivity in the red-haired genetic background. We validated the periaqueductal grey area (PAG) in the brainstem as one of the central areas of opioid/melanocortin antagonism in red-haired mice using microcannulation of the PAG and pharmacologic approaches.

This work demonstrates the physiologic role of MC1R, melanocytes and circulating melanocortins in the regulation of pain and provides a mechanistic framework for the altered opioid signaling and pain sensitivity in red-haired individuals.

While our study focuses on the red hair phenotype, the underlying melanocortin/ opioid signaling balance may also account for pain variations among non-red-haired individuals. Individuals with MC4R polymorphisms may also have elevated pain thresholds and altered sensitivities to analgesics similar to those reported in red-haired individuals. Lastly, modulation of physiologic MC4R signaling might offer a novel therapeutic approach to modulate pain—even in non-red-haired individuals.

P087 | Subcellular compartmentalization of STIM1 may distinguish Darier disease from Hailey-Hailey disease

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Darier disease (DD) and Hailey-Hailey disease (HHD) are autosomal dominant keratinization disorders. They are caused by mutations in the ATPase, Sarcoplasmic/ Endoplasmic Reticulum Ca2+ Transporting 2 (ATP2A2) and ATPase Ca2+ Transporting Type 2C, Member 1 (ATP2C1) gene, respectively. The dysfunction of these calcium pumps disturbs calcium metabolism and homeostasis in keratinocytes, which interferes with keratinization and adhesion molecule functions. Clinically, DD is characterized by keratotic papules developing primarily in the seborrheic areas of the trunk, extending to confluent verrucous and macerated plaques. HHD usually manifests in the intertriginous body sites with oozing erythematous plaques covered with greasy scales. Histologically, acantholysis with variable degrees of dyskeratosis and parakeratosis can be observed in both disorders. Although both diseases can usually be differentiated clinically and histopathologically, their routine distinction is not always easy. In these instances, molecular genetic diagnostics can be helpful. However, not all affected patients harbor ATP2A2 or ATP2C1 mutations.

To solve this diagnostic challenge, we studied the differential expression of two proteins of store-operated calcium entry (SOCE), stromal interaction molecule 1 (STIM1) and calcium release-activated calcium modulator 1 (ORAI1) to examine if they might serve as a novel diagnostic approach and biomarker for the unambiguous differentiation of the two disorders.

Five individuals (P01-P05) who presented to our outpatient clinic with symptoms of DD and/or HHD and ambiguous diagnostic findings were examined. Patients with a clear diagnosis of DD or HHD, respectively, served as controls. Clinical examination, Histopathology, Molecular genetic analysis and Immunohistochemistry for STIM1 and ORAI1 was performed.

In patients with genetically confirmed DD, STIM1 expression was consistently observed in the cytoplasm. In contrast, patients with genetically confirmed HHD revealed a shift of the otherwise homogeneous cytoplasmic expression towards a membrane-bound staining pattern.

Our results suggest that subcellular compartmentalization of STIM1 may be a novel biomarker for the distinction of the two disorders.

P088 (OP05/05) | Morpholino-mediated knockdown of γ-secretase-subunit Nicastrin causes hypopigmentation due to disturbed melanoblast development and indicates evolutionarily distinct roles in zebrafish and humans

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Mutations in the genes that encode the human γ-secretase subunits Presenilin-1, Presenilin Enhancer Protein 2, and Nicastrin (NCSTN) are associated with familial hidradenitis suppurativa (HS). Recently, we have shown that, (i) mutations in the gene PSENEN, which encodes Presenilin Enhancer Protein 2, can also give rise to the hereditary pigmentation disorder Dowling-Degos disease, with or without comorbid HS and (ii) inactivation of the zebrafish homologue psenen causes generalized hypopigmentation through disturbed melanophore distribution. To investigate the consequences of targeted inactivation of ncstn, the zebrafish homologue of human NCSTN, morpholino (MO)-mediated ncstn-knockdown was performed and led to generalized hypopigmentation. Two-photon excitation microscopy and time lapse in vivo-imaging revealed that this phenotype resulted from melanoblasts and melanocytes that were of irregular size and shape, formed atypical aggregates of pigmented cells in the head region, were unphysiologically stuck in uncommon anatomical sites, and had imperfectly developed dendritic cell protrusions, as compared to wild-type larvae. Next, we sought to rescue the phenotype through co-injection of ncstn-MO with wild type zebrafish ncstn or human NCSTN mRNA and to determine the effects of co-injection of four human NCSTN mutations with a previously reported causal association with familial HS: c.632C>G (p.P211R), c.497C>A (p.S166X), c.278delC (p.93Lfs*15), and c.1101+1G>A (p.E333_Q367del). MO-mediated ncstn-knockdown resulted in generalized hypopigmentation through a significant reduction in melanophore size and number, and alterations in their patterns of migration and distribution. This phenotype was rescued by co-injection of zebrafish ncstn RNA, human NCSTN RNA, or a construct encoding the human NCSTN missense mutation c.632C>G (p.P211R). Our results provide evidence that the evolutionarily conserved homologous ncstn protein regions are sufficient in terms of phenotype rescue. Human NCSTN mutations encoding null alleles confer loss-of-function regarding pigmentation homeostasis in zebrafisch. In contrast, the human missense mutation p.P211R was less harmful, asserting sufficient residual ncstn activity to maintain

pigmentation in zebrafish. Since fish lack the anatomical structures, which are affected by HS our data suggest that the zebrafish ncstn gene and the human NCSTN gene have most likely acquired different functions during the course of evolution. In fish, one major role of ncstn is the maintenance of pigmentation homeostasis. In contrast, one of the roles of NCSTN in humans is the prevention of inflammatory processes in the adnexal structures of the skin, as seen in familial HS.

P089 (OP06/01) | Blood transcriptome profiling identifies two candidate endotypes of atopic dermatitis

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Despite robust evidence for a systemic inflammatory component of atopic dermatitis (AD) such as altered serum protein, metabolome and NK cell profiles, only a very limited number of blood transcriptome analyses are available for the disease.

Whole blood samples before and 12 weeks after initiation of treatment with dupilumab from 49 adults with moderate to severe AD from the TREATgermany registry and an independent case-control cohort of 31 AD patients and 40 healthy controls were subjected to mRNA sequencing.

Based on a machine learning approach, we determined a subset of 19 genes that classified patients into two distinct clusters with striking differences in particular for transcripts involved in eosinophil signalling. Compared to healthy controls and in contrast to the "eosinophil-low" endotype, the "eosinophil-high" endotype was characterised by a pronounced global transcriptomic dysregulation, and disease activity as well as intensity of itch positively correlated with eosinophil signatures centred to IL5 signalling. Overall, clinical improvement under treatment with dupilumab was accompanied by a decrease of innate immune response, and an increase of lymphocyte signatures including B cell activation and NK cell composition and/or function. The proportion of super-responders reaching EASI90 after 3 months was slightly higher in the eosinophil-low endotype (32% vs. 11%). Continued downregulation of IL18RAP, interferon gamma, and granzyme A in the eosinophil-high endotype suggests a residual disturbance of NK cell function despite clinical improvement.

The results of this first large-scale analysis of the blood transcriptome show that moderate-to-severe AD has a strong systemic inflammatory component and provides preliminary evidence for blood biomarker-stratified endotypes.

P090 | Generation and characterization of different CRISPR/Cas9-mediated XPA knockouts in A375 melanoma cells

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Introduction: The treatment of melanoma has been revolutionized by the discovery of immune checkpoint inhibitors (ICIs), in particular anti-PD-1 antibodies. A reliable predictive biomarker for response to ICIs does not yet exist. In addition to specific parameters, such as an increased PD-L1 expression or interferon-gamma signature, DNA repair defects have been associated with a better response to anti-PD-1 ICIs.

Xeroderma pigmentosum (XP) is an inherited DNA repair defect syndrome of the nucleotide excision repair (NER), that is exclusively responsible for the repair of UV-induced DNA lesions. Case reports of the use of anti-PD-1 ICIs for the treatment of melanoma have shown impressive responses of XP patients, however, the exact underlying mechanisms are not yet clear.

Hence, to explore these mechanisms, we generated different knockouts of XPA, the central coordinator of the NER, in A375 melanoma cells using the innovative CRISPR/Cas9 method.

Methods and preliminary results: Target sequences in different exons of XPA were determined and cloned into vectors. By transient transfection of A375 cells with these vectors and subsequent antibiotic selection, polyclonal cell lines were generated, which was followed by single clone expansion.

Sequencing showed that homozygous and compound heterozygous knockout cell lines were generated.

Further examinations by Western blot and immunofluorescence were able to prove the knockout and supported the sequencing results.

The abolished expression of full-length XPA was further reflected by a diminished repair of UV-C induced DNA lesions in the host cell reactivation and the post-UV cell survival assays.

Outlook: Further experiments will include investigations of the immunological effects of these XPA-knockouts. We already generated promising first results by flow cytometry and Western blot, which will be repeated and further evaluated.

In a following step, we will correlate functional effects of the XPA-knockouts with the different CRISPR target sequences and the resulting types of DNA defects.

The most promising candidates will be used for further research on the role of the NER for the response to anti-PD-1 ICIs.

P091 | Treatment of Netherton syndrome with omalizumab

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Netherton syndrome (NS) is a rare autosomal recessive ichthyosis caused by biallelic loss-of-function mutations in the SPINK5 gene encoding the lymphoepithelial Kazal-Type-related protease inhibitor (LEKTI). NS is characterized by ichthyosiform erythroderma or ichthyosis linearis circumflexa, hair shaft abnormalities and atopic manifestations with high IgE levels.

We evaluated the clinical and immunological response of NS to treatment with omalizumab, a recombinant, humanized, monoclonal antibody against human IgE. Two patients (one 48-year-old male, one 17-year-old female) were treated with omalizumab for 8 months and monitored in a subsequent 2-month follow-up.

Treatment response was assessed by using the Eczema Area and Severity Index (EASI), the Ichthyosis Area and Severity Index (IASI), the Dermatology Life Quality Index (DLQI), degree of erythema and serum IgE levels. Furthermore, immunohistofluorescene, immunohistochemistry and real-time PCR were used to evaluate immune cell infiltration and cytokine expression in skin.

Although clinical scores were reduced during the first 3–4 months of treatment, this effect was transient and the improvement was not sustained during the second half of the 8 months monitoring period and the follow-up period. Infiltration of mast cells, T cells, neutrophils and macrophages as well as expression of pro-inflammatory cytokines TNF-α, IL-1β, IL-6, IL-23A and IL-17A were decreased in the male patient with more severe phenotype, but not in the female patient. Serum IgE levels remained unchanged.

Our case series on the use of anti-IgE therapy in NS shows initial clinical improvement with reduced skin inflammation, particularly in the patient with more severe disease, but no long-lasting therapeutic benefit. Further studies including additional patients are required.

Health Services Research

P092 | 3D human skin models of inflammatory skin diseases psoriasis and atopic dermatitis

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Aim: Psoriasis (Ps) and atopic dermatitis (AD) are prominent skin diseases accompanied by inflammatory events. While Ps is associated with an imbalanced immune response toward TH1 and TH17 immune responses, AD is associated with enhanced TH2 immunity. Because mouse and human skin differ in cellular architecture and physiology it is difficult to extrapolate from mouse studies to humans. Using disease-associated cytokines, our group has established 3D human skin equivalents (HSE) that mimic Ps and AD well.

Material and methods: HSEs are generated out of primary human keratinocytes and fibroblasts derived from fore skin. Inflammatory conditions were induced treating HSEs with cytokine cocktails (TH17: IL-17 and -22 (Ps-model) or TH2: IL-4, -5 and -13 (AD-model)) for 3 days during airlift cultivation. Disease phenotype formation was validated histologically and by qPCR.

Results: HSEs consist of a cellular dermis and comprise the epidermis with stratified differentiated keratinocytes and a well-developed stratum corneum. The Ps and AD phenotype is successfully induced by treating HSEs with cytokines. Cytokine stimulation results in abnormal terminal differentiation and induces changes in structural protein expression. In both skin disease models a thickening of epidermis is detectable and elafin expression is clearly increased. In the Ps-model expression of further structural proteins including psoriasin, hBD1, hBD2 and involucrin is increased, while involucrin reduction is detectable in the AD model.

Discussion: Inflammatory HSEs mimic in vivo changes quite well and can be reliably used as an alternative to animal testing to study pathogenesis and new treatment strategies. We will further improve HSEs by integrating TH1-, TH17- or TH2-polarized T cells to more closely simulate the physiological situation of both skin diseases.

P093 | Automatic skin area measure and classification of dermatologic diseases due artificial intelligence

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Dermatologists estimate the affected skin region to track progression or regression of a skin disease to decide about further treatment. In classic dermatological diseases such as atopic dermatitis, psoriasis and cutaneous T-cell lymphomas, the affected area plays

a decisive role in the severity of the disease. Various scores of the individual dermatological diseases take into account the proportion of the skin surface affected to a large extent. This estimation is often arbitrary and, especially in an ambulant setting with alternating dermatologists, unstandardized. An automatic segmentation using machine learning algorithms can tackle this problem. Using a diverse dataset of skin photographs, we show that a segmentation model can be trained in a semi-supervised fashion to precisely outline the affected skin area and therefore can be used to determine the affected area. Our model is capable of exploiting standardized and unstandardized, as well as close-up and total photographs. We validate the clinical benefit by tracking patients and quantifying the disease over the course of time. We further use the model to characterize dermatological diseases using a contrastive learning approach. This combination of segmentation and classification of dermatological diseases could be used to perform presorting and prioritization by artificial intelligence as the number of patients in dermatological outpatient departments continues to increase.

Immunology

P094 | Overexpression of S100A9 in obesity impairs macrophage differentiation via TLR4-NFkB-signaling worsening inflammation and wound healing

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In obesity the fine-tuned balance of macrophage phenotypes is disturbed towards a dominance of pro-inflammatory macrophages resulting in exacerbation and persistence of inflammation and impaired tissue repair. However, the mechanisms of obesity-mediated dysregulation of macrophage differentiation are still poorly understood. Herein, we demonstrate an overexpression of S100A9 in conditions of obesity-associated disturbed macrophage differentiation in the skin. We show that saturated free fatty acids (SFA), which are increased in obesity, together with S100A9 induce inflammasome-dependent IL-1β release in macrophages that in turn amplifies S100A9 expression in skin inflammation in obesity. We reveal a yet unrecognized impact of obesity-associated S100A9 overexpression on macrophage differentiation. S100A9 binding to TLR4 and activation of NFkB attenuates development of M2-like macrophage and induces pro-inflammatory functions in these cells. Consequently, inhibition of S100A9 restores disturbed M2-like macrophage differentiation in mouse models of obesity-associated skin inflammation and wound repair. Breaking the viscous cycle of S100A9 overexpression by reduction of SFA has similar effects. Improvement of skin inflammation and wound repair underlines the pathogenic role of S100A9 overexpression in obesity.

Collectively, this study identifies S100A9 as a previously unrecognized vital component in obesity-associated disturbed macrophage differentiation. The findings open new opportunities for therapeutic implications for inflammatory diseases and wound repair in obesity.

P095 | Stress signaling and STAT1 activation characterize the keratinocytic gene expression pattern in Hidradenitis suppurativa

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The underlying pathogenetic factors generating the innate immune signal necessary for T cell activation, initiation and chronification of Hidradenitis suppurativa (HS, also known as Acne inversa) are still poorly understood. Emerging evidence suggests that defective keratinocyte function critically contributes to HS disease development and progression. To elucidate the role of keratinocytes in HS lesion formation, we compared the transcriptomes of isolated lesional and perilesional HS epidermis by RNA sequencing.

We show that HS is characterized by a strong epidermal stress state as evident by a significant overrepresentation of an AP-1-driven stress signature in the overall gene expression pattern of lesional keratinocytes and a substantial activation of the stress-activated cJun N-terminal kinase (JNK) pathway in lesional HS epidermis. Additionally, our data reveal a strong induction of STAT1 activation in lesional HS epidermis that likely results from IFNγ production and governs the expression of key inflammatory genes that coordinate activation of innate immunity and the adaptive T cell response in HS. Taken together, these data implicate a new role of combined stress signaling and JAK/STAT1 pathway activation in disease progression of HS suggesting interference with JAK/STAT1 signaling as a potentially promising therapeutic approach for HS.

P096 | The transcription factor CEBPB is a novel hub gene and multi-functional disease driver in skin inflammation

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Transcription factors represent key nodes that integrate signaling pathways to drive a plethora of downstream cellular responses. In chronic inflammatory skin diseases (CISD), different transcription factors have emerged as crucial players in the pathogenesis. The CCAAT/

enhancer-binding protein beta (CEBPB) is a well-known transcription factor that is sensitive to various immunogenic stimuli. In this study, we investigate CEBPB as a novel master transcription factor in keratinocytes and aim at dissecting its functional role in skin inflammation.
Using a novel approach that combines transcriptomic data with deep clinical phenotyping of CISD patients, we identified CEBPB as novel factor associated with skin inflammation. In spatial transcriptomics, bulk RNA Seq and IHC analysis, CEBPB was significantly upregulated in the lesional skin of Lichen planus, atopic dermatitis and Psoriasis (Pso) patients compared to non-lesional skin, with the strongest levels in Pso. Similarly, in vitro stimulated primary human keratinocytes showed the strongest CEBPB induction under stimulation with Pso-relevant cytokines on both RNA and protein level, hence implying a potential role of CEBPB in Psoriasis. Using a 3D psoriatic skin model, we show that loss of CEBPB by CRISPR/ Cas9 knockout (KO) completely inhibited the development of acanthosis, diminished the number of Ki67+ proliferating keratinocytes, reduced mitochondrial density and ATP levels indicating a downregulated metabolism. Moreover, CEBPB KO reduced secretion of neutrophil attracting chemokines. In line, CEBPB levels positively correlated with clinical scores of acanthosis and neutrophil infiltration. Lastly, we generated a CEBPB target gene signature under different inflammatory conditions allowing to evaluate the therapeutic potential of CEBPB.
In summary, we show that CEBPB is associated with various pathogenic hallmarks of inflammatory skin diseases, hence proposing it as a novel regulatory node in skin inflammation and a control point for pathogenic epithelial response. Our findings hold substantial promise for the use of CEBPB as new therapeutic target in skin inflammation.

P097 (OP02/03) | Low numbers of cytokine transcripts drive inflammatory skin diseases by initiating amplification cascades in localized epidermal clusters

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Abundant heterogeneous T cells infiltrate chronic inflammatory diseases and characterization of these cells is needed to distinguish disease-driving from bystander immune cells. Here, we investigated the landscape of non-communicable inflammatory skin diseases (ncISD) by spatial transcriptomics resulting in a large repository of 52,000 spatially defined human cutaneous transcriptomes of 18 patients. As expected, T cells infiltrated lesional skin, however, they produced a rather low frequency of only 1–10 pathogenic T cell transcripts of IL-17, IL-13 or IFN-g per skin section. Nevertheless, the cytokine expression presented in a disease-specific pattern and could be validated using single cell RNAseq and in situ hybridization. Despite their low frequency, cytokine transcripts evoked specific responder signatures in direct proximity showing that single cytokine transcripts initiate amplification cascades of thousands of specific responder transcripts forming localized epidermal clusters. Thus, within the abundant and heterogeneous T cell infiltrates of ncISD, only a few T cells drive disease by initiating an inflammatory amplification cascade in their local microenvironment.

P098 | Immunovirotherapy drives a convergent evolution towards interferon responsive, dedifferentiated cell states in cancer

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Tumor growth is associated with genomic heterogeneity and phenotypic plasticity that enable proliferation and survival under varied microenvironmental conditions [Zahir et al Nat Gen 2020, Bai et al 2019 Nat Rev Clin Oncol 2019; Marine et al Nat Rev Cancer 2020]. Cellular anti-tumor immunity promotes the emergence of cancer cell subpopulations with heightened resistance to cytotoxic destruction through a shift towards dedifferentiated cell states in inflammatory environments [Landsberg et al Nat 2012; Tumeh et al Nat 2014; Mehta et al Cancer Discov 2018] and genetic selection of specific mutations that impair cytokine responsiveness and antigen presentation [Zaretsky et al NEJM 2018; Keenan et al Nat Med 2019]. The evolutionary dynamics and the relative importance of inflammation-induced reprogramming versus the acquisition of genomic alterations in cancer cells during combinatorial immunotherapeutic interventions is incompletely understood. Here we show using immuno-virotherapy of melanoma as a paradigm, that the evolutionary trajectory of cytokine-dependent genetic- and non-genetic resistance mechanisms converges towards interferon responsive, de-differentiated cell state. We generated a model, where tumors could resist therapy by both genetic- and non-genetic means and observed that interferon signaling deficient MHC-Ilow

cancer cells were targeted by endogenous NK cells and MHC-Ihigh cells by T cells. T cell therapies selected for the mutated MHC-Ilow subpopulation, but unexpectedly, antigen loss via dedifferentiation was noted only after eradicating the subpopulation with oncolytic virotherapy indicating hierarchical evolution of resistance. Finally, undifferentiated melanoma cells were found to resist oncolysis through increased type I interferon responsiveness, which was epigenetically regulated by cross-antagonism of MITF and c-JUN transcription factors. The evolved mechanisms counteracting the loss of interferon responsiveness in MHC-Ilow and MHC-Ihigh cells provide explanation why their genetic selection is rare. Moreover, our work demonstrates that insights into the evolutionary dynamics of therapy resistance can guide the future design of personalized combination therapies.

P099 | The T-cell compartment of the human anal mucosa

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The anal mucosa constitutes an unique immunological environment, involved in a variety of sexually transmitted infections. Thus, host protection in this tissue is crucial for human health. A defective immune response in the anal mucosa is assumed, for instance, in the development of human papillomavirus (HPV)-induced anal cancer, precancer (anal intraepithelial neoplasia) and benign condylomata acuminata in HIV-infected (HIV+) individuals. However, knowledge regarding the unique immunological environment of the anal mucosa is limited. In this project, we aim to understand the memory T cell response in this environment. For our investigations, we have established protocols to generate single-cell suspensions from HPV-infected mucosa samples from HIV- and HIV+ individuals and analyze them by multicolor flow cytometry. Subpopulations of tissue-resident T memory cells (TRM) as well as T cells from peripheral blood are characterized using a panel of T cell markers (CD4, CD8, CD69, CD103, CD27, CD45RA) in combination with functional markers, including IFN-γ, IL-17A and IL-10. Data from our interim analysis reveal differences in the CD69 and CD103 pattern of CD8+ T cells in the anal mucosa compared to normal skin. The majority (mean 52%) of CD8+ T cells in the anal mucosa is CD69+CD103+, while in normal skin the majority is CD69+CD103-. CD8+CD103+ T cells have been associated with improved prognosis in several tumor entities, which makes further investigation of this subpopulation interesting. In this regard, our study can be important to determine, if a defective T memory cell response, in particular in the tissue-resident T memory cell (TRM) compartment of the anal mucosa, contributes to a failure of HPV clearance in HIV.

P100 | Suppression of cAMP formation by inhibitor-loaded polypept(o)ide micelles inhibits melanoma growth by preserving an inflammatory myeloid cell phenotype

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The acidic tumor microenvironment in melanoma drives immune evasion by upregulating cyclic adenosine monophosphate (cAMP) in tumor-infiltrating monocytes. Release of non-toxic concentrations of the adenylate cyclase (AC) inhibitor MDL-12 from peritumorally injected poly(sarcosine)-block-poly(L-glutamic acid γ-benzyl ester) (polypept(o)id) copolymer micelles represses melanoma growth. In combination with selective, non-therapeutic regulatory T cell depletion, AC inhibitor micelles achieve a complete remission of established B16 melanoma. Single-cell sequencing of melanoma-infiltrating immune cells shows that AC inhibitor micelles reduce the number of anti-inflammatory myeloid cells and checkpoint receptor expression on T cells. AC inhibitor micelles thus represent an immunotherapeutic measure to counteract myeloid cell-driven melanoma immune escape.

P101 (OP05/04) | Cytokine-induced senescence in melanoma cells is associated with a high proinflammatory secretome

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The incidence of malignant melanoma is increasing worldwide, making it a growing healthcare issue. Less than 10 years ago, biochemotherapy allowed for an overall survival of around 10 months in disseminated malignant melanoma. Fortunately, therapeutic options for management of malignant melanoma are plentiful today. The central pillar is the immune checkpoint blockade (ICB) with anti-PD-1 and anti- CTLA-4 antibodies, which is mediated by the T helper 1 cell cytokines interferon (IFN)-γ and tumor necrosis factor (TNF). Although 57.6% of melanoma patients respond to ICB, there remains an urgent need for additional effective therapeutic options. Cell cycle inhibitors like palbociclib are FDA approved for different tumor entities and are already being used in off-label curative trials in melanoma patients. An important commonality of chemotherapy, ICB and cell cycle inhibition is that they induce not only tumor cell killing but also cellular senescence.

Senescence is a cellular stress response and is primarily characterized by a stable growth arrest. Other features of senescent cells are an enlarged and flattened cell body or the secretion of cytokines, chemokines and growth factors which is termed senescence-associated secretory phenotype (SASP). Senescence can be induced in several different ways, e.g. via oncogenes, ionizing radiation or replicative stress. It is known that the SASP depends on the inducer. In this study, we induced senescence in two human melanoma cell lines with the cytokines IFN-γ + TNF, the chemotherapeutic agent doxorubicin, and the cell cycle inhibitor palbociclib. We characterized the different senescence phenotypes and SASPs in cytokine-induced senescent (CIS) and therapy-induced senescent (TIS) cells. Also, we portrayed the maintenance of senescence by performing most measurements at two different time points.

To validate senescence induction and characterize the senescence phenotype, we determined the activity of the senescence-associated β-galactosidase (SA-β- gal) and performed different cell cycle analyses with Western Blot and FACS. For SASP analyses, we measured the regulation and secretion of several common SASP factors using qPCR arrays, proteome profiler arrays and ELISA. Each treatment initiated a stable growth arrest, enhanced SA-β-gal activity, diminished the proportion of cells in the S phase and, except palbociclib, induced increased expression of p21. PCR array analyses revealed that gene expression in CIS was manifold stronger than in TIS. The protein array and ELISA analyses confirmed that CIS caused a much more pronounced release of several inflammation- and stress-associated factors as compared to TIS. Prominent factors were IL-6 and IL-8, which have been reported to induce senescence. Thus, we conclude that senescence induction via cytokines may lead to a self-sustaining senescence surveillance of melanoma cells mediated by the SASP.

P102 | Laminin 332 is the predominant autoantigen in Orf-induced immunobullous disease

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Ecthyma contagiosum or Orf is a zoonotic viral infection caused by Parapoxviridae, primarily affecting small ruminants such as sheep and goats. Although Orf virus infection is deemed to follow a self-limited course in humans, there is increasing evidence that it may trigger immunological sequelae such as autoimmune bullous diseases (AIBD). Most studies to date failed to specify the antigenic target in Orfinduced AIBD. This study aimed to determine the major target antigen and further characterize this rare entity. We performed serological analyses of the index patient and 4 patients with previously reported Orf-induced AIBD. By immunoblotting with extracellular matrix and a recently established indirect immunofluorescence assay for detection of serum anti-laminin 332 IgG, we identified IgG1 and/or IgG3 autoantibodies against laminin 332 in all 5 patient sera.

Our data led us to propose that Orf-induced anti-laminin 332 pemphigoid is a clinically and immunologically distinct entity characterized by (i) predominant skin lesions with tense blisters and erythema, (ii) younger disease onset compared to bullous pemphigoid and mucous membrane pemphigoid, (iii) limited disease course which mostly necessitates a milder and shorter immunomodulatory treatment, and (iv) IgG1 and IgG3 as main autoantibody subclasses. Hence, this study highlights the importance of testing for anti-laminin 332 IgG1 and IgG3 autoantibodies in patients with suspected Orf-induced pemphigoid.

P103 | Ionic signals determine the pathogenicity of human Th17 cells

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Flexible environmental stimuli such as diet are able to impact our immune function apart from the action of typical immunological factors. Sodium chloride is one such stimulus. It has recently been identified as a tissue factor with substantial enrichment in peripheral tissues and significant immunomodulatory properties.

Here we demonstrate that NaCl—a flexible immunomodulatory tissue factor—is able to awaken anti-inflammatory properties in Th17, hereby mitigating the disease severity of Multiple Sclerosis in mice. We have shown that NaCl not only induces Th17 properties in naïve T-helper cells, but actually enhances the Th17-phenotype in already differentiated Th17 cells which was exerted via the osmosensitive factors NFAT5 and SGK-1. Notably, in the presence of an anti-inflammatory cytokine micromilieu, NaCl induces a robust anti-inflammatory T cell character beyond lone cytokine effects. In an EAE mouse model, the adoptive T cell transfer of Th17 cells, polarized by high NaCl concentrations AND an anti-inflammatory cytokine milieu, leads to a significantly attenuated disease course. In contrast, high NaCl concentrations aggravated EAE disease severity if priming took place in a proinflammatory cytokine context. We

have identified the tissue cytokines IL-1beta and TGF-beta as critical switch-factors determining these NaCl-induced pro- and anti-inflammatory Th17 phenotypes.

Therefore, the cytokine microenvironment seems to act as a crucial determinator of the bipolar action of NaCl mediating immunotolerance under steady-state conditions but enhancing inflammation in settings of infection or autoimmune events. The modulation of cytokine microenvironments by cytokine-blocking drugs may be exploited for new therapeutic strategies in the treatment of autoimmune Th17-driven diseases, such as Multiple Sclerosis.

P104 (OP04/03) | Th17 cell intrinsic engagement of the NLRP3 inflammasomes promotes IL-1a production and autocrine pathogenicity

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Over the last few years Th17 cells have been recognized as major drivers of several inflammatory diseases. Th17 cells also display heterogeneity and plasticity, which translates into distinct functions in settings of health and disease and which can be exploited for therapeutic purposes. In our previous studies, we've validated the existence of pro-inflammatory and anti-inflammatory human Th17 cell subsets. They differ in their ability of IL-10 expression, their microbial antigen specificities and their priming requirement for IL-1beta. Novel insights indicate that the proinflammatory Th17 cell subset can also produce IL-1α, an innate danger signal and alarmin that might confer pathogenicity to this T cell population. The expression of IL-1α by an adaptive immune cell subset therefore prompts us to investigate its characteristics and regulation in detail as well as to explore innate properties of human T helper cells in general. We found that Th17 cell inducing cytokines and transcriptional networks promote IL-1α production. Surprisingly, we could demonstrate a role for the NLRP3 inflammasome for the unconventional secretion IL-1α. It was selectively expressed in the Th17 cell subset and correlated with IL-1a release. Interestingly, Th17 cell restricted NLRP3 activation coopted caspase-8 but not caspase-1 for the extracellular release of IL-1α, thus engaging in an alternative pathway of inflammasome activation. IL-1α was released via Gasdermin pores independently of cell death. Taken together, we demonstrate that IL-1α represents a novel, so far overlooked effector cytokine of human Th17 cells that is regulated by an alternative mode of inflammasome activation. This demonstrates that innate signaling mechanisms can be adopted by adaptive T cells to exert pro-inflammatory functions. Future work will reveal the impact of this T cell program for human health and disease.

P105 | Expression of the ectonucleotidase CD73 by regulatory T cells is important for their suppressive function in DNFB-induced contact hypersensitivity reactions

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Regulatory T cells (Tregs) play a crucial role in immune regulation, whereby degradation of ATP to adenosine by CD39 and CD73 on Tregs is one suppressive mechanism among others. To investigate the role(s) of Tregs in tolerance induction, we used a Dinitrofluorobenzene (DNFB) driven contact hypersensitivity model, in that tolerance can be induced by pretreatment of C57/bl6 (WT) mice with Dinitrothiocyanobenzene (DNTB). Of interest, CD73 deficient (CD73-/-) mice were resistant to tolerance induction. When investigating the underlying mechanisms, we found after tolerization and sensitization of WT mice more activated CD73+ Tregs (as marked by Ki67 and CTLA4 expression) in draining lymph nodes, and more effector memory Tregs (being CCR4+CD103hi+) trafficking into ears, as compared to non-tolerized animals. Blocking the suppressive function of Tregs right after sensitization by the well-established method of anti-CD25 antibody injection, we were able to abrogate tolerance in WT mice. Application of anti-CD73 antibodies recapitulated this effect and prevented induction of tolerance too. Moreover, transfer of WT Tregs into CD73-/- mice reestablished tolerance, whereas CD73-/- Tregs failed to suppress ear swelling when given right after sensitization. This underlines the importance of CD73 expression by Tregs for their suppressive function during CHS reactions. As for the underlying mechanisms, in skin we observed reduced mRNA levels of the pro-inflammatory cytokines/chemokines IL1, CXCL5 and CXCL2 after tolerance induction, which was nullified by anti-CD73 antibodies. Moreover, injection of adenosine deaminase that degrades the enhanced adenosine concentrations generated by CD73+ Tregs into ears of DNTB-treated mice, led to ear swelling reactions similar to that obtained in control (i.e. non tolerized) animals. In a nutshell, our data indicate that CD73+, adenosine producing Tregs are crucial for regulating CHS reactions and tolerance induction in skin and manipulating function(s) of CD73 in cells may offer a tool to affect autoimmunity and inflammation.

P106 | TH9 cells depend on cystine uptake and PPAR-γ signaling to prevent unchecked lipid ROS and cell death

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γTH9 cells, also known as pathogenic TH2 cells, are a subset of pro-inflammatory effector TH cells characterized by high levels of IL-9 expression. They share many properties with conventional TH2 cells

but are distinguished by their expression of the transcription factor PPAR-γ, on which they depend for full effector function.

PPAR-γ is best known for its role in controlling lipid and glucose metabolism but is increasingly implicated in type 2 inflammation. However, the role of PPAR-γ in TH9 cells remains unclear.

Transcriptional profiling of human TH9 cells in presence or absence of the PPAR-γ inhibitor, GW9662, showed strong upregulation of genes involved in cystine transport and redox control of lipid peroxidation. One of the top upregulated genes was SLC7A11, which encodes a cystine/glutamate transporter.

Interestingly, we found that the chemical inhibition of SLC7A11 by erastin and cystine starvation in cystine free medium leads to an increase in lipid ROS levels and cell death in TH9 cells but not in conventional TH2 cells.

Moreover, PPAR-γ inhibition promotes a further increase in lipid ROS and cell death upon erastin treatment only in TH9 cells, and this effect was not associated with augmented cellular ROS or mitochondrial ROS. Importantly, the anti-oxidant Nacetylcysteine completely rescued the effect of erastin, alone or in combination with GW9662, highlighting the importance of cystine for TH9 cells metabolism and survival. To this end, we showed that TH9 cells require exogenous cystine for proliferation and IL-9 expression, and this mechanism of cystine dependency goes through mTORC1 activation.

Erastin and/or GW9662 were also able to selectively deplete the CCR8+CRTh2+ effector memory T cells isolated from allergic contact dermatitis skin biopsies, which share phenotypic and functional features with Th9 cells.

These preliminary data suggest that PPAR-γ plays a role in protecting pathogenic TH2 cells from unchecked lipid ROS and consecutive cell death. Our findings open up new therapeutic avenues to selectively target pathogenic TH2 cells in the treatment of allergic diseases by leveraging their particular dependency on cystine to prevent cell death from unchecked lipid ROS.

P107 | Investigation of intracellular kinase activity in Pemphigus vulgaris using a human skin organ culture model

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Pemphigus vulgaris (PV) is an autoimmune skin blistering disease. Autoantibodies against desmoglein (Dsg)1 and Dsg3 are formed, leading to acantholysis in the skin and mucous membranes, which is very painful and potentially life-threatening for patients and has a huge impact on their quality of life. Treatment options are mainly limited to systemic cortisone therapy and a few others. For more extensive treatment, new therapeutic targets need to be identified. In this work, the activity of kinases in PV was investigated in more detail.

For this purpose, an anti-Dsg1/ 3 single-chain variable fragment (scFv) was injected in skin, using an ex vivo human skin organ culture model, resulting in intraepidermal blister formation after 24 h of incubation. Hematoxylin-eosin stainings were performed on the skin samples, after which quantitative histometric measurements of intraepidermal split formations were performed. In addition, immunohistochemical stainings were performed, in which the binding pattern of the scFv were demonstrated. Proteins were extracted from skin samples, whereupon the activity of the kinases containing in those proteins was detected in a device called Pamgene. A significant increase in activity was measured for overall eight kinases, including several representatives of the Src family.

In the following, specific inhibitors for those kinases were selected, and cell culture experiments (keratinocyte dissociation assay) were preformed. The effect of inhibiting these kinases on acantholysis was evaluated. For some kinases, it was shown that there was a significant reduction in acantholysis in the keratinocyte dissociation assay due to their inhibition. The experiments are still ongoing and final results will be available soon.

The results of this work may serve as a foundation in the development of new, innovative drug and treatment options for PV.

P108 | HCA2 signaling regulates the suppressive activity of regulatory T cells

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It was recently observed that the suppressive activity of regulatory T cells (Treg) in the absence of the G-protein coupled receptor 109a (HCA2) is significantly reduced. This was demonstrated in the contact hypersensitivity model in which Treg obtained from HCA2-knock-out mice (HCA2-KO) were less potent in suppressing the contact hypersensitivity response in comparison to Treg obtained from wild type (WT) donors. Here, we asked whether the same phenomenon can be observed in other skin inflammatory models. To address this issue, we used the psoriasis like imiquimod (IMQ)-induced skin inflammation. Topical application of IMQ on the skin of HCA2-KO or WT mice resulted in psoriasiform inflammation (skin thickening, erythema, scales). However, the inflammatory response was much stronger pronounced in HCA2-KO. To prove whether this is due to a reduced activity of Treg, Treg obtained from HCA2-KO or WT mice were injected i.v. in IMQ-treated WT recipients. Whereas Treg from WT donors mitigated IMQ-induced inflammation in the recipients, Treg from HCA2-KO did not reduce but unexpectedly even enhanced the IMQ reaction in the recipients. To get more insight into this "paradox" reaction, lymph nodes and spleens were obtained from the recipients and the expression of IL-6, IL-17 and IL-23 was analyzed by qRT-PCR. The expression of all these cytokines was significantly upregulated in the recipients of HCA2-KO Treg. Taken together, this suggests that in the absence of HCA2 Treg switch from a suppressive into a proinflammatory type. The mode of action by

which this shift is mediated remains to be determined. Furthermore, analysis of abundance of bacterial phyla revealed altered skin microbiota on HCA2-KO in comparison to WT mice. Especially, the Clostridium class which is known to induce Treg in the colon was reduced on the skin of HCA2-KO. Since the microbiome exerts its effects partially via HCA2, we speculate that the microbiome in the absence of HCA2 is unable to mediate its well know capacity to induce/activate Treg.

P109 | Demonstration of T cell redirection and immune activation in skin rash following tebentafusp treatment

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Background: Tebentafusp (tebe) is an investigational TCR-anti-CD3 bispecific fusion protein that targets gp100 and activates T cells. In a recent phase 3 study, tebe improved overall survival (OS) in metastatic uveal melanoma patients (pts)1. Most pts developed skin rash after dose 1, an expected adverse event since melanocytes also express gp100; however, rash was not an independent predictor of OS benefit2.

Methods: 12/14 pts developed >1 grade rash within 7 days post 1st dose. Paired skin biopsies from pts with metastatic melanoma (NCT01211262) were collected pre and post the 1st (N = 12) or 4th (N = 2) dose of tebe. Fixed samples were assessed by hematoxylin and eosin and CD3 immunohistochemistry (IHC). On 5 additional skin biopsies, dual IHC for Melan-A and CD4/8 was performed. RNAlater® samples were analyzed by RNAseq. Differential gene expression, pathway and cell type composition analysis were performed using R software.

Results: Biopsies from these pts showed increase in lymphocytic infiltrate, exocytosis of lymphocytes and basal cell vacuolization (p = 0.002) along the dermo-epidermal junction (DEJ) and upper dermal perivascular inflammatory infiltrate (p = 0.01) post treatment compared to baseline. The DEJ inflammatory infiltrate consisted of CD3+ T cells, which were predominantly CD8+ and localized close to melanocytes. RNAseq identified 1786 differentially expressed genes (threshold: p-adj ≤0.05, log2-ratio ≥0.75), with enrichment of innate and adaptive pathways, including IFNγ signaling, post treatment compared to baseline (p-adj <10–17). An increase in macrophage and CD8+ T cell genes was accompanied by upregulation of the cytotoxic genes GZMB and PRF1 (p-adj <10–6). Chemokines CXCL9/10/11 and cytokines IL-10/15/32 were overexpressed (p-adj <10–7). Expression of melanocyte markers, PMEL, MLANA and DCT were decreased.

Conclusions: Tebe induced rapid recruitment of T cells in the proximity of intraepidermal melanocytes in addition to macrophage activation. Thus tebe-induced skin rash is an on-target effect and may be an important pharmacodynamic tool to better understand the precise molecular and cellular cascades of T cell redirection in the tumor.

P110 | IL-13 mediated ILC-DC signalling in naive skin imprints dermal dendritic cells to T helper 2 induction

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Type 2 Dendritic Cells (DC2) play a crucial role in informing the adaptive immune system about infectious, innocuous or self-antigens, but display tissue specific signatures. To assess if DC2 adapt to their specific tissue environment and modify their functional characteristics, we assessed DC2 subsets in different barrier and mucosal sites by spectral flow cytometry and RNA sequencing. We found that a subset of dermal DC2, which expressed low levels of CD11b, displayed a unique signature for STAT6 signalling. Strikingly, CD11b-low DC2 did not develop in STAT6- KO mice, while DC2 development in all other tissues was STAT6 independent.

CD11b-low DC2 were also absent from IL-13, IL-13Ra1 and IL-4Ra knock-out mice, indicating that STAT6 was activated through IL-13 signalling in the naïve skin. IL-13 was furthermore sufficient to drive CD11b-low DC2 development in vivo and in vitro and was produced by a unique population of dermal Innate Lymphoid Cells (ILC) at the steady state. In naïve skin, IL-13 production was not dependent on alarmins or the microbiota, and IL-13+ dermal ILC did not express the IL-2 or IL-33 receptor.

We could furthermore show in several in vivo models that Th2 responses were diminished, while Th17 responses increased, when the development of CD11b-low DC2 was impaired.

Our results therefore suggest that the IL-13 mediated development of dermal DC2 fosters a protective non-inflammatory environment at the steady state, but might also contribute to its pro-allergic conditioning upon dysregulation.

P111 | Implementation of primary immune cells into a human skin organoid

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The prevention of sensitisation to allergens and the therapy of contact dermatitis are of great medical and economic importance, since 15% of Germans develop an allergic contact dermatitis during their lifetime and 27% of Europeans present with cutaneous sensitizations against potential allergens. Particularly since the EU's ban on animal experiments for testing the compatibility of cosmetics in 2013, enormous efforts have been made to find suitable alternative test methods. Immunocompetent 3D skin models are presumably the most promising replacement to animal testing. In this project, we aimed to integrate human primary immunocompetent cells into a human skin organoid consisting of a dermis equivalent and primary keratinocytes. We established a 3D-skin model using human primary fibroblasts in combination with human primary keratinocytes and in combination with the human N/TERT-1 keratinocyte cell-line. In both cases, we were able to induce epidermal growth and differentiation; however models with primary keratinocytes were histologically more similar to regular human skin than models with the N/TERT-1 cell line. Subsequently we established Filaggrin, Involucrin and Loricrin tissue stainings, all demonstrating a well-built skin barrier. In recent experiments, we co-seeded organoids with different cell counts of Peripheral Blood Mononuclear Cell (PBMCs) and supplied them with diverse cytokine cocktails for 2 days to support dendritic cell-like differentiation (IL-4, GM-CSF and/or TGF-b). Furthermore, using the plastic-adherent fraction of PBMCs, we generated monocyte-derived dendritic cells (Mo DC) as well as monocyte-derived Langerhans cells (Mo-LC) and integrated both into our fullthickness skin equivalents. The successful integration of human leukocytes was confirmed by immunohistochemical CD45-staining. We were able to identify the leukocytes and found well integrated CD68+ Mo-DCs and CD3+ T-cells in our human skin organoids. Our next step will be to assess the level of activation of seeded DCs using immunohistochemistry (e.g. CD83 expression) and to stimulate the immune cells with various contact allergens.

P112 | PPAR-gamma promotes proliferation of pathogenic Th2 cells through regulation of IL-2 signaling

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Recently, a subset of allergen-specific Th2 cells has been identified and termed "pathogenic" Th2 (pTh2) cells, based on their crucial role in mediating type-2- mediated immunopathology. pTh2 cells express high levels of the ligand-activated transcription factor peroxisome proliferator activated receptor gamma (PPAR-g). The functional role of PPAR-g for pTh2 cells, however, remains incompletely understood. Here, we analyzed the effect of PPAR-g inhibition on basic T cell functions such as IL-2- or T cell receptor (TCR)-induced proliferation in pTh2 cells isolated from peripheral blood.

Strikingly, PPAR-g inhibition strongly reduced IL-2-induced proliferation, but not TCR-induced proliferation, suggesting specific control of cytokine signaling events by PPAR-g.

To investigate the underlying mechanisms, we performed transcriptomic analysis of T cell clones treated with a chemical inhibitor (GW9962) of PPAR-g. Pathway analysis revealed that the IL-2 signaling pathway is affected by PPAR-g-inhibition, in line with our observation from the proliferation data.

To assess the impact of PPAR-g inhibition on IL-2 signaling, we systematically measured the effect on the expression of the IL-2R chains and the phosphorylation of signal transducer and activator of transcription (STAT) molecules. After chemical inhibition of PPAR-gamma in pTh2, IL-2R alpha and IL-2R gamma were significantly downregulated and IL-2R beta remained unchanged compared to the untreated control. Furthermore, cells treated with GW9662 showed a significantly reduced phosphorylation of STAT3 and STAT5, while phosphorylation of STAT6 remained unaffected.

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

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

P113 | The G-protein coupled receptor EBI2 is involved in the pathogenesis of murine TNCB-induced contact hypersensitivity

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Patients with allergic contact dermatitis (ACD) suffer from allergic reactions to specific antigens that come in contact with the skin. In the murine ACD model, contact hypersensitivity (CHS), application

of a hapten onto the skin elicits a local T cell-mediated inflammation. We found an attenuated TNCB-induced CHS response in animals that lack the G-protein coupled receptor EBI2 (GPR183). EBI2 has been shown to be involved in the activation of T cells in the periphery as well as to promote T cell immigration into inflamed tissue. Its main ligand 7α,25-dihydroxycholesterol (7α,25-OHC) is generated via sequential hydroxylation of cholesterol by CH25H and CYB7B1. We found these ligand generating enzymes to be upregulated in the inflamed ears of the mice, just as they are in lesions of patients. EBI2 deficient mice showed ameliorated CHS responses in an acute, chronic and memory model, and adoptive T cell transfer experiments highlighted the role of EBI2 on T cells in the development of the disease reaction. Our data therefore indicate that EBI2 is involved in the pathogenesis of the CHS reaction and ACD.

P114 | Comparison of Janus kinase and phosphodiesterase 4 inhibition in terms of antiviral responses in vitro

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The Janus kinase (JAK) inhibitor tofacitinib as well as apremilast, an inhibitor of the enzyme phosphodiesterase 4 (PDE4) are approved for the treatment of psoriatic arthritis. Both oral therapies have been associated with nasopharyngitis, and upper respiratory tract infections. In addition, increased incidences of herpes zoster have been observed under treatment with tofacitinib but not with apremilast. JAKs are found downstream of the type II cytokine receptor family used by a number of TH17 cell-associated cytokines for signal transduction. Inhibition of PDE4 leads to the accumulation of intracellular cAMP, and subsequent activation of the protein kinase A which is followed by a reduction of a variety of innate and adaptive immune responses including a lower expression of TH17 cell-associated cytokines. These cytokines induce the secretion of antiviral and antimicrobial peptides by keratinocytes. To investigate antiviral effects, primary human keratinocytes were treated with tofacitinib or apremilast and various cytokines and/or bacterial surface proteins and analysed by RT-qPCR. CD69 expression on tofacitinib or apremilast-treated PBMCs was investigated via flow cytometry.

We found that in contrast to apremilast, tofacitinib markedly reduced the gene expression of antiviral peptides such as MX1 or ISG15 in keratinocytes in vitro. Additionally, JAK inhibition but not PDE4 inhibition reduced the activation of T cells stimulated with viral VZV gE. Using both substances we did not observed significant effects on antimicrobial responses.

To conclude, we report that tofacitinib reduced the expression of antiviral peptides as well as the activation of T cells by viral antigens in vitro while apremilast did not significantly influence the antiviral immunity. These results are in line with the clinical observation of increased numbers for zoster in patients treated with tofacitinib.

P115 | Co-localization of Ki-67 and DNA in neutrophils: Investigating the role of mitotic surfactant proteins in NETosis

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Neutrophils are the first responders to sites of injury and are critical in the inflammatory process and tissue homeostasis. Highly versatile and plastic cells, neutrophils can eliminate pathogens through phagocytosis, reactive oxygen species (ROS) generation, and importantly through production of neutrophil extracellular traps (NETs). These NETs are arguably one of the neutrophil's most powerful tools for controlling infection, composed of nuclear DNA studded with cellular and antimicrobial proteins. However, these are a double-edged sword as they are implicated in a number of diseases when dysregulated, including psoriasis and systemic lupus erythematosus. Careful control of this process is therefore necessary for sufficient neutrophil activation without leading to excessive or prolonged inflammation. The Ki-67 protein provides a surfactant function during mitosis, coating chromosomes following disassembly of the nuclear membrane and preventing their collapse into a single mass. Preliminary data has shown that Ki-67 similarly colocalizes with DNA during the process of NETosis, however it has not been studied how this might translate into regulation of the process. Therefore, we have studied the localization and role of Ki-67 during and in NET formation and have explored the role of other nucleolar proteins such as Nucleophosmin. This will generate insight into the regulation of NETosis, uncovering a new mechanistic pathway and potential therapeutic targets for the management of inflammatory diseases including numerous skin diseases.

P116 | Topical application of adenosine receptor agonists to skin prevents contact hypersensitivity reactions by reducing migration and activation of skin migratory dendritic cells

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We have recently shown that production of Adenosine (Ado) by skin and skin migratory (sm) CD73+ dendritic cells (DCs) is critical for tolerance development in a contact hypersensitivity (CHS) model. Since Ado has well documented immunosuppressive properties, we investigated the use of Ado receptor (AdoR) agonists for treatment of CHS.

We choose the A2A (CGS21680; CGS) and A2B (BAY60-6583; BAY) AdoR agonists, as both induce cAMP mediated signalling that has

been shown to exert immunosuppressive functions in various cell types.

Both AdoR agonists were epicutaneously applied to skin prior to sensitization and challenge with the hapten DNFB, and ear swelling and the immunologic outcome was analyzed in skin and draining lymph nodes (dLNs). We found that animals treated with AdoR agonists showed a reduced ear swelling as compared to solvent controls. Consistently, fewer activated T cells were found in skin after challenge. While the numbers of Tregs remained the same within all the groups, we recorded higher numbers of CD4+CD25- T cells expressing anergic markers, such as Lag-3, CD137, PD-1, CD272 and Tim-3 in dLNs in CGS-treated group, as compared to solvent and BAY-groups. In ear tissue, AdoR agonists reduced the production of proinflammatory cytokines and chemokines as well as the infiltration of neutrophils upon sensitization.

As possible targets for AdoR agonists we identified skin DCs, as they expressed high levels of A2A and A2B AdoR (by qRT-PCR) when compared to keratinocytes. Moreover, sensitization is critically dependent on skin migratory (sm) DCs, therefore, we analysed numbers and phenotypes of skin-to-LN migrated smDCs, 1–3 days after sensitization. Here, we found that less CD207+ DCs migrated to the dLN from CGS and BAY treated ears as compared to solvent controls. Additionally, the smDCs expressed less CD80 and CD86 on their surface and secreted less IL-12. After FACS sorting and co-incubation of these smDCs with OT-I or OT-II T cells and their respective OVA peptides, reduced proliferation and inferior production of proinflammatory cytokines was detected in cultures with smDCs from CGS-treated animals as compared to solvent controls.

In conclusion, topical application of AdoR agonists to skin prevents sensitization of T cells against haptens by reducing migration and activation of smDCs.

P117 | Interaction of neutrophil extracellular traps with macrophages instigates exacerbated inflammation in wound healing

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Chronic wound healing poses an enormous problem, especially in elderly people and diabetic patients. Often the wounds display signs of inadequate inflammation, which are brought by neutrophils and macrophages ensembles that sustain it furthermore. Factors that regulate timely neutrophils clearance and resolution of inflammation are not fully understood, and a new role of neutrophil extracellular traps (NETs) in the skin requires more attention. Aim of the study is to reveal the role of neutrophil and NETs in wound healing, which signal to macrophages and regulate further fate of inflammation or resolution.

We compared spatiotemporal organization of neutrophils and NETs in the full thickness wound healing model in wild-type and type 2 diabetic db/db mice. In wildtype wounds, neutrophils infiltrate quickly into the tissue, compact in the upper part, where most of NETosis takes place. Later the upper part with condensed layer of NETosed neutrophils is moved outwards and becomes a thick layer of scab that function as a protection shield, which has high level of elastase activity. By this the proteolytic activity is constrained in the outer surrounding, while a new epidermal barrier is formed below.

In opposite, neutrophils in diabetic wounds behave differently. Despite that overall neutrophil Ly6G gene expression is lower, neutrophils infiltrate with delay and spread in the wound bed as well as form more NETs within the tissue. Subsequently, elastase and other neutrophils-derived proteases are higher in diabetic wounds, measured in substrate cleavage assays. Consistent to mice, human chronic diabetic wounds showed more NETs in the wound bed, while acute wounds have NETs at open wound margins.

It appears that in normal wound healing most of the neutrophils are physically concentrated in the upper part and are moved to the scab, thus avoiding close interaction with macrophages, which is not the case in diabetic wounds. To reveal mechanism of how NETs contribute to inflammation, NETs were co-cultured *in vitro* with macrophages. NETs themselves release IL-1β, also NETs induce IL-1β and suppress IL-10 release by human macrophage in the presence of LPS, suggesting a role of NETs in macrophage inflammasome activation and in change of macrophage activation states in chronic wounds

In conclusion, our data suggest different behaviors of neutrophil and NETs in normal and diabetic wounds and different clearance mechanism involving scab formation or macrophage recognition. Abundant NETs found in diabetic wound may induce macrophage inflammasome activation, which in turn exacerbate further inflammation in diabetic wounds.

P118 | Soluble CD83 promotes cutaneous wound healing in an IDO- and beta-catenindependent manner

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Wound healing is a complex and tightly synchronized process with distinct cellular and cytokine profiles. Dysregulations in the course of the wound healing process result in delayed wound closure and/or chronification of wounds. In addition, specific factors such as age and diabetes, are also associated with impairment of wound healing, and patients require intensive and time consuming wound care strategies. Moreover, the treatment with anti-inflammatory agents, such as corticosteroids in the context of organ transplantation, negatively

affect the wound healing process and delay wound closure. Thus, novel pro-resolving treatment options with reduced negative side effects are urgently needed for the treatment of chronic and hard-to-heal wounds. Previously, we have shown that sCD83 possesses immune modulatory properties, inducing resolution of inflammation in murine autoimmune models, including antigen-induced arthritis (AIA), experimental autoimmune encephalomyelitis (EAE), inflammatory bowel disease (IBD) and Systemic lupus erythematosus (SLE) as well as transplantation models for cornea, heart and skin.

In the present study, we assessed the pro-regenerative properties of sCD83 in cutaneous wound healing. Not only accelerated sCD83 wound healing after systemic but also after topical treatment, which would be therapeutically more feasible. Cytokine profile analyses revealed an initial upregulation of inflammatory mediators, followed by a switch towards pro-resolving factors, known to be important for tissue repair and resolution of inflammation. Mechanistically, we show that sCD83- accelerated wound healing processes absolutely rely on the enzymatic IDO1 activity. Moreover, sCD83 modulates epidermal stem cell differentiation as indicated by reduced beta-catenin protein levels and increases blood vessel formation within treated wound areas. Thus, our results represent a very interesting basis for future therapeutic options for tissue regeneration as well as chronic- and hard-to-heal wounds.

P119 | Dysfunction in regulatory T cells leads to AIBD-related antigen-specific B cells and pathogenic anti-Col7 autoantibody causing blisters independently of immune cells

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Different autoimmune diseases develop due to dysfunctional regulatory T cells (Treg). A missense mutation in the transcription factor foxp3 leads to the absence of functional Treg in scurfy mice. The resulting uncontrolled expansion of autoreactive CD4+ T cells and high titers of skin reactive autoantibodies cause blister formation in the skin and the development of autoimmune blistering diseases (AIBD) in scurfy mice.

Previously, we generated hybridomas of spontaneously activated B cells from scurfy mice and could show that one of these autoantibodies (H510) is pathogenic in vivo after injection in neonatal WT mice by targeting the murine von-Willebrand-Factor- A-like domain 2 (vWFA2) of Collagen Type VII (Col7). Moreover, the injection of this IgG1 autoantibody represents an experimental mouse model for epidermolysis bullosa acquisita (EBA) based on direct autoantibody transfer. To get further insight into the mechanism of blister formation induced by H510, we injected the anti- Col7 antibody in Fcγ-receptor knock-out (KO) mice and complement C3-KO mice. We found subepidermal blisters in the majority of injected KO-mice indicating a pathomechanism independent of inflammatory immune cells and the complement system.

To further understand the production of pathogenic autoantibodies and antigen-specific B cell responses in the context of AIBD, we analyzed the development and frequency of antigen-specific B cells in scurfy mice. Performing Enzyme-linked immuno- spot (ELISpot) assays with scurfy splenocytes and different known AIBD antigens, we found elevated frequencies of specific B cells reactive against AIBD-related proteins in sick scurfy mice. For further studies of the AIBD onset in the absence of functional Treg, we aim to characterize different B cell subsets in scurfy mice associated with frequently developed autoantigen-specific B cells.

P120 (OP03/02) | Neurotransmitters as modulators of immune functions in neutrophils

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The neurotransmitter dopamine is essential for intercellular communication within the nervous system. Additionally, the expression of enzymes for dopamine production, as well as of proteins for dopamine release and signaling has been described in a variety of immune cells, including B- and T-cells. However, the role of dopamine particularly in the innate system remains enigmatic. Here, we have investigated the dopaminergic machinery in human neutrophils and have been able to identify enzymes for dopamine production and transport. Furthermore, we have found direct evidence for the production and storage of dopamine by neutrophils. For the first time, dopamine exocytosis from neutrophils was directly imaged in real time with a high spatiotemporal resolution using a fluorescent dopamine nanosensor. Ca2+ mobilization was visualized in parallel with a fluorescent Ca2+ indicator showing an increase in intracellular Ca2+ concentration prior to dopamine exocytosis. Dopamine exocytosis was induced by platelet-derived serotonin, another neurotransmitter with immune-modulatory function. Furthermore, dopamine as well as serotonin inhibited the formation of neutrophil extracellular traps (NETs), an important immune defense mechanism, in a concentration-dependent manner. These findings show that dopamine is an inhibitory immune modulator that most likely exerts its effect via auto- and paracrine pathways. These findings illustrate the importance of a neuro-immunological axis within the (innate) immune system, which merits further studies and could revolutionize our understanding of how inflammatory processes are modified in a context-dependent manner.

P121 | Effective anti-melanoma immune response by peritumoral delivery of the heat shock protein 70 (Hsp70)

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Immune checkpoint inhibitors increased overall survival for patients with advanced and metastatic melanoma, but long-term treatment responses are still limited to few patients with tumor specific effector T-cells at the tumor sites as predictors for treatment success. Thus, new treatment strategies are required. In preclinical models we could recently show, that targeted activation of melanoma-associated mast cells (MC) with lipopolysaccharide (LPS) induces an effective anti-melanoma immune response in a TLR4-dependent manner. Tumor control was mediated by MC-derived CXCL10, a T cell recruiting chemokine whose expression positively correlates with survival of melanoma patients. Like LPS, Hsp70 can also activate the TLR4 pathway and extracellular Hsp70 was shown to stimulate immune responses in a variety of cancers. We therefore investigated the impact of Hsp70 on melanoma and its environmental immune cells. In in vitro studies we could demonstrate that Hsp70 activates mast cells in a TLR4-dependent manner and leads to secretion of CXCL10. Repeated peritumoral application of Hsp70 in a B16 mouse melanoma model resulted in tumor control with enhanced tumor-infiltrating lymphocytes and in increase of TNF-a and IFNy producing T cells in the draining lymph nodes. The combination of the Hsp70 treatment with anti-CTLA-4 immunotherapy further augmented anti-melanoma efficacy. Collectively, combined Hsp70 administration with immune checkpoint therapy represents a highly efficient anti-tumor modality, and is promising for clinical translation.

P122 | Neutrophil extracellular traps promote enhanced *S. aureus* skin colonization via TLR4/RAGE signaling and induction of oxidative stress in keratinocytes

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Introduction: Although *Staphylococcus aureus* can cause life threatening infections, about 20–30% of the human population shows asymptomatic colonization mostly in the nose. It is known that a disrupted skin barrier leads to rapid infiltration of neutrophils, resulting in enhanced *S. aureus* skin colonization. Previous experiments of our group showed that neutrophil extracellular traps (NETs) can promote enhanced *S. aureus* skin colonization by interacting with keratinocytes [1]. However, it is not yet understood how neutrophils or NETs influence keratinocytes in a way that favors *S. aureus* colonization.
Objectives: In this work, we investigated the timing of NET formation after coincubation of neutrophils with keratinocytes and *S. aureus* infection as well as the signaling pathways induced in keratinocytes by neutrophils and NETs which might play a role in the colonizing-enhancing effect.
Material and Methods: Using an in vitro co-culture model with human primary keratinocytes and neutrophils, we analyzed how long neutrophils and keratinocytes must be pre-incubated prior to *S. aureus* infection to see a colonizing enhancing effect using colony-formation units (CFU) assays. We analysed the formation of NETs by SYTOX Green staining and live cell imaging. Furthermore, the induction of pro-inflammatory mediators in keratinocytes co-cultured with neutrophils/NETs were analysed via ELISA and qPCR and activated signaling pathways were elucidated by western blot and blockade studies. The induction of oxidative stress was measured using dichlorofluorescein substrate.
Results: We show that NET formation in the co-culture and the resulting enhanced *S. aureus* colonization correlates with the activation status of the neutrophils. Moreover, depending on the time of co-culture, distinct signaling pathways are activated in keratinocytes leading to the expression and release of pro-inflammatory cytokines. Furthermore, oxidative stress is induced in keratinocytes co-cultured with neutrophils/ NETs. We could show that the induction of this pro-inflammatory state and oxidative stress is mediated by TLR4/RAGE signaling in keratinocytes. Blocking TLR4 and RAGE prevents this pro-inflammatory state and the enhanced *S. aureus* skin colonization.
Conclusion: Out data suggest, that during the co-incubation of neutrophils with keratinocytes, neutrophils are activated and primed to form neutrophil extracellular traps after concomitant *S. aureus* infection. Moreover, the *S. aureus* colonizing-enhancing effect is mediated by the interaction of neutrophils/NETs and keratinocytes via TLR4/ RAGE signaling, which induces a pro-inflammatory state and oxidative stress in the keratinocytes.
[1] Bitschar K. et al. *Staphylococcus aureus* Skin Colonization Is Enhanced by the Interaction of Neutrophil Extracellular Traps with Keratinocytes. J Invest Dermatol. 2020 May;140(5):1054-1065.e4

P123 | Bacterial membrane vesicles shape *S. aureus* skin colonization and induction of innate immune responses

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Introduction: The human skin is constantly exposed to pathogens but is only rarely colonized by them. *Staphylococcus aureus* is a pathogenic bacterium that causes human infections like mild skin lesions up to invasive, life threatening infections. *S. aureus* produces membrane vesicles (MVs), which can contribute to skin inflammation. Under homeostatic conditions skin commensals prevent *S. aureus* from skin colonization.
Objectives: The aim of this work was to investigate the role of MVs of *S. aureus* and skin commensals in *S. aureus* skin colonization.

Materials and Methods: We used a protocol for the efficient isolation of MVs from Gram-positive bacteria using size-fractionation and enrichment by a MV-precipitation reagent (ExoQuickTC). Cytokine induction in primary human keratinocytes was analyzed using ELISA or LegendPlex analysis. Furthermore we used our skin explants protocol to simulate MVs studies on the human skin. In addition, an established co-culture system of keratinocytes and neutrophils was taken for the analysis of neutrophil migration and neutrophil extracellular traps formation.

Results: Different staphylococci strains produce membrane vesicles (MVs), the MVs of skin commensals show a protective effect on *S. aureus* skin colonization. In contrast, MVs released by *S. aureus* are able to induce CXCL8 and TNF-α in primary human keratinocytes, recruit neutrophils and induce neutrophil extracellular traps, which enhance *S. aureus* skin colonization. The membrane lipid and protein A content of the MVs correlate with the induction level of CXCL8 depending on TLR2 and NFkB. Moreover MVs of *S. aureus* strains from the lesional skin of AD patients show an enhanced membrane lipid and protein A content compared to the strains from the non-lesional sites and have an enhanced proinflammatory potential, which correlates with the enhanced skin colonization ability of the lesional AD strains.

Conclusion: Our data underline the complex interplay in host- and bacterial derived factors in *S. aureus* skin colonization and the important role of bacterial derived MVs, their membrane lipid and protein A content in skin inflammatory disorders.

P124 | TNF is partially required for cell death triggered skin inflammation upon acute loss of cFLIP

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cFLIP is required for epidermal integrity and skin inflammation silencing via protection from TNF-induced keratinocyte apoptosis. Here we have generated and analyzed cFLIP epidermal KO mice with additional TNF deficiency. Intriguingly the ablation of TNF rescued the pathological phenotype of epidermal cFLIP KO from characteristic weight loss and increased mortality. Moreover, the lack of TNF in these animals strongly reduced and delayed the epidermal hyperkeratosis and the increase of apoptosis in keratinocytes. Our data demonstrate that TNF signaling in cFLIP deficient keratinocytes is the critical factor for the regulation of skin inflammation via modulated cytokines and chemokines expression and thus the attraction of immune cells. Our data suggest that autocrine TNF loop activation upon cFLIP deletion is dispensable for T cells, but is critical for the neutrophils attraction. Our findings provide evidence for a negative regulatory role of cFLIP for TNF-dependent apoptosis and partially for epidermal inflammation. However, alternative signalling pathways may contribute to the development of the dramatic skin disease upon cFLIP deletion. Our data warrant future studies of the regulatory mechanism controlling the development of skin disease upon cFLIP deficiency and the role of cFLIP/TNF in a number of inflammatory skin diseases, including toxic epidermal necrolysis (TEN).

P125 (OP04/01) | Evidence for direct binding of complement components to the immunodominant NC16A domain of collagen XVII

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Bullous pemphigoid (BP) is the major autoimmune blistering skin condition worldwide, characterized by tissue-bound autoantibodies that typically fix complement. In that context, autoantibodies binding collagen XVII (COL17, BP180), a hemidesmosomal structure protein that mediates adhesion of basal keratinocytes to the dermis, promote complement fixation, inflammation and the formation of tense blisters. In contrast to the rather detailed understanding of the BP effector phase, mechanisms enabling the emergence of self-directed antibodies against COL17 remain much less understood. Insights into these early steps in BP immunopathogenesis may identify new treatment avenues for this expanding and fragile patient group.

We here report evidence for covalent, direct binding of C3b, the first opsonizing fragment of activated complement component C3, to the immunodominant NC16A domain of collagen XVII. Incubating recombinant COL17-NC16A antigen with a source of fresh complement in the presence or absence of complement-inhibitory amounts of tinzaparin sodium, we performed immunoprecipitation and reducing SDS-PAGE. These experiments resulted in the appearance of novel bands under conditions that permit complement activation. Selected bands were subjected to tandem mass spectrometry analysis, identifying a range of unique tryptic peptides that confidently identify the C3b alpha' chain and the C3b beta chain as being present at respective bands examined. The fact that C3b alpha' co-precipitated with the COL17-NC16A antigen is consistent with its ability to form a covalent bond with the protein antigen, as demonstrated by a specific tryptic peptide we here identified.

Our results point toward direct opsonization of the immunodominant COL17-NC16A antigen with activated complement C3 fragments, in the absence of COL17-directed autoantibodies. Against the well-documented immunogenicity-boosting effect of C3- derived fragments when bound to protein antigens, we interpret our in vitro findings to represent a previously underexplored key step in the early immunopathogenesis of BP. It has been shown that decoration with complement C3-derived moieties improves the efficacy of

protein antigens to elicit antibody responses by more than 10,000-fold. In the scenario of BP, C3b opsonization can be equally expected to increase the normally low immunogenic potential of COL17-NC16A self-antigen, possibly to a point where tolerance cannot be maintained. Confirming this proteomic revelation, we can show by direct immunofluorescence microscopy that in states of skin inflammation granular C3 deposits (as frequently seen in cases of granular C3 dermatosis) do in fact co-localize with BP180 at the basement-membrane zone.

We propose that binding of complement factors to skin autoantigens may constitute one of the earliest processes in the pathogenesis of autoimmune skin blistering diseases such as BP, mucous membrane pemphigoid or epidermolysis bullosa acquisita. It implies that these processes are normally kept in check by complement regulatory or inhibitory mechanisms that may lose their protective capacity during local inflammatory or infective episodes, or for genetic or age-related reasons. Our evidence warrants further exploration of its relevance and contribution in developing autoimmune skin disease.

P126 | Notable differences in the gene expression profile of different types of lichenoid skin inflammation

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Lichenoid inflammation accounts for the majority of appearing clinical and histological inflammation patterns in dermatology. Accordingly, a vast number of clinically diverse and poorly understood inflammatory skin diseases shows lichenoid characteristics in histology. Naturally or "artificially" initiated cell-mediated cytotoxicity is considered one major mechanism of action underlying lichenoid inflammation. The naturally occurring inflammatory skin disease is Lichen planus (LP), eponymous to the several subtypes of lichenoid skin diseases. Besides, lichenoid inflammation of the skin can be induced "artificially" by immune checkpoint inhibitor therapy of metastasizing malignant melanoma (MM). Immune checkpoint inhibitor therapy is a well-established treatment concept for MM patients, e.g., employing anti-PD1 antibodies. However, during therapy patients frequently develop lichenoid skin lesions ranging from mild rashes to severe mucocutaneous reactions (artificial lichenoid skin reactions; ALSR).

Aberrant gene expression profiles might be fundamental to variance in disease manifestation of the different forms of LP and ALSR. Thus, we performed a transcriptomic analysis using the Nanostring(TM) technology, revealing hierarchical clustering of differentially expressed genes in classical, oral and genital LP, LP planopilaris, and anti-PD1 skin reactions. A good segregation of the different conditions was observed based on the gene expression profile of individual samples and the magnitude of change in gene expression. Moreover, CIBERSORT analysis of the relative levels of distinct cell types further substantiated heterogeneity of the different disease groups. Looking at specific pathway-related genes of cytotoxicity, we observed clustering of oral and genital LP with anti-PD1 lesions along with upregulation of several cytotoxicity-associated genes including HLA-A/B/C, PRF1 (Perforin), GZMB (Granzyme B) and GNLY (Granulysin). In contrast, the clearly from other lichenoid skin lesions distinct gene expression pattern in LP planopilaris is indicative for a significant variability in the pathogenesis of different subtypes of LP. Further, multiplex immunohistochemistry Imaging (Akoya Biosciences) revealed an increased percentage of IL-17A-positive cells among the T cell population in LP planopilaris.

These results underline the need for a detailed mechanistic characterization of the molecular pathways in which the distinct forms of LP and ALSR deviate. Long-term, we aim to contribute to providing remedy for millions of patients suffering from a much distressing condition.

P127 | Keratinocyte-intrinsic BCL10/MALT1 activity initiates and amplifies psoriasiform skin inflammation

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Psoriasis is a chronic inflammatory skin disease arising from ill-defined pathological crosstalk between keratinocytes and the immune system. BCL10 and MALT1 are ubiquitously expressed inflammatory signaling proteins that can interact with the psoriasis susceptibility factor CARD14, but their functions in psoriasis are insufficiently understood. We report that although keratinocyte-intrinsic BCL10/ MALT1 deletions completely rescue inflammatory skin pathology triggered by germline Card14 gain-of-function mutation in mice, the BCL10/MALT1 signalosome is surprisingly not involved in the CARD14-dependent IL-17R proximal pathway. Instead, it plays a more pleiotropic role by amplifying keratinocyte responses

to a series of inflammatory cytokines, including IL-17A, IL-1β and TNF. Moreover, selective keratinocyte-intrinsic activation of BCL10/MALT1 signaling with an artificial engager molecule is sufficient to initiate lymphocyte-mediated psoriasiform skin inflammation, and aberrant BCL10/MALT1 activity is frequently detected in the skin of human sporadic psoriasis. Together, these results establish that BCL10/MALT1 signalosomes can act as initiators and crucial amplifiers of psoriatic skin inflammation and indicate a critical function for this complex in sporadic psoriasis.

P128 | Selective inhibition of tyrosine kinase 2 prevents and restores interleukin-12- induced hair follicle immune privilege collapse: a novel approach to alopecia areata therapy?

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Alopecia areata (AA) is an immune-mediated hair loss disorder characterized by elevated levels of IFNγ and Th1-driven inflammatory responses toward the hair follicle (HF) bulb. This results in immune privilege (IP) collapse, premature catagen development, and HF dystrophy. Given the critical role of interleukin (IL)-12 in priming Th1 responses, we investigated whether IL-12 could be directly involved in inducing HF-IP collapse and whether the selective tyrosine kinase 2 (TYK2) inhibitor, BMS-986202, could prevent or reverse the process. By quantitative immunohistomorphometry, we showed that ex vivo treatment of microdissected HFs with IL-12 (3 ng/ml) + IL-18 (20 ng/ml) upregulated MHC-I and II as well as MICA/B expression in the hair bulb (cardinal features of IP collapse), increased the numbers of CD3+ or CD56+ cells in HF epithelium and mesenchyme, and selectively enriched IFNγ-inducible genes. In addition, more peribulbar IL-12RB2+ cells were found in acute lesional scalp skin samples of AA patients than healthy controls. We further confirmed the role of IL-12 in the HF-IP collapse by selectively blocking IL-12 receptor signaling. BMS-986202 (300 nM), when administered to microdissected HFs before or after IL-12 + IL-18 stimulation, prevented or attenuated the expression of MHC-I and II as well as secretion of IFNγ. Therefore, our data demonstrate that local IL-12 directly promotes perifollicular immune cell expansion, IFNγ secretion, and HF-IP collapse. These findings support a potential role of IL-12 signaling in AA pathogenesis and highlight IL-12 as a potential new target for pharmacologic AA therapy.

P129 (OP01/02) | PDE4 inhibition improves clinical lesions in a novel immunization-induced mouse model of mucous membrane pemphigoid

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Mucous membrane pemphigoid (MMP) is a subepithelial blistering autoimmune disease with predominant mucosal lesions. Autoantibodies are directed against structural proteins of the basement membrane zone of epidermis and orifice-close epithelia. Therapeutic options are limited due to the lack of randomized controlled trials. Here, for the first time, we established a mouse model in which repetitive immunization of adult B6.S and SJL/J mice with recombinant murine laminin alpha 3 (mLAMA3) led to the generation of murine anti-mLAMA3 antibodies and, 8–14 weeks after immunization, to erosions and blisters in the oral cavity as demonstrated by endoscopy, corneal lesions visualized by fluorescein staining, and crusts and erosions on the skin in up to 90%, 60% and 20% of animals, respectively. By direct immunofluorescence microscopy, linear deposits of IgG, IgA, and C3 were detected in biopsies of palpebral conjunctiva, cornea, buccal mucosa, esophagus, bladder, colon, small intestine, genital mucosa, and perilesional skin. By histopathology of lesional biopsies, subepithelial splitting was seen in the cornea, palpebral conjunctiva, buccal mucosa, esophagus, and skin. We then explored the clinical efficacy of PDE4 inhibition, an approach currently used for the treatment of Behet disease and psoriasis. B6.S mice were immunized with mLAMα3 and after reaching a score of either 7% affected eye area or an oral score of 2, mice were randomized into 4 study groups to receive the PDE4 inhibitor roflumilast (n = 15, 5 mg/kg/day), dapsone (n = 15, 100 mg/kg/day), a combination of roflumilast and dapsone (n = 15), or vehicle (n = 15, methocel 2% w/v) over a period of 8 weeks. In mice of the three treatment groups, oral lesions significantly decreased compared to vehicle-treated mice. The strongest effect had the combination of roflumilast and dapsone, followed by monotherapy with roflumilast or with dapsone. Roflumilast also led to a significant reduction of eye disease and affected body surface area as well as significantly less split formation in the palpebral conjunctiva compared to vehicle-treated mice. The latter disease score was also improved by dapsone, although not statistically significant. In summary, the immunization-induced MMP mouse model reflects major immunopathological and clinical characteristics of the human disease including response to dapsone and will be a valuable tool for the preclinical validation of antiinflammatory compounds in cutaneous, oral, and conjunctival disease. By the use of this mouse model, PDE4 inhibition emerged as potential novel treatment option for patients with MMP.

P130 | Impact of HCA2/Gpr109a receptor signaling on allergic inflammation in the skin

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The hydroxycarbolic acid receptor HCA2/Gpr109a is expressed in white and brown adipocytes as well as in immune cells including neutrophils, macrophages, and epidermal Langerhans cells. Intestinal epithelial cells, keratinocytes, and microglia also express HCA2. Endogenous agonists of this G-protein coupled receptor are the short chain fatty acid (SCFA) butyrate and the hydroxycarbolic acid 3-hydroxy-butyrate. HCA2 also is a target for niacin (nicotinic acid). Nutritional or pharmacological activation of HCA2 is important for the inhibition of insulin production, regulation of blood pressure and reduction of lipolysis. Furthermore, it attenuates inflammation in atherosclerosis or inflammatory bowel disease. In an experimental model of colonic inflammation Hcar2-/- mice showed increased intestinal inflammation and tumorigenesis compared to wild type mice. In a mouse model of psoriasis, topical application of sodium butyrate attenuated the inflammatory immune response by inducing Treg and IL-10. The detailed mechanisms of the antiinflammatory effects of HCA2 receptor signaling for the regulation of cutaneous inflammation are still not fully understood.

To evaluate the role of HCA2 signaling in the regulation of allergic immune responses in the skin, we used the experimental mouse model of contact hypersensitivity. Mice were sensitized with the obligate contact sensitizer 2,4-dinitro-1-fluorobenzene (DNFB). Five days later contact allergy was induced by application of DNFB on one ear. Contact allergic ear swelling was measured and immunological analyses (histology, FACS) as well as RT-PCR were performed on inflamed skin. We found that Hcar2-/- mice displayed a strong allergic immune response as shown by increased ear swelling that was significantly enhanced and prolonged in comparison to wild type animals. The number of infiltrating CD45+ immune cells, Ly6G/Ly6C+ neutrophils and CD3+ lymphocytes also was significantly enhanced in inflamed ear tissue of Hcar2-/- mice. RT-PCR analyses revealed increased levels of TNFα, IL-4 and CCL8 in Hcar2 knockout in comparison to wild type animals.

Based on our observations we hypothesize that the HCA2 receptor plays an important role in limiting excessive cellular immune activation in the skin and in promoting a return to homeostatic conditions. Further experiments will elucidate on which cell subtype HCA2 receptor signaling is of major importance for the attenuation of cutaneous immune responses.

P131 | Antibodies to the BP180 C-terminus cause subepithelial autoimmune blistering disease through activation of the C5a/C5aR1 axis

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Bullous pemphigoid (BP) and mucous membrane pemphigoid (MMP) are rare autoimmune blistering diseases characterized by autoantibodies targeting BP180 (collagen XVII) in the majority of cases. The 16th non-collagenous (NC16A) domain of the BP180 ectodomain has been identified as the immunodominant site in BP. Additional extracellular targets of the BP180 C-terminal domain have been described in both BP and MMP; however, their role in disease development has not yet been unequivocally demonstrated. In this study, we generated a human fusion peptide consisting of the 15 non-collagenous domains of the BP180 ectodomain (NC1-15), located downstream (towards the C-terminus) of the NC16A domain. IgG reactivity against the NC1-15 polypeptide was detected by Western blot analysis in patients with BP (15.6%, n = 83) and MMP (4.2%, n = 65). In addition, BP180 ectodomainreactive sera (n = 4) depleted for NC16A IgG induced dermal-epidermal separation in cryosections of normal human skin after incubation with normal human leukocytes indicating the pathogenic potential of antibodies directed against BP180 C-terminal epitopes. To further corroborate our in-vitro findings, we generated a new pemphigoid mouse model by antibody transfer of rabbit IgG against the murine homologue of NC1-15, namely NC1-14. Following subcutaneous injection of 10 mg of anti- NC1-14 IgG every other day over a period of 12 days, C57BL/6J mice presented with erythematous lesions, erosions, and crusts, particularly around the ears, head, neck, and forepaws recapitulating a BP-like phenotype. Mucosal lesions in the oral cavity were only rarely detected in these mice. Direct immunofluorescence microscopy and histopathological analyses of skin biopsies showed linear IgG and complement C3b deposits along the basement membrane as well subepidermal cleavage with inflammatory infiltrates. Following administration of anti-NC1-14 IgG to C5aR1-deficient and wildtype C57BL/6J mice (n = 17/ group) a significant reduction of skin lesions, which was already apparent on Day 4, was observed in knock-out vs. control mice. This finding demonstrates a critical role of complement activation, i.e. the C5a/C5aR1 axis, for tissue destruction by antibodies against the C-terminal stretch of BP180. The new BP-like pemphigoid mouse model will be a valuable tool to further characterize the pathogenic role of antibodies against BP180 C-terminal epitopes and to test new and more efficient therapeutic

approaches for treatment of pemphigoid diseases targeting the BP180 C-terminus.

P132 | Identification of bullous pemphigoid (BP) patients with sole IgG-reactivity against BP230 strongly indicates that autoantibodies against intracellular BP230 contribute to skin damage in BP

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Bullous pemphigoid as severe and most common autoimmune blistering disease is characterised by IgG autoantibodies against the hemidesmosomal proteins BP180 and BP230. Several studies revealed that IgG autoantibodies against BP180, in particular the extracellular domain Nc16a, have the potential to induce blisters. However, it is controversial, if autoantibodies against the intracellular antigen BP230 have a pathogenic function or arise just as a bystander effect. Therefore, we systemically studied sera of patients with only anti-BP230 autoantibodies using ELISA, indirect immunofluorescence staining and immunoblotting to exclude the presence of any IgG autoantibodies, in particular against BP180. Combining all results, we indeed identified 8 patients, who were characterised by sole IgG autoantibodies against BP230. Further profiling analyses using indirect immunofluorescence revealed that the present autoantibodies in this collective were mainly directed against the C-terminal region of BP230. This observation was confirmed by immunoblotting analysing IgG reactivity against 7 overlapping fragments spanning the whole BP230 molecule. Remarkably, all 8 BP samples identified had in common that they reacted with the C-terminal fragments C2 and C3, proposing that autoantibodies bound to this region might interfere with the physiological connection between BP230 and keratin and thereby cause skin damage. Autoantibodies against the N-terminal region were occasionally present, whereas autoreactivity against the central domain of BP230 was not detected. Clinical data of 5 BP patients with sole reactivity to BP230 revealed a mild clinical phenotype with focal blister formation (2 patients) or a non-bullous disease manifestation (3 patients). This clinical observation indicates that BP230 autoantibodies have pathogenic potential but convey a distinct clinical phenotype. In summary, our results strongly suggest that IgG autoantibodies against BP230 have the potential to cause skin damage independently from other autoantibodies; however, the exact mechanisms blister formation remains elusive.

P133 | Metabolic reprogramming coordinates dynamics of myeloid cell function in skin repair

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Skin injury induces a complex, dynamic cellular program proceeding in sequential stages of inflammation, tissue growth and differentiation. Cells of the monocytemacrophage lineage are an essential component of the body's innate ability to restore tissue function after injury. The molecular determinants that control the dynamics of macrophage functional plasticity during healing progression are largely unknown and are just beginning to emerge. Here we demonstrate that skin injury in mice induces different metabolic programs in wound macrophages by profiling their early versus late stages at both the transcriptional and functional levels. We show that early phase wound macrophages activate glycolytic metabolism, which, however, is not sufficient to ensure a productive repair response. Instead, combining conditional disruption of the electron transport chain in myeloid cells by deletion of mitochondrial aspartyl-tRNA synthetase (DARS2) with single cell sequencing analysis of wound macrophages, we find that at early stage a defined subpopulation of macrophages requires repurposing of mitochondrial activity to initiate a cascade of mitochondrial ROS production, HIF1alpha stabilization, ultimately driving an effective pro-angiogenic transcriptional program essential for timely healing. In contrast, we show that late phase wound macrophages increase their mitochondrial mass and activate oxidative metabolism in mitochondria, both dependent on type 2 immune signals. Interestingly, our study uncovers that IL-4/IL-13 signaling orchestrates mitochondrial stress responses in late stage wound macrophages including the integrated mitochondrial stress response and the mitochondria-specific unfolded protein response. We conclude that to convey late phase wound macrophages into repair mode they depend on IL-4Ralpha-mediated mitochondrial respiration and so far unknown regulation of mitohormesis. Our findings are likely to have clinical impact in such that perturbed mitochondrial stress in myeloid cells might contribute to pathological wound healing scenarios, thereby modulating mitochondrial stress responses might provide a target for therapeutic benefit.

P134 (OP03/05) | Imiquimod induced psoriasis requires non-classical monocytes

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Psoriasis is a chronic inflammatory skin disease caused by an IL-23- and IL-17 dominated immune response. Monocytes are regarded as an important leukocyte population in psoriasis. Classical monocytes (cMo) give rise to non-classical monocytes (ncMo), and the two populations exit the bone marrow and circulate in blood. cMo enter skin under inflammatory conditions to become macrophages, dermal dendritic cells or Langerhans cells. ncMo are known to perform an immune surveillance function in the vasculature best portrayed in mice by an extensive patrolling function along the vascular endothelium. The function of ncMo in skin inflammation remains largely unknown. Yet, in our previous studies we defined the population of human slan (6-sulfoLacNAc) expressing ncMo in skin and blood. We reported on their proinflammatory capacity in response to TLR4/7/8 ligation and their presence in psoriasis skin lesions. Here we provide evidence for an important immune stimulatory role of ncMo for the in-vivo development of skin lesions in a psoriasis mouse model with topical imiquimod (IMQ) treatment. In wild type (WT) mice application of the TLR7-ligand IMQ induced an early influx of ncMo to the skin, while cMo arrived later during disease development. This immediate activation of ncMo is in line with their known high sensitivity to TLR7-ligand stimulation. We next studied the IMQ-dependent psoriasis model in mice lacking ncMo. Mice with a deletion in the Nr4a1 super-enhancer subdomain (Nr4a1se-/-) were reported to lack Ly6Clow ncMo, while retaining both Nr4a1 expression in tissue macrophages and normal macrophage responses to inflammatory stimuli. Mice lacking ncMo (Nr4a1se-/-) showed a strongly reduced dermatitis development in response to topical IMQ. In the absence of ncMo IMQ treated skin samples showed a reduction of Th17 T cells, IL-17A-producing γδT cells, NK cells and macrophages. In addition, expression of IL-17A, IL-23, TNF-alpha and IL-1beta was reduced. Early ncMo infiltration coincided with neutrophil influx and expression of CXCL1 and CCL1, both of which were largely reduced in IMQ-treated Nr4a1se-/- mice. Reconstitution of Nr4a1se-/- mice with FACS-sorted ncMo of WT mice completely reversed the psoriasis-deficient pheno- and immunotype of Nr4a1se-/- mice. We conclude that ncMo are critical for the early recruitment of neutrophils and subsequent development of an IL-23- and IL-17 driven psoriasis like skin inflammation.

P135 | Tofacitinib suppresses IL-10/IL-10R signaling and modulates host defense responses in human macrophages

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JAK inhibitors are increasingly used in dermatology. Despite broad inhibitory effects on cytokine signaling cascades, they only modestly increase the risk for infectious diseases. To address molecular mechanisms underlying this unexpected clinical observation, we investigated how tofacintib, a first-in-class JAK inhibitor, regulates host defense responses in TLR4-activated human macrophages. Specifically, we asked if tofacitinib inhibits anti-inflammatory IL-10 signaling, thereby counteracting downregulation of inflammatory, host-protective pathways. We found that tofacitinib blocked macrophage responses to IL-10 at the level of STAT3 phosphorylation. Furthermore, TLR4-induced, auto-/paracrine IL-10/IL-10R activation promoted expression of hepcidin, the master regulator of iron metabolism, resulting in intracellular iron sequestration. In contrast, auto-/paracrine IL-10/IL-10R activation repressed expression of cathelicidin antimicrobial peptide, as well as antigen-presenting molecules, thus together, inducing a pathogen-favoring environment. While tofacitinib further repressed cathelicidin, it prevented induction of intracellular hepcidin, and restored expression of antigen-presentation molecules in TLR4- activated macrophages. Our study supports the concept that induction of IL-10/ IL-10R signaling drives a complex immune evasion strategy of intracellular microbes. Moreover, we conclude that tofacitinib has diverging effects on macrophage host response pathways, and we identify the TLR4-IL-10-STAT3-hepcidin axis as a potential therapeutic target to counteract immune evasion.

P136 | Sphingosine 1-phosphate receptor signalling promotes hair growth and inhibits perifollicular T-cell expansion and immune privilege collapse ex vivo

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Sphingosine 1-phosphate (S1P) is a bioactive phospholipid that signals through five cell surface receptors (S1P1-5). Despite the role of S1P receptors in lymphocyte trafficking and epidermal keratinocyte proliferation and differentiation, their role in hair follicle (HF) physiology remains obscure. We hypothesized that S1P receptor activation may be involved in alopecia areata (AA), a T cell-mediated hair

loss disorder characterized by immune privilege (IP) collapse in the hair bulb.

RNAseq revelated S1P1,3,5 are expressed in human scalp HFs. By in situ hybridization and immunostaining, we confirmed the expression of S1P1,5 in HF epithelium and mesenchyme. Ex vivo treatment of human scalp HFs with the selective S1P1,4,5 modulator etrasimod led to enrichment of keratinization-associated genes, tendential prolongation of anagen, and preservation of IP. The anagen-prolonging effect of etrasimod was reproduced in human scalp skin organ culture, even under pro-inflammatory conditions induced with IFNγ, the key pathological cytokine in AA. Etrasimod prevented IFNγ;-dependent IP collapse and inhibited the increase of perifollicular CD8+ lymphocytes. Supporting the potential role in AA, S1P1,5 were expressed in immune cells within the infiltrate around the bulb, and the intrafollicular epithelial expression of S1P1,5 was increased in the HFs of patients compared to healthy controls.

Taken together, these results suggest that S1P receptor signaling is involved in the regulation of hair growth and preservation of IP in HF, and may also be involved in IP collapse and T cell recruitment in AA. These preliminary findings invite the investigation of targeting S1P receptors for AA management.

P137 | Immunological monitoring of CD4+ T cells in patients with stage III/IV melanoma during treatment with immune checkpoint inhibitors

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Over the past decade, immune checkpoint Inhibitors (ICIs), more specifically the PD(L)-1 and CTLA-4 Inhibitors have improved the outcome of the patients with advanced melanoma and other skin cancers dramatically. However, the intensified and combined use has also resulted in increased reports of immune related adverse events (irAEs) that can involve any organ and grade from low-grade to potentially life threatening. One organ affected by irAEs is the skin. Most of the irAEs are mediated by abnormal T cell reactions, yet the exact role of specialized T cell subsets in irAEs, especially of the skin are not fully understood. In our study, we aim to shed light on immunological effects of ICIs on CD4+ T cells to better understand immune cell composition and T cells involved in irAEs.

In this prospective observational study we studied peripheral blood mononuclear cells (PBMCs) from patients with stage III and IV melanoma at baseline and during the first 4 months of ICI treatment (pembrolizumab or nivolumab alone, or nivolumab combined with ipilimumab). Immunotherapy-naivety was the main inclusion requirement. The cells were analyzed by flow cytometry based on the expression of surface markers for T-helper or T-regulatory cells and CLA as a skin-specific marker.

Currently, we enrolled 51 patients in the study. Seventeen patients have completed the observation period of 3 months and are part of the data analysis. Ten patients developed irAEs. During the course of the therapy, we could observe different changes in the T cell compartment. As expected, we observed a significant decrease of naive T cells and an increase of memory T cells. Detailed analysis of the individual CD4+ T cell subsets were performed. Here, we found a significant increase of the non-follicular Th1 cells and T-regulatory cells in both, CLA+ and CLA- populations. Additionally, among the CLA- T cells, a significant increase of the follicular T regulatory cells could be determined.

So far, we could establish that ICI-therapy significantly affects the non-follicular CD4+ T cell compartment. Our results by immunological monitoring permit a better understanding of mechanisms of ICI and of irAEs and may help to identify predictive factors of irAEs.

P138 (OP03/01) | Type 2 immunity regulates dermal white adipocyte function

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Type 2 immunity has emerged as critical regulator of tissue repair and regeneration in various organs. Mechanistic understanding of how type 2 immune responses regulate tissue homeostasis and regenerative processes in skin is not entirely understood. In this study we demonstrate that the type 2 cytokines IL-4/IL-13 are critical regulators of dermal white adipose tissue (dWAT) homeostasis and function. dWAT is a distinct fat compartment that is integrally linked with tissue homeostasis and self-renewing processes in the skin such as hair follicle growth, wound healing and thermoregulation. To elucidate whether type 2 immunity is involved in maintaining skin homeostasis under physiological conditions, we systematically analysed oscillations of dWAT during first postnatal hair cycling in back skin of mice with global deficiency of interleukin-4 receptor alpha (Il4ra -/-). Interestingly, at P21 the dWAT layer in Il4ra -/- mice was significantly thicker and size of dermal adipocytes was increased compared to littermate controls. Yet, we did not observe alterations in hair cycling in Il4ra -/- mice compared to controls, indicating that type 2 cytokines regulate dermal adipocyte homeostasis in a hair cycle-independent manner. We hypothesized that lipolysis-driven regression of dWAT in first catagen phase (P18-P21) is impaired in Il4ra -/- mice contributing to dWAT hypertrophy. To address this hypothesis, we analysed back skin of P21 old Il4ra -/- and control mice by immunohistochemistry, western blot analysis and qRT-PCR.

Interestingly, immunofluorescence staining and western blot analysis of adipocytes from Il4ra -/- mice revealed virtually absent activity of hormone-sensitive lipase (HSL) by reduced phosphorylation at serine residue 660 (Ser660). Notably, HSL transcripts were not different in mutant and control mice. Thus, our findings suggest that deficiency of IL-4/IL-13 signalling impedes regression of dWAT by reduced and/or delayed enzymatic activation and lipolytic activity of HSL. Further, to identify type 2 cytokine producing cells during postnatal skin development, we monitored temporal and spatial expression of IL-4 in skin during dWAT development and hair cycling in Il4- eGFP (4get) reporter mice. By immunofluorescence staining and flow cytometry, we found that CD11b+ Siglec-F+ eosinophils are the predominant IL-4 expressing cells in unchallenged skin. Thus, regulatory functions of type 2 cytokine mediated signalling on dermal adipocytes in early postnatal life might be driven by IL-4 producing eosinophils in the skin. Collectively, we provide novel mechanistic insights into the regulation of dWAT homeostasis and function by type 2 cytokine signalling. Our findings are likely to be relevant for the mechanistic understanding of type 2- driven skin diseases including Atopic dermatitis and skin fibrosis.

P139 | Pre-metastatic and immunomodulatory conditioning of the hepatic vascular niche to prevent melanoma liver metastasis

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With the approval of immunotherapy for advanced melanoma patients a new era in melanoma therapy was heralded. Meanwhile not only stage IV patients receive immunotherapy (in the palliative setting) but also patients with locally advanced stage III melanomas are treated with adjuvant immunotherapy to prevent disease progression. However, liver metastasis, which is detected in ~10 20% of stage 4 patients, gained special emphasis, as it recently evolved as decisive indicator of treatment resistance to immune checkpoint inhibition. Our project focusses on analyzing pre-metastatic immunologic conditioning of the hepatic vascular niche to prevent liver metastasis formation and to overcome therapy resistance.

In our mouse model primary melanomas were simulated by intradermal injection of WT31 or B16F10 luc2 melanoma cells. Neo-adjuvant, adjuvant or palliative therapy regimens were then performed. Both the hepatic vascular niche and the immune cell infiltration were comparatively analyzed. Moreover, hepatic metastases were induced by intrasplenic injection of these melanoma cells to check for liver metastasis susceptibility among the varying therapy modalities.

First, the premetastatic niche of the liver was analyzed. The presence of intradermal melanomas did not influence hepatic metastasis formation as compared to PBS controls. Besides, RNA-seq of isolated liver sinusoidal endothelial cells of both groups was performed. Despite no significant differences in gene expression, overall representation analysis showed predominant regulation of immune-related pathways. Histomorphologic analysis of hepatic metastases in pre-conditioned livers revealed no differences as compared to the control group. Immune cell infiltration in preconditioned and control livers is currently analyzed. Besides, comparisons of neoadjuvant, adjuvant and palliative therapy regimens showed significant influences on the number of liver metastases and therapy response to immune checkpoint inhibition. These differences are currently further analyzed by flow cytometry, immunofluorescence stainings and RNA-seq.

Our data indicate that the choice of the therapeutic regimen is an important factor influencing not only susceptibility of the hepatic vascular niche to liver metastasis but also therapy response to immune checkpoint inhibition.

P140 | The role of granzyme B and granulysin expressing human DCs in the treatment response to melanoma

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Melanomas are malignant tumors derived from melanocytes, usually developing on UV-exposed areas of the skin. Despite impressive treatment results obtained in stage IV melanoma patients with immune checkpoint inhibitors (anti-PD-1 +/- anti- CTLA-4 antibodies) therapy resistance (primary or acquired) is frequently found. Therefore it is important to study and understand the different immune compartments involved in a successful activation of the immune system by immune checkpoint inhibitor (ICI) therapy. Due to the high tumor mutation burden and the associated high immunogenicity, melanomas are predestined for therapy with ICI. A successful immune response consists of a fast nonspecific phase, which activates the innate response and a second specific adaptive response. However, when the activation of the immune system fails, e.g. due to immune escape mechanism of the tumor, the disease will progress.

In this present study, we are investigating the initial innate immune activation and tumor antigen presentation to T cells through dendritic cells (DCs). By studying a melanoma cell line (MeWo) and an immortalized keratinocyte cell line (HaCaT) invitro, we show that DCs mediate a cytotoxic effect on the melanoma cell line MeWo in co-culture compared to HaCat cells as normal control, where the cytotoxicity was not detected. When we activated the DCs with

lipopolysaccharide (LPS) for 24 h prior to the incubation with MeWo, their cytotoxic capacity was even increased. However, the incubation with LPS pre-activated DCs also resulted in some dead cells in HaCaT co-culture. In contrast to HaCaT-DCs co-culture, we found that the costimulatory molecules CD83, CD86, CD40, CD80, and HLA-DR were upregulated solely on DCs that were co-cultured with MeWo after 24 h without LPS pre-activation. Interestingly, we also found that the expression of granzyme B but not granulysin was elevated in DCs following co-culture with MeWo without LPS treatment. These protein levels were not increased in the HaCaT-DCs co-culture. We are testing the co-culture for the expression of chemokines and cytokines. We are especially interested factors activating CD4 and CD8 T lymphocytes, including interleukin 6 and 12, interferon α, CXCR3, and CXCL10.

To identify the cytotoxicity mediated by human CD11c+ DCs, peripheral blood and tissue specimens are now collected from patients with metastatic melanoma before, during, and after the infusion of ICIs. We are characterizing the phenotype and activation of human DCs and T lymphocytes. In addition, we are interested in the detection of the expression of granzyme B and granulysin in DCs. Our studies will show the possible impact of DCs with cytotoxic characteristics on stage IV melanoma survival and response to ICI.

P141 | Deciphering the presence and function of T regulatory subsets in pemphigus

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Pemphigus is a severe blistering disorder of skin and mucosa characterized by autoantibodies against desmosomal proteins of the skin. The interaction between T (follicular) helper cells and autoantibody producing B cells is important in disease pathogenesis. We are interested in the role of T regulatory cells (Treg) and T follicular regulatory cells (Tf-reg) as critical cellular checkpoints leading to tolerance or autoimmunity.

By the use of surface markers, a flow cytometry panel was established to investigate Treg (and Tf-reg cell subsets based on the expression of chemokine receptors: type 1 (CXCR3+CCR6-), type 2 (CXCR3-CCR6-), type 17 (CXCR3-CCR6+) and type 17.1 (CXCR3+CCR6+)). First, we established the presence of these subsets in peripheral blood of human individuals and isolated them from PBMCs. Sorted Treg/Tf-reg subsets were then analyzed for the expression of cytokines and transcription factors by qPCR. Finally, PBMCs from pemphigus patients (n = 63) and healthy donors (n = 19) were used for flow cytometry analysis, in order to establish comparisons for Treg and Tf-reg subsets in health and disease.

From the expression analysis of cytokines and transcription factors (IFNγ, IL17, IL21, IL10, IL4, IL6, TGFB, FOXP3) we found a consistent level of FOXP3 and TGFB, confirming the regulatory characteristics of Treg/Tf-reg cells. We observed some common but also some different expression levels of lineage-defining cytokines between specific cell subsets. Importantly, Treg/Tf reg cell subset quantification via flow cytometry unraveled a significant lower percentage of Treg 17.1 and Tf reg 2 cells in pemphigus patients compared to healthy individuals.

Our flow cytometry panel allowed us to characterize Treg and Tf reg cell subsets in an autoantibody-mediated blistering skin disease, where we specifically found a lower presence of Treg17.1 and Tfr2 cells. Further analysis of these subsets to investigate the phenotype and functional differences like suppression of T cell proliferation in health and disease is ongoing.

P142 | Diminished expression of tissue residency markers in skin lesions of patients with pemphigus vulgaris

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Pemphigus vulgaris (PV) is an autoimmune blistering disease, in which autoantibodies against desmoglein (Dsg) 1 and 3 interfere with epidermal cell-cell adhesion, thereby causing blister formation and erosions of the skin and/or mucous membranes. The underlying loss of epidermal adhesion is caused by IgG autoantibodies against Dsg3. Even though, Dsg-specific T cells play a central role in the PV pathogenesis, little is known about the presence of other T cell subsets in PV skin lesions since detailed phenotypical and functional analyses are currently missing. Therefore, we established a reliable protocol for the isolation of leukocytes from 4 mm punch biopsies of the skin, which are routinely taken for diagnostic purposes in the clinic. Using this protocol, we aimed to comprehensively characterize T cells residing in lesional and perilesional skin as well as matched peripheral blood of PV patients in comparison to healthy control samples (HC).

Skin biopsies of 4 mm were enzymatically digested followed by filtration, staining and phenotypic analysis using multicolor flow cytometry. Cutaneous T cells possessed signs of tissue-residency and chemokine receptor expression profile known for skin homing. Results suggested a predominance of CD4+ T cells as well as an enrichment of regulatory T cells in the skin of PV patients compared to HC, whereas MAIT cells and gamma-delta T cells showed no differences between the groups. Investigating the permanent residence of these cells, we found all investigated tissue-residency markers (i.e. CD69, CD103, CD49a) to be expressed on T cells in the skin. However, the expression of such markers, particularly CD69 and CD103, was significantly diminished in T cells from PV lesions, suggesting an impact of inflammation and epithelial layer destruction on T cell immunity. Furthermore, tissue-resident T cells exhibited

a chemokine receptor profile known for skin-homing. Among T cells in general, CD4+ effector memory T cells dominated in the skin and these tissue-resident memory T cells possessed a distinct chemokine receptor profile with CXCR3, CLA, CCR4 and CCR8 being dominantly expressed. In conclusion, this study contributes to a comprehensive characterization of T cells in affected skin of PV patients, which is of particular interest for disease monitoring as well as for the identification of potential therapeutic targets. Future experiments shall address functional differences between T cells from lesional and healthy skin including detailed information about the cellular status of these cells using single-cell sequencing.

P143 | Isolation of skin-derived lymphocytes from human skin and murine tissues: A rapid and epitope-preserving approach

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Tissue-resident immune cells have been shown to play an important role in skin health and disease. It is estimated that healthy adult skin contains about 20 billion T cells, but also smaller numbers of other innate and adaptive leukocytes. However, due to the limited access to skin samples of patients and healthy individuals, the characterization of these cells remains challenging. Therefore, we aimed to establish a protocol for the rapid isolation of lymphocytes from the skin using 4 mm punch biopsies routinely taken for diagnostic purposes in the clinic. Following the manual disruption of the skin tissue with scalpels, the samples were enzymatically digested at 37°C. Using different enzymatic concentrations of type IV collagenase and DNase, we established the optimal combination and digestion time for the highest possible cellular yield as well as marker preservation in leukocytes stained for multicolor flow cytometry. In terms of cellular yield, an incubation time of 60 min resulted in on average of 6.000 live CD45+ lymphocytes and allowed the detection of T cells, B cells, whereas the best results for NK cells and innate lymphoid cells were obtained after 30 min and 45 min of digestion, respectively. Taking epitope preservation into account, we showed that our protocol was best after a 30 min digestion for various lymphocyte subsets. This protocol could be utilized for a detailed phenotypic characterization of T cells, including chemokine receptors, which are otherwise easily affected by digestion. Furthermore, isolated T cells retained their functional capacities upon a polyclonal stimulation with PMA/ionomycin. We further report that the optimized rapid isolation protocol could be used in the same manner for murine skin and mucosa. In summary, this study opens up the possibility of gaining rapid access to skin-derived lymphocytes from human or murine tissues. The isolated cells can subsequently be used for a comprehensive analysis of lymphocyte subpopulations for both disease surveillance and identification of potential therapeutic targets or other downstream applications, such as cell culture or RNA sequencing.

P144 | Testing of novel therapeutic targets for blocking acantholysis in pemphigus vulgaris by using an ex vivo porcine skin organ model

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Pemphigus is an autoimmune disease, leading to painful skin blistering. These blisters are mediated by autoantibodies targeting the desmogleins (Dsg) 1 or 3 in the keratinocytes. There are three different clinical presentations, depending on the autoantibody target. Until today a satisfactory, specific treatment is not available. To understand the mechanism of the acantholysis in pemphigus vulgaris and elucidate new treatment targets, a standardized ex vivo human skin organ culture (HSOC) model has been established by our lab. However, the human skin samples are highly dependent on skin donations from plastic elective surgery. Thus, the human skin is inconsistent in its availability as well as age and the body part it's from. This variance might impair the results. There is a need for a more standardizable and reliable skin organ culture model. In fact, porcine skin has a high similarity with human skin regarding thickness and hair density. This makes porcine skin a promising candidate for a new model system.
We performed a porcine skin organ culture (PSOC) model, treating the porcine skin with an anti-Dsg 1/3 antibody fragment (scFv) in the absence and presence of six different low molecular weight (MW) inhibitors. We could show a similar immunohistological structure in porcine compared to human skin regarding the distribution of Dsg 1 and Dsg 3. Additionally, we detected a specific, to human skin comparable binding of the scFv to the porcine Dsg 1 and 3. In fact, the scFv binding leads to a specific intraepidermal split formation in both, the HSOC and PSOC. The treatment with two different low MW inhibitors leads to a significant reduction in the split formation. There are Rigosertib, a PLK1 Inhibitor as well as BIRB 796, a MAPK inhibitor which are both promising candidates for further investigation using both, the HSOC and PSOC.

P145 | Cellular stress due to RNA repeat expansions in myotonic dystrophy type II

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Background: Myotonic dystrophy (MD) type II is characterized by autosomal dominant progressive myopathy and multiorgan involvement including an increased risk for developing autoimmune disorders. The disease is caused by (CCTG)n expansion in CNBP (Cellular Nucleic Acid-Binding) leading to stable CCUG RNA repeat

expansions. Their impact on cellular function and role for disease manifestation is incompletely understood.

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

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

Results: Using RNA FISH technique we demonstrated that fibroblasts of MDII patients accumulate CCUG RNA repeat expansions in the nuclear and cytoplasmic compartment. The cytoplasmic repeats are translated by a mechanism called repeat associated non-AUG (RAN) translation, which led to RAN protein accumulation in the skin of MDII patients. In response to this proteins the cells had chronically elevated mRNA levels of BiP. This protein centrally regulates the unfolded protein response of the ER. Interestingly, we detected a chronic ER stress response on mRNA and protein level and RNA-sequencing analysis in patient cells. This was associated with elevated levels of ROS that correlated with the intensity of repeat expansions in the cell. ROS formation in addition correlated with mitochondrial stress indicated by a reduced membrane potential. The chronic stress response was associated with an enhanced type I interferon response that could predispose to autoimmune diseases.

Conclusion: The chronic ER stress response and mitochondrial stress in fibroblasts demonstrate an ubiquitous reaction to RNA repeat expansions in patients with MDII and may provide an explanation for the non-myopathic disease manifestation such as autoimmune disorders.

P146 | Comparison of molecular signatures in macro- and microbiopsies of psoriasis and atopic eczema

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The most common chronic inflammatory skin diseases are psoriasis and atopic eczema. As more specific and effective therapies have been developed over the last years, correct diagnosis is indispensable. In some cases, however, it remains challenging to differentiate between psoriasis and atopic eczema as both can share several features in clinical and histological presentation. Therefore, molecular classifiers (MC) were developed to predict correct diagnoses with high sensitivity and specificity based on the gene expressions of NOS2, CCL27 or IL36. Until now, MC were only performed with tissue samples of 4–6 mm skin biopsies ("macrobiopsies") or paraffin embedded tissue. Microbiopsies of 1 mm in size offer a new possibility to collect tissue samples in a less invasive and painful manner than conventional biopsies. Additionally, sutures or local anesthesia are not required, saving valuable time in clinical routine.

To test whether microbiopsies are suitable to perform MC tests, we analysed the gene expression of NOS2, CCL27 and IL-36G in 51 paired micro- and macro-biopsy samples (27 eczema, 19 psoriasis and 5 mixed phenotypes). Here, we detected a high correlation of MC results between micro- and macro-biopsy in atopic eczema. To investigate the correlation of gene expression between micro- and macrobiopsy in more detail, we performed RNA-seq analysis of 10 atopic eczema (5 macro- and 5 microbiopsies), 10 psoriasis (5 macro- and 5 microbiopsies) and 4 unclear phenotype (2 macro- and 4 microbiopsies) samples. We found no significant difference between differentially regulated genes in macro- and microbiopsies, indicating that 1 mm biopsies contain sufficient RNA material to capture as much information about transcriptome changes as in 4–6 mm biopsies. Pathway analysis confirmed an upregulation of Th17 related pathways in psoriasis and Th2 related pathways in atopic eczema samples.

Thus, microbiopsies appear to be a viable alternative to conventional biopsies in performing gene expression analysis and MC tests.

P147 | Inhibition of IFN-gamma and administration of IL-4 ameliorates the induction of experimental epidermolysis bullosa acquisita

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Pemphigoid diseases (PDs) are a group of severe and potentially life-threatening autoimmune diseases defined by an autoantibody-mediated immune response against distinct components of the hemidesmosomal anchoring complex at the dermal-epidermal junction of skin and mucous membranes. Systemic corticosteroids remain the gold standard treatment but the patients suffer from multiple adverse events, including death. Therefore, and due to the rising incidence, there is a clear and so far, unmet medical need for the development of effective and safe therapeutic strategies for these patients. Furthermore, pathogenic mechanisms that are involved in the disease progression are only incompletely understood. A detailed understanding of PD pathogenesis may be the key for the development of novel therapeutic strategies. In recent studies, focusing on the contribution of T cells and monocytes/macrophages to PD skin inflammation, we also observed an increase of the expression of the classical CD4+T helper type (Th) 2 cytokines IL-4/IL-13 and the pro-inflammatory Th1 cytokine IFN-gamma in the experimental PD epidermolysis bullosa acquisita (EBA). Based on these observations, we here aimed to investigate their contribution to disease progression in detail. For this, we induced experimental EBA in

mice by transfer of antibodies targeting type VII collagen (COL7), the autoantigen in EBA. Here, a function-blocking IFN-gamma antibody reduced clinical disease severity by 50% Unexpectedly, blockade of IL-4 had no impact on clinical disease manifestation in this model. By contrast, treatment with recombinant IL4 (complexed with an IL4 antibody) led to an almost complete protection from clinical disease manifestation in this model. These findings are seemingly in contrast to emerging case reports documenting a good clinical response of patients with the PD bullous pemphigoid to IL4R inhibition. Yet, we believe that these seemingly contrasting results reflect diverse functions of IL4. More specifically, we hypothesize that IL4 is required for the generation of autoantibodies, but possesses potent anti-inflammatory functions in autoantibody-mediated skin inflammation in PD. Overall, this provides further insights into cytokine biology in PD, contributing to the understanding of autoantibody-driven skin inflammation.

P148 (OP06/02) | Sebaceous glands are actively and differentially involved in the pathogenesis of atopic dermatitis and psoriasis as revealed by spatial transcriptomics

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Background: The primary function of sebaceous glands (SGs) is to produce the lipid rich sebum and thereby contribute to skin lubrication. Recent findings however revealed that sebum lipids may also have other functions like protection from UV light or immunoregulation.

Methods: We performed spatial transcriptomics on lesional and autologous nonlesional human skin samples of patients suffering from psoriasis (PsO) (n = 3), atopic dermatitis (AD) (n = 5), and on nonlesional samples of patients with lichen planus (n = 5). Annotation was conducted in a standardized matter using H&E stains of the samples, considering general morphology, specific cell types, and anatomical structures including SGs. After normalization and batch correction of RNAseq data, differentially expressed genes were identified followed by pathway enrichment analysis. Finally, a meta-analysis was done using available RNAseq data from the SZ95 human sebocyte cell line treated with AD-like stimuli.

Results: Besides confirming that SGs contribute to skin homeostasis with an active lipid metabolism, we identified a large set of genes that were so far not known to be expressed in in vivo SGs of healthy skin. Our analysis using AD and PsO samples further revealed that while in SGs of AD samples lipid production, in SGs of PsO samples keratinization and neutrophil activation related genes showed an altered expression. Altogether we found that gene signatures of immune functions, host defense and lipid metabolism distinguished SGs of PsO from SGs of AD to which a disease-associated cytokine milieu may contribute to.

Conclusions: SG are not bystanders in chronic inflammatory skin diseases like PsO and AD, but actively and differentially contribute to lipid production and inflammation depending on their surrounding micromilieu.

P149 (OP01/03) | Single-cell and spatial architecture of human tissue granulomas reveals an aberrant immune-regulatory program underlying sarcoidosis

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Background: Granulomatous disorders include a wide variety of infectious and non-infectious conditions with heterogeneous clinical courses and considerable constraints of affected organs. They evolved as protective mechanisms and imply dynamic interactions of various immune and non-immune cell populations leading to compact, well organized structures. Sarcoidosis belongs to the group of granulomatous disorders of unknown etiology where persistent inflammation can lead to severe organ destruction. In our study, we take cutaneous sarcoidosis as model disease to acquire new insights into the pathomechanisms of non-infectious granuloma formation and maintenance.

Objectives: To dissect the gene-regulatory programs and cell-cell interactions underlying granuloma formation, we combined single-cell RNA-seq and spatial transcriptomics with fluorescently labeled antibody detection for protein expression in granulomatous and non-affected skin from 12 patients with chronic cutaneous sarcoidosis.

Results: We found granuloma-associated T cells adopting a Th17.1 phenotype. They activate macrophages, fibroblasts and endothelial cells by producing GM-CSF and interferon-gamma. Macrophages and fibroblasts adopt a pro-inflammatory, longlived phenotype that allows them to attract and retain immune and structural cells within granuloma structures by secreting chemokines and remodeling the extra cellular matrix. Small blood vessels are surrounding

granuloma structures, facilitating leukocyte trafficking to the site of inflammation.

Conclusion: We performed the first single-cell characterization of sarcoidosis granuloma tissue and showed T cells, macrophages and fibroblasts triggering local inflammation and forming a disease-promoting triangle of adaptive, innate and structural immunity in non-infectious granulomatous disease.

P150 | Single-cell sequencing defines the molecular and cellular underpinnings in spatially-resolved Non-Langerhans cell histiocytosis

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Non-Langerhans cell histiocytosis (NLCH) comprises a group of rare, proliferative disorders, driven by histiocytes such as macrophages and dendritic cells, which are not classified as Langerhans cells and do not belong to the group of hemophagocytic lymphohistiocytosis. Here, we followed a systems biological, integrative approach to characterize and explore the molecular underpinnings underlying the pathophysiology of this disease in a case with a severe and progressive form classified as malignant histiocytosis. The patient's epidermal skin was found to be invaded by proliferative monocyte/macrophage populations additionally detectable in sporadically developing, granulomatous lesions. Single cell RNA-seq of skin and granuloma biopsies identified highly proliferative myeloid cells with stem-cell-like characteristics, displaying an inflammatory and tissue-disruptive phenotype including genes with known roles in osteoclast function. The identified gene signatures include high levels of SIGLEC15, CTSK (encoding cathepsin K), SLC9B2, ACP5, RACK1, MMP9 and MMP14 expression. This finding provides a possible explanation for the highly invasive and skin-barrier-disruptive characteristics of the identified population, which could be further demonstrated by integrative analysis using spatial transcriptomics. Our spatial data reveal the pro-liferative macrophage signature to be manifested throughout epidermal skin. The invasive character of the here identified macrophage cell subset was further supported by scRNA-seq profiling of the patients PBMCs confirming the population to be present systems-wide. Taken together, our data characterize a macrophage population with neoplastic, stem-cell-like characteristics in NLCH with gene-signatures supporting their invasive and disruptive properties in skin and peripheral blood. Our findings identify critical signaling pathways and gene regulatory factors providing a possible starting point for therapeutic intervention to interfere with the inflammatory and tissue-disruptive phenotype of macrophages driving malignant NLCH.

P151 | Delayed antiretroviral therapy in HIV-infected individuals leads to irreversible depletion of skin- and mucosa-resident memory T cells

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People living with HIV (PLWH) are at increased risk of developing skin and mucosal malignancies despite systemic reconstitution of CD4+ T cells upon antiretroviral therapy (ART). The underlying mechanism of chronic tissue-related immunodeficiency in HIV is unclear. We collected longitudinal skin biopsies from early-presenting HIV+ individuals before and after 1 year of ART and compared them to HIV late presenters with initial low systemic levels of CD4+ T cells. We found that skin CD4+ tissue-resident memory T (Trm) cells were depleted after HIV infection and replenished only upon early ART initiation. TCR clonal analysis following early ART suggested a systemic origin for reconstituting CD4+ Trm cells. Single-cell RNA-sequencing of PLWH that received late ART treatment revealed a loss of CXCR3+ Trm cells and a tolerogenic skin immune environment. In biopsies of human papilloma virus-induced precancerous lesions, the frequency of CXCR3+ Trm cells in the mucosa was reduced in PLWH versus HIV- individuals. These results reveal an irreversible loss of CXCR3+ Trm cells confined to skin and mucosa in PLWH that received late ART treatment, which may be a precipitating factor in the development of HPV-related cancer.

P152 | Type I interferon activation in dermatomyositis

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Background: Dermatomyositis (DM) is a rare autoimmune disease affecting patients' skin and muscle. Symptoms include violaceous erythema in sun exposed areas, dermal atrophy, ulcerations and proximal muscle weakness. The disease is characterized by an elevated type I interferon (IFN) signature in patients' blood. The origin and pathogenesis of the disease remain incompletely understood.

Objective: To investigate the type I IFN activation in fibroblasts of DM patients and the effects of JAK inhibition.

Methods: Fibroblasts isolated from patients' skin tissue were cultivated and analyzed for their ISG expression using RT-qPCR. RNA Sequencing was performed in native as well as irradiated fibroblasts. Solar simulated radiation was used to stimulate the cells. The Sequencing data was evaluated using principal component and KEGG-pathway analysis. Patients were treated and in vitro blood samples incubated with Baricitinib. The ISG expression in blood samples was then analyzed using RT-qPCR. Patients' symptoms were assessed using the Cutaneous Dermatomyositis Disease Area and Severity Index (CDASI).

Results: Fibroblasts were isolated from skin biopsies and cultured in vitro. Using RT-qPCR, we found that most of the patient fibroblast cell lines had a higher expression of type I interferon stimulated genes (ISGs) than healthy controls (26 patients and 7 controls). There was a positive correlation of the height of ISG expression and the severity of their skin symptoms. RNA Sequencing data showed differential gene expression for fibroblasts of DM patients and healthy controls. 45% of significantly upregulated genes in DM patient cells were type I IFN stimulated genes. Genes connected to cytosolic DNA sensing, TLR signaling, nucleotide excision repair, mitophagy, apoptosis, necroptosis, cellular senescence or protein processing were significantly upregulated. UV-irradiation stimulated significant upregulation in these pathways and increased the number of upregulated ISGs. In vitro incubation of blood samples from DM patients with the JAK1/2 inhibitor Baricitinib significantly reduced the expression of type I IFN stimulated genes indicating a therapeutic potential of the drug in DM. This finding is in line with the clinical finding demonstrating reduction of cutaneous lesions and systemic disease activity in patients with DM and treatment with JAK inhibitors.

Conclusion: Here we demonstrate that elevated expression of type I interferon stimulated genes in fibroblasts of DM patients is maintained in cell culture and the number of upregulated ISGs was enhanced by UV irradiation. This finding can be relevant for the explanation of UV induced flares of DM. It further implies a therapeutic potential of JAK inhibition in patients with DM.

P153 (OP05/03) | Desmoglein 3 extracellular domain 5 specific IgG is pathogenic in pemphigus vulgaris

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Introduction: Pemphigus vulgaris (PV) represents a severe autoimmune blistering disease that is characterized by autoantibodies (auto-ab) against the desmosomal adhesion molecules desmoglein3 (Dsg3) and Dsg1. Binding of Dsg-specific autoab to their target structures induces an disruption of the desmosomal integrity leading to an intraepidermal loss of keratinocyte adhesion, which results in the clinical manifestation of flaccid blisters and erosions in PV patients. The underlying mechanisms that induces blister formation upon binding of Dsg-specific auto-ab are largely unknown. Numerous studies demonstrated the pathogenicity of auto-ab specific for the amino-terminal region (extracellular domain 1, EC1) of Dsg3, such as the monoclonal antibody AK23. However, the Dsg3 specific autoab response in PV patients is polyclonal, including auto-ab directed against both aminoterminal- and membrane proximal epitopes.

Methods: In this study the pathogenicity of an antibody directed against the membrane-proximal region (EC5) of the Dsg3 ectodomain was analysed. A novel monoclonal EC5-specific antibody was isolated from the supernatant of a Dsg3- specific B-cell hybridoma and tested in various specificity and functional assays including immunofluorescence, keratinocyte dissociation assay, by atomic force microscopy and in an in vivo passive transfer mouse model.

Results: Results clearly demonstrate that the auto-ab is capable of inhibiting intercellular keratinocyte adhesion accompanied by the activation of the p38 MAPK signal transduction pathway. Here, for the first time, we demonstrate that a monoconal antibody directed against the membrane-proximal EC5 subdomain of human Dsg3 exhibits a pathogenic activity similar to the well characterized EC1- specific antibody AK23, yet without Ca2+ dependency. Passive transfer analysis into neonatal mice revealed acantholytic properties in vivo. Our results reveal new aspects of a more defined understanding of auto-ab-induced loss of epidermal adhesion in PV.

P154 | Detection of skin-homing receptors on circulating CD4+ T lymphocytes in patients with chronic spontaneous urticaria

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Chronic spontaneous urticaria (CSU) is a skin disease characterized by recurrent, itchy wheals. The pathomechanisms of CSU and, in particular, the extent of Tcell involvement in these processes, have not been clarified to date. Investigating T-cell responses in CSU patients by measuring the secretion of different marker cytokines after unspecific activation with anti-CD3/CD28 antibodies using ELISPOT analysis, we recently observed that CSU patients had significantly reduced numbers of peripheral IFN-γ-, IL-17- and IL-10-secreting T-cells compared to a control group of healthy controls (HC). Furthermore, it has been shown by others that CSU patients have significantly more CD4+ T-cells in their lesional and non-lesional skin compared to biopsies of HC skin. Therefore, we investigated the

migratory capacity of T-cells into the skin by analyzing the expression of the skin-homing receptors CCR4, CCR8, CCR10 and cutaneous lymphocyte-associated antigen (CLA) on different T-cell subsets using multi-parametric flow cytometry. In addition, patients can be further classified based on CSU endotypes, i.e. type I (characterized by IgE autoantibodies to autoallergens), type IIb (characterized by IgG autoantibodies targeting the high-affinity IgE receptor FcεRI or IgE bound to its receptor on mast cells and basophils) or overlap (showing signs of type I and type IIb). Accordingly, in this study autoallergic type I patients were identified by the presence of IgE against thyroid peroxidase (TPO) or IL-24, whereas the autoimmune subgroup IIb was specified by positive serum autoreactivity.

Our results showed for the overall population of patients with CSU (n = 59) a significantly higher expression of the skin-homing marker CLA on CD4+ T-cells compared to the HC group (n = 11). Additionally, a trend towards increased CCR10 expression was observed in the CSU cohort. Furthermore, stratification of the CSU patients according to their CSU endotype and analysis of the different T-cell subpopulations revealed that autoallergic CSU patients had an increased CLA expression on Th2 and Th17 cells, whereas patients of the type IIb subgroup were characterized by higher expression of CLA on Th1 and Th17.1 cells. In addition, functional T-cell analysis by ELISPOT showed increased IL-10- and IFN-γ-secretion in autoimmune type IIb patients suggesting a more reactive phenotype than in the type I and overlap cohort.

In conclusion, based on both skin-homing receptor expression and functional cytokine secretion analyses, we were able to determine the different potential of distinct T-cell subsets to migrate into the skin which seems to be dependent on the CSU endotype. These findings confirm our assumption that individual T-cell subsets are involved to different extents in the various pathophysiological processes of CSU.

P155 | The role of the NLRP3 inflammasome in Langerhans cells for pathogenesis of vitiligo

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Vitiligo is an autoimmune disease leading to progressive destruction of melanocytes by autoreactive CD8+ T cells. Environmental influences, like sun exposure or skincontact with chemical substances, leading to melanocyte-stress are known causes for vitiligo induction and disease progression. Damage-associated molecular patterns (DAMP) were found to be upregulated in stressed melanocytes and are involved in vitiligo. However, the role of the innate immune system, especially of dendritic cells (DC) in vitiligo is still unclear.

Due to the fact that epidermal Langerhans cells (LC), a specific subset of DC in the epidermis, express NLRP1 in lesional skin of vitiligo patients and mouse LC express high amounts of NLRP3, we hypothesize that LC are activated by melanocyte-stress and present melanocyte-antigens to induce autoreactive T cells.

In order to investigate the importance of NLRP1/3 in LC, we will establish chemically induced vitiligo models in mouse and human skin. Melanocyte stress and early activation of the innate immunity will be determined by using multicolor flow cytometry, microscopy and RT-qPCR. We will study the activation of inflammasome in skin DC with specific focus on LC mediated by DAMPs released by melanocytes. Moreover, the subsequent T cell responses leading to depigmentation will be analyzed in LC-depletion mouse model, bone marrow chimera models lacking NLRP3 in LC and human skin explants. A novel LC-based vaccine platform will be evaluated for its potential for vitiligo treatment in mouse models.

Taken together, this project will shed light on the role of NLRP1/3 in LC for vitiligo pathogenesis.

P156 | T cell deficiency associated with downregulation of GPI-anchored proteins

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Glycosylphosphatidylinositol (GPI) is a common posttranscriptional modification of cell surface proteins by which the protein is anchored to the plasma membrane. Defective GPI biosynthesis in hematopoietic stem cells was shown to cause loss of the complement inhibitors CD55 and CD59 on erythrocytes, leading to complement dependent intravascular hemolysis and thrombosis. We describe a patient with chronic lymphocytic inflammation of the skin, massive T cell lymphopenia and global loss of GPI-anchored proteins (GPI-AP) on a large proportion of T cells in blood and skin lesions.

We aim to identify the cause of the GPI-AP defect of this patient and its link to T cell deficiency in peripheral blood and infiltration in the skin by flow cytometry, wholeexome and bulk RNA sequencing, proteomics, immunofluorescence, western blotting and T cell culture.

Whole-exome sequencing did not reveal any mutations classically associated with GPI anchor deficiency. On RNA level, we could not observe a difference in expression levels of GPI biosynthesis genes between GPI anchor positive and negative T cells and also expression levels of GPI-AP were similar. We further performed western blot analysis of CD48, a GPI-AP expressed on T cells, to assess if GPI-AP are trapped within the ER-Golgi transport. As GPI-AP were completely absent in western blot, a defect at an early stage of GPI-AP biosynthesis is likely. By flow cytometry of T cell subsets in the blood we found CD8 T cells to predominate with an almost diminished naïve T cell population. Around 60 % of blood-derived T cells express cutaneous lymphocyte antigen and CCR4- and CCR10-expressing T cells are more abundant than in healthy donors. When

analyzing T cells in skin sections, we observed comparable numbers of GPI-AP deficient T cells in skin and blood. However, T cells in the skin are predominantly CD4 positive, suggesting a migration imbalance in the T cell compartment. By performing proteomics of GPI positive and negative T cell populations we will get further insight why translation of GPI-AP is disturbed and if there is a connection to the skin homing properties of T cells.

Together, we describe for the first time a patient with a GPI-anchored protein defect exclusively on T cells presenting with a skin phenotype, which will give insights into the relevance of GPI-anchored proteins for T cell function, migration and maturation.

P157 | Impact of UVB-induced IL-33 on skin immunity

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IL-33 is a danger-associated molecular pattern (DAMP) that is released upon cell stress and activates immune cells, thereby mediating inflammation. It is associated with several types of inflammatory diseases, for example asthma, atopic dermatitis and psoriasis. UVB irradiation is also known to regulate IL-33 expression in keratinocytes and fibroblasts of the skin and these IL-33 expressing fibroblasts were shown to co-localize with dermal mast cells. Skin-resident mast cells are important for the mediation of UV-induced immunosuppression.

We could show increased IL-33 levels in the skin after chronic UVB irradiation of mice, together with greatly elevated dermal mast cell numbers. In addition, we identified IL-33 to be crucial for mast cell activation and survival in vitro, therefore, we aimed to understand the role of IL-33 on mast cells and skin immunity in vivo.

To test the impact of IL-33 signaling in UVB irradiation, wildtype mice were irradiated with chronic, low doses of UVB over a period of 6 weeks and IL-33 signaling was blocked by the injection of anti-IL-33 receptor antibodies. IL-33 blocking did not affect mast cell numbers, but interestingly UVB-induced keratinocyte proliferation was reduced, resulting in a decreased epidermal thickening. Moreover, innate immune cell numbers in the skin such as macrophages, neutrophils and monocytes were decreased, indicating a reduced inflammatory response when IL-33 signaling was blocked in UVB-irradiated skin. In contrast, adaptive immune cells in lymphoid organs remained unaltered in the absence of IL-33, revealing that IL-33 rather acts locally in the skin than in the periphery.

Next, we plan to analyze how increased IL-33 levels might affect immune cells, especially mast cells, and the epidermal barrier independent of UVB irradiation by administration of recombinant IL-33 in vivo. With this, we aim to gain further knowledge about the role of the IL-33/ST2 axis in skin immunity.

P158 | Disease development of experimental epidermolysis bullosa acquisita is independent of Mlkl, CD93 and Trem1 despite their elevated expression in lesional skin

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Hub-genes are defined as genes with a central position in gene interaction networks, thereby being potential regulators of disease activity. Recently, several hub-genes for the development of experimental epidermolysis bullosa acquisita (EBA) have been identified. Amongst these, the regulatory function of Sykb has been validated in vivo, using different murine models of EBA. However, validation of other hub genes has not been performed. So far, a central role in disease regulation has been proposed for Mlkl, CD93, as well as Trem-1 and -3. Mixed lineage kinase domain like pseudokinase (Mlkl) interacts with RIP-3 and plays a critical role in TNF-a induced necroptosis. Increased expression is also found in inflammatory bowels disease (IBD) in children. CD93, previously believed to be the complement C1q-receptor, is thought to play an important role in intercellular adhesion. Lastly, the triggering receptors expressed on myeloid cells -1 and -3 are involved in the pathogenesis of IBD and blockage provides protections against septic shock. However, the role of these molecules in autoimmune blistering diseases is unknown.

To address this knowledge gap, we investigated the expression of these hub-genes in lesional skin of antibody-transfer induced EBA, in a first attempt to validate the functional relevance. Here, we could show a significantly increased expression of Mlkl, CD93 and Trem-1. We previously published that Trem-1 did not have an impact on neutrophil effector functions important for the pathogenesis of EBA. Thus, disease development of experimental EBA was independent of Trem-1. In the present study, we therefore evaluated the functional role of other hub-genes, namely CD93 and Mlkl, on neutrophil effector functions. However, the release of reactive oxygen species was independent of the CD93 or Mlkl expression. Furthermore, we evaluated the in vivo relevance of CD93 and Mlkl on disease development using knockout mice. After induction of experimental EBA, no difference in disease manifestation between knockout and wildtype mice could be observed. Functional validation of simultaneous blockage of Trem-1 and -3, thereby taking the possible redundant function of both in the murine immune system into account, is a this point still ongoing.

In summary, despite being potentially important regulatory genes which are overexpressed in lesional skin, functional validation showed that disease development of experimental EBA is independent of CD93 and Mlkl. This study highlights the importance of functional validation of potential hub-genes.

P159 | Adoptive transfer of regulatory T cells ameliorates autoantibody-induced inflammation in experimental epidermolysis bullosa acquisita

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Intriguingly, different lymphocyte populations regulate recruitment and activation of different myeloid cells in the effector phase of pemphigoid disorders (PDs). In particular, disease-promoting and disease-suppressing functions were demonstrated. Regulatory T cells (Tregs) are among the latter. Most of these discoveries were made in the antibody transfer-induced mouse model of epidermolysis bullosa acquisita (EBA), a PD characterized by autoantibodies targeting type VII collagen (COL7). Due to their anti-inflammatory and thus disease alleviating effects, Tregs may be used to treat autoantibody-induced tissue damage in PDs and in EBA in particular. To study the role and potential therapeutic impact of Tregs in PDs, 2 independent approaches were pursued in the antibody transfer-induced EBA: As IL-2/anti- IL-2 mAb JES6-1 complex has been demonstrated to induce Tregs and thus alleviate disease in other inflammatory diseases, the complex was administered in a preventive treatment regime in antibody transfer-induced EBA model. Second, freshly isolated Tregs from healthy mice were adoptively transferred intra-venously 1 day before disease induction. Among the clinical phenotype, the number and percentage of Tregs and T-effector cells in spleen, lymph nodes, blood and skin were assessed by flow cytometry using FoxP3 as a Treg-specific marker.

Administration of IL-2/JES6-1 showed no amelioration of the clinical score in experimental EBA. On the cellular level, IL-2/JES6-1 led to an increase of Tregs in the spleen, but also an increase of T-effector cells were observed. Of note, the adoptive transfer of Tregs before disease induction showed a significant reduction of clinical disease manifestation compared to an untreated mice group. More specifically, treatment of mice with Treg reduced the clinical score by 50 % at the last day of experiment. Molecular analysis to better understand the mode of action of Treg therapy is currently underway. Based on previous results, where Treg were depleted in this model, we expect an increased local concentration of IL-4, IL-5 and IL-10.

In a translational arm of the project, Tregs from peripheral blood of bullous pemphigoid (BP) patients were analysed by flow cytometry. To investigate if frequencies of Tregs change during the course of PDs, blood from patients was taken and the cells were analyzed at diagnosis as wells as 3, 6 and 12 months follow-up. Interestingly, no difference of Tregs in peripheral blood of BP patients compared to an age-matched control group at all the investigated time points was observed.

Taken together, these findings suggest that adoptive Treg transfer, but not induction of Treg by IL-2/JES6-1, can modulate antibody-induced inflammation in EBA. The unchanged number of Tregs in BP patients suggest a functional defect in Treg in BP patients. However, this needs to be experimentally addressed in the future. Importantly, this indicates that Tregs not only contribute to the loss of tolerance in PD, but also have a significant impact on autoantibody-induced inflammation.

P160 | Application of an inflammatory skin model with induced pluripotent stem cell-derived macrophages to study granuloma formation

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Introduction: Currently available in vitro models for inflammatory skin diseases are often based on two-dimensional cell culture and stimulation of patientderived cells. However, this does not account for cellular interactions within tissue microenvironment. Sarcoidosis is an inflammatory skin disease of unknown etiology, characterized by the aggregation of macrophages, lymphoid cells and fibroblasts. These granulomas represent the pathological hallmark of sarcoidosis, yet the process of granuloma formation is not known. Therefore, we aim to engineer an immunocompetent skin model allowing us to mimic and study the inflammatory niche occurring in vivo.

Results: As the access to patient samples is often limited we focused on the potential use of induced pluripotent stem cells (iPSCs) and their ability to differentiate into every other cell type. For our approach, we established a protocol to successfully differentiate macrophages from healthy and patient-specific iPSCs. In a twostep process, monolayer differentiation of monocytes is induced followed by their differentiation into macrophage subtypes using IL-4 or INF-γ and LPS. iPSC-derived macrophages (iPSDM) are compared to monocyte-derived macrophages (MoDM) from the same individuals. iPSDM and MoDM expressed the canonical markers CD45, CD14 and CD68, macrophage subtype specific markers CD80 (M1) and CD206 (M2) as well as the respective cytokines TNFα and IL-4. Next, we incorporated MoDMs from patients or healthy controls into human skin equivalents to assess their behavior in a three-dimensional setting. We were able to localize the cells by immunofluorescence staining and fluorescent cell tagging and track their migration behavior. Interestingly, patient-derived MoDMs tend to migrate all into a similar direction similar to granuloma formation, whereas control cells were more evenly distributed within the matrix. In the next steps, we will further characterize iPSDMs and study their interaction with other cell types in this 3D in vitro inflammatory skin model.

Discussion: A proper immunocompetent skin model reflecting the complex inflammatory niche occurring in vivo allows us to identify cellular disease drivers. Two-dimensional cell culture systems are useful to study basic cellular functions but fail to display many key

factors such as cell-cell interaction and nutrient excess. To overcome such limitations, we combine primary cell culture, direct differentiation, bioprinting and the intrinsically driven self-assembly of cells to decipher cellular processes involved in inflammatory skin diseases.

P161 | Exploratory study to evaluate changes in inflammatory pattern in patients with active, moderate-to-severe hidradenitis suppurativa

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Patients with hidradenitis suppurativa (HS) suffer from a chronic inflammatory disease characterized by painful inflammatory nodules, abscesses, fistulas, and scars. Currently, little is known about the pathophysiology of HS. Different factors like genetic, environment, lifestyle, hormone balance, or microbiome play a significant role. A negative imbalance of these factors lead to an immune activation around the hair follicle and hyperkeratosis as initial steps. The infiltrate of HS regions includes neutrophilic granulocytes, plasma cells, dendritic cells and immune cells, which release enhanced several inflammatory modulators such as tumor necrosis factor (TNF) -alpha, Interleukin (IL) -1, IL-6 and IL-23 in the early phase.
According to the unknown availability of data about the cell infiltrate residing in HS lesions our study aims to examine different cell types such as dendritic cells, mast cells, B- and T-cells and many other immune cells in skin biopsies of HS patients. Therefore, biopsies were taken from lesional and non-lesional skin of 15 patients. These skin biopsies were cryoconserved and will be analyzed via multi-epitopeligand- cartography (MELK) to investigate inflammatory cell network changes in lesional and non-lesional skin areas.

P162 | Pityriasis rubra pilaris: Patient's characteristics and immunological mechanisms

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Pityriasis rubra pilaris (PRP) is a rare, chronic inflammatory skin disease, which manifests with erythemato-squamous skin alterations. In contrast to psoriasis vulgaris, the aetiopathophysiology of PRP is largely unknown and there are no disease-targeted approved therapies.
The aim of our project was the characterization of the PRP patients (retrospective collection of data based on the medical records) and the determination of markers of the main immunological pathways skin and blood of the patients. Comparison with patients with psoriasis was done to meaningfully interpret the data.
Based on data from over 100 PRP patient records, we found that the curve of the age of the first manifestation of PRP shows three peaks, with a clear one in the first decade of life. The skin lesions that appeared most frequently in initial PRP manifestation were erythroderma and palmoplantar hyperkeratosis. Contrary to the current view, the first symptoms most often appeared on the trunk. The proportion of patients with coronary artery disease and hypertension in our PRP cohort was much higher than that in our psoriasis patient cohort, although the proportion of PRP patients who were smokers or overweight was significantly lower or similar. Interestingly, a comparatively high proportion of PRP patients showed malignant tumors. Analyses of immunological parameters in the skin lesions and the blood of individual PRP patients suggested that the T1, T17 and T22 cell pathways are active in PRP, but do not achieve the extent observed in patients with psoriasis. Analysis of IL-1b (the main producers of which are myeloid cells), as well as IL-36 (mainly produced by tissue cells) in the PRP lesions demonstrated significant activation of the innate immune system, comparable to that observed in psoriasis. Moreover, cooperation of the T17 and IL-1b pathways with involvement of neutrophils was suggested by the lesional expression pattern of chemokines and antibacterial proteins.
Our study shows novel characteristics both of epidemiological and immunological aspects of PRP.

P163 | Select hyperactivating NLRP3 ligands enhance the TH1- and TH17-inducing potential of human type 2 conventional dendritic cells

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The detection of microorganisms and danger signals by pattern recognition receptors on dendritic cells (DCs) and the consequent formation of inflammasomes are pivotal for initiating protective immune responses. Typically, the activation of inflammasomes leads to IL-1β secretion accompanied by pyroptotic cell death. However, dependent on the cell type and the inflammasome ligands used, some cells can survive inflammasome

activation and exist in a state of hyperactivation (defined by IL-1β secretion from living cells along with other pro-inflammatory cytokines). Here, we report that the conventional type 2 DC (cDC2) subset is the major human DC subset that is transcriptionally and functionally able to induce inflammasome formation and enter a state of hyperactivation. When cDC2 were stimulated with ligands that relatively weakly activated the inflammasome, the cells did not enter pyroptosis but instead secreted IL-12 family cytokines together with IL-1β. Hyperactivated cDC2 induced prominent T helper type 1 (TH1) and TH17 responses that were superior to those seen in response to Toll-like receptor (TLR) stimulation alone or to stronger, classical pyroptosis-inducing inflammasome ligands. These findings not only define the human cDC2 subpopulation as a prime target for the treatment of inflammasome-dependent inflammatory diseases but may also enable new approaches for adjuvant and vaccine development.

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P164 | Antigen targeting of Fc receptors induces strong and functional relevant T cell responses in vivo independent of ITAM signaling but dependent on dendritic cell subsets

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Dendritic cells (DCs) are important antigen presenting cells (APCs) and induce immune responses, but also preserve peripheral tolerance. We showed the preferential induction of either CD4+ or CD8+ T cell responses by DC subpopulations in vivo by targeting antigens to endocytic C-type lectin receptors. The also highly endocytic active Fc receptors (FcRs) enable APCs to take up antigens in form of immune complexes. As they are expressed on various APCs, we aimed to identify responsible APCs for primary and secondary immune responses by using our antigen delivery by recombinant antibodies to activating and inhibitory FcRs. This targeting induced CD4+ and CD8+ T cell responses independent the receptor's type. Moreover and in contrast to DEC205 and DCIR2 targeting, especially antigen delivery to FcγRIV was superior in inducing simultaneously CD4+ and CD8+ T cell responses, not only in a transgenic setting, but also in naïve mice. As FcγRIV is expressed on both splenic cDC subsets, we used it to verify the subset intrinsic preferences to trigger either CD4+ or CD8+ T cell responses. Thereby we could clearly show the induction of CD4+ T cell responses by splenic CD8- DCs, whereas the CD8+ DCs induced CD8+ T cell responses.

The naïve CD8+ T cell responses were of functional relevance, as we demonstrated the effective dose-dependent killing of peptide loaded target cells in vivo. Therefore, we suggest antigen targeting to FcRs as useful tool to induce de novo as well as the modulation of immune responses for future therapeutic applications. Additionally, we could demonstrate the responses to be effective in a murine melanoma model (in a preventive as well as a therapeutic setting). We now further investigate, which mechanisms play a role after antigen targeting to CD11b+CD8- DCs, which adjuvant is most promising, and if the concomitant induction of a CD4+ T cell response is beneficial to the anti-tumor CD8+ T cell response in our system.

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Immunology

P165 (OP01/01) | Intratumoral interaction dynamics of CD4+ T cells with myeloid cells during tumor regression

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Introduction: Current melanoma immunotherapies, such as immune checkpoint blockade, utilize cytotoxic effector functions of CD8+ T

cells. However, tumors frequently evade immune control, by loss of MHC-I expression or becoming unresponsive to IFN, resulting in impaired CD8+ T cell control. In recent years, the potential of CD4+ T cells for melanoma immunotherapy has become increasingly apparent. Tumor-specific CD4+ T cells in the tumor microenvironment can facilitate tumor regression by both direct and indirect mechanisms. In this project, we developed an adoptive cell transfer (ACT) therapy protocol that enables a direct comparison of CD8+ and CD4+ anti-tumor functions.

Materials and methods: Using the transplantable mouse melanoma cell line HCmel12, we established an ACT therapy protocol for T cell receptor transgenic CD8+ and CD4+ T cells that specifically target the melanocytic antigens gp100 and TRP1, respectively. The ACT protocol includes chemotherapeutic preconditioning with cyclophosphamide, an adenoviral vaccine expressing both the TRP1 and gp100 antigen and local injections of TLR agonists poly(I:C) and CpG to stimulate innate immunity. Using this model, we analyze tumor growth and survival of mice challenged with CRISPR/Cas9 induced HCmel12 knockout variants for TRP1, MHCI, MHC-II and Jak1-KO. We investigate T cell dynamics via intravital microscopy and complement our findings with standard immunological techniques such as flow cytometry and immunofluorescence imaging.

Results: We found that anti-tumor CD4+ T cells are able to control established HCmel12 melanomas as efficient as CD8+ T cells. While tumor-derived antigen was crucial for CD4+ T cell-mediated tumor rejection, CD4+ T cell ACT worked independently of cytotoxic CD8+ T cells and direct tumor cell recognition via MHCII. Intravital microscopy revealed that CD4+ T cells in the tumor microenvironment (TME) decelerate and form stable contacts with CD11c-eYFP+ cells in an antigen-dependent manner. The CD4+ T cell-mediated anti-tumor effect was found to be dependent on IFNg. Surprisingly, IFN-unresponsive Jak1-KO tumors were still largely controlled by CD4+ T cell ACT. Instead, we found strong recruitment and activation of iNOS+ inflammatory monocytes in the TME. When we inhibited iNOS using L-NIL, we demonstrated the loss of CD4+ T cell-mediated tumor control in Jak1-KO tumors.

Conclusion: Our results highlight the pleiotropic role of anti-tumor CD4+ T cells. While tumor-specific CD4+ T cells can work independent of CD8+ T cells and direct tumor cell recognition, therapeutic success critically depends on IFNg. Interestingly, IFN-unresponsive tumors can be controlled through an alternative mechanism. Here, inflammatory monocytes were recruited and activated, leading to iNOS-dependent tumor control in IFN-unresponsive tumors. These data demonstrate the vast potential of anti-tumor CD4+ T cells and could launch new strategies for treatment of melanoma, especially those that evade standard CD8+ T cell focused immunotherapies.

P166 | Cellular immune dysregulation after tick feeding in human skin increases susceptibility to tick-borne infections

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As a consequence of global temperature rise, ticks (Ixodida) and tick-borne diseases are emerging. During tick attachment to human skin, the feeding cavity becomes a site of transmission for immunomodulatory salivary compounds, which can exert local immunosuppressive effects. Tick-borne pathogens, such as Borrelia burgdorferi, may benefit from dampened local immune cell activation.

Here we identify changes in key immune cell populations in human blood and skin samples taken after a tick bite, providing insights to the early immune reaction to compounds of tick saliva and their immunomodulatory capacity. For this purpose, leukocytes were isolated from skin biopsies and blood taken after tick bites without borrelia infection (n = 16). Borrelia-infected skin samples, as determined by PCR, were excluded from further analysis (n = 1). Isolated cells were stimulated with cell activation cocktail and analyzed by flow cytometry. In addition, immunofluorescent staining of skin sections for T cells including tissue-resident memory T cells (TRM) in tick bite lesions was performed.

The skin infiltrate at the tick feeding site was characterized by an increase in T cells, B cells and neutrophils compared to intraindividual control biopsies, while dendritic cells and Langerhans cells were reduced after tick bites without signs of cutaneous borreliosis.

Although the number of lymphocytes including TRM increased after tick bite, IFNγ- and IL-4-producing T cells were reduced, indicating impaired Th1 and Th2 responses. In peripheral blood, decreased numbers of T cells, NK cells, NKT cells and ILC3 were found in tick bite patients compared to healthy controls, suggesting that tick bites induce discreet systemic immune effects.

Collectively, we show that tick feeding exerts profound changes on the skin immune network and even alters immune cells of the peripheral blood. This detailed map of cell-specific changes may allow designing effective prevention and treatment strategies for tick-borne diseases in the future.

P167 | Unraveling the kinetics and cellular contributions in type 2 immune responses in atopic dermatitis

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Atopic dermatitis (AD) is a chronic inflammatory skin disease associated with skin barrier defects and microbial dysbiosis. The development and progression of AD critically depends on the action of type 2 immune cells and mediators. However, the identity of these immune cells as well as their chronological appearance in the establishment of AD are still elusive. This project aims to disclose the kinetics and cellular contributors of type 2 immune responses in AD, focusing on the dynamics of interleukin (IL)-4 induction during skin inflammation in pre-clinical models. To mimic skin barrier impairment, mice were subjected to repeated tape stripping (TS) of their shaved back skin. Medium containing *Staphylococcus aureus* (*S. aureus*) was applied to induce microbial dysbiosis. Both conditions were induced either alone or in combination at defined time points. Using IL-4 reporter mice, we found that TS as well as the combination of TS and *S. aureus* increased the appearance of IL-4- producing cell types such as T helper type 2 cells, γδ T cells and natural killer T cells in the skin, accompanied by increased mRNA expression of IL-13 and CCL22, 6 days after treatment. Skin-draining lymph nodes partially mimicked the previously described dynamics of IL-4-producing cell types. Moreover, increased mRNA expression of IL-33 and CXCL1 was detected at early time points, accompanied by downregulation of skin barrier-forming proteins within the combined treatment with TS and *S. aureus*. Analyses of the skin microbiome of WT mice affirm this finding by pointing to a shift within the microbial homeostasis characterized by a predominance of Staphylococci after TS. This shows that skin barrier impairment and microbial dysbiosis act synergistically to induce the increased appearance of specific IL-4-producing cell types, as well as other features of AD. In the long term, this project will clarify the role of different type 2 cytokine-producing cells in the development of AD beyond Th2 cells and develop new treatment strategies.

P168 | Xenobiotic metabolism is triggered in atopic dermatitis

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Atopic dermatitis (AD) is a chronic inflammatory skin disease whose detailed pathogenesis remains elusive despite a clear involvement of epidermal barrier impairment and immune dysregulation. Xenobiotic metabolism is an important detoxification mechanism utilized by organs, including skin, to eliminate noxious exogenous and endogenous compounds and includes phase I and phase II enzymes as well as membrane transporters. Transcriptional control of xenobiotic metabolism is mediated via aryl hydrocarbon receptor (AhR), pregnane X receptor (PXR), constitutive androstane receptor (CAR) and peroxisome proliferator-activated receptors (PPARs). Previous work from our laboratory suggested that AD patients might have increased xenobiotic metabolism in the epidermis. Furthermore, transgenic mice overexpressing the constitutively activated human PXR displayed a Th2/Th17 skin inflammation and barrier dysfunction resembling AD. The goal of the current study is to decipher the role of xenobiotic metabolism in AD pathogenesis. Results show an increased expression of phase I and II enzymes, such as CYP1A1, CYP1B1 and CYP3A4, UGT1A1, UGT1A6, UGT1A9 and UGT1A10 in AD human epidermal equivalents (HEEs) when compared to control (CTL) HEEs. This is in line with the upregulation of upstream receptors namely AHR, PPARA and PPARG. In contrast, the transcription of membrane transporters (ABCC1, ABCC2, ABCB1) is downregulated. Immunofluorescence staining confirmed increased CYP1A1, CYP3A and UGT1A1 protein levels in AD HEEs. These results suggest a constitutive activation of the xenobiotic metabolism in AD, which can cause oxidative stress and inflammation. Accordingly, SOD2 is upregulated in AD HEEs when compared to CTL HEEs, indicating mitochondrial stress. Thus, upregulation of xenobiotic metabolism in nonlesional AD might contribute to disease pathogenesis.

P169 | Impaired clearance of senescent fibroblasts from aging skin: Towards therapeutic implications

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Fibroblasts constitute the principal component of the connective tissue and play a pivotal role in organ homeostasis and aging. When cells like fibroblast go through stress, they prefer to undergo cellular senescence. Thereby preventing the cell to become cancerous. Aging is defined as progressive loss of physiological integrity, leading to impaired tissue/organ function and increase susceptibility to age-related diseases. Cellular senescence, a permanent state of cell cycle arrest, is thought to be one of the causative processes of tissue aging and aging -related disorders. We previously showed that senescent fibroblasts profoundly accumulate in the skin during aging. Under some transient conditions in mammals and amphibians with high regenerative potentials, senescent cells can be successfully removed by cells of the innate immune system such as Natural Killer (NK) cells. We here set out to understand whether senescent fibroblasts in aging skin are resistant to removal by NK cells or, alternatively, NK cells themselves are compromised to remove senescent fibroblasts. Using a newly developed NK cell mediated killing assay,

we found that NK-92 cell line and primary NK cells preferentially kill senescent fibroblasts. These data imply that, in an in-vitro settings senescent fibroblasts are not resistant to the innate immune cell mediated clearance. Of note, although there is no significant change in the absolute number of NK cells in peripheral blood and skin between young (~23 years) and old (~70 years) healthy individuals, primary NK cells isolated from old donor are profoundly less efficient in killing human dermal fibroblasts as opposed to those isolated from young donor. Furthermore, at the synapse between target cells and NK cells the release of granzyme B, the effector molecule, which through pores formed by perforin on the target cell membrane enter the target cell to initiate their apoptosis, is significantly reduced in NK cells isolated from old individuals compared to that of NK cells from young individuals. Also, on the protein level, there is significant reduction in the content of granzyme B and perforin in NK cells isolated from old donors compared to their young counterpart. These data contribute to advancing our understanding of mechanisms underlying senescent cell accumulation, and may be exploited for future senolytic therapies.

Therefore, our main scientific goal, is to find out, whether natural killer (NK) cells loss their efficiency in the removal or clearance of senescent fibroblast in aged skin. Depending on the result, we will try to enhance the removal capacity of senescent fibroblast by cells of the innate immune system.

Infectious Diseases

P170 | Natural history of cutaneous human polyomavirus infection in healthy individuals

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Several human polyomaviruses (HPyVs) were recently discovered. Merkel cell polyomavirus (MCPyV) induces Merkel cell carcinoma. HPyV6, HPyV7, and TSPyV have been associated with rare skin lesions in immunosuppressed patients. HPyV9, HPyV10, and STLPyV have not been convincingly associated with any disease. The aim of this prospective study was to evaluate cutaneous prevalence, persistence and viral load of HPyVs in healthy individuals. 870 forehead and hand swabs were collected from 109 volunteers 4–6 weeks apart (collection period-1). 59 participants were available for follow-up a decade later (collection period-2). HPyV-DNA prevalence and viral loads of MCPyV, HPyV6, HPyV7, TSPyV, HPyV9, HPyV10, and STLPyV were determined by virus-specific real-time PCRs. Risk factors for HPyV prevalence, short- and long-term persistence were explored by logistic regression analyses. Baseline prevalence rates were similar for forehead and hand: MCPyV 67.9/67.0%, HPyV6 31.2/25.7%, HPyV7 13.8/11.0%, HPyV10 11.9/15.6%, STLPyV 7.3/8.3%, TSPyV 0.9/0.9%, HPyV9 0.9/0.9%. Short-term persistence in period-1 was found in 59.6% (MCPyV), 23.9% (HPyV6), 10.1% (HPyV7), 6.4% (HPyV10), 5.5% (STLPyV) and 0% (TSPyV and HPyV9) on the forehead, with similar values for the hand. Long-term persistence for 9–12 years occurred only for MCPyV (forehead/hand 39.0%/44.1% of volunteers), HPyV6 (16.9%/11.9%), and HPyV7 (3.4%/5.1%). Individuals with short-term persistence had significantly higher viral loads at baseline compared to those with transient DNA-positivity ($p < 0.001$ for MCPyV, HPyV6, HPyV7, HPyV10, respectively). This was also true for median viral loads in period-1 of MCPyV, HPyV6 and HPyV7 of volunteers with long-term persistence. Multiplicity (two or more different HPyVs) was a risk factor for prevalence and persistence for most HPyVs. Further risk factors were older age for HPyV6 and male sex for MCPyV on the forehead. Smoking was not a risk factor. In contrast to MCPyV, HPyV6, HPyV7, and rarely STLPyV, polyomaviruses TSPyV, HPyV9, and HPyV10 do not seem to be long-term constituents of the human skin virome of healthy individuals. Furthermore, this study showed that higher viral loads are associated with both short- and long-term persistence of HPyVs on the skin. HPyV multiplicity is a risk factor for prevalence, short-term and/or long-term persistence of MCPyV, HPyV6, HPyV7 and HPyV10.

P171 | Adaptation of *Staphylococci* to the human skin environment identified using an ex vivo tissue model

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Staphylococci such as *Staphylococcus aureus* or *S. epidermidis* interact with their human host in two different ways: as harmless members of the microbiota or as invasive pathogens once the epithelial barrier is compromised. Staphylococci are equipped with several interactive regulatory systems, which might orchestrate appropriate virulence gene expression during colonization and infection. We hypothesize that gene expression changes significantly as soon as, for example, *S. aureus* changes its habitat from the nose to the skin. Therefore, we established an ex vivo skin explants model to investigate *S. aureus* adaption to human skin and we compare the transcription of key virulence factors with transcription in the nose. On the long-run, knowledge on the specific gene expression pattern can give major insights into the importance of highly expressed factors and the importance of underlying regulatory systems.

From the analysis of regulatory loci we found evidence for a significant down regulation of the global virulence regulator agr directly after the initial contact with skin, regardless of the growth phase from which *S. aureus* originated. In contrast, the alterative sigma factor B (sigB) and the antimicrobial peptide-sensing system (graRS) were actively transcribed 6 h after epidermal contact suggesting a role of these regulatory elements. Accordingly, tissue adherence was primarily mediated by the sigB target genes clumping factor A (clfA) and the fibrinogen and fibronectin binding protein A (fnbA). At later timepoints, wall teichoic acid (WTA) also contributed to the adhesion process. In agreement with other studies, we detected a strong involvement of proteases from all three catalytic classes during the entire colonization process.
In summary, *S. aureus* gene expression during colonization of human skin differs significantly from nasal colonization. In both scenarios, however, defined individual factors are expressed. Furthermore, colonization of healthy skin led to a uniform response of the pathogen to the surrounding milieu independently of the human host and regardless of the growth phase from which *S. aureus* originates.

P172 | Snapshot of the expression pattern of *Staphylococcus epidermidis* in the nose and on the skin of healthy individuals

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Staphylococcus epidermidis is recognized as a principle component of skin microbiota. In addition, the species possess a selective pathogenic potential to cause nosocomial infections related to implanted medical devices. While the beneficial function of S. epidermidis is to interact with our immune system to promote skin health and homeostasis, recent evidence suggests that certain strains can also damage the skin barrier and that skin integrity itself plays a critical role in whether S. epidermidis colonizes the skin as friend or foe.
To address this bimodal function of S. epidermidis, we performed direct transcript analysis of nasal and skin swabs by qPCR and compared the transcriptional profile during colonization of its natural environments, the epidermis and the nose, to that of the native isolates grown in vitro. In total, nasal and skin swabs were collected from eleven healthy individuals and genes from the following categories were analyzed: i.) global regulators, ii.) toxins, iii.) metabolic genes, iv.) biofilm and capsule, v.) adhesion, vi.) cell wall enzymes and vii.) immune evasion. The global regulators studied were differentially expressed among individuals, with the exception of sarA, which was strongly transcribed in both nose and skin, suggesting a role for this regulatory protein during colonization. Interestingly, the beta-hemolysin encoding gene hlb was strongly transcribed in the nose and even more strongly on the skin. In contrast, metabolic genes involved in the TCA-cycle were not expressed during colonization. For the permanent colonization, factors for tissue adherence, immune evasion and cell wall metabolism seem to be important.
In summary, we could show that the adaptation of S. epidermidis in its authentic environment is a multifactorial process which greatly differs from the expression profile in vitro.

P173 | Evolution of T. indotinae mutants showing resistance to terbinafine and azoles

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Background: The T. indotineae population shows a high amount of terbinafine resistant isolates based on different point mutations of the squalene epoxidase erg1 (ergosterol) gene. Moreover, a significant proportion of these isolates also exhibit azole resistance of to date unknown mechanism. It is hence of interest, to elucidate the molecular mechanism for azole resistance, especially the identification of mutations in the sterol 14-α demethylase Erg11 genes, which encode for enzymes interacting with azoles.
Methods: Clinical isolates of T. indotinae from Jena university hospital were included in the study. Sequencing of putative Erg11 genes and analysis of phenotypic resistance pattern using a microplate-laser-nephelometry (MLN)-based growth assay was performed.
Results: Erg1 mutants with Phe397Leu and Leu393Ser exhibited resistance against terbinafine. One of the two Erg1 Ala448Thr mutant strain exhibited high azole resistance based of an unknown mechanism. Five different types of Erg11B mutants were detected; two double mutants, one Ala230Thr/Asp441Gly, the second Ala230Thr/Tyr444Cys and single mutants with Gly443Glu, Tyr444Cys and Tyr444His. Erg11B double mutants demonstrated an increased resistance for specific azoles. All isolates featured the wild type genotype of Erg11A. All strains demonstrated different combinations of Erg1 and Erg11 genotypes.
Conclusion: It could be shown that resistance against terbinafine and azoles evolved several times independently within the T. indotineae population. In accordance, the challenge for fungal treatment lies beyond species identification, as it is not enough to predict therapeutic efficacy of antifungals. In future, it will also become important to analyze genes involved in resistance mechanisms to choose the right antimycotic treatment.

P174 | Histologic investigation of COVID-19 associated perniosis like skin lesions

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COVID-19 still has a big impact on health care and economics all over the world. The major task is to protect the most vulnerable

persons from being infected. Skin lesions have been reported since the beginning of the pandemic more or less systematically. Data to their general prevalence differ a lot. A wide variety of lesions have been described: early appearing popular or pustular rash, maculopapulous or urticarial rash during the course of the disease and late appearing perniosis like acral lesions. The latter are associated with childhood, a mild course of the disease and appear around 4 weeks after the onset of COVID-19 symptoms.

Here we report histologic results of perniosis like acral lesions and the comparison of immune histochemic results in these cases. Skin biopsies were formalin fixed and paraffin embedded sections were stained with hematoxylin/eosine and immune histochemically with antibodies against lymphocytic antigens. Kryosections were analysed by immunofluorescence for pathologic deposits of immunglobulins or complement.

We could confirm association of perniosis like lesions with mild COVID-19. The inflammatory differs a lot in the specimens of different patients with being nearly absent in some cases whereas showing dense dermal lymphocytic infiltrates with a pronouncement around adnexial structures in others. IgG deposits in small blood vessels could be detected in one patient.

In general our data are in line with others that perniosis like lesions are more common in young patients, after mild disease and that there are no specific histologic signs for the diagnosis, hence histology must be interpreted in the context of anamnestic data.

Pharmacology

P175 | Identification of plant extracts with anti-inflammatory and antioxidative activity as potential ingredients for cosmetic products applying a dedicated screening platform

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Nowadays, consumers are increasingly demanding for natural active ingredients and excipients in cosmetic products. Plants are an attractive natural source of such active ingredients. Plant extracts do not only contain numerous functional bioactive compounds but are also a sustainable raw material.

The availability of many potential drugs as source for such extracts in combination with various possible extraction methods results in a high number of potential extracts for application as cosmetic active ingredients. Thus, there is need for a screening algorithm which allows to discriminate between these extracts regarding anti-inflammatory and antioxidative activity in parallel testing.

A basic screen was applied to screen 24 proprietary plant extracts of different polarities (aqueous extraction to extraction with hypercritical CO2) for potential biological activities in primary cells (NHDF) and cell lines (HaCaT, B16 melanocytes, HEK293t, RAW 264.1 macrophages) and in an enzyme assay. Non-toxic dose range was determined as first step. Cell proliferation, antioxidative effects (8- isoprostane formation, ROS formation, NO production), glucose uptake, MMP1, MMP9, TIMP1, anti-inflammatory activity (IL-6, IL-8, PGE2, NF-κB transcriptional activity), melanin production and hyaluronidase activity were assessed. Initially, all extracts were screened at three concentrations. A scoring system was applied for overall evaluation. Based on the scoring, extracts of interest were selected for confirmation of the screening results (5 conc., $n = 3$). A high reproducibility of the screening results was shown.

Extract 1 was shown to exhibit potent activity at low concentrations. A maximum concentration of 20 µg/ml was chosen based on viability data obtained in HaCaT, NHDF and RAW macrophages. A dose-dependent sign. reduction of IL-6 and PGE2- release from NHDF stimulated with IL-1β was observed starting from 5 µg/ml. H2O2- induced ROS formation in HaCaT was reduced to more then 50%, comparable to the positive control vit. C, in a U-shaped dose response curve. Concentrations of 0.6 and 2 µg/ml showed the highest antioxidant activity. A sign. reduction of ROS (>30%) was also measured for 6 and 20 µg/ml extract. LPS-induced nitric oxide production in RAW macrophages was significantly reduced in a dose-dependent manner with a max. effect of 80% compared to LPS control.

Effects on NF-κB transcriptional activity initially observed in HEK cells were confirmed in HaCaT. HaCaT NF-κB Luc cells were either stimulated with TNF-α or irradiated with UVB. Based on cytotox. results 25 µg/ml was chosen as max. concentration. NF-κB activity was determined in parallel to viability. Following stimulation with TNF-α extract 1 showed sign., dose dependent inhibition of NF-κB activation (>50%) after 6 h. Cell viability was not affected. In the case of UVB irradiation a dose-dependent, sign. inhibition of NF-κB activation (>50%) was shown after 24 h. Cell viability was reduced to ~70% by irradiation with UVB without additional sign. cytotoxic effect of the plant extract. Results were confirmed by determination of cytokines and chemokine (GM-CSF, IL-1α, IL-6, IL-8, IL-10, TNF-α, IP-10, TSLP, MCP-1, and RANTES) in the supernatants.

Application of a screening algorithm to identify anti-inflammatory and antioxidative activity in different plant extracts followed by confirmation of the results and additional in-depth investigations resulted in an active ingredient for a cosmetic product for very dry or eczema-prone skin. This extract was shown to exhibit dose-dependent potent anti-inflammatory and antioxidative activity in several primary cells and cell lines.

P176 | Distinct metabolite profile in pemphigus vulgaris

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Background: Pemphigus vulgaris (PV) is a severe and difficult-to-treat autoimmune skin disease, characterized and caused by autoantibodies predominately targeting desmoglein (Dsg) 3, and often also Dsg 1. Molecular understanding of human PV pathogenesis is still incomplete, partially due to the lack of multidimensional data.
Objective: Add to the molecular understanding of human PV by contrasting plasma metabolites and lipids in PV patients before/after therapy and to healthy volunteers.
Methods: We longitudinally collected plasma from PV patients at change of therapy due to relapse or lack of disease control (inclusion) and 12 months later (remission), and from age/sex-matched controls. All patients were treated with rituximab with/without additional immunosuppressants. Metabolomics and lipidomics was performed using liquid chromatography coupled to tandem mass spectrometry followed by multilevel partial least squares-discriminant analysis.
Results: During the 12-months observation period, all patients improved clinically. Metabolite profiles of PV patients were distinct between the two time points. Specifically, metabolite profiles of PV patients at remission were similar to those of healthy individuals. Among metabolites distinguishing PV patients at inclusion from those at remission and healthy individuals, were pantothenic acid, taurine, hypoxanthine and pyridoxine. Regarding lipids, a separation between patients at inclusion and remission was observed; whilst the latter lipid profiles of patients did not differ from controls.
Conclusion: We here describe distinct metabolite profiles in human PV patients, which may have therapeutic implications as, for example, pantothenic acid supplementation has therapeutic effects in inflammatory diseases.

P177 | Skin inflammatory models based on ex vivo skin/T cell co-culture to investigate redox-sensitive formulations for the topical delivery of rapamycin

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Alternative skin models reproducing features of inflammatory skin diseases represent useful tools to develop more efficient and safer topical therapies while reducing the number of animals used in experimental testing. In this study, we developed short-term culture models based on ex vivo human skin co-cultured in a trans-well setup with T cell lines. In order to introduce features of inflamed skin, the ex vivo tissue was treated with low-dose proteases and lipopolysaccharide (LPS), while different agents like phytohaemagglutinin (PHA), ionomycin, or Th17 cytokines (IL-17 and IL-22) were tested to activate the co-cultured T cells. The models were used to investigate oxidative-sensitive CMS core multi-shell (osCMS) nanocarriers to be used as transporters for a more efficient and selective topical delivery of the mTOR inhibitor rapamycin to inflamed skin. Rapamycin is a highly promising compound for the treatment of inflammatory, hyperproliferative skin disorders like psoriasis. However, its topical use is hampered by the limited penetration across the skin barrier due to its high molecular weight (MW of 914.172 g/mol) and high lipophilicity.
The skin inflammatory model enabled the assessment of biological readouts at both the tissue (e.g., IL-1, IL-6, IL-8 profiles in epidermis and dermis extracts) and T cell level (e.g., mTOR activity and IL-2, IL-17A release). All investigated formulations successfully delivered rapamycin across the skin as revealed by the T cell inhibitory effects. Downregulation of mTOR activity and low levels of inflammatory markers were detected in skin extracts, pointing to local anti-proliferative activity and low irritative potential of the formulations. Thus, environment-sensitive CMS nanocarriers seem to be promising drug delivery formulations for the topical treatment of inflammatory skin diseases.
We conclude that the ex vivo skin/T-cell co-culture setup is a promising alternative to animal models for the assessment of the efficacy and safety of novel antiinflammatory formulations.

Photobiology

P178 | Filtered 222nm UVC—The future of skin disinfection?

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Irradiation with 222nm UVC is a promising strategy to neutralize bacteria and viruses like the coronavirus. It is a simple and relatively easy to apply method that could be implemented in a variety of fields such as means to disinfect hospital rooms, appliances, or even in hand sanitization. This is particularly important, considering the increasing number of hospital-acquired infections. Surgical site infections are the most frequently occurring ones and can not only prolong the post-operative recovery time but also result in increased medical and financial burden both for the patient and the medical staff. In some cases, such infections may lead to limb amputation or even have a lethal outcome. With the large amount of antibiotics applied in human healthcare and animal farming, the number of

multidrug-resistant pathogens is on the rise, which can potentially further aggravate the outcome of surgical site infection.

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

To exclude potential mutagenic effects on human skin during clinical application, this study investigated the DNA damage in form of cyclobutylpyrimidine dimers (CPD) caused by 222nm UVC on human skin reconstructs. Furthermore, the antimicrobial capacity of filtered 222nm UVC was investigated as well.

In brief, human full-thickness skin reconstructs were irradiated with 222nm UVC without and with filters (blocking range of 230–270 nm) with different single doses (100 mJ/cm^2, 500 mJ/cm^2) and with repetitive treatment (3 × 500 mJ/cm^2) or with UVB (308 nm). The administered doses had no significant effect on the viability of the skin reconstructs. The treatment with UVB and non-filtered UVC irradiation induced a significant amount of CPDs, compared to non-treated samples. When filtered 222 nm UVC was applied, the amount of CPD was lower compared to unfiltered UVC treatment and UVB treatment. In addition, an antimicrobial effect (99.9% reduction of colony forming units) of such filtered UVC 222 nm against E. coli, *S. aureus*, and Candida albicans could be demonstrated. This means that a therapeutic window has been identified in which microorganisms are killed but tissue is alive and not damaged.

Overall this study shows both an antimicrobial effect against bacteria and fungi and the protective potential of filters against DNA damage induced by UVC 222 nm irradiation, which could give rise to clinical applications in the future.

P179 | NOD-2 and TLR-5 gene polymorphisms are associated with polymorphous light eruption

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Polymorphous light eruption (PLE) is the most common form of immunologically mediated photosensitivity dermatoses with genetic susceptibility. PLE patients show a reduced Langerhans cell (LC) depletion in the epidermis after ultraviolet B (UV-B) irradiation resulting in a non-suppressive microenvironment in the skin. In stem cell transplanted and UV-B-irradiated patients with incomplete or no depletion of LCs after UV-B irradiation, a severe acute graft-versus-host disease (GvHD) was observed. Genetic variation in nucleotide-binding oligomerization domain 2 (NOD-2) and toll like receptor 5 (TLR-5) genes also confers susceptibility to GvHD. We hypothesized, that these polymorphisms (SNPs) could also play a role in the pathogenesis of PLE.

We investigated single nucleotide polymorphisms (SNPs) of NOD-2 (R702W, G908R, 3020Cins) and TLR-5 (A592S, P616L, N392STOP) in skin biopsies of patients (n = 143) with PLE and in blood of healthy controls (n = 104) via restriction fragment length polymorphism (RFLP) analysis.

The NOD-2 SNP R702W and 3020Cins and the TLR-5 SNP N392STOP showed significant differences between controls and PLE patients. Moreover, there were significantly more homozygous and heterozygous genotypes in PLE patients in the NOD-2 SNP R702W than in samples from healthy donors. The genotypes of the NOD-2 SNP G908R and the TLR-5 SNPs A592S and P616L showed no significant difference between controls and PLE patients.

The present study revealed GvHD associated SNPs in NOD-2 and TLR-5 in patients with PLE.

The functional relevance of these findings needs to be further investigated including the question, whether PLE patients might be more susceptible for GvHD after stem cell transplantation.

P180 | The anti-fibrotic effect of cold atmospheric plasma on localized scleroderma in vitro and in vivo

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Cold Atmospheric Plasma (CAP) has shown promising results in the treatment of various skin diseases. The therapeutic effect of CAP on localized scleroderma (LS), however, has not yet been evaluated. We investigated the effects of CAP on LS by comparing human normal fibroblasts (hNF), human TGF-β-activated fibroblasts (hAF), and human localized scleroderma-derived fibroblasts (hLSF) after direct CAP treatment, co-cultured with plasma-treated human epidermal keratinocytes (hEK) and with an experimental murine model of scleroderma. In hAF and hLSF, 2- min CAP treatment with the MicroPlaSterβ® plasma torch did not affect pro-fibrotic gene expression of alpha smooth muscle actin, fibroblast activating protein, and collagen type I, however, it promoted re-expression of matrix metalloproteinase 1. Functionally, CAP treatment reduced cell migration and stress fiber formation in hAF and hLSF. The relevance of CAP treatment was confirmed in an in vivo model of bleomycin-induced dermal fibrosis. In this model, CAP-treated mice showed significantly reduced dermal thickness and collagen deposition as well as a decrease in both alpha smooth muscle actin-positive myofibroblasts and CD68- positive macrophages in the affected skin in comparison to untreated fibrotic tissue. In conclusion, this study provides the first evidence for the successful use of CAP for treating LS and may be the basis for clinical trials including patients with LS.

P181 | Langerhans cells are essential regulators of the early immune response to UVB-induced DNA damage

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Mutations induced by UV radiation can lead to melanoma and non-melanoma skin cancer. Skin cells can undergo sunburn cell (SBC) formation subsequently leading to programmed cell death (apoptosis) of irreversible DNA-damaged cells when damage repair is insufficient. These mechanisms prevent the introduction of mutations caused by UV skin irradiation and ensuing photocarcinogenesis. The early immunological response by skin resident innate immune cells after high dose UVB irradiation of skin, representing acute sunburn, is indistinct as previous work focused mainly on adaptive immune responses in the skin-draining lymph nodes. The aim of this project was to gain new insights into the role of skin resident innate immune cells in the immunosurveillance of UVB-irradiated skin. For this purpose, we exposed the back skin of mice to one high dose (sunburn dose) of UVB and analysed the skin for alterations of resident innate immune cells. Acute sunburn led to DNA-damage, SBC formation and apoptosis of keratinocytes. These processes were accompanied by a strong activation of Langerhans cells (LC) and a massively increased TNFα level in the skin. In the absence of LC or after TNFα neutralization less keratinocytes underwent apoptosis. Moreover, the inflammatory process of SBC formation was strongly reduced due to an impaired recruitment of neutrophils. A better understanding of the immunological events in early photocarcinogenesis is crucial to develop novel treatment options for skin cancer.

P182 | Investigations on the relevance of the interactions between the transcription factors AhR and HIF-1alpha in the context of UV-inducible cutaneous signaling response

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The aryl hydrocarbon receptor (AhR) and hypoxia-induced factor 1alpha (HIF-1alpha) are structurally related transcription factors and their activation in the epidermis can be induced by exposure to UVB irradiation. Both dimerize with the same cofactor ARNT (AhR nuclear translocator) but then activate different target genes that can regulate inflammation and repair processes. Prominent examples of target genes would be e.g. VEGF (HIF-1alpha) and Cyp1A1 (AhR). The studying of the interaction between these two proteins and the resulting effects on the skin's response to UVB exposure are of special scientific interest.

For this purpose, a new mouse line with simultaneous keratinocyte-specific deficiency for AhR and HIF-1alpha ("DcKO"—double conditional knockout) was generated by Cre-lox-mediated deletion dependent on Keratin 5 which is expressed in basal keratinocytes. The mice are phenotypically healthy and breed at a normal rate.

First results revealed that they are apparently not hypersensitive to UVB exposure compared to WT or respective single conditional KO as might have been expected, given the loss of two UVB-responsive transcription factors orchestrating inflammation and repair processes. CPD removal and epidermal barrier function were overall comparable to littermates.

However, upon closer examination, naive DcKO mice showed distinct differences compared to WT littermates which would normally be expected of UVB-exposed mice. These include dorsal epidermal/dermal thickening and increased pigmentation. Furthermore, both major immune cell types, Langerhans cells and dendritic epidermal T cells, displayed an activation-like morphology and reduced density within the tissue, suggesting intrinsic functional aberrations compared to control. In addition, we could detect elevated frequencies of regulatory T cells in skin-draining lymph nodes, which would also be expected in UVB-exposed WT mice as a hallmark of UVB-induced immunosuppression.

Therefore, this mouse model is a valuable source to investigate processes connected to epidermal homeostasis which includes immune functions.

Interestingly, these mice exhibited a lower body weight than WT littermates (within a healthy range), emphasizing the relevance of inter-organ cross-talk research. Further research will address the important question whether these findings are the consequence of beneficial or maladaptive intracellular signaling shifts due to the mutual deletion of HIF-1alpha and AhR in keratinocytes.

Taken together, these findings indicate complex interactions between skin and organism and offer new descriptive insights into the epidermal regulation of responses to environmental stressors, which could also be relevant for human preventative medicine and therapy in the field of photodermatology.

P183 | Two in one filter foil: Opportunities for protection and therapy

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It is well known that exposing human skin to solar UVA (320–400 nm) radiation can have damaging effects ranging from accelerated skin aging (photoaging) to skin cancer. UVA-induced reactive oxygen species (ROS) can cause oxidative damage to DNA like 8-oxoguanosine (8-HdG), DNA double-strand breaks, and lipid and protein oxidation.

Despite its potential adverse effects, UV radiation in form of light- and heliotherapy has a continuously increasing spectrum of medical applications. Light therapy can be employed for the treatment of seasonal affective disorder, depression, burn out, sick headache and sleeping disturbance, while heliotherapy can be used for the treatment of psoriasis, atopic dermatitis, cardiovascular disease (improving endurance performance), osteoporosis, seasonal depression. However, one major disadvantage of many forms of irradiation therapy is its mutagenic effect in cells. This damaging effect of UV irradiation can have long-term consequences, which can be seen firstly years after treatment. Therefore, new UV protective strategies, have to be tested for their efficiency to shield against UV induced damage without reducing its therapeutic potential.

A field that can greatly benefit from improved protection strategies against UVA-induced damage is the Photodynamic Therapy (PDT), a treatment usually prescribed in cases of actinic keratosis (AK), Bowen disease, and certain types of basal cell carcinoma. In recent years, Daylight-Mediated Photodynamic Therapy (daylight PDT) has been proposed as an alternative to classical PDT. It substitutes specialized therapeutic devices with a regulated outdoor sun exposure. Daylight PDT proved effective against AK independent of weather conditions and even irradiation reduction by 83% due to cloud-cover resulted in successful treatment as long as a minimal irradiation dose of 3.5–8 J/cm^2 was achieved. However, patients have reported increased pain load when treatment was performed on a sunny day, with the lowest pain-score recorded on rainy or cloudy days. Furthermore, there are indications that cloudy weather conditions do not always correspond to reduction of solar irradiance. Depending on cloud formation and composition, it is possible to receive higher irradiation doses when the weather is cloudy compared to clear-sky conditions.

In this work, we investigated the protective effects of HelioVital filter foil against UVA irradiation in skin cells. We could shown that HelioVital sun protection filter foil has protective effects against UVA irradiation induced changes in cell proliferation, MMP expression and against UVA-induced ROS production and DNA damage. These results could pave the way for clinical studies with HelioVital filter foil shielding against the damaging effects of phototherapy and other forms of irradiation therapy, thereby increasing the safety and treatment opportunities of these forms of therapy.

Patients wishing to perform daylight PDT could do so in "mock outdoor conditions" by receiving the therapy in special greenhouse-gazebos covered with HelioVital filters. Since the HelioVital filter foil absorbs approximately 60% of solar UV irradiation, a range mimicking cloudy or partially cloudy weather conditions, it could be applied as sun-shielding during daylight PDT, giving the patients the benefit of reduced pain load even on sunny days without the variable cloud formation.

P184 | Reducing the burden of atopic dermatitis: Combination therapy of curcumin and light reduces inflammation in-vitro

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Background: With a global prevalence of over 20% amongst children, Atopic dermatitis (AD) is the predominant form of chronic, inflammatory skin disorders in children. Cardinal signs of AD are Xersosis and reoccurring intense itching, resulting in a significant disease burden. New treatment modalities that focus on reducing the skin inflammation could thus be beneficial. Therefore, we investigated whether we can establish an in-vitro AD- like cell culture model and if the combination therapy of curcumin (Cur) and visible light (Vis) could reduce induced inflammation.

Material & Methods: In order to generate an AD-like environment in-vitro, Human keratinocytes (HaCaT and primary keratinocytes) were cultured in monolayers and stimulated with a predefined cytokine cocktail (IL-4, IL-13, IL-31, TNF-α) prior Cur/Vis treatment, with Cur concentrations ranging from 0.1 to 0.3 μM.

Effects of cytokine simulation and Cur/Vis treatment on proliferation, inflammation, cell viability and apoptosis-induction were analysed by BrdU-Assay, IL-6/ IL-8 Elisa Assays, quantification of DNA fragmentation and Annexin V/PI staining via flow cytometry (FACS) as well as immunoblotting. We investigated Thymic stromal lymphopoietin (TSLP) expression as marker for AD-specific gene- and protein expression by RT-PCR analysis and immunoblotting. Furthermore, effects of the applied cytokine mix on differentiation and stratification were investigated by haematoxylin-eosin staining of an epidermis model.

Results: Our results show, that the applied cytokine mix induces inflammation in HaCaT, which could be reduced by our Cur/Vis treatment regime in a dose-dependent manner. In primary keratinocytes, cell proliferation was not affected by Cur/Vis treatment and neither Cell-Death-Detection assay nor FACS analysis could detect apoptosis when applying low doses of Cur. However, higher doses of Cur led to detectable early and late stage apoptosis. Moreover, we were able to detect a curcumin dependent regulation of cytokine induced signal transduction from zero to 24 h after Cur/Vis treatment. Interestingly, the regulation was not in all cases concertation dependent. Baseline levels of TSLP-RNA could be detected by RTPCR, however no TSLP protein could be detected in our 2D cell-culture model. Furthermore, we detected a disruption of differentiation within the cytokine treated epidermis models compared to the control group epidermis models.

Conclusion: The presented data suggests an anti-inflammatory effect of Cur/Vis treatment in primary keratinocytes after stimulation with AD specific cytokines and may contribute to the establishment of a novel treatment approach of topical AD in the future. However, further confirmation of the results both by more advanced 3D skin models as well as in clinical trials is needed.

P185 | Impact of the photodynamic treatment with curcumin and visible light irradiation is wavelength specific

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Background: Curcumin--a rhizomal phytochemical from the plant Curcuma longa--is well known to inhibit cell proliferation and to induce apoptosis in a broad range of cell lines. In previous studies, we showed that combining low curcumin concentrations and subsequent UVA or VIS irradiation induced anti-proliferative and pro-apoptotic effects. Differentiation between these two light qualities revealed that VIS irradiation was more potent than UVA irradiation. In this study, we aimed to investigate which influence various specific wavelengths within the visible spectrum have as single treatment or combinatory treatment on e.g. cell morphology, cell viability and proliferation.

Material and Methods: The human keratinocyte cell line (HaCaT) and the human lung carcinoma epithelial cell line (A549) were treated with 0.2–1.0 µg/ml curcumin for 1 h prior to irradiation with 3 J/cm2 of either one of the following wavelengths (420 nm, 435 and 585 nm) or combinations of these wavelengths using a daylight PDT device (MultiLite®, GME). After 24 h cell morphology was monitored with the incubator microscope IncuCyte, proliferation was monitored by quantitative determination of BrdU incorporation during mitosis.

Results and Conclusions: None of the applied wavelengths influenced cell morphology or cell integrity. Monitoring the influence on proliferation showed that irradiation of curcumin treated cultures was clearly wavelength specifically influenced. Whereas irradiation with 435 nm did not influence proliferation, a moderate curcumin concentration dependent proliferation reduction was observed after irradiation with 585 nm. The most prominent curcumin concentration dependent anti-proliferative effect was observed after irradiation with 420 nm. Combination of the different wavelengths showed that not only the applied wavelength was important but also the sequence of their application. Whereas no or only a moderate anti-proliferative effect could be observed after irradiation with 420 nm followed by 585 nm a very distinct curcumin concentration dependent influence on proliferation was observed after irradiating in reverse order—585 nm followed by 420 nm. A comparable impact could be observed when monitoring the irradiation order of 420 and 435 nm. Combining all three wavelengths showed that the most promising combination was either 585 nm–420 nm–435 nm or 585 nm–435 nm–420 nm.

These results indicate that curcumin treatment and irradiation with 420 nm as single wavelength treatment or the three-wavelength combination of 585 nm–420 nm–435 nm or 585 nm–435 nm–420 nm are the most effective treatment regiments. They should be further investigated along with other wavelengths related to the application of curcumin during a potential photodynamic therapy.

Pruritus

P186 | The aryl hydrocarbon receptor in chronic pruritus

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The aryl hydrocarbon receptor (AhR) is a ubiquitously abundant receptor and belongs to the family of biosensor bHLH-PAS proteins. The AhR is a transcription factor localized in the cytosol that, upon binding to a ligand, leads to extensive regulatory mechanisms, depending on the nature of the ligand. Among other functions, it plays a crucial role in the maintenance of the skin barrier, but also shows direct influence on proliferation of peripheral neuronal cells. In the context of atopic dermatitis, the functions of AhR have already been investigated in separate studies, although the results were occasionally controversial. Thus, studies showed a relief of atopic dermatitis by ligand-based AhR activation, whereas studies in mice showed the development of atopic dermatitis after AhR activation. Here, we took a closer look at the function of AhR in the context of pruritus development in atopic dermatitis as a candidate for dermatologic inflammatory pruritus and brachioradial pruritus as a candidate for neuropathic pruritus. For this purpose, we treated human primary keratinocytes from patients with atopic dermatitis, brachioradial pruritus and healthy controls with different ligands of AhR and analyzed the expression of genes involved in the pro-inflammatory immune response such as IL6 as well as pruritus-associated Th2 cytokines such as IL4 and IL13. Furthermore, we used cryosections to examine AhR expression in skin biopsies of the two pruritus entities. Doing so, we found that activation of AhR has a direct influence on the expression of pruritus-associated genes Furthermore, expression pattern of AhR in the skin of chronic pruritus patients showed abnormal pattern. Taken together, AhR seems to play a previously underestimated role in the development or maintenance of pruritus and should be investigated into depth in further studies.

P187 | In vitro modeling of scratching in chronic pruritus

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Scratching as a reaction to acute pruritus usually has no negative effect on the skin. However, in the case of chronic pruritus, scratching is practiced far more excessively by those affected and represents an enormous mechanical stress for the exposed skin area. This stress can lead to a worsening of the itch and one quickly enters an itch-scratch cycle. Simulation of scratching in pruritus research has been very limited and often involved only treating cells with known trigger

factors or animal experiments. Using a cytostretcher, we subjected human primary keratinocytes to mechanical stress. With an optimized stress protocol consisting of periods of mechanical deflection and resting periods, we were thus able to simulate acute and chronic scratching in vitro. The cells showed not only cytoskeletal remodeling but also induction of gene expression of pruritus-associated genes as found in pruritic lesional skin. Using this application we have the opportunity to shed new light on the factor of mechanical stress in pruritus development and maintenance, as wells as in the context of chronification.

P188 | Pruritic lesional skin in chronic nodular prurigo exhibits specific DNA methylation signatures

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Chronic nodular prurigo (CNPG) develops from chronic pruritus due to continuous scratching that leads to multiple itchy nodules. It is still unclear whether scratching itself, by initiating the itch-scratch cycle, is responsible for the development and maintenance of chronicity, or whether other mechanisms also play a role. The epigenetic DNA methylation is a promising candidate for such a mechanism as it is one of the most stable modifications that can endure multiple cell divisions. Thus, deregulated expression of relevant pruritogenic genes may remain for a long period to sustain chronicity.

Therefore, we performed global DNA methylation profiles in five CNPG patients and matched healthy controls (HC). Biopsies were obtained from pruritic lesional (PL) and non-pruritic non-lesional (NPNL) skin of patients and from healthy skin of controls (HC). Methylation profiles were generated using Illumina Infinium Methylation EPIC arrays (Diagonede, Belgium). Distance clustering was done with all samples before normalization and principal component analysis (PCA) with differentially methylated probes (DMP). Distance clustering as well as PCA clearly differentiated PL from NPNL and HC. This was also seen in the presence of DMPs. We found significant DMP counts between PL and NPNL (n = 28879) and PL and HC (n = 24994). In contrast none of the probes was shown to be significantly differentially methylated between NPNL and HC. Interestingly, in both settings (PL vs NPNL and vs HC), the highest percentages of DMPs were found in openseas, shores and shelfs (vs NPNL: 4.08, 3.56, 3.04; vs HC: 3.64, 2.92, 2.62) and significantly fewer in islands (0.86 and 0.55). Further analyses of the data and correlation with gene expression profiles are underway and may provide a deeper knowledge of mechanisms of chronicity in CNPG.

P189 | Chronic pruritus patients reveal distinct expression signatures in chronically scratched skin

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Chronic pruritus (CP) is a prevalent symptom of several different diseases. The multitude of origins of CP includes particularly inflammatory (e.g. atopic dermatitis; AD) and neuropathic (e.g. brachioradial pruritus; BRP) pruritus. Even though the origin of CP differs, the hallmark of chronic scratching affects all CP entities equally. Characteristics of chronically scratched skin are epidermal hyperplasia, neuronal damage and inflammation resulting in lichenification. Although the itchscratch cycle and the visible skin alterations have a high impact on patients, the influence of scratching on the course of pruritus is not entirely clear and effective treatments are insufficient. A deeper insight into involved genes and pathways by means of expression analyses may help to improve our knowledge of the underlying mechanisms and to define new therapeutic targets.

Accordingly, we systematically investigated cutaneous expression profiles of pruritic, lichenified skin (PLi), pruritic, non-lichenified skin (PNLi) and non-pruritic, nonlichenified skin (NPNL) from CP patients. Biopsies were obtained from patients suffering from AD (n = 32), BRP (n = 33) and healthy controls (HC; only NPNL, n = 64). RNA was isolated and subsequently analyzed by NGS-based mRNA-Sequencing (in collaboration with the Core Facility Genomics Münster). For that, mRNA was enriched using NEBNext® mRNA Isolation Kit. Multiplexing was done with NEBNext® Mulitplex Oligos and library preparation was carried out using NEBNext Ultra RNA. Single read sequencing (72 cycles) was done on the NextSeq2000 system targeting on 25 million reads per sample. Differentially expressed genes (DEGs) were defined with DESeq2, requiring a shrunken log2 fold change >1 and svalue <0.01. Subsequently, pathway analyses were performed by means of the free online database Reactome.

Principal component analyses (PCA) revealed differences between PLi vs. PNLi and NPNL, indicating scratch-induced differential transcriptomes. Next, we calculated DEGs between the three different localizations to deepen our first findings. Most DEGs were found between PLi and NPNL (AD n = 807; BRP n = 2346). Comparison of PLi and PNLi revealed 1527 DEGs in BRP but only 91 in AD. Finally, PNLi and NPNL showed similar expression profiles with low abundance of DEGs (AD n = 144; BRP n = 61). Comparing all localizations from patients to NPNL from HC, the most DEGs were found in PLi (n = 2317) of BRP patients and in both PLi (n = 2674) and PNLi (n = 1387) of AD patients. In contrast, PNLi in BRP (n = 136) and NPNL of both patient groups (AD n = 142; BRP n = 66) showed less DEGs compared to HC. Pathway analysis of DEGs revealed various pathways like formation

of the cornified envelope, FCGR activation and others to be enriched in PLi skin. Additionally, pathways of collagen signaling were predominantly enriched in PLi of BRP whereas pathways of FCERI and BCR signaling were seen in PLi of AD patients.

All these results show differences due to lichenification underlining considerable impact of scratching on the course of CP. Further analyses of the expression profiles are in progress and may deepen the knowledge of scratch specific molecules and pathways involved in CP pathology.

Tumor Biology

P190 | Crucial role of reactive oxygen species (ROS) for the proapoptotic effects of indirubin derivatives in cutaneous SCC cells

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Efficient drugs are needed for countering the worldwide high incidence of cutaneous squamous cell carcinoma (cSCC) and actinic keratosis. Indirubin derivatives represent promising candidates, but their effects in cSCC cells have not been reported before. Here, we investigated the efficacy of three indirubin derivatives (DKP-071, -073 and -184) in four cSCC cell lines. High efficacy was seen in SCLI, SCL-II, SCC-12 and SCC-13 resulting in up to 80% loss of cell proliferation, 60% loss of cell viability and 30% induced apoptosis (10 μM). Apoptosis was further enhanced in combinations with TNF-related apoptosis-inducing ligand (TRAIL). Induction of reactive oxygen species (ROS) appeared as critical for these effects. Thus, antioxidative pretreatment completely abolished apoptosis as well as restored cell proliferation and viability. As concerning the pathways, complete activation of caspases cascades (caspases-3, -4, -6, -7, -8 and -9), loss of mitochondrial membrane potential, activation of proapoptotic PKCδ, inhibition of STAT3, downregulation of antiapoptotic XIAP and survivin as well as upregulation of the proapoptotic Bcl-2 protein Puma and the cell cycle inhibitor p21 were obtained. Importantly, all activation steps were prevented by antioxidants, thus proving ROS as a master regulator of indirubins´ antitumor effects. ROS induction presently develops as an important issue in anticancer therapy.

P191 | Targeting cutaneous T-cell lymphoma cells by ingenol mebutate (PEP005) correlates with PKC-delta activation, ROS induction as well as downregulation of XIAP and c-FLIP

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New therapeutic strategies are needed for cutaneous T-cell lymphoma (CTCL), and the plant extract ingenol mebutate (PEP005) may be considered. PEP005 has been approved for actinic keratosis, and proapoptotic activities were described in different cancer cells. Here, we aimed to investigate its efficacy in four CTCL cell lines and its mode of action. While HuT-78 and HH corresponded with induced apoptosis as well as the loss of cell viability and cell proliferation, MyLa and SeAx remained resistant. Interestingly, both sensitive and resistant cells showed caspase-8 activation and enhanced levels of reactive oxygen species (ROS), while final caspase-3 activation was restricted to sensitive cells. Apoptosis induction was prevented by the caspase inhibitor QVD-Oph as well as by the antioxidant vitamin E. Caspase activation by PEP005 may be explained to some extent by the downregulation of the caspase antagonistic proteins c-FLIP and XIAP in sensitive cells, whereas both proteins were strongly expressed in resistant cells. Finally, PEP005 resulted in the activation of proapoptotic PKC-delta, and the PKC inhibitor bisindolylmaleimide I reduced apoptosis, caspase-3 processing and ROS production, as well as restored cell viability. In conclusion, PKC-delta appeared to be a central player in apoptosis regulation in CTCL cells, also suggesting its therapeutic targeting.

P192 | ERK inhibition by Sch-772984 in combination with the Mcl-1 inhibitor S63845 results in induction of apoptosis and loss of cell viability in cutaneous melanoma cell lines

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Introduction: Activation of the MAP kinase cascade via BRAF-MEK-ERK represents a critical step in cutaneous melanoma. Thus, targeting BRAF and MEK by selective inhibitors has dramatically improved standard care of melanoma patients in recent years. Nevertheless, inevitable emergence of drug resistance is still critically limiting the clinical efficiency. The inhibition of the MAP kinase pathway downstream of BRAF, e.g. by direct inhibition of ERK may circumvent resistance problems based on RAF kinases themselves. Furthermore, limited apoptosis induction by MAPK inhibition may depend on suppressed apoptosis pathways, e.g. by overexpression or activation of antiapoptotic Bcl-2 family proteins. The antiapoptotic Bcl-2 protein Mcl-1 is amplified in several cancer types including melanoma, and

high Mcl-1 expression was correlated with tumor progression and resistance to BRAF/MEK inhibitors.

Materials and Methods: The effects of S63845 and Sch-772984 were investigated in BRAF-mutated melanoma cell lines A-375 and A-2058 as well as in the BRAF-WT cell line MeWo. Apoptosis was determined by propidium iodide staining and cell cycle analysis. Cell viability was determined by calcein staining and flow cytometry. Cell proliferation was quantified by ELISA after WST-1 staining. Mitochondrial membrane potential was determined by TMRM+ staining and flow cytometry, while production of reactive oxygen species was investigated by H2DCF-DA staining. Western blotting was performed for pERK, Mcl-1, caspase-3 and the caspase-3 antagonist XIAP.

Results: Whereas single treatment with S63845 and Sch-772984 revealed only limited effects on melanoma cells, the combination of both inhibitors resulted in strong induction of apoptosis in all three cell lines after 48 h (>60%). This went along with strong loss of cell proliferation and loss of cell viability (<10%). Loss of mitochondrial membrane potential (MMP) as well as induced levels of reactive oxygen species (ROS) were indicative for an activation of proapoptotic mitochondrial pathways. Comparable findings were obtained by combination of the BRAF inhibitor vemurafenib and S63845 in BRAF-mutated cell lines. The high activity of Sch-772984 was proven by complete downregulation of pERK (Western blotting). In contrast, S63845 resulted in upregulation of Mcl-1, which indicates a cellular compensation of its inhibition. Clearly indicating the strong combination effects, the main effector caspase-3 was activated, seen by its 17 kDa and 19 kDa activated cleavage products, while the caspase-3 antagonist XIAP was downregulated in combinations.

Conclusions: Inhibition of pERK, as by the here applied inhibitor Sch-772984, represents an efficient strategy for targeting the MAPK cascade in melanoma cells, including BRAF-WT cells. However, the direct effects of MAPK inhibition on apoptosis and loss of cell viability were only limited. This could be significantly enhanced by combination with Mcl-1 inhibition, which may open new therapeutic options for melanoma.

P194 | Persister state-directed transitioning and vulnerability in melanoma

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Melanoma is a highly plastic tumor characterized by dynamic interconversion of different cell identities depending on the biological context. For example, melanoma cells with high expression of the H3K4 demethylase KDM5B (JARID1B) rest in a slow-cycling, yet reversible persister state. Over time, KDM5Bhigh cells can promote rapid tumor repopulation with equilibrated KDM5B expression heterogeneity. The cellular identity of KDM5Bhigh persister cells has not been studied so far, missing an important cell state-directed treatment opportunity in melanoma. Here, we have established a doxycycline-titratable system for genetic induction of permanent intratumor expression of KDM5B and screened for chemical agents that phenocopy this effect. Transcriptional profiling and cell functional assays confirmed that the dihydropyridine phenoxyethyl 4-(2-fluorophenyl)-2,7,7-trimethyl-5-oxo-1,4,5,6,7,8- hexa-hydroquinoline-3-carboxylate (termed Cpd1) supports high KDM5B expression and directs melanoma cells towards differentiation along the melanocytic lineage and to cell cycle-arrest. The high KDM5B state additionally prevents cell proliferation through negative regulation of cytokinetic abscission. Moreover, treatment with Cpd1 promoted the expression of the melanocyte-specific tyrosinase gene specifically sensitizing melanoma cells for the tyrosinase-processed antifolate prodrug 3-O- (3,4,5-trimethoxybenzoyl)-(-)-epicatechin (TMECG). In summary, our study provides proof-of-concept for a new dual hit strategy in melanoma, in which persister state-directed transitioning limits tumor growth and plasticity and primes melanoma cells towards lineage-specific elimination.

P195 | Genes involved in keratinocyte-melanoma cell interaction

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The early stages of melanoma development are poorly understood, but a substantial number of studies support the notion of a major contribution of epidermal keratinocytes. Recently, it could be shown that loss of Par-3 in keratinocytes, a gene involved in cell division and polarized cell growth, plays a central role in this process. In the present study, we addressed the question whether genes induced in melanoma cells and keratinocytes in co-culture might help to identify genes involved in melanoma development. In these experiments, immortalized keratinocytes were co-cultured with A375 melanoma cells. Next-generation sequencing was performed (RNA-seq) to analyse differential gene expression. By this means, a number of genes were identified that differed in their expression between co-culture and cultures of keratinocytes or melanoma cells alone. Gene expression candidates were subsequently validated after separation of different cell types using cell sorting. Among top genes that were upregulated in melanoma cells were COX2, CSF3 and HAS2, genes upregulated in keratinocytes were CCL2 and TNFAIP6. Among top gene ontology terms were metabolic pathways and cell adhesion. Thus, we conclude that keratinocytes influence gene expression in melanoma cells and vice versa and may thereby impact on melanoma cell biology. Next we performed laser microdissection of melanoma-associated epidermis. Based on the high mutational burden of epidermal keratinocytes, we hypothesized that mutationally impaired intermediate filament proteins, adhesion molecules and signaling pathways in keratinocytes might promote early melanoma development. Epidermal keratinocytes from regions in close proximity to melanoma tumours were collected and sequenced in a set of 10 samples. These analyses identified a plethora of genetic variants in each sample. Pathways or biological processes enriched for mutational variants included cell adhesion, epidermis development, and integrin signaling. Among top mutated genes was an atypical cadherin, which is known to be involved in cell adhesion.

Taken together, we provide evidence that keratinocyte-melanoma cell interaction induces a specific set of genes, and mutations in keratinocyte genes might be involved in early melanoma development.

P196 (OP04/04) | Spatial transcriptomics of primary melanoma lesions

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In recent years, single-cell RNA sequencing (scRNA-seq) analyses have been performed for a number of different cancers in order to identify clonal and subclonal structures. Moreover, single-cell analyses allowed a more in depth characterization of the tumor microenvironment (TME) in solid tumors with a particular emphasis on tumor-immune cell interactions. However, in classical scRNA-seq experiments, the spatial relationships between the cells still remain unclear, which means that concrete cell-cell interactions remain hard to define.

In the present study, we analysed a number of primary melanoma lesions by spatial transcriptomics technology (10x Genomics) to identify gene expression profiles associated with particular cell types in a spatial context. Primary melanomas were of different clinical subtypes and tumor thicknesses (low to high-risk tumors). For spatial gene expression analysis using 10x Genomics technology, tissue sections were immobilized on specific glass slides, which carry capture oligonucleotides with poly-dT tails and barcode sequences with spatial information. Captured RNAs are reverse-transcribed and sequenced using next-generation sequencing (NGS). We used NicheNet in order to identify gene patterns of putative ligand-receptor interactions between neighboring cells.

By this means, we identified a large number of receptor-ligand pairs expressed at the tumor-immune cell interface, some of which have already been shown to be of functional relevance in cancer, such as APOE-SCARB1. APOE variants are linked to melanoma treatment response, and mice expressing the human APOE4 allele exhibit reduced melanoma progression and metastasis. It is further known that APOE4 mice exhibit enhanced anti-tumor immune activation relative to APOE2 mice. Scavenger receptor B 1 (SCARB1) defective (SR-BI null mice) have an over-activated T and B cell immunity, implicating that SCARB1 per se is an immune-inactivator. A further example of the present study is MIF-CD74, which may be regarded as a kind of positive control for spatial transcriptomics technology. Macrophage migration inhibitory factor (MIF) is a lymphokine involved in cell-mediated immunity and an immunosuppressive factor secreted in the TME of melanomas. Moreover, it has been shown in a recent experimental study that blockade of the MIF-CD74 signaling on macrophages and dendritic cells restored the anti-tumor immune response against melanoma.

Taken together, spatial transcriptomics may identify new target structures at the interface between the tumor and immune cell infiltrate but also between immune cells within the tumor microenvironment and point at new targets for immunotherapy.

P197 | Individualized treatment of melanoma cell cultures based on the mutational landscapes

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Melanoma is solid tumor with high mutational burden. A majority of melanoma tumors carry mutations in the Ras-Raf-MEK-ERK pathway. Consequently, BRAF inhibition (BRAFi) treatment using small molecules inhibitors against mutated BRAF alone or in combination with MEK inhibition (MEKi) have been shown to be highly effective and significantly improved overall survival of patients. However, relapse rates are high an affect a majority of patients. Based on data from high-throughput sequencing studies, many melanomas carry additional mutations in signaling pathways that may be targeted by highly active inhibitors not yet approved for clinical use.

In the present project, we aimed at an individualized melanoma treatment using new targeted treatment options based on individual mutational patterns. For this purpose, a pre-clinical in vitro model was set up using the IncuCyte® life cell imaging system to analyze a large number of melanoma cell cultures in a 2D model. To select individual treatment combinations, a panel of 83 target genes was sequenced by next-generation sequencing (panel sequencing). Depending on the mutational profile, the biological effects of different drug combinations were subsequently tested to examine the optimal treatment strategy. Mutations were identified in BRAF, NRAS and NF1 as well as in PTEN, CDKN2A, ARID2 and ARID1B, and a number of other genes. Among substances used for targeted treatment were dabrafenib, trametinib, palbociclib, apitolisib and stattic. Overall, mutationally activated pathways showed a good response to pathway-targeted substances, and combination therapy was often more effective than individual substances.

By treating a BRAF-mutated cell line carrying further mutations in PTEN and ARID1B, classical treatment with BRAFi and MEKi resulted in a CI (combinatorial index) value of ~ 2, indicating even an antagonistic effect, while a combination of dabrafenib and apitolisib (PI3K/mTOR inhibitor) led to a CI value of 0.44, arguing for a strong synergistic effect. A second BRAF-mutated cell line with additional mutations in DCC (netrin 1 receptor) showed a high CI value for combined treatment with BRAFi and MEKi, whereas dabrafenib in combination with again apitolisib resulted in a value of 0.45, indicating a synergistic effect. DCC signals through Src kinase pathway and subsequent PI3K activation. Based on these results and other BRAF-mutated cell lines, we found out that the standard therapy with a BRAFi and a MEKi may be less effective than a combination with an inhibitor of another pathway. Interestingly, treating a cell line with mutations in NRAS, ARID2 and PTEN with a combination of trametinib and apitolisib resulted in a CI value of 0.25, suggesting again a synergistic effect. Taken together, these results may help to develop new treatment strategies for metastatic melanoma.

P198 | GARP as a potential shared uniform marker for melanoma and glioblastoma stem-like cells

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Gliomas have a higher incidence rate among melanoma patients compared to healthy population (10.46 vs. 6.13 cases per 100,000 person-years, SIR = 1.42 (1.22–1.62)). Glioblastomas are notorious for their extremely aggressive behavior and highly infiltrative phenotype, which renders the complete resection of these tumors by surgery virtually impossible. In addition to surgery, standard therapy consists of radiotherapy and non-targeted chemotherapy with DNA alkylating agent temozolomide. Despite aggressive multimodal therapy, patients with glioblastoma have a poor prognosis with a median survival of 12–15 months.

It has been hypothesized that the correlation between melanoma and glioblastoma is due to common genetic susceptibilities. This connection is further supported by the responsiveness to temozolomide by both melanoma and glioblastoma, indicating the existence of common pathophysiologic pathways.

Recently, we found that Glycoprotein A repetitions predominant (GARP) is a common marker of melanoma and glioblastoma. GARP is a protein, which is known to be expressed on the surface of activated regulatory T cells and platelets. In our previous investigations, we demonstrated that GARP has strong regulatory and antiinflammatory properties in vitro and in vivo. GARP led to the induction of peripheral Treg and the inhibition of tumor antigen-specific CD8+ T cells.

Therapeutic targeting of glioblastomas is particularly challenging due to the high degree of both inherent and acquired therapeutic resistance and intratumoral heterogeneity manifest by coexistence of distinct types of glioma cells with varying degrees of differentiation. Therapeutic resistance of glioblastomas has been attributed to glioblastoma stem-like cells (GSCs) that comprise a specialized population of tumor cells possessing some fundamental properties

of stem cells such as unlimited self-renewal and differentiation and inherent plasticity. These properties render GSCs capable to adapt to and survive cytotoxic insults that are otherwise lethal to non-stem glioma cells and reconstitute the tumor after (or under) initial nontargeted therapy. GSCs are currently considered as the main determinants of therapy resistance in glioblastoma and drivers of tumor recurrence. Targeting these cells remains a major challenge due to the lack of stable markers that would enable to distinguish GSCs from non-stem glioblastoma cells. Several markers like CD133, CD44, and CD15, were associated with GSCs in the past but failed to universally identify GSCs.

In this study, we addressed the suitability of GARP as a potential marker for GSCs using a multi-dimensional experimental setup consisting of a panel of patient-derived GSCs derived from (1) different regions of the same tumor, (2) newly diagnosed or recurrent glioblastomas, and (3) isogenic GSCs with varying differentiation and self-renewal capacity. In total, 12 GSC lines were analyzed. The methodological approach consisted of flow cytometry, confocal microscopy, and by immunoprecipitation western blot. As a reference for comparison, GSC marker CD133 was assessed in parallel with GARP.

We found that GARP is expressed in all GSC lines tested. Compared to CD133, GARP expression was considerably more stable and persisted in GSCs propagating either in vitro or in vivo. In contrast to CD133, GARP expression was found unaffected by changes in the cellular state with both self-renewing and differentiating GSCs showing comparable.

P199 | Inhibition of the mTOR inhibitory protein sirtuin 1 leads to faster tumor growth under PD1-based immune checkpoint blockade

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The introduction of immunocheckpoint inhibitors (ICI) has led to significant improvement in the prognosis of advanced melanoma. Unfortunately, up to 60% of patients show primary resistance to therapy. Previous data indicate that high tumor infiltration with CD8+ lymphocytes correlates positively with better patient outcome. However, the underlying tumorintrinsic mechanisms that influence the mostly heterogeneous immune cell infiltration are still poorly defined. By combining modern mass spectroscopic methods such as MALDI-MSI and LC-MS/MS we investigated the spatial protein composition in relation to CD8+ T cell infiltrate.

First, seven melanoma samples were conventionally immunohistochemically stained with anti-CD8. Tumors were then semiquantitatively divided into CD8high and CD8low regions. Consecutive tumor sections were analyzed by MALDI-MSI and LC-MS/MS. Using the newly described SPRING algorithm, we were able to dissect tumor compartment-specific protein profiles in an unsupervised manner and detect over 80 proteins that were significantly more frequently expressed in CD8high areas. A Gene Ontology-supported pathway enrichment analysis of the proteins showed functional group affiliations with negative regulation of cellular senescence, negative regulation of the TOR pathway, positive regulation of the ERBB pathway, protein phosphatase 1 binding, and the nBAF complex, among others. Interestingly, among the top thirty regulated candidates in the Cytoscape/ClueGO and GSEA analysis, we found the mTOR signaling-associated protein Sirtuin1 (SIRT1) which has recently been shown to affect immune cell infiltration, energy metabolism, and cell death in tumors and also acts as an epigenetic regulator. To further investigate the role of SIRT1 in melanoma, the protein was correlated with overall survival (OS) and immune cells (e.g. T cells) in the RNAseq data set of The Cancer Genome Atlas (TCGA) and Riaz et. al.. Survival of patients with high SIRT1 levels in melanoma was significantly higher compared with patients with low SIRT1 levels in TCGA (p = 0.007) and Riaz et. al. (p = 0.028). Furthermore a positive spearman's correlation of SIRT1 with T cell gene signatures in TGCA SKM (rho = 0.15, p = 0.001) and Riaz et al. (rho = 0.39, p = 0.016) was found. Finally, the effect of SIRT1 modulation on the anti-tumor efficacy of ICI therapy was examined in vivo in a syngeneic melanoma mouse model known to respond to experimentally administered a-PD1 antibodies. As expected, drug inhibition of SIRT1 using ex-527 in combination with a-PD1 resulted in faster tumor growth than under a-PD1 monotherapy (p = 0.04, slope = 30.94 vs. 14.54). Currently, the composition of tumor microenviroment (e.g. CD4, CD8) of drug-treated mouse tumors is analyzed ex vivo using multiplex immunohistochemical staining.

P200 | Establishment and characterization of primary keratinocyte cultures from tumor and healthy tissues

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Individualized and targeted treatment strategies considering cellular and molecular properties of tumors are becoming more and more important for cancer treatment. Routine establishment of primary cell cultures of tumor and corresponding healthy tissue of the same patient would allow individualized therapy testing on this material in vitro. Therefore, the aim of this study was to establish and characterize primary keratinocyte cell cultures of keratinocyte derived skin tumors and their healthy counterparts using a conditioned medium and Rho kinase inhibition. So far, two pairs (tumor/healthy) of cell cultures from patients with basal cell carcinomas could be

established. These cells can be cultured for about 50 days and 12 passages. Immunofluorescence analyses of cell surface markers confirmed a pure keratinocyte culture without any fibroblast contamination. Further characterization with respect to cell morphology, proliferation, migration, kinetics, and expression of genes frequently altered in basal cell carcinomas revealed significant differences between healthy and tumor cells. A comparison of early and late passages showed changes in morphology as well as in proliferation and migration rate. A comparison of cultured cells with original tissue is still pending, however, our results suggest that these cultures can be used for in vitro comparison of tumor and healthy cells but one should always compare cells from identical passages. Routine establishment of such pairs of tumor and healthy cell cultures from the same patients will provide an excellent tool for testing innovative treatment strategies such as the targeted exploitation of synthetic lethal interactions or testing the selective efficacy of novel small molecules or cold atmospheric pressure plasma.

P201 | Ionic signals promote anti-tumor T cell cytotoxicity

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The ability of T cells to elicit efficient anti-tumor immune responses within the immune environment of cancer cells is restrained by the induction of dysfunctional T cell responses by a multitude of mechanisms. Tonicity signals have not been taken into consideration as immunomodulatory factors of the tumour microenvironment so far. This is mostly due to the prevailing concept that osmolarity is tightly regulated by renal function and that fluctuations of certain osmolytes such as sodium chloride (NaCl) are thought to be incompatible with basic physiological processes. However, new experimental evidence has recently challenged this concept and demonstrated profound variability of NaCl concentrations in specific tissues and an impact of ionic signals for T cell functions (Matthias et al. Sci Transl Med 2019 & J Clin Invest 2020).

We found that tumours are highly enriched in NaCl relative to surrounding nonmalignant tissues. NaCl strongly affected memory CD8 T cells functions. CD8 T cell cytotoxicity was significantly increased upon exposure to elevated extracellular NaCl concentrations as seen by elevated secretion of cytotoxic molecules and pro-inflammatory cytokines. NaCl also shifted the threshold of TCR activation. It increased the metabolic fitness of human CD8 T cells and even reversed states of T cell exhaustion as observed by metabolomic and transcriptomic investigations. These effects were conferred to T cells via the p38-NFAT5-SGKI signalling axis. The overall anti-tumor effect of NaCl was further supported by real-time cytotoxicity assays, which demonstrated increased killing of melanoma cells by antigen-specific CD8 T cells, which have been preconditioned in elevated NaCl conditions.

Together, these data suggest that ionic signalling in the tumor microenvironment strongly affects anti-tumor cytotoxicity. This has implications for future therapeutic strategies and for empowering the cytotoxicity of adoptively transferred T cells in settings of cancer or chronic infections.

P202 | Enhanced expression of p21 promotes sensitivity of melanoma cells towards targeted therapies

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Metastatic melanoma patients benefit from the approved targeted BRAF inhibitor (BRAFi) therapy. Despite the great progress in the therapeutic approach to combat metastatic melanoma, fast emerging drug resistance in patients limits its longterm efficacy. In this study we aimed to unravel the role of p53 and its target gene CDKN1A/p21 in the response of melanoma cells towards BRAFi. We noticed that the sensitivity towards the mouse double minute 2 inhibitor (MDM2i) differed between the melanoma cell lines and did not correlate with the p53 mutational status. We show that p53 activation increases BRAFi sensitivity in a synergistic manner exclusively in cells with a high expression of CDKN1A/p21. In a similar way high expression of p21 was associated with a better response towards the MDM2i compared to those with low p21 expression. Indeed, p21 knockdown decreased the sensitivity towards both targeted therapies. The results indicate that the sensitivity of melanoma cells towards targeted therapies (BRAFi and MDM2i) is dependent on the p21 protein level in the cells. These findings offer new potential strategies for the improvement of targeted therapies with BRAFi by increasing treatment efficacy using combination therapies with p53 activating substances, which are able to increase p21 expression levels. Furthermore, the data suggest that the expression and induction level of p21 could be used as a predictive biomarker in melanoma patients to forecast the outcome of a treatment with p53 activating substances and BRAFi.

P203 | Resistance to BRAF inhibitors: EZH2 and its downstream targets as potential therapeutic options in melanoma

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Introduction: Malignant melanoma is one of the most aggressive tumors with an increasing incidence worldwide. Approximately 60% of melanomas are associated with BRAF mutations, which lead to a constitutive activation of the RAF-MEKMAPK signaling pathway. In various solid tumors, high activity of the histone methyltransferase EZH2, which epigenetically modifies H3K27, is associated with a

poor prognosis and cancer progression. We and others have shown that EZH2 is a downstream target of the BRAF signaling. However, EZH2 has not yet been studied in BRAF mutant melanoma and in connection with BRAFi resistance.

Methods: BRAF mutated melanoma cell-line A375 and vemurafenib resistant cell-line A375R were treated with different concentrations of vemurafenib, the EZH2-inhibitor tazemetostat (EPZ-6438) or a combination of both. To investigate if EZH2 influences the emergence of resistance of melanoma cells to vemurafenib, cell viability assays were carried out and apoptosis and the cell cycle were analyzed using FACS. EZH2 expression and H3k27me3 were detected by western blot. To investigate downstream targets of EZH2, next-generation sequencing analyses (NGS) were performed on A375R cells treated with DMSO, vemurafenib, tazemetostat or vemurafenib and tazemetostat in combination and A375 cells as controls.

Results: Treatment of A375 and A375R with vemurafenib resulted in decreased level of EZH2 in the BRAFi sensitive A375 cells, whereas EZH2 expression in resistant melanoma cells was not affected. Functional Inhibition of EZH2 by Tazemetostat did not decrease the level of EZH2, but inhibited H3K27 trimethylation in different melanoma cell lines, independent of BRAFi resistance status. Cell viability was decreased when A375R were treated with a combination of vemurafenib and tazemetostat in comparison to vemurafenib monotherapy. This was also observed in flow cytometric analyses, which showed increased apoptosis and a G0/G1 phase arrest in the cell cycle of A375R cells treated with a combination of vemurafenib and tazemetostat.

Conclusion: EZH2 contributes to BRAFi resistance in melanoma. Thus addition of tazemetostat to vemurafenib or influencing the expression of downstream targets of EZH2 could represent new therapeutic options in melanoma.

P204 | Dissecting the IFNγ-mediated transcriptomic alterations in Merkel cell carcinoma cell lines using Nanopore sequencing

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Merkel cell carcinoma (MCC) is a rare and highly aggressive skin cancer, mainly caused by the genomic integration of Merkel cell polyomavirus. A UV-induced point-mutation leads to expression of a truncated form of the virus' large T antigen (truncLT). As an immunogenic tumor, MCC escapes immune surveillance via disturbance of the human leukocyte antigen (HLA) class I expression or by hijacking the hosts immune control mechanisms like checkpoints such as programmed cell death-protein 1 (PD-1) and PD-ligand 1 (PD-L1). Immune evasion in MCC is of high clinical relevance regarding immunotherapy resistance; however, the exact mechanism is not fully understood yet.

Interferons are of great interest in context of MCC as they beneficially influence antiviral immune responses and possess anti-MCC activity. Especially IFNγ as a key player of cellular immunity could play a crucial role in MCC as it exerts a variety of anti-tumoral effects but also immunomodulatory functions, which are misused by the tumor.

To date, the influence of IFNγ on MCC cell lines regarding transcriptomic alterations has not been studied comprehensively. In melanoma, IFNγ-related mRNA profiles have been shown to predict clinical response to PD-1 treatment, underlining the high informational value of sequencing studies in cancer research.

In this project, we analyse the IFNγ-induced transcriptomic program in MCC cells using the nanopore sequencing platform. Therefore, we studied the effect of IFNγ on three MCC cell lines (WaGa, MKL-1, and MKL-2) regarding gene expression in presence or absence of IFNγ. We could detect differential gene expression in all three cell lines, whereby the highest number of significantly altered genes was detected in MKL-1. Some of the detected genes were even differentially expressed in all three cell lines. Subsequent pathway analysis and manual annotation showed a clear up-regulation in genes involved in tumor immune escape due to IFNγ treatment. Additionally, the identified genes were categorized into groups with anti-tumoral or protumoral effects in their detected regulation. Our results indicate that Nanopore sequencing is a suitable tool to study the mRNA profile of MCC and delivers detailed information about transcriptomic alterations under IFNγ influence. In the next steps, the identified differentially expressed genes can be analysed for their role in immune escape of MCC and their in vivo relevance. These findings could contribute to a better understanding how MCC resists immunotherapy and to optimize clinical treatment of MCC patients accordingly.

P205 | Evaluation of the G protein-coupled estrogen receptor as a potential new therapy target in cutaneous T-cell lymphoma

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Introduction: As other non-Hodgkin lymphoma (NHL) Mycosis fungoides (MF) and Sézary syndrome (SS) have a greater incidence rate in males than females. The endocrine contribution to this gender difference is yet unknown. In general, it is hypothesized that the reduced rate of NHL among females might be based on a protective role of estrogens in lymphomagenesis. Basically estrogens exert their effects through estrogen receptor alpha (ERα) estrogen receptor beta (ERβ) and the G protein-coupled estrogen receptor (GPER).

A tumor suppressive effect of different ERβ and GPER agonists in several cancer in vitro and in vivo models could be shown. We could recently demonstrate a distinct tumor suppressive effect of ERβ agonists in CTCL. However, GPERs role on NHL lymphomagenesis and its impact on CTCL is yet unknown.

Material & Methods: Here, we analyzed GPER and G-1, a synthetic, highly selective and potent GPER agonist in four different CTCL cell lines (Hut-78, MyLa, SeAx, HH). We performed 3-(4,5-dimeth ylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4- sulfophenyl)-2H-tetrazolium (MTS) proliferation assays and lactate dehydrogenase (LDH) assays. To analyze the effects on cell cycle distribution we performed flow cytometry of Propidium Iodide (PI) stained cells. For hormone receptor detection in the investigated cell lines and to determine influence on apoptotic pathways we performed qPCR, western blot and FACS analysis. We further investigated the effect of G-1 on PBMCs and CD4+ T cells isolated from healthy patients.

Results: We could show that all lymphoid cell lines express GPER. We further demonstrate that targeting GPER with the selective synthetic GPER agonists G-1 significantly reduced cell proliferation of all four CTCL cell lines in a dose-dependent manner. Flow cytometry analysis revealed that G-1 promotes apoptosis and elicits G2/M cell cycle arrest of CTCL cells. Further, western blot analysis revealed that GPER stimulation promoted several pathways related to apoptosis (e.g. upregulation of p53 and PARP cleavage) and inhibited anti-apoptotic proteins such as Mcl-1 and XIAP that are linked to survival of lymphoma cells. However, PBMCs and CD4+ T cells isolated from healthy patients were not affected by G-1 treatment.

Conclusion: Altogether, our preclinical results suggest that targeting GPER might be potential novelty in the targeted treatment of CTCL.

P206 | CRISPR/Cas9-based multiplex gene editing to determine the impact of heparan sulfate on the melanoma microenvironment

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The composition of the tumor microenvironment defines the malignancies and therapy response of melanoma. Glycosaminoglycans (GAGs) such as heparan sulphate (HS) are major regulators of the tumor microenvironment. In the last years, it becomes evident that HS is actively involved in signaling processes and the recruitment of immune cells. Most cytokines, growth factors and second messengers such as vascular endothelial growth factor A (VEGF-A), CXCL9 and CCL2 that contribute to the tumor microenvironment bear HS binding sites. The biosynthesis of HS involves the consecutive action of 11 different enzymes. At each modification step only a fraction of the potential substrates are modified. The end result is a linear polysaccharide of considerable structural heterogeneity and different biological activities. On a cellular level, this can produce an enormous range of distinct phenotypes such as different cell adhesive properties or migratory potentials, which is further reflected by a heterogeneous tumor tissue. In approximately 40% of melanoma patients, at least one gene coding for an HS building enzyme is genetically altered. To better evaluate the pathophysiological relevance of HS heterogeneity for tumor biology, we established a gene technological shotgun approach that enables CRISPR/Cas9-based multiplex gene editing in single cells (CRISplex). The CRISPR/Cas9 technology revolutionized genomic engineering. Defined positions within the genome are targeted through guiding RNAs (gRNAs), followed by Cas9 directed (CRISPR associated protein 9) DNA double strand breaks. DNA repair of the affected cell can produce deletions or insertions within the targeted gene section which can result in gene destruction.

We generated a gRNA library targeting the most frequently genetically altered enzymes of HS biosynthesis in melanoma patients. CRISplex introduces multiple gene editing events into the genome of B16F10 cells constitutively expressing Cas9 through the combinatorial and transient transfection with synthetic gRNAs. This enables a high rate of genetic engineering while maintaining low off-target levels. To find the optimal transfection strategy for multiplex gene editing, B16F10 melanoma cells were transfected using either lipo- or nucleofection. Confocal microscopy imaging revealed that the uptake of fluorescence-labeled gRNA through lipofection is the superior choice regarding transfection efficiency. In dependence of the transfection efficiency, gene editing events were stochastically distributed, which resulted intentionally in heterogeneous cell populations. Knockouts were analyzed by genomic cleavage detection assays and next generation sequencing (NGS). In further experiments, we will analyze the CRISplex engineered melanoma cells in microfluidic experiments mimicking the hematogenous dissemination of melanoma cells and murine animal models. Perspectively, identification of tumor supportive patterns in HS biosynthesis may offer novel targets for current cancer therapies.

In conclusion, CRISplex is a scalable, cost effective and easy to follow tool that enabled the targeting of multiple genes of the HS biosynthesis. However, our approach can further be applied to other cell lines and different functionally connected pathways.

P207 | Regulatory mechanisms of extracellular vesicle delivered miR-92b-3p on generation of carcinoma-associated fibroblasts in melanoma

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Background: Extracellular vesicles (EVs) contribute to intercellular cell-cell communication within the tumor microenvironment Released by melanoma cells they interact with a variety of cells (e.g. immune cells and stroma cells), thereby creating a tumorpromoting

microenvironment. EVs are small, cell-derived membrane nanovesicles loaded with proteins, mRNAs and noncoding RNAs e.g. miRNAs. Carcinomaassociated fibroblasts (CAF) represent the most common stromal cells in the tumor microenvironment, supporting tumor growth, and facilitating metastatic spreading of malignant cells. We hypothesize that EVs derived by melanoma cells contribute to CAF formation by delivering miRNAs.

Aim: We investigated the molecular mechanisms how EV delivered miRNAs from melanoma cells induce CAFs and thereby supports melanoma progression.

Methods: We isolated EVs from melanoma cell lines and normal melanocytes by ultracentrifugation and size exclusion chromatography. To trigger CAF formation, we stimulated normal human dermal fibroblasts (NHDFs) with different EVs. Further, we analyzed the biological functions and the gene expression profile of induced CAFs. Additionally, the miRNA cargo of EVs derived from melanoma cell lines and normal melanocytes was investigated by next generation sequencing

Results: We demonstrated the uptake of melanoma-derived EVs by NHDFs, resulting in induced cell viability, proliferation and motility. Analyses by qRT-PCR revealed that melanoma EVs induced the expression of CAF marker genes (e.g. aSMA and FAP). Moreover, tumor-promoting factors such as IL-6 and IL-8 showed an increased expression in NHDFs treated with EVs. By next generation sequencing, we have identified a differential miRNA cargo in a comparison of EVs that were released by melanoma cell lines or by normal melanocytes. We found miR-92b-3p highly enriched in melanoma cells and their corresponding EVs. Besides, we demonstrated that NHDFs incubated with EVs derived from melanoma cells also showed an accumulation of miR-92b-3p.

Conclusion: Melanoma specific miRNAs are delivered into fibroblasts by EVs, and induce a tumor promoting CAF phenotype.

P208 (OP06/04) | Deficiency of Lyve1 protects against hepatic melanoma metastasis

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During metastasis tumor cells directly interact with organ-specific endothelial cells. Lyve1 is a membrane glycoprotein that is expressed on liver sinusoidal endothelial cells (LSEC), lymphatic endothelial cells and macrophage subpopulations. Functionally, it is described as an endocytotic receptor of Hyaluronan, shows analogies to CD44 and is also involved in the adhesion of leukocytes and tumor cells to endothelial cells. Therefore, we here analyzed the role of Lyve1 during melanoma liver metastasis.

In this study constitutional Lyve1 deficient mice (Lyve1-/-) were used. Liver metastasis was induced by spleen injection of B16F10 luc2 or intravenous injection of WT31 melanoma cells.

Hepatic metastasis of B16F10 luc2 and WT31 melanoma was significantly reduced in Lyve1-/- as compared to wildtype (WT) mice. Therefore, both groups were analyzed in detail, but no difference in neither endothelial differentiation nor metabolic zonation was detected in livers of untreated mice. Interestingly, Lyve1-/- mice present with increased Hyaluronan plasma levels. Due to its role for tumor cell adhesion, initial tumor cell adhesion and retention was studied in Lyve1-/- and WT mice, but no differences were observed. Since Lyve1 is also expressed on tumor associated macrophages, hepatic immune cell infiltration was analyzed. By both immunofluorescence stainings and flow cytometry increased numbers of CD4+, CD8+ and regulatory T cells were detected in untreated Lyve1-/- livers. These then decreased with the presence of hepatic metastases of B16F10 or WT31 melanoma.

Lyve1 significantly reduced hepatic metastasis in two models of melanoma liver metastasis. Functionally, Lyve1 did not act on hepatic endothelial melanoma cell adhesion but influenced immune cell composition after liver colonization of melanoma cells.

P209 (OP01/04) | nAngiopoietin-like 4 inhibits metastasis formation

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Cancer is the 2nd leading cause of death worldwide. Early-stage detection, primary tumor excision and novel therapies have

significantly improved the prognosis of cancer patients. Yet, metastatic patients have poor survival rates and 90% of cancer-associated deaths occur in metastatic patients. Thus, in order to develop novel curative alternatives for patients with metastatic disease, there is an urgent need to decipher the molecular mechanisms regulating the metastasis process. Recent studies have shown that tumor cells may disseminate from the primary site at a very early stage and primary tumor may modulate the growth of tumor cells at the secondary site. However, the underlying mechanisms involved in control of metastatic outgrowth by primary tumor cells remain mysterious.

Here, we identified that ANGPTL4, a secreted glycoprotein and its cleavage fragments (cANGPLT4 and nANGLT4) are actively involved in primary tumor mediated control of metastatic growth. Employing a wide array of preclinical in vivo models and correlation analyses with clinical human specimens, we found that the n- and cANGPTL4 exert distinctly opposing functions during tumor progression. cANGPTL4 enhanced sprouting angiogenesis by binding to integrins and enhances primary tumor growth and metastatic progression in a post-surgical metastasis model. In contrast, nANGPTL4 interacted with endothelial cell-expressed SDC4, leading to a reduction of Wnt-signaling and inhibition of sprouting angiogenesis. Moreover, nANGPTL4 significantly enhanced overall survival in a post-surgical metastasis model. In agreement, analysis of indexed clinical primary tumor and serum samples of lung cancer and melanoma patients showed that uncleaved full-length ANGPTL4 and cANGPTL4 were primarily detected locally in tumor tissues, whereas nANGPTL4 predominated the systemic circulation. Furthermore, longitudinal studies proved that nANGPLT4 concentrations correlated inversely with disease progression in cancer patients. Therapeutic administration of either recombinant nANGPTL4 protein or AAV (adeno-associated virus) induced overexpression of nANGPTL4 severely impaired lung metastasis in mice.

In summary, we identified proteolytic processing of tumor-derived ANGPTL4 as a molecular switch converting a locally pro-angiogenic/pro-tumorigenic molecule into a systemically acting inhibitor of metastasis and angiogenesis. Further, the findings identify nANGPTL4 as a potential novel diagnostic and anti-metastatic therapy agent.

P210 | Does aberrant glutamate metabolism in melanoma affect dendritic cell function?

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Recent advances in the understanding of the metabolic state of the tumor microenvironment illustrate that tumor immunity can be negatively regulated by metabolites secreted in the tumor tissue. How this affects dendritic cell (DC) function and their central role in anti-tumor immunity in melanoma remains unexplored. We are working on the transgenic melanoma mouse model tg(Grm1)EPv, which spontaneously develops melanoma in the ear and tail skin due to an overexpression of the metabotropic glutamate receptor 1 (Grm1) in melanocytes. This aberrant glutamate metabolism might not only cause tumor formation but could also affect immune cell function in the tumor microenvironment. Thus, the goal of this PhD thesis is to investigate the metabolic changes in progressing melanoma with a specific focus on glutamate metabolism, and how this affects tumor-infiltrating DC and subsequent T cell responses.

Currently, we are analyzing the impact of elevated glutamate and glutamine levels on the differentiation and maturation of bone marrow-derived DC (BMDC) and human monocyte-derived DC (moDC) using flow cytometry. This will be followed by functional analysis of their potential to stimulate T cell proliferation. Within vitro co-cultures of Grm1-overexpressing melanoma cell lines and DC we will further investigate alterations in the DC function. To confirm these findings in vivo, we will use the tg(Grm-1)EPv mice, and screen them for metabolic changes at different stages of the disease (tumor free 6–10 weeks, tumor early 4–6 months, tumor advanced 8–10 months) with a glutamate assay kit and LC/MS analysis of metabolites. Moreover, we will analyze changes in DC subsets during tumor progression by multiparameter flow cytometry. Additional immunological assays will assist to understand phenotypical and functional alterations in tumor-infiltrating DC.

Acquired knowledge can benefit in the design of novel therapeutic strategies for cancer patients involving potential modification of tumor glutamate metabolism. Combination therapies with inhibitors of the glutamate pathway might help to improve response rates in cancer patients.

P211 | Extracellular vesicles potentiate invasiveness of melanoma cell subpopulations via transfer of miR-1246

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Background: The underlying molecular processes of melanoma metastasis are incompletely understood. At the cellular level, several steps must be taken to engage metastatic processes. Intratumoral plasticity and heterogeneity markedly influences the metastatic potential of cells by enabling durability to different stress factors. This versatility is mediated by cooperation of subpopulations of tumor cells by virtue of the exchange of genetic material or functional molecules via extracellular vesicles (EVs), which contributes to tissue invasion and metastasis to distant organs.

Aim: To investigate molecular mechanisms by which miRNAs delivered by extracellular vesicles contribute to melanoma invasion and metastasis.

Methods and results: By invasion assays, we isolated a highly invasive subpopulation (BLM-HI) of the melanoma cell line BLM. Incubation

of the less invasive parental BLM cells with conditioned medium of BLM-HI resulted in a higher invasive capacity in a 3D spheroid model. Based on these results, we wondered if EVs isolated from the medium of the BLM-HI subpopulation would mediate this invasive quality. We found that these EVs indeed increased the invasive ability of parental BLM cells. By next generation sequencing (NGS) we observed a differential gene expression in BLM cells treated with BLM HI-EVs compared to untreated cells. Transcriptome analysis revealed significant upregulations of pathways related to extracellular matrix organization and pathways connected to the hallmark of epithelial-mesenchymal transition (EMT)—a key process for metastasis. These findings support our thesis that EVs and their contents are able to promote invasion and phenotype switching within tumor cell subpopulations.

Since one major function of EVs is intercellular transport of miRNAs, we searched for and identified, by small RNA sequencing, differential enrichment for specific miRNAs in EVs derived from BLM-HI subpopulation compared to parental BLM cells. We found miR-1246 significantly enriched in EVs from BLM-HI cells. Treatment of parental BLM cells with EVs released by BLM-HI resulted in increased intracellular levels of miR-1246.

Conclusion: Transfer of EV from melanoma cells is a key process regarding intercellular cooperation and transformation of cellular phenotypes, including increasing invasive potential. We suppose that miR-1246 contributes to a pro-invasive phenotype of melanoma cells and could serve as a potential prognostic marker for melanoma progression.

P212 | Comprehensive analysis of melanoma cell-derived miRNA cargo in extracellular vesicles

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Background: In recent years, research has increasingly focused on the influence of cell-cell communication within the tumor microenvironment. Extracellular vesicles (EVs) are important mediators of this intercellular communication. By transporting functional molecules derived from melanoma cells (e.g. miRNAs) EVs influence the function and phenotype of tumor cells, immune cells and stromal cells and thus promote tumor progression.

Aim: The aim of this study was to compare miRNA expression in melanoma cells and normal melanocytes with miRNA profiles of their respective EVs.

Methods: EVs were isolated from conditioned medium by differential centrifugation steps including ultracentrifugation. Isolated EVs were characterized by western blot and nanoparticle tracking analysis (NTA). Total RNA of EVs and cells was isolated by TRIZOL based preparation. miRNA profiles were analyzed by a small RNA specific next generation sequencing approach. Data analyses and functional network generation were performed using Qlucore Omics Explorer software and MIENTURNET (MIcroRNAENrichmentTURnedNETwork) online tool.

Results: The isolated EVs from MM and melanocytes both showed the surface markers CD81, CD63 and a similar size distribution, but melanoma cells released higher numbers of EVs compared to normal melanocytes. As to the miRNA profiles, we found the highest correlation between EVs from melanoma cells and melanocytes, followed by EVs to their corresponding cells, and the lowest correlation was found between endogenous miRNA expression from melanoma cells and normal melanocytes. By comprehensive analyses, eight miRNAs (miR-17-3p, miR-92b-3p, miR-125b-5p, miR-140-5p, miR-182-5p, miR-183-5p, miR-221-3p and miR-584-5p) were identified to be enriched in both melanoma cells and melanoma-derived EVs. Functional network generation and pathway enrichment analysis revealed that these miRNAs are associated with different tumor entities and signaling pathways contributing melanoma progression.

Conclusion: miRNA cargo of melanoma-derived EVs could potentially serve as prognostic and diagnostic markers.

P213 | GARP, a novel driver of proliferation and growth in 2D and 3D cultured melanoma cells

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Malignant melanoma is the deadliest form of skin cancer. Despite recent therapeutic advances, especially using immune checkpoint inhibitors, late-stage patients still have limited treatment options—highlighting the great need to discover and characterize potential novel therapeutic targets.

One promising target, a transmembrane protein by the name of glycoprotein A repetitions predominant (GARP), is highly expressed on both the surface of melanoma cells and on tumor promoting immune cell populations, including regulatory T cells (Treg) and platelets. Acting as a regulator and activator of latent-TGF-beta, GARP is well known to exhibit suppressive functions in the tumor microenvironment, namely by inducing peripheral Treg and by inhibiting the proliferation and cytokine production of effector T cells.

However, relatively little is known about the effects of GARP on cancer cells themselves. Recent findings have begun to link elevated GARP levels to metastasis (oral squamous carcinoma), invasion (breast cancer), and proliferation (bone sarcoma) of cancer cells. Altogether indicating that GARP may play a greater role in tumorigenesis than previously described.

This study aimed to characterize the effects of GARP on the proliferation and growth of melanoma cells in both 2D and 3D cell culture settings. This was accomplished by generating stably transfected GARP overexpression (GARP+) human melanoma cell lines (surface overexpression confirmed via flow cytometry) and by monitoring their proliferation (via flow cytometry) in both 2D monolayer cell

culture and in 3D melanoma spheroids, a more physiologically relevant in vitro tumor model.

It was found that GARP+ cell lines divided significantly faster than the wild type (WT) control cell line in both 2D and 3D cell culture settings. GARP+ spheroids also generated significantly larger melanoma spheroids than WT spheroids. As a therapeutic approach, WT spheroids were treated with a blocking GARP antibody, which effectively resulted in significantly smaller spheroids. Interestingly, this study also observed for the first time that melanoma spheroids exhibited an outer layer or "corona" of loosely attached cells resembling superficial spreading—a clinical manifestation of melanoma. When this corona was further analyzed to determine its area, it was found that not only did the GARP+ spheroids develop coronas faster, but they were significantly larger in size in comparison to the WT control spheroids.

In future studies, 3D invasion assays will be performed to evaluate whether GARP plays a role in the invasiveness of melanoma spheroids and if these coronas are linked to enhanced invasive potential. Whether GARP promotes the proliferation of melanoma cells via a TGF-beta dependent or independent mechanism remains unclear and will be further explored in future work.

In summary, this study identified GARP as a novel driver of proliferation and growth of melanoma cells in both 2D and 3D cell culture settings. Notably, we also observed for the first time that melanoma spheroids form cellular "coronas", a previously uncharacterized structure of melanoma spheroids, in a GARP-dependent manner. Lastly, this study demonstrated the promise of targeting GARP, a protein with multiple tumor-promoting functions, including immunosuppression and proliferation, for the treatment of melanoma via antibody-based therapeutic strategies.

P214 | miR-129-5p from extracellular vesicles presents a potential marker for response to BRAF/MEK inhibitors

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Background: Almost 50–60% of all malignant melanomas harbor constitutively activating mutations in BRAF. Potent BRAF inhibitors (BRAFi) and MEK inhibitors (MEKi) are clinically approved. However, patients rapidly develop mechanisms of resistance to these targeted therapies, leading to tumor progression. Because there is a delay between tumor progression at the molecular or cellular level and tumor progression confirmed by CT scans, there is an urgent need for new rapid and reliable methods of monitoring response and resistance to treatment in order to detect disease progression at its earliest stage.
Aim: The aim of the project is to evaluate principally whether miR-129-5p is a reliable and robust liquid biopsy marker for patient response to BRAF/ MEK inhibitors.
Methods: Extracellular vesicles (EVs) were isolated from conditioned medium by differential centrifugation steps including ultracentrifugation. Size exclusion chromatography (SEC) was used for EV isolation from patient serum. Isolated EVs were characterized by western blot and nanoparticle tracking analysis (NTA). Total RNA of EVs and cells was isolated by TRIZOL based preparation. The miRNA expression was determined by qRT-PCR, using the synthetic miRNA mimic cel-miR-39 for normalization.
Results: We confirmed that miR-129-5p is downregulated by constitutive activated BRAF signaling. However, BRAFi and MEKi treatment of melanoma cell lines strongly induces miR-129-5p expression (60–400 fold) in BRAF mutant cell lines. In contrast, treatment with BRAFi or MEKi of resistant cell lines, BRAF wildtype cell lines or normal melanocytes showed no increase in miR-129-5p expression. The expression of miR-129-5p was even reduced in cell lines that were resistant to BRAFi or MEKi. Since EVs and their contents can be detected in patients' blood, we investigated whether miR-129-5p is a reliable and robust liquid biopsy marker for patient response to BRAF/MEK inhibition.
To this end, we established isolation and analysis of extracellular vesicles from conditioned cell culture medium and patient derived blood samples. Our results show an enrichment for miR-129-5p in EVs derived from different melanoma cell lines treated with BRAFi or MEKi. Initial analyses of EVs isolated from blood samples from patients showed an increased accumulation of miR-129-5p during treatment with BRAF/MEK inhibitor.
Conclusion: Our study suggests that the EV-enveloped miR-129-5p could potentially serve as a diagnostic marker to monitor the response to BRAF/MEK inhibition in melanoma.

P215 | Type I cytokine immunotherapy induce senescence and clearance of melanoma cells

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Immune responses can counteract the development of tumors by eliminating tumor cells. Insufficient immigration of immune cells into the tumor microenvironment is one cause of uncontrolled tumor growth. In addition to immune cells mediating cellular immune responses, immune cells act via soluble factors. We have shown that the cytokines of CD4+ TH1 cells, interferon-γ (IFN-γ) and tumor necrosis factor (TNF), block the cell cycle through activation of the CDK (cyclin dependent kinase) inhibitor p16Ink4a. Tumor control exhibits basic features of senescence and has been established as cytokine-induced senescence (CIS) in tumor cells. In some tumor entities, CIS is also mediated by the combination of IFN-α with TNF. Here, we show that intra-peritoneal injection of IFN-α induces tumor growth during rescue treatment of life-threatening malignant ascites. After IFN-α application, melanoma cells began to strongly express senescent-β-galactosidase (SA-β-gal) and cell cycle inhibitors p16Ink4a or nuclear phospho-p21. Simultaneously,

even in the absence of the cytokines, melanoma cells were induced into permanent growth arrest and removed from the peritoneum. To validate the therapeutic potential of IFN-α in vivo, we transferred the treatment protocol to a mouse model of peritoneal carcinomatosis in NOD-SCID mice. During the four cycles of IFN-α, tumor burden was efficiently controlled by arresting cancer cell proliferation. We demonstrated that IFN-α induced cancer cell senescence is a functional immunotherapy and contributes critically to therapeutic cancer immune control in addition to cancer cell killing.

P216 | An AGO2 variant affects the function of miRNAs and influences the viability of melanoma cells

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Argonaute 2 (AGO2) is the effector protein of RNA-interference (RNAi) binding to small regulatory RNAs (miRNAs or siRNAs) and mediating the inhibition of translation of complementary target mRNAs. The mechanism of RNAi influences all important cellular processes, such as proliferation or invasion, and plays a decisive role during melanoma development. Using RNA sequencing (RNA-seq), we showed that a wide range of miRNAs is deregulated only during melanoma tumorigenesis whereas no regulation in embryonic development from melanoblasts to melanocytes can be observed. In melanoma cells a plenty of miRNAs is strongly enriched compared normal human epidermal melanocytes. This upregulation is melanoma specific and contrary to the miRNA expression patterns in many other types of cancer where miRNAs are mostly downregulated.

In addition, using affinity purification followed by mass spectrometry we found that the expression of AGO2, the most abundant human AGO protein, is significantly reduced in melanoma compared to other cancer cell lines.

In RNA-seq data, we identified the expression of another, yet unknown mature AGO2 mRNA variant in melanoma cells and patient samples. The translation of this variant results in an N-terminally truncated protein version of AGO2. A specific siRNA-mediated knockdown of this AGO2 variant leads to reduced proliferation of melanoma cell lines, which was analyzed by various proliferation assays as XTTassay, clonogenic assay or real time cell analysis. Using flow cytometry, we identified that the reduced proliferation is caused by an increase in apoptosis. Performing RNA-Seq after knockdown of the AGO2 variant, we detected a major part of miRNA target genes upregulated in melanoma cells indicating a critical role of this variant for miRNA function.

Our data offer new knowledge about a previously unknown AGO2 variant and a potential functional significance of this variant for RNAi in melanoma cells. Further, our results provide the basis for a potential, promising therapeutic tool via decreasing the survival of melanoma cells.

P217 | High-dose vitamin C in BRAF inhibitor resistant melanoma cells in vitro and in vivo

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Introduction: In recent years, we and others have discovered that high-dose vitamin C paradoxically acts as a pro-oxidant, causing large amounts of hydrogen peroxide that cannot be compensated by tumor cells, especially melanoma cells. Therefore, high-dose vitamin C may be an attractive approach for supportive care of refractory melanoma resistant to established modern therapies, such as BRAF inhibitors in BRAF-mutated melanoma.

Methods: Several BRAF-mutated melanoma cell lines were treated with either pharmacological doses of vitamin C alone or in combination with the BRAF inhibitor vemurafenib. Viability, cell cycle distribution, formation of reactive oxygen species and protein levels of GLUT-1 and HIF1alpha as well as phosphorylation levels of ERK1/2 were assessed. To study in vivo effects, BL6 mice subcutaneously bearing a D4M.3A (BRAFV600E) melanoma were treated with intraperitoneal injections of vitamin C (2 g/kg body weight), vemurafenib (30 mg/kg body weight), or a combination thereof.

Results: Both, vemurafenib sensitive and resistant BRAF mutated melanoma cell lines were sensitive to the treatment with pharmaceutically active amounts of vitamin C. Treatment of mice with BRAFV600E melanoma resulted in short-term ascorbate serum levels in the millimolar range. Furthermore, the therapeutic effect of BRAFV600E inhibition was markedly improved by high-dose vitamin C in terms of reduced tumor growth, which was reflected in a lower proliferation rate and an increased proportion of apoptotic cells.

Conclusion: High-dose vitamin C therapy can be combined with standard targeted melanoma therapies without severe toxicities or loss of efficacy.

P218 | Preclinical evaluation of Braftovi (encorafenib) plus Mektovi (binimetinib) in NRAS mutated patient-derived xenografts (PDXs)

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Background: Patients with NRAS-mutant metastatic melanoma often have aggressive disease requiring a fast acting, effective therapy. The MEK inhibitor binimetinib shows an overall response rate of 15% in patients with NRAS-mutant melanoma, providing a backbone for combination strategies. Our previous studies demonstrated that in NRAS-mutant melanoma the antitumor activity of the MEK inhibitor binimetinib is significantly potentiated by the BRAF inhibitor encorafenib through induction of ER stress, leading to melanoma cell death by apoptotic mechanisms. BRAFi increased pERK, but also significantly increased growth inhibition and apoptosis induced by the MEKi in monolayer, spheroids, organotypic and patient-derived tissue slice cultures of NRAS-mutant melanoma. BRAFi such as encorafenib induced an ER stress response via the PERK pathway, as detected by phosphorylation of eIF2α and 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, the latter of which was enhanced by combination with encorafenib. The increased apoptotic rates caused by the combination treatment were significantly reduced through siRNA knockdown of ATF4 and BIM, confirming its critical roles in this process. Encorafenib combined with binimetinib was well tolerated in a phase III trial showing potent antitumor activity in BRAF-mutant melanoma, making rapid evaluation in NRAS-mutant melanoma imminently feasible. These data provide a mechanistic rationale for evaluation of binimetinib combined with encorafenib in preclinical and clinical studies on NRAS-mutant metastatic melanoma.

Methods: NRAS-mutant melanoma cells, as well as tissue slice cultures of patient tumors are treated with BRAF and MEK inhibitors alone and in combination. In addition to the investigation of cell cytotoxicity and cell cycle remainder, the altered signal transmission is detected. Furthermore, the patient cells were injected into NSG mice and treated in vivo with BRAFi or/and MEKi. Subsequently, the tumors were further analyzed ex vivo and the expression of MAPK Siganl pathway proteins, as well as various ER stress associated proteins was evaluated.

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. In vivo the combination therapy of encorafenib with binimetinib resulted in significantly reduced tumor growth compared to the control and encorafenib groups; but the best effect in terms of tumor growth inhibition was measured in the binimetinib therapy group.

Conclusions: In vitro and ex vivo a tendency towards a better response to the combination therapy was detected. Surprisingly, the in vivo data showed no increased combinatorial effect. However, the in vivo effect of binimetinib as monotherapy was unexpectedly high in the tested regimen. Therefore, binimetinib proved to be advantageous in the treatment of melanoma in vivo and could be a good base for combinational therapies in melanoma treatment.

P219 | Phenotypic melanoma heterogeneity is regulated through cell-matrix interaction-dependent changes in tumor microarchitecture

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Phenotypic heterogeneity of solid cancer plays a critical role in shaping treatment response. This type of heterogeneity is organized spatially with specific phenotypes, such as distinct clusters of proliferating and cell cycle-arrested cells within a tumor. What determines the occurrence of these phenotypically distinct domains in solid cancers is poorly understood. Utilizing in vitro and in vivo three-dimensional models, we show that in melanoma spatial organization of phenotypic heterogeneity is dictated by the expression and activity of the lineage-survival oncogene MITF. Mechanistically, we reveal that MITF controls extracellular matrix (ECM) composition and decreases ECM organization. This leads to reduction of Rho-ROCK-myosin signaling-driven mechanotransduction through poor focal adhesion maturation and reduced contractility of the actin cytoskeleton. The resulting altered

tumor microarchitecture and structural relaxation decrease tumor solid stress and subsequently p27Kip1 expression, ultimately reducing phenotypic heterogeneity. Consequently, selective inhibition of ROCK phenocopies the effect of MITF overexpression, demonstrating the importance of cell-ECM crosstalk in this process.

In summary, our findings place tumor cell-ECM crosstalk resulting in altered tumor microarchitecture and ROCK-driven mechanotransduction as a central driver of phenotypic tumor heterogeneity. Melanoma shares these physical properties with other solid cancers underscoring the potential for therapeutically targeting this phenomenon in cancer, beyond melanoma.

P220 | Nanocarrier systems targeting the tumor microenvironment: From liposome functionalization to the choice of antibody attachment

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The immunosuppressive tumor microenvironment has a major impact on the dysregulation of immune reactions in tumor patients. An approach to overcome this can be performed with immune checkpoint inhibitors (mainly for inflamed tumor phenotypes). Nevertheless, only around 50 % of melanoma patients even react to checkpoint inhibitors and they often come with severe immune related adverse events. Therefore, more research has to be conducted to both, improve our understanding of the tumor microenvironment and to address immune escape mechanisms even better.

Nanoparticle carrier systems are actually being developed to address this purpose. Previous studies have found that the transmembrane protein glycoprotein A repetition predominant (GARP) is highly expressed on the surface of various tumor entities, including melanoma as well as on immune modulating cells in the tumor microenvironment, such as activated Treg and platelets. We could show that GARP suppresses anti-tumor immune responses by inducing peripheral Treg and, at the same time, inhibiting the proliferation and cytokine production of T effector cells—making it an ideal target structure for nanoparticles to address.

Herein, we improve the selective targeting of nanocarriers to enable active accumulation in the tumor environment. We tested different liposomal nanocarriers with varying amounts of surface functionalization of PEG and/or biotin. Measured by flow cytometry, different amounts of fluorescent streptavidin-silica particles were bound depending on the percentage of functionalization. Parallel incubation with bovine serum albumin particles and a preincubation with unfunctionalized liposomes increased streptavidin binding concentrations, which indicates a starting position for the linkage of an anti-GARP-antibody biotin functionalization. In future studies, we will identify a suitable anti-GARP-antibody for targeted delivery in melanoma cells.

In addition, we showed by flow cytometry, that in the PEG/biotin functionalized liposomes encapsulated fluorescent model cargos (DiR, DiD, Calcein, Sulforhodamine B) were accumulated inside of human melanoma cells (MaMel-19).

Additional experiments performed with doxorubicin (chemotherapeutic agent) encapsulated polymersomes, (polymer capsules with hydrophilic core) support these findings. Hereby, in 3D spheroids, through microscopy monitoring, the fluorescent agent was seen to accumulate inside the cells and demonstrated a high cytotoxicity indicated by growth inhibition and severe damage to spheroid structure. In 2D cell culture, measured by flow cytometry, likewise doxorubicin was found to accumulate inside the cells and therefore the cells were less viable.

In summary, we developed a liposomal carrier system to address the tumor microenvironment. PEG/biotin functionalized liposomal nanocarriers were found to be a good starting point for the attachment of an anti-GARP-antibody to increase selectivity. In addition, the uptake of encapsulated model cargos in MaMel-19 cells was successfully shown for liposomal and polymersomal carrier systems.

P221 | Molecular alterations in drug-resistant melanoma cells

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Malignant melanoma is highly invasive and has a high metastatic potential, accounting for the most extensive skin cancer-related deaths. Improvement of life expectancy in melanoma patients came with the use of targeted therapies, with BRAF and MEK inhibitors representing an efficient therapeutic option for BRAF-mutant metastatic melanoma. Despite that, many patients develop resistance over time due to several resistance mechanisms, including genetic and epigenetic mechanisms. Modifications in the tumor microenvironment have also been involved in the acquisition of resistance. Still, the direct role of melanoma cells in modifying the extracellular matrix in resistance is less clear. We used A375 and Skmel28 harboring a BRAF mutation and BLM and MV3 carrying an NRAS mutation to address this issue. We generated resistant cell lines by maintaining them under BRAF- and MEK-inhibitors pressure until resistance occurred. Acquisition of resistance was accompanied by cellular changes reflecting a mesenchymal phenotype, with elongated cell morphology and enhanced vimentin transcription, signs of an epithelial to mesenchymal transition. Analysis of the secretome of resistant cells identified enhanced expression of factors such as CCL2, CCL20, and osteopontin, necessary for generating a proinflammatory microenvironment.

Moreover, resistant cells showed an altered expression of extracellular matrix molecules and matrix modifying enzymes, including MMP8, -14, -15, -16, and ADAM10. Among the extracellular matrix

molecules, the tenascin C (TNC) expression, although downregulated in most melanoma cells responding to the inhibitors, was high in melanoma cells after resistance occurred. In these cells, we also found enhanced fibronectin transcription.

In human melanoma tissue samples from patients pre- and post-treatment, we could detect high TNC expression in the peritumoral areas before treatment. In resistant patients' metastatic tissues TNC was highly expressed in both peritumoral and tumor areas. TNC was significantly higher in sera from stage IV melanoma patients than healthy individuals, independent of the mutation status and the S100 tumor marker. Furthermore, high TNC was in the sera of stage IV melanoma patients before therapy and after the acquisition of resistance, but in the latter, a significant reduction was detected. The current data suggest that serum levels of TNC are not predictive of BRAF/MEK inhibitors-therapy efficacy.

In summary, factors produced by resistant melanoma cells might foster resistance generating a peritumoral environment, which might facilitate the crosstalk between melanoma cells, fibroblasts, macrophages, and inflammatory cells and drive, in combination with the ECM and proteolytic enzymes, tumor resistance, invasion, and metastasis.

P222 (OP03/04) | Mutational evolution in the Hgf-Cdk4 mouse melanoma model

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In the last decade, large sequencing studies have generated in-depth insights into the genomes of cancer cells. Despite these advances, the genetic evolution during metastatic tumor progression remains poorly understood. Whereas driver mutations alone are insufficient to cause malignant transformation or induce metastatic spread, genomic instability is increasingly recognized as a critical element of tumor progression. Genetically engineered mouse models have proven to be indispensable in melanoma research. However, most GEMMs artificially introduce key mutations before or early during tumor progression.

In the current project, we aim to analyze the evolution of mutations in the Hgf- Cdk4 mouse melanoma model established in our laboratory. From this model, we have successfully generated metastasizing melanoma cell lines. In comparative histopathological analyses, the morphology of primary and transplanted Hgf- Cdk4 melanomas closely resembles the phenotype of blue nevus-like melanomas observed in humans. Since this melanoma subtype is known to harbor activating mutations in the genes for Gnaq or Gna11, we performed amplicon-based nextgeneration sequencing of our cell lines for described mutational hotspots. In this, we detected Gnaq/11 Q209 mutations in all analyzed Hgf-Cdk4 cell lines. Previous work has identified also recurrent Trp53 mutations in the Hgf-Cdk4 cell line HCmel12. We therefore additionally sequenced the catalytic regions of the Trp53 gene and observed heterozygous Trp53 mutations in all analyzed Hgf-Cdk4 cell lines. To understand when these mutations occur during tumorigenesis, we sequenced primary Hgf-Cdk4 melanomas and identified high-frequency Gnaq/11 Q209 mutations in 30% of spontaneous melanomas. Interestingly, all melanomas induced by the model carcinogen DMBA harbored Gnaq/11 Q209 mutations. Trp53 mutations of primary melanomas were non-recurring and showed low allele frequencies. Our data suggests a model in which Hgf-Cdk4 melanomas acquire activating Gnaq/11 mutations early during primary tumor development, whereas the Trp53 mutations occur late during serial transplantation or in vitro culture. As serial transplantation also increases the metastatic potential of cell lines, we hypothesize that genomic instability plays a critical role in metastasis also in the Hgf-Cdk4 mouse melanoma model, making it an ideal tool to understand melanoma metastasis.

P223 (OP02/02) | Role of the damage-associated molecular pattern molecule S100A8/A9 and neutrophils in the peripheral blood and the tumor microenvironment in malignant melanoma

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The role of the tumor microenvironment (TME) in the progression of melanoma and in the response to immune-checkpoint inhibition (ICI) is still only partially understood. In particular, cells of the innate immune system such as neutrophil granulocytes are assumed to play an important role in the establishment of an immunosuppressive TME. Our previous work identified damage-associated molecular patterns (DAMP) such as the heterodimer S100A8/A9 as mediators of chemotaxis and activation of neutrophils. High expression in TME and high serum levels of S100A8/A9 in melanoma patients correlate with an unfavorable prognosis and non-response to immune-checkpoint inhibition (ICI).

To test the relevance of S100A8/A9 and neutrophils as possible predictive biomarkers for ICI of melanoma, the expression of the relevant factors in the peripheral blood of 45 stage IV melanoma patients (serum levels before and during immunotherapy, i.e. baseline (BE) and before the 4th infusion (C4) concentrations) were analyzed. We found significant overall survival (OS) shortening effects of S100A8/A9 before as well as during immunotherapy (BE $p < 0.01$; C4 $p < 0.05$). However, patients with high baseline and 4th cycle S100A8/9 serum levels achieved a prolonged progression-free survival (PFS) (BE $p < 0.05$; C4 $p < 0.01$). At baseline, the opposite effect was shown for neutrophils. A higher expression of neutrophils had significant overall survival prolonging effects ($p < 0.001$) and a negative impact on progression-free survival ($p < 0.05$).

Using immunofluorescence staining of neutrophils on 293 tissue sections of 114 melanoma patients, i.e. 44 naevi, 86 primary tumors and 163 metastases, we demonstrated significantly higher neutrophil counts in primary melanomas versus naevi ($p < 0.001$), in metastases versus naevi ($p < 0.001$) and in primary tumors versus metastases ($p < 0.05$). Further, high neutrophil count was correlated with a better prognosis in terms of PFS: We showed anti-tumor effects of neutrophils detected in the TME of primary melanomas ($p < 0.05$). This finding further needs to be explained in the context of previous research.

Microenvironment-derived S100A8/A9 has been established as a clinically meaningful blood-based marker of unfavorable prognosis and non-response to immune-checkpoint inhibition (ICI) in melanoma. The elucidation of its specific role within the TME and interaction with neutrophils will require further experimental research. Last but not least, the tumor-stage specific polarization of neutrophils as potential explanation of ambivalent neutrophil impact on tumor progression will need to be examined in greater detail by future research.

P224 | Functional analysis of tumor-homing eosinophils in melanoma progression and response to immune-checkpoint inhibition

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Over the last years immune-checkpoint inhibition (ICI) such as anti-PD-1 or anti-CTLA-4 antibody therapies have revolutionized the treatment of metastatic melanoma and emerged as gold standard treatment. Although ICI leads to a significant increase in survival of melanoma patients, only 58% respond to combined anti-PD-1 and anti-CTLA-4 therapy. In addition, more than half of the melanoma patients have severe immune-related adverse events (irAEs). However, the detailed mode of action of ICI and the specific role of innate immune cells in response or nonresponse to ICI are not yet fully understood. It seems that T cells are pivotal for tumor growth suppression and successful treatment. Recently, it has been described that eosinophils orchestrate the anti-melanoma T cell response. A prerequisite might be their activated functional state. We hypothesize that activated eosinophils have an impact on tumor progression and on the benefit of ICI by promoting antitumoral T cell response. Whether eosinophils could serve as prognostic biomarkers and whether their presence might predict the efficacy of ICI were investigated.

We performed a localization analysis using immunofluorescence co-staining of eosinophils and T cells in tissue samples of primary melanomas and associated metastases from a total of 119 patients. To detect eosinophils, we utilized the eosinophil marker SIGLEC-8 and to identify activity status, two activation markers: eosinophil cationic protein (ECP) and eosinophil peroxidase (EPX). Moreover, colocalization analysis of eosinophils with cytotoxic CD8+ effector T cells has been performed.

For systemic analyses, blood samples from 45 patients with metastatic melanoma who responded or did not respond to ICI were included. Using established ELISA assays, serum levels of eosinophil activation markers ECP and EPX were measured before initiation of ICI (baseline) and before the fourth infusion of therapeutic drugs (4th cycle).

Local expression analysis showed a highly significant correlation between expressed eosinophil markers (SIGLEC-8, ECP and EPX) and T cell marker (CD8) among all tissue samples ($p < 0.0001$). In over half of all tissue samples, co-localized eosinophils and T cells were detectable. Additionally, melanoma patients with higher amounts of ECP+ eosinophils ($p = 0.0098$) as well as CD8+ T cells ($p = 0.0025$) in their primary melanoma had a significantly improved progression-free survival (PFS).

Regarding systemic effects, higher baseline serum levels of eosinophils (cutoff 0.13 Mrd/l, $p = 0.026$) and their activity markers ECP (cutoff 37.58 ng/ml, $p = 0.012$) and EPX (cutoff 1.07 ng/ml, $p = 0.048$) were associated with prolonged PFS in stage IV melanoma. Patients with relatively constant to decreasing absolute eosinophil count (cutoff -0.06, $p = 0.0047$) and ECP serum level (cutoff 3.46, $p = 0.0005$) between baseline and 4th cycle achieved a longer PFS than those with increasing levels. Responders tended to present slightly higher baseline serum levels of eosinophils, ECP and EPX, without a significant difference ($p > 0.05$).

Our data reveal that activated eosinophils are associated with local T cell infiltration and linked to better prognosis in malignant melanoma. In addition, higher baseline serum levels of eosinophils, ECP and EPX are related to delayed relapse and may serve as reliable prognostic biomarkers. The precise molecular mechanisms which are responsible for the antitumor effects of eosinophils in malignant melanoma and their impact on T cell response require further investigation.

P225 | Hepatic steatosis and NASH-associated liver fibrosis promote adhesion and growth of melanoma cells in the hepatic vascular niche

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Background: Malignant melanoma (MM) preferentially metastasize to the lymph nodes, lungs, central nervous system, bones, and to the liver. When considering the frequency of MM metastases, around 20% are observed in the liver, which is associated with a

poor prognosis. This preferential metastazation phenomenon is also known as metastatic tropism. Paget first described this in the 19th century and established the "seed" and "soil" theory, that different seeds (tumor cells) are thriving only in distinct soils (metastatic organ). It has been shown that distant organs are pre-conditioning themselves, and especially their vasculature, whenever there is a tumor present. In the liver, sinusoidal endothelial cells (LSECs) represent unique microvascular EC that may upregulate cell contact and adhesion molecules prior to metastazation. Furthermore, pathological changes of the liver during liver steatosis, fibrosis and/or cirrhosis, which are associated with alterations in the hepatic vascular niche, may further affect tumor cell adhesion and growth.

Hypothesis: We hypothesize that pathologic changes of the liver microvasculature during steatosis and NASH-associated liver fibrosis have a considerable influence on the hepatic MM metastazation process.

Methods: We injected B16-F10-Luc2 and Wt31 MM cells into a mouse model of nonalcoholic steatohepatitis (NASH) induced by feeding a choline-deficient amino acid defined (CDAA)-diet. Next to melanoma metastasis quantification, histological stainings, and transcriptomic analyses were performed in order to search for candidate genes that might influence the metastazation process.

Results: Experiments with short (1-, 2-, 4-weeks) and long-term (10 weeks) CDAAdiet, either representing liver steatosis or NASH-associated liver fibrosis, showed increased liver metastases of B16-F10-Luc2 and Wt31 melanoma cells during steatosis and liver fibrosis. Furthermore, we confirmed increased initial melanoma adherence of B16-F10-Luc2 cells in steatotic or fibrotic livers of 2- and 10 weeks CDAA-diet fed mice, when compared to controls. These results prompted us to look at adhesion molecules that might serve as potential therapeutic targets. Therefore, we isolated EC from CDAA-diet fed mice and performed transcriptomic analyses. RNASeq results rendered highly upregulated genes, such as EpCAM, Osteopontin and matrix metalloproteinases (MMPs). EpCAM plays an important role in metastasis through modulation of NF-kappaB, c-myc, and E-FABP and through binding to CD9. Osteopontin has an anti-apoptotic and proliferative effect on tumor cells and MMPs mediate cellular migration by breaking down the extracellular matrix barrier.

Outlook: Future experiments consist in confirmation of the above mentioned candidate genes on protein level, in order to initiate potential therapeutic targeting in vivo. Moreover, we will employ additional mouse models with high fat diet and a genetic model with perisinusoidal liver fibrosis, as the CDAA model not fully recapitulates the biology of the human disease.

P226 | Identification and validation of proteins and core signaling pathways associated with therapy resistance of melanoma brain metastases

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Since 2011, effective targeted and immune therapies are available to treat metastasized melanoma. However, therapies still lack long-term responses and recovery for patients with melanoma brain metastases, showing either short response duration or low response rates in especially symptomatic brain metastases, likely due to brain-specific resistance mechanisms. Uncovering the differences between intracranial and extracranial metastases thus becomes an important approach to find urgently needed brain-specific therapies. The mutational landscape appears to be highly similar between the two subgroups, whereas epigenetic regulation is diverging. We collected a unique FFPE cohort composed of 16 melanoma patients with matched intracranial and extracranial metastases. Analyzing methylome and transcriptome data revealed 38 protein candidates with different methylation and corresponding RNA expression levels in the respective metastases pairs. Nine candidates were already verified by immunohistochemistry examination. By including expression levels in normal tissue, protein kinase C zeta (PRKCz) was identified as a promising candidate, displaying the highest expression level in the intracranial metastases. It is a downstream effector of PI3K and activates the MAPK/ ERK-signaling cascade as well as NFB, facilitating cell polarity, proliferation and migration. We have so far validated increased PRKCz and PRKCz phosphorylated (activated) levels in a melanoma brain metastasis cell line. Additionally, PRKCz levels were found further increased when cultured together with astrocytes, representing normal brain tissue. We have seen no differences between melanoma cells in monoculture vs transwell-cultured cells, having no direct contact but sharing culture media with normal tissue cells (fibroblasts or astrocytes). This indicates

that direct cell-cell contact between melanoma cells and normal tissue cells may be essential in any intracranial metastasis model. Future experiments will investigate the role of PRKCz in various melanoma—normal tissue 2D and 3D co-culture setups. In addition, computational experiments will expand our knowledge about deregulated candidates in melanoma brain metastases, getting a broader view of PRKCz involvement. Taken together, our approach is promising to unravel therapy resistance of brain metastases on an epigenetic level.

P227 | The relationship between coagulation and tumor progression in malignant melanoma and its prognostic value

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In the pathogenesis of malignant melanoma, the development of a procoagulant microenvironment through the activation of platelets and endothelial cells leads to metastasis and the occurrence of thromboembolism. It has been shown that the interaction between tumor cell and endothelial cell required for peripheral metastasis is mediated by VEGF-A and Thrombin. During this process, Von Willebrand factor (VWF) is released from endothelial cells into the vascular lumen. VWF promotes the development of thromboembolism due its platelet adhesion effect. This is promoted by a downregulated activity of "a disintegrin-like and metalloproteinase with thrombospondin type I repeats 13" (ADAMTS-13), a VWF-cleaving metalloproteinase. In plasma samples from melanoma patients, increasing VWF levels indicated progression of metastatic melanoma. So far, no correlation is known in melanoma patients in tumor stages I to III. Platelets facilitate the migration of metastatic tumor cells from the vascular lumen to peripheral tissue to form metastases. Activated platelets secrete platelet factor 4 (PF4). PF4 has been previously studied in other solid tumor entities and high plasma PF4 levels are associated with poorer prognosis. VWF, PF4, and ADAMTS13 activity in plasma of stage 0-IV melanoma patients before therapy is determined in comparison to basal cell carcinoma with very less metastatic tendency and healthy controls. ADAMTS-13 activity is measured using a newly established and validated ELISA activity assay. Our results show a significant difference in the values of VWF and VWF/ ADAMTS-13 activity ratio between 0-III stage melanoma compared to metastatic melanoma patient. Our results of significant increased PF4 in metastatic melanoma patients suggest that platelet activation plays a crucial role in tumor progression and that this is diagnostically useful by measuring platelet activity parameters such as PF4. Further studies, particularly mechanistic studies, are needed to understand the relationship between endothelial cell and platelet activation leading to increased coagulation and metastatic tendency. Promising results make further investigation within larger clinical trials reasonable.

P228 | Liquid biopsies: Potential and challenges

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The analysis of tumor cells or tumor cell products obtained from blood or other body fluids ("liquid biopsy" [LB]) provides a broad range of opportunities in the field of oncology. Clinical application areas include early detection of cancer or tumor recurrence, individual risk assessment and therapy monitoring. LB allows to portray the entire disease as tumor cells or tumor cell products are released from all metastatic or primary tumor sites, providing comprehensive and real-time information on tumor cell evolution, therapeutic targets and mechanisms of resistance to therapy. Here, I focus on the most prominent LB markers, circulating tumor cells (CTCs) and circulating tumor-derived DNA (ctDNA), in the blood of melanoma patients. After a brief introduction of key technologies used to detect CTCs and ctDNA, I discuss recent clinical studies on these biomarkers for early detection and prognostication of cancer as well as prediction and monitoring of cancer therapies. I also point out current methodological and biological limitations that still hamper the implementation of LB into clinical practice.

P229 | Downregulation of gamma-Secretase subunit Nicastrin reduces melanoma cell migration and proliferation and exerts a regulatory function on cancer-associated store-operated calcium entry

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Melanoma is a highly aggressive skin cancer derived from melanocytes and responsible for about 90 % of deaths from cutaneous malignancies. To date, several studies have implicated an involvement of gamma-Secretase, a membrane protease complex that has a pivotal role in the canonical Notch signaling pathway, in the development of distinct tumor entities. In up to 12 % of the melanoma samples included in the cBioPortal database, mutations were shown to occur

in the genes encoding one of the four gamma-Secretase subunits Nicastrin (Nct), Presenilin, Presenilin enhancer 2, and Anterior pharynx-defective 1. Using a zebrafish model, we and others have recently shown that downregulation of Nct, the gate-keeping subunit of gamma-Secretase, leads to a disturbance of melanocyte ontogenesis and results in dysfunctional melanocyte proliferation, differentiation, migration and maturation.

In order to investigate the specific role of gamma-Secretase subunit Nct in melanoma, we sought to (i) assess the immediate effects of Nct inactivation on melanoma propagation and (ii) investigate the consequences of Nct suppression on store-operated calcium entry (SOCE), in which depletion of endoplasmic reticulum (ER) calcium (Ca2+) stores triggers the influx of Ca2+ from the extracellular space under the control of two proteins, STIM and Orai, of which different isoforms have been described. To this aim, we performed short interfering ribonucleic acid (siRNA)- mediated knockdown in various melanoma cell lines.

Downregulation of Nct led to reduced melanoma cell migration and proliferation. Fluorescence activated cell scanning (FACS) analysis and staining for the proliferation marker Proliferating-Cell-Nuclear-Antigen (PCNA) showed that the decreased proliferation was due to cell cycle arrest in the G0/G1 phase. Mechanistic studies using single cell Ca2+ imaging revealed that downregulation of Nct increased Ca2+ levels in the ER and enhanced refilling of Ca2+ stores after depletion. Although Nct-downregulation had no effect on total STIM1 protein levels, STIM1 cleavage was reduced, which resulted in decreased levels of a 60 kDa cleavage fragment that might act as a negative regulator of SOCE

Taken together, our data not only suggest an immediate impact of Nct dysfunction on melanoma propagation but also indicate an indirect role of this gamma-Secretase subunit conferred by dysfunctional regulation of SOCE, a major mechanism in the preservation of cellular Ca2+ homeostasis that governs melanoma cell migration and proliferation.

P230 | The role of adhesion molecules in melanoma cell—Endothelial interactions

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Cutaneous melanoma is the type of skin cancer with the highest mortality rate (Larkin et al., 2019). One prominent clinical feature of melanoma is its ability to form distant metastasis in almost every organ of the human body. In previous work, our group has shown an important role for the inflammation-induced interaction between melanoma cells and blood vessels in the metastatic progression of melanoma (Bald et al., 2014). As mediator of cellular interactions, adhesion molecules are increasingly recognized as central players in this process (Hamidi et al., 2018). The underlying molecular mechanisms remain unclear.

In the current project, we hypothesize that the adhesion molecules ICAM-1 and VCAM-1 regulate the interaction between melanoma cells and endothelial cells. For this, we used the metastasizing mouse melanoma cell line HCmel12 and generated knockouts for both genes via CRISPR-Cas9 genome editing and confirmed successful disruption by next-generation sequencing. ICAM-1 and VCAM-1 knockout cells showed no difference in their proliferation compared to CRISPR-ctrl cells. Next, we sought to analyze the effect of melanoma-endothelial co-culture on the differentiation state of melanoma cells. In this, HCmel12 cells retained a more differentiated phenotype under inflammatory stimulation with TNF-α when co-cultured on bEND endothelial cells compared to cells co-cultured on XB2 keratinocytes. Surprisingly, both ICAM-1 and VCAM-1 knockout cells revealed a more differentiated phenotype with no difference between co-culture conditions.

In our further work, we will perform time-lapse video microscopy of fluorescently labeled.

ICAM-1 and VCAM-1 knockout melanoma cells on endothelial cells to assess their migratory potential.

P231 | Circulating cytokines as predictive biomarkers for the occurrence of immunerelated adverse events under checkpoint inhibition

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The use of checkpoint inhibitors (CPI) has significantly improved the treatment of metastatic melanoma. However, approximately 40–60% of patients develop immune-related adverse events (irAEs), some of which are severe. The aim of this study was to identify biomarker cytokines in the blood of patients to predict the occurrence of irAEs at an early stage. Therefore, in this prospective study, serum samples from skin cancer patients treated with CPI were collected prior to initiation and during the course of therapy. Electrochemiluminescence was used to determine the concentration of 57 different cytokines/chemokines and expression was correlated with the occurrence of irAEs.

From 81 patients, which were recruited, 74% (n = 60) received an anti-PD-1 antibody, 2.5% (n = 2) were treated with an anti-CTLA-4 antibody alone and 23.5% (n = 19) received a combination therapy of both. 67.7% (n = 54) of patients developed irAEs in the first 6 months after starting therapy, whereby 22.2% of these patients experienced severe adverse events (grade 3 and 4). Gastroenterological, dermatological and endocrine irAEs were the most common adverse events, occurring on average 9–11 weeks after the start of therapy.

We detected almost no significant differences in cytokine concentrations measured at baseline between patients who developed irAE in the first 6 month and patients without toxicities. However, we found several cytokines (such as IL-7, Eotaxin, MCP-1, BCA) which were significantly higher upregulated during CPI treatment in patients with irAE compared to patients without irAE. Thus, we could show that especially the early individual reaction of the patients' immune system, in particular the dynamic of the parameters from baseline to 3–4 weeks after initiation of therapy should be taken into account for a risk assessment. In combination with further biomarker studies, our study could help to establish an immune profile with individual patient monitoring that can predict potential toxicity with a high degree of precision.

P232 | Investigating the role of dendritic cells in tumor-targeted therapy-mediated anti-tumor immunity

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Melanoma belongs to the 10 most common cancer types and its incidence is increasing. It has a high mutational load with driver mutations affecting genes regulating critical signaling pathways involved in proliferation and cell growth such as in the mitogen-activated protein kinase (MAPK) pathway (e.g., BRAF and NRAS), or in the phosphoinositide 3-kinase (PI3K) pathway (e.g., PTEN). Half of the melanoma patients carry a specific point mutation affecting BRAF, leading to an amino acid substitution of valine to glutamic acid in position 600 (BRAF V600E), which constitutively activates the MAPK pathway. Targeted therapy using inhibitors specific for mutant BRAF (BRAFi) elicts high response rates in melanoma patients. However, patients frequently relapse within the first year of treatment due to the development of therapy resistance, which led to development of a combination therapy with MEK inhibitors (MEKi).

Besides inducing programmed cell death of BRAF V600E-mutant melanoma cells, BRAFi and MEKi modulate the tumor immune microenvironment and induce antitumor immune responses. Until now, the studies on immunological effects of tumor-targeted therapy mainly focused on effector T cells and NK cells. However, the functional role of dendritic cells (DC) in anti-tumor responses by BRAFi and MEKi remains elusive. As DC are crucial to initiate T and NK cell responses, we want to address which DC subsets are involved in immune modulation during treatment. We hypothesize that tumor-targeted therapy boosts T cell responses by improving DC function. In order to understand the complexity and functionality of these cells, we designed a multicolor flow cytometry panel to clearly discriminate the DC compartment from other myeloid cells. This panel provides a deep insight into the different DC subsets, including cDC1, cDC2, plasmacytoid DC (pDC), Langerhans cells (LC) and monocyte-derived DC (moDC). Using this optimized panel, we can show that BRAFi and also combination with MEKi lead to a decrease in immunosuppressive myeloid cells from tumors. Furthermore, this targeted therapy recruits DC to the tumor and effects the phenotype of DC subsets and we observed an increased frequency of migratory DC in tumor draining lymph nodes.

Further characterization of tumor-infiltrating as well as migratory DC subsets in the draining lymph nodes will give us valuable insights whether alterations in DC function contribute to resistance development.

P233 | Examination of tumor control in immunotherapy with immune checkpoint blockade combined with CDK4/6 inhibition

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The adaptive immune system possesses the ability to eradicate tumour cells through natural immune responses or supported by immune checkpoint blockade therapy (ICB). However, tumour cell reduction by cytotoxicity is frequently incomplete and insufficient for a permanent cancer control and in clinical context a large portion of patients experience relapse after an initial therapy response.

After an effective treatment of cancers, remaining tumor cells can be retained in a senescent state, which is dependent on the TH-1 cytokines INF-γ and TNF (Cytokineinduced senescence, CIS). The underlying mechanism is reliant on the activation of the cell cycle regulators p16Ink4a/p19Arf (Cdkn2a) and p21Cip1 (Cdkn1a) in the tumour cells and relapses are often associated with defects either in the cytokine pathways or in these regulators.

In our ongoing project we use knock-out tumour cell lines (Stat1-/-; Tr55-/-; Cdkn2a -/-; Cdkn1a -/-), which are unable to undergo senescence upon stimulation with TH-1 cytokines and we aim to compensate for these defects by stimulating an immune response with an ICB therapy and combining it with a CDK4/6 inhibitor (Palbociclib; Pfizer Inc.) to examine if senescence can be reinstated in those cells. CDK4 and CDK6 are main regulators of the G1-S cell-cycle transition and act downstream of p16Ink4a/p19Arf and p21Cip1 and recently CDK-Inhibitors opened the possibility to intervene in these pathways when p16Ink4a/p19Arf or p21Cip1 are defective.

In vitro we investigate if a combined cytokine and CDK4/6 inhibition treatment can induce senescence in these knockout cell lines and examine for common proliferation and senescence markers.

In a syngenic in vivo mouse model we use these cells lines to investigate the direct effects of this combinational treatment. We treat the mice with monoclonal antibodies against the checkpoint inhibitors PD-L1 and LAG-3 combined with Palbociclib for several weeks and investigate the tumour growth dynamics and the long-term effects of this regimen.

P234 | Artesunate induces cell death in cutaneous T-cell lymphoma

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Mycosis fungoides and Sézary Syndrome are the most common forms of cutaneous T-cell lymphoma (CTCL). Despite all progress in clinical research in the recent years, there are still only limited treatment options.

Artesunate (ART) a semi-synthetic drug, which is used worldwide, in the guidelinebased therapy of resistant malaria has been reported to have anti-proliferative activity in tumor cells.

Therefore, we investigated the effects of ART on four CTCL cell lines (HUT-78, SeAx, MyLa and HH) and found a distinct reduction of cell viability and cell survival (MTS, Annexin/7AAD). These effects were increased by the addition of iron and were almost reversed by the iron-chelator DFOM. Lipid-ROS was not increased and GPX4 was upregulated. Furthermore, ART treatment led to an increase of ROS and Caspase 3/7 activity. Interestingly, the ROS-Inhibitor NAC could only partly abrogate the anti-proliferative effect of ART.

Different mechanisms seem to contribute to the observed effects. Western-blot analysis provided further evidence for classical apoptosis such as PARP cleavage and the upregulation of Noxa, as well as the downregulation of Survivin and XIAP. However, there is also evidence for autophagy, as for example the upregulation of LC3B protein.

In summary, our data provide first evidence that ART decreases CTCL cell viability and drives CTCL cells towards cell death. Autophagy and apoptosis seem to be involved rather than the commonly reported ferroptotic mechanism of action of ART.

P235 (OP01/05) | Repurposing bortezomib for improved treatment of melanoma by exploiting immunogenic cell death

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Immunogenic cell death (ICD) constitutes a prominent pathway for the activation of the immune system against cancer, which in turn determines the long-term success of anticancer therapies. Only a few agents can elicit bona fide ICD, including some clinically established chemotherapeutics such as the proteasome inhibitor bortezomib, as demonstrated in malignant myeloma and mantle cell lymphoma, but not yet in melanoma. We have shown in melanoma that bortezomib induces NOXA-dependent apoptosis. Here, we show that bortezomib indeed causes ICD in vitro through induction of endoplasmic reticulum stress, autophagy and apoptosis and through translocation and/or secretion of damage-associated molecular patterns (DAMPs). Vaccination with bortezomib-treated dead melanoma cells induced tumour immunogenicity in vivo, as evidenced in a significant reduction/delay after challenge with live cells. Intralesional injection of bortezomib synergised with subsequent systemic treatment with immune checkpoint inhibition using CTLA-4 and PD-1 antagonists. Re-challenge demonstrated long-term protection through bortezomib combined with immune checkpoint inhibition. Polyfunctional T cell assays revealed that intralesional bortezomib injection generates a tumour-specific T cell response. Finally, immune checkpoint inhibitor-resistance was reverted by bortezomib-induced immunogenicity. In summary, bortezomib induces ER stress and apoptosis, enhances ICD markers (DAMPs) in vitro and is immunogenic in vivo. Bortezomib-induced ICD is a good strategy to recruit the inflammatory immune response. Bortezomib-induced ICD enhances response to immune checkpoint inhibitors, even in ICI-resistant tumours. We propose intralesional injection of bortezomib combined with systemic CTLA-4 and PD-1 antagonists to improve immune therapy in melanoma.

P236 (OP02/01) | Oncolytic virus therapy as a powerful promoter of a combined tumor antigen specific Th1 cell and immune checkpoint inhibitor-based cancer immunotherapy

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Introduction: Over the last few decades, cancer research is engaged to understand how tumor cells evade the immune system. This yielded in novel and very efficient immune checkpoint inhibitor- and oncolytic virus-based treatment approaches. We recently have established an effective combined cancer immunotherapy (COMBO) with progressed endogenous insular cell carcinomas in RIP1-Tag2 mice combining tumorantigen specific IFN-γ producing CD4+ T cells (TA-Th1) with immune checkpoint blockade (ICB: anti-PD-L1 and anti-LAG-3 monoclonal antibodies (mAbs)) and 2 Gy total body irradiation (TBI). In this study we aim to uncover whether addition of oncolytic virus treatment (OncoVEX® (AMGEN); a genetically modified oncolytic HSV-1 that selectively infects and replicates in tumor

cells, applied intraperitoneally (i.p.)), well known for its selectivity for cancer cells and for its capability in boosting an anti-cancer immune response, is capable to enhance the treatment efficiency.

Methods: We started OncoVEX® (i.p.) & COMBO treatment in 10 wks old mice with progressed carcinomas, initiated by a 2 Gy TBI followed by a twice weekly ICB injection and a weekly TA-Th1 and OncoVEX® administration. Blood glucose levels (BGL) were measured twice weekly since a decrease in the BGL correlates directly with tumor progression. At the end of the study, we performed H&E histology, immunohistochemistry (IHC; CD3+) as well as immuno¬fluorescence analyses of the tumor tissues and the spleens. In addition, we conducted in vitro [18F]FHBG cancer cell uptake studies to get a measure for the susceptibility of RIP1-Tag2 insular cell- and MC38 adenocarcinomas for OncoVEX infection as [18F]FHBG is phosphorylated by the HSV-1 thymidine kinase and thereby trapped within thymidine kinase expressing cells.

Results/Discussion: Addition of OncoVEX® to our COMBO treatment resulted in an impressive increase in BGL over a few days indicating tumor regression. The therapeutic effect in the COMBO treatment group was less pronounced, while sham-treatment, solo OncoVEX® or ICB treatment as well as combining OncoVEX® & ICB did not exhibit therapeutic effects. Analysis of the serum of OncoVEX®-treated RIP1-Tag2 mice confirmed presence of HSV-1 DNA indicating successful tumor cell infection and tumor cell lysis. Addition of OncoVEX® to our COMBO treatment resulted in a pronounced CD3+ T cell infiltration within the insular cell carcinomas of RIP1-Tag2 mice. Thus, OncoVEX® & COMBO-treated RIP1-Tag2 mice exhibited smaller tumors compared to solo COMBO-treatment mice. Moreover, we analyzed the [18F]FHBG uptake of OncoVEX® infected RIP1-Tag2 and MC38 carcinoma cells in vitro and determined a significantly higher [18F]FHBG uptake exclusively in the OncoVEX® infected cancer cells thus enabling non-invasive in vivo visualization of OncoVEX®-infected cancer cells with simultaneous [18F]FHBG positron emission tomography and magnetic resonance tomography. In conclusion, this novel multimodal immunotherapeutic approach warrants further investigation and then the translation into a phase I trial setting.

P237 | Induction of anti-tumor responses via antigen targeting of dendritic cells in vivo

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Multiple groups have shown that antigen targeting by using recombinant antigenconjugated antibodies against different endocytic receptors specifically expressed on DCs is an excellent method to trigger and modulate immune responses in vivo. By taking advantage of the subset-specifically expressed endocytic C-type lectin receptors DEC205 present on splenic CD8+ DCs or DCIR2 on CD11c+CD8- DCs, it is possible to predominantly trigger antigen-specific CD8+ or CD4+ T cell responses, respectively. By this targeting approach the survival of tumor challenged mice is prolonged accompanied by a reduced tumor growth. Recently, we demonstrated FcγRIV, present on both classical DC subsets in murine spleen, to be effective in the induction of concomitant CD8+ and CD4+ T cell responses. In this project, we focus on two central aspects:

1. Potential of FcγRIV targeting: Even though this receptor is expressed on an array of immune cells, the induction of immune responses by targeting of FcγRIV is solely dependent on DCs. Since this receptor is found on both, CD8+ and CD8- DCs, and induced potent T cell responses, we set out to determine, if the targeting of this receptor is superior compared to targeting approaches via DEC205 and DCIR2 in murine tumor models in vivo.
2. Mechanism of tumor protection by DCIR2 targeting: Antigen delivery via DEC205 induces CD8+ T cells capable of killing tumor cells directly. In contrast, DCIR2 targeting triggers only minor T cell responses in naïve mice. In the current project, we want to obtain mechanistic insights in the anti-tumor responses induced by DCIR2 targeting. We hypothesize that the minor induction of CD8+ T cells by DCIR2 targeting is sufficient to kill a small number of tumor cells. As a consequence, other tumor specific antigens are released and trigger de novo immune responses (CD8+ T cells and/or antibodies), which can in turn explain the observed protection. To investigate this, we are using several tumor cell lines to study cross-reactivity and the tumor-associated-epitope spreading after the initial immune response.

Miscellaneous

P238 | Searching for novel drug candidates to promote wound healing

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Tissue injury, especially injury of the skin, is an almost everyday occurrence. Usually, the highly complex and tightly regulated process of wound healing runs automatically without the need of external assistance. However, wounds can get "stuck" in one of the phases of wound healing, resulting in chronic, non-healing wounds. Chronic wounds pose a huge threat to the health of those affected. Furthermore, the quality of life of affected patients decreases severely and the treatment of chronic wounds places an immense burden on the healthcare system. Due to our aging society, non-optimal wound healing will be an even increasing problem, since it can be a side effect of specific diseases (e.g. Type two Diabetes mellitus) that

become more frequent in old age. Current treatment strategies of badly healing wounds are unsatisfactory. Thus, there is an immense demand of finding new drug candidates. In our established ex vivo wound healing organ culture (WHOC), we screened 141 candidates of an inhibitor-library. The effects of these inhibitors were analyzed with regard to their influence on wound area, wound perimeter, and wound volume. Area and perimeter were assessed based on two-dimensional reflected light microscopy images. For volume determination three-dimensional, depth-resolved tomograms were acquired using optical coherence tomography (OCT). Over the entire screening, we found eleven promising candidates, which accelerated wound healing by at least 15 % (relative wound area treated compared to untreated wounds after 7 days of incubation). These candidates are now further validated, by the macroscopic parameters mentioned above and additionally by histochemical analysis of the length and area of epithelial tongues and of the microscopic wound area and diameter. This analysis will be followed by immunohistochemical analyses to investigate how the inhibitors contribute to wound healing. With some of the inhibitors showing promising macroscopic results in the validation, the determination of new drug candidates amongst them is feasible.

P239 | Complete depletion of hyaluronan synthase activity in murine skin leads to delayed wound healing

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Hyaluronic (HA) acid is a main structural component of the extracellular matrix (ECM) and plays a key role in homeostasis, inflammation and repair of the skin. HA is synthesized by the three membrane-bound isoenzymes Has1-3, while Has2 synthesizes the majority of HA in the skin. Additionally, HA plays a crucial role during skin aging, whereby the HA concentration decreases, especially in the epidermis, with increasing age. In parallel, the risk of wound healing disorders increases with age. This leads to the question of whether there is a connection between the decreased HA content and the increased incidence of wound healing disorders.

To investigate our hypothesis, we established an inducible total Has-KO mouse model in which, in addition to the permanent Has1 and Has3-KO (1), a Has2 knockout can be induced under control of the UbiquitinC-Promoter. Consequently, the ablation of all known HA synthases may simulate the diminished HA content in aging human skin as best it can, despite the general differences between human and murine skin. In this mouse model (C57/BL6- Has1,3KO-HAS2fl/fl-UBC-CreERT2), an inducible reduction of the HA content in the skin by about 80–95% can be achieved. Thus, the impact on resting ECM structure as well as on wound healing can be investigated.

Interestingly, the animals with Has-tripleKO do not show macroscopic or behavioral abnormalities. However, the proportion of dermal white adipose tissue (dWAT) in the skin increases significantly in the animals with double or triple KO. The extent to which these changes in skin structure influence wound healing was part of the following investigations.

Wound healing is a complex process divided into four major overlapping phases: hemostasis, inflammation, proliferation and remodeling. HA is particularly important during the inflammatory phase, as it is required for the formation of a fibrin clot and to build a woven structure to guide attracted cells to the wound site. During healing, the three HA synthases are regulated differently, with Has1 showing a short mRNA expression peak after 6 h and then falling. Has2 and Has3 mRNA show a clear increase after 24 and 48 h as investigated in wildtype mouse skin.

To investigate the influence of HA loss on wound healing, full-thickness skin wounds of 1 square centimeter were established on the back of the mice. Initial experiments revealed that animals with complete HA depletion (Has-tripleKO) show delayed wound closure in comparison to wildtype and Has1/3-double knock-out animals. We detected significantly decreased expression of myofibroblast genes such as Coll1, Coll3, ED-A-FN and alpha-SMA in the newly formed skin of mice lacking HA (Has1/3-doubleKO & Has-triple-KO) at day 9 post wounding. Since myofibroblasts are extremely important for effective tissue restoration, future experiments will investigate how the lack of these affects the structural and mechanical properties of the healed skin. Detailed analysis of early phases of wound healing in our model will reveal, whether diminished HA synthesis impairs early infiltrating immune cells and thus defines the fate of the wounds.

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P240 | Synthesis, characterization and biocompatibility of antimicrobial hybrid materials: SiO2/fumaric-and SiO2/vanillic acid

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In recent years, the demand for cost -effective antimicrobial surfaces and medical products has steadily increased in the health care sector due to a rise of antimicrobial resistance. Organic compounds with antimicrobial activity were incorporated into a silica network, which releases the active compound by contact with fluid. The toxicity for human contact was tested simultaneous to the antimicrobial activity to ensure the safety of such coatings.

Effective inhibition of microorganisms without possible toxicity for humans is a necessity for medical products and their application. Safe antimicrobial functionalization of the surface is difficult to obtain, accomplished by integrating active compounds already used as additives in pharmaceutical industry into the inorganic network.

Hybrid coatings with two organic compounds, fumaric and vanillic acid, incorporated in a silica matrix with 5, 15, 30 and 50 wt% were synthesized and characterized. The toxicity of the hybrid materials for human skin keratinocyte cell line (HaCaT) was investigated following EN ISO 10993-5. Moreover, SiO2/fumaric acid and SiO2/vanillic acid surfaces were tested according to ISO 22196 using Escherichia coli DSM 5923 as testing microorganism.

The data showed no indication that incorporation of vanillic or fumaric acid up to 50 wt% exerts toxic effects on human keratinocytes. In addition, SiO2/fumaric acid coatings synthesized with ≥15 wt% acid as well as SiO2/vanillic acid coatings of ≥30 wt% acid exhibited a high antimicrobial activity against E.coli DSM 5923.

The results indicate a safe functionalization of surfaces with organic/inorganic coatings, which could potentially be used as an antimicrobial modification for medical products and surfaces in the health care sector.

P241 | Tattoo removal using a novel radiofrequency (RF) device—A feasibility study

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Introduction: About ten million Germans are estimated to have tattoos. However, the number of those who no longer feel comfortable with their tattoo is growing steadily, by almost 40 percent in recent years. The current gold standard for tattoo removal is laser treatment. However, this method is time-consuming, costly, not always successful and not without risk. The superficial treatment with a high-energy laser can leave scars or permanent pigment disorders. Moreover, the tattoo color is not eliminated, but shattered into tiny particles, which are eliminated via the lymphatic system and can accumulate in the lymph nodes and exert a carcinogenic effect. The development of new, more gentle tattoo removal methods is therefore very important.

Methods: As part of a research project on minimally invasive tattoo removal, a medical device has been developed that uses radiofrequency (RF) technology to remove tattoos. The combination of direct current and the radiofrequency is supposed to loosen cellular adherence in the skin layers allowing direct removal of the tattoo dye from the tissue without the generation of residual, potentially toxic products and decrease side effects such as scarring and inflammation. As part of a feasibility study, the newly developed procedure was tested on 10 volunteers for the removal of tattoos (ethics approval 2020-1951_1-BO). In all participating volunteers, 1 cm^2 of a tattooed skin was treated once in each of four consecutive treatment sessions.

Results: The feasibility study showed that the newly developed medical procedure for minimally invasive tattoo removal using radiofrequency is a tolerable procedure that does not cause any visible epidermal tissue damage. The scab formed at the treatment site was clearly pigmented with the removed tattoo ink. After healing of the treatment site, tattoo removal between the different study participants was very heterogeneous ranging from incredibly effective to disappointingly little pigment removal. Nonetheless, the overall participants' satisfaction with the treatment outcome was predominantly good.

Conclusions: The newly developed medical procedure for minimally invasive removal of tattoos using radiofrequency technology is a promising approach for a gentle removal of tattoo pigments.

P242 | Vitamin D deficiency sensitizes to UV/endorphin and opioid addiction

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While prior data demonstrated a key role for UV-triggered skin β-endorphin in addictive effects of UV radiation, it also highlighted a striking paradox: a pathway exists which promotes exposure to UV--which is responsible for more human malignancies than any other carcinogen.

Evolutionarily, developing addiction to UVB radiation, a common carcinogen whose effects manifest dominantly in post-reproductive years, could enhance population fitness if accompanied by positive effects earlier in childhood or in early adulthood. Vitamin D deficiency has been suggested to contribute as a recent evolutionary driver for light skin pigmentation in humans. Therefore, it is plausible that additional mechanisms, like UV-seeking behavior, may also contribute to maintenance of Vitamin D levels.

Here we extend our understanding by experimentally demonstrating that vitamin D deficiency increases UV/opioid-seeking behavior until its levels are corrected—after which vitamin D actually represses UV/opioid seeking behavior. Such an adaptive negative feedback loop could increase population fitness by preventing vitamin D deficiency and rickets, balancing negative consequences of UV damage. A terribly important consequence of this newly discovered vitamin D role is the increased sensitivity to addictive propensity of exogenous opioids, which we demonstrate in genetic and pharmacologic preclinical models, and also using unbiased human database analytics.

We also delve into mechanistic means through which vitamin D modulates opiate signaling within the CNS. Mechanistically, transcriptomic analyses identified a pathway through which vitamin D signaling modulates opioid-induced c-Fos expression in the nucleus accumbens, a brain region important for motivational states and addictive behaviors, suggesting that reward repression by vitamin D might not be restricted to opioids, a finding that we thus extended to nicotine responses. Importantly we observed that replenishment of vitamin D in deficient mice both prevented and reversed these effects.

Our findings thus have major public health implications: (1) that vitamin D deficiency may exacerbate opioid dependency (including both UV-induced endorphin and exogenous opioid addiction), and (2) this effect of vitamin D deficiency may be reversible/preventable by vitamin D supplementation.

P243 | Laser-capture microdissection-coupled metagenomics: A powerful method for analysis of the HF microbiome

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Human hair follicles (HFs) are home to a diverse community of microorganisms, which have been suggested to play an important role in proper induction of the immune system and regulating immune function. Recent evidence has also proposed a role of this community in re-populating the skin surface, indicating a higher impact of the HF microbiota on skin homeostasis. Despite, traditional evaluations characterise this microbiome as a constituent of the skin, inadvertently examining the HF microbiome indistinctly from the skin surface community, with methods (e.g., swabbing, pore stripping and whole punch biopsy analysis) that fail to sample the deeper compartments of the HF. Here, we describe a laser-capture microdissection and metagenomics-coupled technique to investigate the microbial communities residing in the diverse regions of the human HF. All main HF colonisers were identified in the lower, middle and upper HF. Surprisingly, whole microbiome community shifts were found between the lower and upper regions, with both inter and intra-individual differences. Further, the microbial community of the lower and middle regions was found to be more diverse compared to the upper region communities. This work highlights the importance of a comprehensive analysis of the HF microbiome, with regional discrimination within the follicle and with clear distinction from the skin surface.

P244 | The functional in vitro impact of lesional versus non-lesional atopic dermatitis microbiota

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Introduction and aim of the study: Genetic predisposition and a type 2 T-cell-immune deviation are the main drivers for atopic dermatitis (AD), but also environmental factors play an important role. Mutations in the filaggrin gene (FLG) are the strongest known heritable predisposing factors for AD. Filaggrin is a key protein in the physiological process of building the stratum corneum, as a consequence FLG mutations lead to a disrupted skin barrier and enable allergens to immigrate in the epidermis. It is known that a dysbiosis of the skin microbiota is characteristic for patients with AD, especially an increased *Staphylococcus aureus* occurrence. It has not been sufficiently elucidated whether the dysbiosis of the cutaneous microbiota is causally related to the occurrence of an inflammatory disease process such as AD, or whether the dysbiosis occurs as a consequence of the disrupted skin barrier. However, evidence to date indicates that impaired microbiota diversity both negatively affects the physiological function of the skin barrier and exacerbates cutaneous inflammatory responses.
Functional approaches to address this question are rare, which we like to address in this study. We sought to decipher this interaction by analyzing the influence of both lesional and non-lesional skin microbiota on various AD relevant factors like differentiation markers in a 3D skin model.
Methods: In our study we performed a standardized skin rinsing setup to harvest the skin microbiota of non-lesional and lesional AD. The skin microbiota of localization-, gender-, and age-matched healthy participants and AD patients were used for stimulation of different model systems. Primary keratinocytes and 3D skin equivalents generated with primary keratinocytes were used as model systems. The isolated microbiota derived from the skin rinsing solutions was applied to the surface of 3D skin equivalents and primary keratinocytes. The expression of various differentiation markers and AD relevant factors by the primary keratinocytes were analyzed by realtime PCR and immunohistochemistry.
Results and Conclusion: In summary, our findings show that the expression of differentiation markers (e.g. keratin-1) was increased by non-lesional AD microbiota and decreased by lesional AD microbiota. In addition to that, expression of AD-relevant factors was increased by lesional microbiota and decreased by non-lesional microbiota. Our observations highlight the functional difference of non-lesional and lesional AD microbiota and strengthen the hypothesis that the lesional AD skin-derived microbiota triggers inflammation and impairs the epidermal barrier. Work is in progress to evaluate the role of the filaggrin status in this scenario.

P245 | Investigation into the effects of stratum corneum acidification on calcium and ammonium ion activity on the skin surface

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The acidification of the stratum corneum is a common procedure to influence structure and function of the skin. With regard to the chemical effects, an influence on the electrostatic or coordinative binding of ions and consequently their activity might be expected.

As measure for such effects, determination of calcium ions and ammonium ions appears one interesting approach. While calcium ions certainly have a role for structure and function of the skin, ammonium ions may reflect a variety of enzymatic and non-enzymatic reactions leading to molecules such as trans-urocanic acid or pyrrolidone carboxylic acid from amino acids. Moreover, transglutaminase activity show even a close functional relation of both ions. To obtain more insights into pH-dependent activity of calcium and ammonium ions, the aim of the present study is to determine the extractable amounts of both ions under acidification of the skin surface.

Overall, 15 volunteers were enrolled after informed written consent. The sites of investigation were the median portions of the forearm of both sides. For acidification of the skin a standard glycolic acid preparation showing a pH of 3,8 was applied to only one side. The corresponding contralateral side served as untreated control. Before and after treatment, skin surface pH was measured using a standard glass electrode. The amounts of calcium and ammonium ions extractable by ion free water were quantified using spectrophotometric tests. Additionally, capacitive measurements of skin hydration and assessments of the transepidermal water loss (TEWL) were performed. The statistical evaluation consisted of the comparison of calcium and ammonium amounts determined before and after treatment using a t test. Furthermore, the relation between ions and skin physiology parameters was investigated exploratory using correlation and regression analysis procedures.

The application of the acid preparation led to a statistically significant decrease of the pH towards values corresponding to the pH of the product. There was no relevant change of skin surface pH in the contralateral corresponding area. Moreover, a statistically significant increase of both the extractable calcium ions as well as the ammonium ions could be found in the treated area, while the increase of the ammonium ions was even stronger than the increase of the calcium ions. There was no relevant increase in the corresponding contralateral non treated control area. The correlation analysis revealed significant inverse correlations between the ions and skin surface pH values as well as a positive correlation between both ions. The comparison between the ions and skin hydration or TEWL revealed significant inverse relations.

The results strongly suggest an increase of calcium and ammonium ion activity due to acidification of the stratum corneum. Given the role of the ions for stratum corneum physiology, it can be concluded that acidification of the stratum corneum exerts its effects partly due to ion release and increase of ion activity. The positive relation between calcium and ammonium ions might indeed be at least in part due to ammonium producing reactions that are calcium dependent. An explanation regarding the inverse relations between the ions and skin hydration or TEWL remains rather speculative, but an increasing amount of free ions might interfere with the dielectric properties of water molecules and at the same time might hold back higher amounts of water. Studies dealing with pH-depending calcium and ammonium ion binding to molecules of the skin might reveal more insights into the effects assessed.

P246 (OP05/01) | DPP4 prevents hair follicle activation and regeneration by modulating Wnt signaling

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The hair follicle (HF) is a mini organ capable of self-renewal (anagen or hair formation), which recapitulates organogenesis in a cyclic fashion. Injury to the skin results in loss of hair follicles most of the times but large wounds on the back of mice can regenerate HF in a central area surrounded by a non-regenerative periphery with fibrosis. This process, wound induced hair follicle neogenesis (WIHN) is facilitated by similar pathways of anagen activation. Identification of modulators of anagen entry and WIHN might aid pinpointing targets for allowing regeneration of skin appendages after trauma or inducing hair growth in individuals affected for example by alopecia. We have identified one of such factors, namely Dipeptidyl peptidase IV (DPP4). We previously showed that DPP4 inhibition improves WIHN. Our current work shows that DPP4/Dpp4 is regulated during HF cycle with downregulation in dermal cell populations during anagen. Additionally, scRNAseq localizes the majority of Dpp4 expressing cells in non-regenerative areas of wounds , which is also confirmed with immunofluorescence and gene expression as well as enzymatic activity analyses. DPP4 inhibition during HF resting phase (telogen) results in faster anagen induction after depilation of dorsal skin in mice. A combination of scRNAseq and studies in vivo with DPP4 inhibition shows that reduction of DPP4 in wounds or in telogen results in increased Wnt signaling, a pathway known to be required HF organogenesis, anagen entry and WIHN. Mechanistically, DPP4 cleaves Wnt ligands required for Wnt activation, so inhibition of this enzyme results in higher availability of Wnt ligands for activation of HF and WIHN. Together our observations support the notion of DPP4 being a inhibitory factor for hair follicle activation and regeneration and a potential target for manipulation to improve HF regeneration and activity.

None

P247 (OP05/02) | Slc7a11 controls fibroblast differentiation through ferroptotic cell death

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Objective: Scientist have discussed the role of oxidatve stress in systemic sclerosis (SSc) for some years now and there is evidence that excessive amounts of reactive oxygen species (ROS) trigger inflammation and subsequent fibroblast activation. Importantly quiescent

fibroblasts from SSc skin compared to skin of healthy donors show high levels of ROS, which can be regulated via the glutamate/cystine antiporter (SLC7a11/ xCT). xCT is a plasma membrane protein that transports glutamate in exchange for cystine. In the cytosol cystine is rapidly reduced to cysteine and subsequently glutathione (GSH), the major antioxidant in the body, regulating ROS induced cell death such as ferroptosis. In this project we set out to identify the contribution of the xCT system and ferroptosis to fibrosis development.

We analyzed CD45- skin cells from scleroderma patients by NGS and as expected found increased collagen 3 and 6 expression in SSc specimen. Among the highly regulated genes in SSc was also an antiporter: the glutamate/cystine antiporter SLC7a11. We postulated due to it´s role in antioxidation that mice lacking xCT (SLC7a11 KO) would show enhanced ROS stress due to the reduced levels of GSH that would in term trigger downstream fibrotic processes.

Fibrosis was induced via daily injection of HOCl (2,6% chloride/inj.) intradermally into the back skin for 28 days in xCT KO versus WT mice. After 4 weeks collagen was quantified by line distance measurement of the dermis and myofibroblast activation was determined by immune histochemistry of α-SMA in the skin sections.

Surprisingly, SLC7a11 KO mice accumulated less collagen compared to WT mice after HOCl exposure and showed less myofibroblast differentiation. Differential gene expression of KO skin revealed less profibrotic transcripts and extracelluar matrix synthesis. Isolated fibroblasts from SLC7a11 KO mice failed to proliferate in vitro supposedly relying on exogenous cystine for survival. This is why we decided to graft KO skin to the backs of WT mice and vice versa. We found that SLC7a11 competent grafts perfectly healed in vivo and responded to HOCl while SLC7a11 incompetent grafts barely closed the wound sites. However, the xCT graft kept it´s fibrotic phenotype in the WT recipients suggesting a skin- related mechanism behind fibrosis resistance in SLC7a11 KO mice. Furthermore, in vitro cultured primary WT fibroblasts in presence of profibrotic TGF-ß showed less collagen-1 and vimentin expression in presence of ferroptosis inhibiting tocopherol-α and more Col-1+Vim+ cells after dimethyl fumarte (DMF) treatment.

As Slc7a11 is known to regulate ferroptotic cell death via GSH we hypothesize that xCT fibroblasts are more prone to ferroptosis leading to a reduced fibrotic phenotype.

P248 | Transcriptional differences of lipid-metabolizing enzymes in sebocytes derived from sebaceous glands of the skin and preputial glands

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Sebaceous glands (SG) are known to significantly contribute to skin homeostasis by producing and secreting lipids and enzymes. Due to their small size, poor accessibility and tissue yield as well as the lack of an adequate culture model SG research faces tremendous challenges. SG resembling tissue structures are found in male rodents in form of preputial glands (PG). Similar to SG, PG are comprised of lipid-specialized sebocytes. Several studies investigating skin sebocytes have been built on data obtained from PG sebocytes. Therefore, we sought to compare both types of sebocytes, using a single cell RNA sequencing approach, to unravel potential similarities and differences between the two sebocyte populations.

scRNAseq revealed that in total more than 600 genes were significantly regulated between skin sebocytes (sSEB) and preputial gland sebocytes (pSEB). Two distinct SEB populations were detected in both tissues which, however, differed significantly from each other. The sSEB specific gene set was highly associated with skin-specific functions, such as epidermal development and gland formation. Contrary, genes highly expressed in pSEB were strongly related to lipid specific functions. Furthermore, we found striking dissimilarities in the differentiation program of sSEB and pSEB. In contrast to pSEB, sSEB precursors expressed genes downstream of Wnt/β-catenin, allowing cell differentiation into hair follicle (HF) keratinocytes. While Blimp1 positive sebocyte-precursors could not be clearly identified in pSEB, a pseudotime trajectory analysis of sSEB nicely showed the lineage decision checkpoints of precursor cells either into a sSEB or hair follicle dedicated differentiation pathway. To analyse the differences in lipid synthesis pathways between sSEB and pSEB, we investigated the expression of key enzymes involved in the production of squalene and sphingolipids. We found that murine sSEB express all enzymes required for squalene synthesis. In contrast, pSEB express 3-Hydroxy-3-Methylglutaryl-Coenzym-A (HMG-CoA) synthase (Hmgcs1), HMG-CoA-reductase (Hmgcr) and farnesyl diphosphate synthase (Fdps) at similar levels as sSEB. The last and critical enzyme for squalene synthesis, squalene synthase (Fdft1), however was almost exclusively expressed by sSEB. Moreover, our scRNAseq data revealed striking differences in the expression levels of genes involved in bioactive sphingolipid synthesis. While Genes associated with de novo sphingolipid synthesis were found to be higher expressed in pSEB, expression of ceramide synthase (Cers4), which is crucial for the recycling of sphingolipids via the salvage pathway, was exclusively found in sSEB.

Together, our data showed tissue-specific differentiation programs of the sebocyte populations in both glands, and significant differences in their ability to produce specific lipids. Further studies deciphering potentially overlapping cell functions are required to elucidate whether the two SEB populations share distinct features, beyond coarse SEB-identifying characteristics, which would allow a use of pSEB as a surrogate model for sSEB.

P249 | Ex vivo and in vitro analysis of an innovative collagen foam for soft and hard tissue regeneration

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Background/aim: Collagen has a sufficient biocompatibility, biodegradability and positive effects on molecular processes, such as cell adhesion, proliferation and differentiation. Therefore, collagen-based wound dressings are a widespread treatment for acute or chronic wounds, as they protect the wound from infection and contamination, reduce scarring and support the skins natural regenerative capacity. The aim of this study was the development and analysis of a new type of collagen-based wound foam made from bovine split skin. The novel biomaterial is intended to combine the regeneration-promoting qualities of collagen with the complete adaptation to wound surface and volume of a wound foam. For this purpose, surface structure, density, pore and bubble size of the foam were analyzed ex vivo and subsequently complemented by in vitro cytocompatibility analyses according to DIN ISO 10993-5/-12.

Materials and Methods: Collagen foams with different protein concentrations (10–50 mg/ml) were produced from homogenized bovine split skin using various chemical and mechanical processes. Size, amount and distribution of the collagen bubbles were recorded using a Dynamic Foam Analyzer (DFA). The surface properties of the collagen foams were analyzed via cryo focused ion beam and scanning electron microscopy. Cytocompatibility analyses were carried out using XTT (viability), BrdU (proliferation) and LDH (toxicity) assays. In vitro, the collagen foams were compared with the established soft and hard tissue materials Cerabone and Jason membrane (both botiss biomaterials GmbH, Zossen, Germany).

Results: Structure and distribution of the collagen bubbles could be detected via DFA. For the foam with a protein concentration of 20 mg/ml, a surface area of 1000–2000 square micrometers was measured for the largest proportion of the collagen bubbles. Furthermore, the average diameter of the foam bubbles was 28–48 µm. In addition, the total number of bubbles measured in the collagen foam remained constant for the entire observation period (= 5 min) of the experiment. In vitro, all foams showed a sufficient cytocompatibility.

Conclusion: In this study, a novel collagen based wound foam was successfully manufactured from bovine split skin. Evaluation of the initially obtained ex vivo data showed that size and distribution of the produced collagen bubbles are mainly homogeneous within the foam. Furthermore, sufficient cytocompatibility of the produced foams could be demonstrated in vitro. The results of the present study are the basis for further investigations and developments of the collagen foam as an innovative therapy option for the treatment of acute and chronic wounds. Future studies could focus on integrating and investigating different additives in the foams.

P250 | 3D printing for soft tissue regeneration and applications in medicine

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Background/aim: Since the development of the first additive manufacturing technologies in the 1980s, 3D printing applications have steadily gained in importance in various areas of modern medicine. This is due to the wide range of possible uses e.g., for the production of surgical instruments or individually adapted mechanical components in medical products. Within the last decade, the possibility of printing even entire soft or hard tissues, optimally adjusted to the patient's wound situation, has gained a lot of interest, as it blazes the trail for an innovative and patient-specific treatment of acute and chronic wounds.

In this context, the aim of the present work was to provide a general overview of the various printing technologies available today and their application for medical use.

Materials and Methods: Initially, the most important additive processes, e.g., photocuring, droplet- or extrusion-based printing, were summarized and assessed, regarding their potential for current and further developments in the field of soft tissue printing. In order to create a detailed overview, previous related publications were researched in particular for advantages, disadvantages, operating principles and areas of application of the various 3D printing processes with special consideration of their previous use for soft tissue regeneration. Furthermore, it was possible to compare the suitability of the respective applications with regard to available biomaterials, influence on cell viability, previous experience with its use in soft tissue regeneration and need for further optimization.

The obtained data was assembled to an overall context and supplemented with own considerations regarding the potential for future research and development concepts.

Results: The use of additive manufacturing processes for the construction of soft tissue structures, fully adapted to the patient´s requirements, is a young and comparatively little-served field of research, but with great potential, especially for surgical or reconstructive-oriented fields of modern medicine. A large number of different printing technologies or the combination of them have enabled a significant progress in the production of biological soft tissues. This includes, e.g., the printing of artificial skin grafts from pure fibroblasts embedded in collagen, the construction of skinlike layered tissue made from keratinocytes and fibroblasts and, most recently, the creation of pigmented skin through the inclusion of

melanocytes within the bioink. However, clear drawbacks are still evident in sufficient vascularization of the created tissue, as well as in the creation of a functional artificial neuronal system. Although the experiments and documented results published so far seem to predict a continuously development and improvement of the various printing applications, further intensive efforts will be required to overcome the remaining issues and to optimize this promising technology for clinical use.

P251 | Poor perifollicular vascularization is associated with nutrient insufficiency and a quiescent metabolic phenotype in intermediate hair follicles from patients with female pattern hair loss

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Female pattern hair loss (FPHL) is a non-scarring form of alopecia, resulting from premature catagen induction leading to telogen effluvium and progressive transformation of terminal (t) scalp hair follicles (HFs) into intermediate/miniaturized (i/m) HFs. Currently, available treatment strategies, are often combined with nutritional supplements. However, data supporting nutrient deficiency in FPHL HFs are lacking. Therefore, we attempted to investigate this by asking whether any changes can be seen in perifollicular vascularization in FPHL and by examining HF nutritional status and metabolism in affected and nonaffected HFs. CD31 quantification revealed significantly reduced numbers of perifollicular blood vessels in the relatively unaffected (occipital) but most prominently in the affected (parietal) scalp from FPHL patients compared to occipital scalp from healthy donors. This was accompanied by changes in the interfollicular expression of angiogenesis associated growth factors VEGF and thrombospondin-1. Metabolic analyses showed that nutrients important for adequate hair growth such as biotin, pantothenic acid, L-cysteine, and L-glutamic acid were decreased in iHFs compared to tHFs in affected FPHL scalp skin. iHFs also displayed a more quiescent metabolic phenotype, characterized by a lower rate of aerobic glycolysis and glutaminolysis during organ culture. Yet, lesional t or i/m FPHL HFs were capable of taking up labeled exogenous nutrients when these were added in excess into the organ culture medium. Taken together, our data suggest that t and i/ m HFs in FPHL affected scalp skin are poorly vascularized which likely compromises nutrient delivery to the HFs and that nutrient supplementation may be capable in principle to correct this deficiency.

P252 | Odorant-dependent Merkel cell chemosensation: Implications for wound healing

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Merkel cells (MCs) are found in the skin and release neuromediators through secretory vesicles. Although MCs have been known so far exclusively for being transducers of mechanical pressure applied to the skin, recent observations suggest that their role may extend beyond pure mechanosensation.

In the present study, we report that over 70% of epidermal K8+/K18+/K20+ MCs co-expressed OR2AT4, hinting that this olfactory receptor may have an important physiological role in these cells. To explore this, we exposed human skin from healthy subjects to the topical application of the specific OR2AT4 agonist Sandalore® ex vivo. Sandalore® did not affect K20+ cell numbers or proliferation nor apoptosis. Instead, Sandalore® up-regulated the number of K20+ cells expressing Piccolo (presynaptic marker), suggesting an increase in neurotransmitter release. In parallel, the intracellular content of nerve growth factor (NGF) in MCs was ablated after exposure to Sandalore®, possibly due to active release upon Sandalore®- mediated cellular depolarization. Corroborating this hypothesis, live-cell imaging showed release of the fluorescent false neurotransmitter, FFN206, from pre-labeled MCs, 5-min after Sandalore® treatment of epidermal sheets from healthy donors.

Therefore, OR2AT4 activation appears to induce MC depolarization, promoting the secretion of vesicles containing neuromediators such as NGF. Because NGF is known to promote wound healing, our results suggest that MCs may support skin healing response previously observed after Sandalore® application. In conclusion, we show that human MCs not only are mechanosensory cells but also operate as important chemosensory cells in human skin that can identify selected odorants, whose stimulation may alter neuromediators secretion by MCs.

P253 | Identification of the epidermal growth factor receptor (EGFR) as a target of ubiquitous organic pollutants

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Several organic pollutants ubiquitously occur in our environment, including dioxins like TCDD that can accumulate in adipose tissue. Due

to their long half-life an accumulation can take place for several years, causing chronic illness. In higher concentrations, those chemicals can also cause acute toxic effects such as chloracne. Until now, the processes evoked by dioxins and other halogenated compounds in the skin leading to this chronic-inflammatory skin disease remain unknown. However, an inhibition of proliferative signaling advantaging differentiation of epidermal keratinocytes seems to be fundamental.

A receptor known to be activated by dioxin-like compounds in keratinocytes is the Arylhydrocarbon receptor (AHR). Since chloracne is only provoked by an acute intoxication with dioxins and other chlorinated aromatic compounds, but not by AHR ligands with different chemical structures, an activation of other pathways could be a reasonable explanation. Renownedly, the Epidermal growth factor receptor (EGFR) plays an important role in proliferation and differentiation of keratinocytes. An inhibition of the EGFR, often medically induced for cancer treatment, leads to adverse dermal effects such as papulopustular rashes on the face and upper body. This is presumed to be precipitated by a switch of epidermal keratinocytes program from proliferation to differentiation. We recently found that dioxins and other halogenated compounds interfere with the growth factor induced phosphorylation and internalization of EGFR. In silico docking analysis predicted that the dioxin-like compounds TCDD and PCB126, but not the polycyclic aromatic hydrocarbon (PAH) B[a]P, bind the EGFRs extracellular domain. Generation of point mutated EGFR plasmids and subsequent western blot analysis confirmed that the residues Q8 and Q408 of the predicted binding site are vital for the compound's interference of EGFR phosphorylation. Investigation of EGFR ligand-induced proliferation in keratinocytes showed that several dioxin-like compounds inhibit this process, while also non-dioxin-like compounds such as PCB47, but not PAHs like B[a]P or B[k]F, depleted proliferation.

These findings could contribute to better understanding of the processes behind the emergence of chloracne and other dioxin-induced skin diseases and therefore, hold potential to help developing novel preventive and therapeutic approaches. Furthermore, a yet undescribed mechanism concerning the extracellular domain of the EGFR as a binding site for several ubiquitous organic pollutants is introduced, expanding the already essential role of EGFR in skin diseases.

P254 | *Staphylococcus* overgrowth and disturbances in skin recolonization are associated with cutaneous graft-versus-host disease

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Patients undergoing allogeneic hematopoietic stem cell transplantation (HSCT) display dysbiosis in the gut microbiome, which is a risk factor for transplant-related mortality and development of graft-versus-host disease (GVHD). Since gut and skin are GVHD target organs, and little is known about the cutaneous microbiome, we investigated perturbations in the skin microbiome and cutaneous immune system in a longitudinal study. We hypothesized that changes in the skin microbiome affect cutaneous GVHD development and outcome. By taking serial biopsies of the skin microbiome and skin tissue before and after HSCT, we found reduced bacterial colonization after transplantation, but overabundance of *Staphylococcus* spp. in patients that developed skin GVHD. To investigate simultaneous changes in the skin immune system we performed bulk RNA sequencing of isolated skin T cells and mononuclear phagocytes. We found signatures of host immune activation after transplantation, particularly an upregulation of genes required for antigen presentation in mononuclear phagocytes and T cell receptor signaling and effector function in T cells. To investigate crosstalk between mononuclear phagocytes and T cells we assessed receptor-ligand interactions and found increased expression of interacting genes involved in chemotaxis and cell migration early after transplantation. To connect microbial dysbiosis with the observed immune signatures, we assessed the co-localization of bacteria and CD45+ leukocytes in the skin of patients. Importantly, bacteria and leukocytes were in closer proximity early after transplantation in patients that later developed GVHD. Taken together, our data suggests that conditioning promotes disturbances in the skin microbiome, particularly among *Staphylococcus* spp., as well as activation of cutaneous host immune cells. These changes associate with the development of cutaneous GVHD. Therefore, targeting the cutaneous microbiome to prevent dysbiosis and *Staphylococcus* overgrowth might present a novel strategy for the prophylaxis of GVHD.

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