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ABSTRACT

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Allergology

P001 | Role of myeloid cells and coagulation proteases in protease-activated receptor 2 (PAR2)-mediated skin inflammation

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Protease-activated receptor (PAR) 2 signaling is mandatory for skin barrier function and cutaneous inflammation, but the function of PAR2-activating proteases and relevant immune cell populations is incompletely defined. Mutation-induced complete insensitivity of PAR2 to proteolytic cleavage markedly attenuated the acute and chronic cutaneous immune response in contact hypersensitivity (CHS), a murine model of the allergic contact dermatitis (ACD) in humans. In skin lesions of ACD patients, numbers of PAR2 positive infiltrating myeloid cells were significantly increased. Accordingly, cell type-specific deletion of PAR2 in myeloid immune cells resulted in a curtailed skin inflammation and hapten-specific T cell response in CHS mice. Pharmacological approaches demonstrated a pivotal role of tissue factor (TF) and coagulation factor Xa (FXa) as upstream activators of PAR2 in both, the induction and effector phase of CHS. Experiments with PAR2 mutant mouse strains with differential cleavage sensitivity for FXa versus skin epithelial cellexpressed proteases uncovered a challenge time course-dependent regulation of CHS development with an important function of FXa induced PAR2 activation for the late phase of skin inflammation. Thus, the TF-FXa-PAR2 axis is a key mediator and potential therapeutic target of inflammatory skin diseases.

P002 (OP02/01) | Effector functions of human TH9 cells depend on PPARg-regulated glucose metabolism

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TH9 cells, a subpopulation of TH2 cells, are crucial mediators of allergic skin inflammation. They are characterized by expression of IL-9/IL-9R and rely on the transcription factor PPAR-g for full effector function. The functional role of PPAR-g in TH9 cells, however, remains unknown. We found that PPAR-g is a positive regulator of glycolysis in human TH9 cells. Accordingly, TH9 cells featured a higher glycolytic activity as compared to TH1 and TH2 cells. In turn, impairment of glycolysis led to downregulation of IL-9, but not IL-13 expression, thus emulating the effects of PPARg antagonism on cytokine production. Conversely, enhancing glycolytic activity by increasing glucose availability increased IL-9 levels, while leaving IL-13 expression unchanged. Mechanistically, PPAR-g- and glycolysis-dependent regulation of IL-9 expression was mediated through mTORC1. Collectively, these observations indicated a dichotomous regulatory role of glycolytic activity on IL-9 and IL-13 expression in

activated TH9 cells that is dependent on PPAR-g-regulated glycolysis and mediated via mTORC1.

In vitro and ex vivo studies on samples of allergic contact dermatitis indicated that this PPARγ/mTORC1/IL-9 pathway was active in pTH2 cells in human skin inflammation. Additionally, we found that tissue glucose levels were dynamically regulated in acute allergic skin inflammation, suggesting that in situ glucose availability might be linked to distinct immunological signals in vivo.

In summary, our data propose that PPAR-g is a positive regulator of glucose metabolism in TH9 cells and that IL-9 expression is specifically dependent on tissue availability of glucose and cellular metabolic activity. These findings highlight a novel link between the metabolic environment in the tissue during inflammation and type 2- driven skin inflammation.

P003 | Responses of human skin mast cells to the G-protein biased MRGPRX2 agonist icatibant – differences in degranulation, activation, and signal transduction vis–vis balanced ligands

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Cutaneous mast cells (MCs) are involved in multiple dermatoses, including urticaria, atopic and contact dermatitis. While believed for decades that the most potent stimulatory route is triggered by the FcεRI/IgE/allergen axis, many inconsistencies remained. After its discovery, the alternative route via MRGPRX2 has gained much attention and is now believed to mediate clinical phenomena that depend on MCs, but for which FcεRI involvement (alone) is implausible. MRGPRX2 is activated by a plethora of ligands, of which several hundred have been unearthed. By their capacity to activate G-proteins and beta-arrestins, they can be classified as balanced (activating both) and biased (activating only G-proteins). While codeine and Substance P (SP) represent balanced agonists, icatibant has been described as biased in cell lines, while it remains to be established whether this holds for skin mast cells (skMCs), the cells operating in skin diseases as well as during injection of icatibant. Icatibant gives rise to local injection-site reactions in almost every patient, while other drugs do so less frequently, even though the same receptor is in play.

Here, using MCs purified from normal human skin, we report that skMCs degranulate with icatibant (measured by beta-hexosaminidase release), but that responses were substantially weaker compared with SP and codeine, reaching only about 47% of the potency of balanced agonists at saturating concentrations. In contrast, differences across ligands were much lower in the MC line LAD2. Icatibant also triggered CD107a exteriorization in skMCs (the best suited activation marker of these cells), yet also less profoundly than SP or codeine, thus paralleling degranulation. On comparison with SP, signal transduction (ERK1/2 and p38 phosphorylation) initiated by icatibant was delayed, and overall weaker in skMCs. Interference with G-proteins, and Ca++ channels revealed that while relevance of Gi and 2-APB-inhibitable Ca ++ channels was comparable for degranulation responses to all ligands, Gq and La3+ inhibitable channels were more relevant in icatibant-elicited granule discharge. This suggests that distinct signalosomes are activated by balanced versus biased MRGPRX2 ligands in skMCs.

Collectively, the biased ligand icatibant differs in many respects from balanced MRGPRX2 agonists regarding signal transduction, degranulation efficiency, and CD107a exteriorization. Possibly, despite the lower degranulability, lack of desensitization favors repetitive stimulation from an icatibant depot, while balanced ligands cancel their further function on first stimulation through induction of refractoriness.

P004 | Mast cell-derived GM-CSF and physical interaction in the skin promotes bidirectional mast cell-eosinophil activation

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Introduction: In the late and chronic phase of allergies, mast cells (MCs) and eosinophils (Eos) enter in a bidirectional crosstalk leading to cell activation driven by physical contact and/or released mediators. This interaction, termed the Allergic Effector Unit (AEU), substantially contributes to chronic allergic inflammation. However, some functional features of the AEU are still incompletely solved.

Objective: To investigate the contribution of individual MC mediators in Eos activation and the physical interaction of primary human skin MC and human blood eosinophils using in vitro and ex vivo models.

Methods: MCs or Eos were treated either with cell culture supernatants or cocultured. β-hexosaminidase or histamine release was used as a readout for MC activation. Eos activation was determined via CD69 expression using flow cytometry. Cytokine blocking antibodies were used to assess the contribution of individual MCderived cytokines to Eos activation. To mimic Eos infiltration into skin, we injected blood Eos into human skin explants and performed ex vivo skin microdialysis.

Results: Supernatants from anti-IgE or cortistatin-14-activated skin MCs increased the expression of CD69 on Eos (% CD69+ Eos: unstimulated = 3.60.9, anti-IgE = 28.610.4, p = 0.014; cortistatin = 13.0317.2). By blocking individual MC-derived cytokines in supernatant, we identified GM-CSF as a crucial cytokine for Eos

activation, as blocking of GM-CSF reduced CD69 to the levels of unstimulated Eos. Co-culture of unstimulated MCs and Eos induced a strong CD69 upregulation on Eos, which was further increased when Eos were co-cultured with previously activated MCs. Finally, injection of eosinophils into ex vivo skin induced strong histamine release by MCs (vehicle: 49 ng/ml 36.7 vs. injected eosinophils: 132 ng/ml 46).

Discussion: Bidirectional crosstalk between MCs and Eos, i.e. the AEU, may result in a reciprocal activation loop that promotes chronic activation of both cell types contributing to disease pathology. Thus, therapeutic disruption of the AEU, for example by targeting GM-CSF or its receptor, may be a valuable approach for the treatment of chronic allergic inflammation.

P005 (OP01/01) | The gut microbiome is decisive in the outcome of allergic contact dermatitis

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Background: Numerous recent studies revealed an association between dysbiosis in the gut and inflammatory diseases, including allergies. However, the role of commensal bacteria in allergic contact dermatitis (ACD), one of the most common occupational skin diseases, is largely unknown. Therefore, we set out to investigate the role of commensal bacteria in the contact hypersensitivity (CHS) reaction, a mouse model of skin inflammation resembling ACD in patients, that is driven by IFN-γ producing CD8+ T cells.

Methods and Results: CHS was carried out by sensitization (abdominal skin) and challenge (ear) with the contact allergen (hapten) trinitrochlorobenzene (TNCB) in conventional (CONV) and germ-free (GF) mice to investigate the effect of the microbiome on T cell-mediated skin inflammation. We found that GF mice exhibited a significant reduction in ear swelling, which serves as an in vivo parameter for the CHS immune reaction, compared to CONV mice. This effect was reversible upon reconstitution of the microbial load through cohousing CONV and GF mice, indicating a pivotal role of the microbiome for allergic skin inflammation. To define whether the skin- or gut- related microbiota affected the CHS reaction, we added an antibiotic cocktail (ABX) to the drinking water, which is known to influence the gut-related microbiome only. As observed in GF mice, ear swelling was significantly impaired after ABX treatment compared to CONV mice, suggesting that the intestinal rather than the cutaneous microbiome plays a role in the skin inflammation of CHS. Next, we analysed the resulting T cell response by hapten-specific restimulation of T cells obtained from skin-draining lymph nodes of GF, ABX and CONV mice. We detected a high proliferation of CD8+ T cells and an increased IFN-γ production, but no significant difference between GF, ABX and the respective CONV control groups, showing unaffected activation and differentiation of hapten-specific T cells in the absence of microbiota. However, we found enhanced IL-10 levels in T cell cultures from GF and ABX compared to CONV mice. As previously demonstrated, CD4+CD25+FoxP3+ regulatory T cells (Tregs) constrain CHS responses by producing IL-10. Therefore, Treg-derived IL-10 could be the cause of decreased ear swelling in GF and ABX mice. CHS induction in ABX-treated transgenic mice (CD4Cre × IL-10flox, FoxP3Cre × IL-10flox) revealed that the absence of IL-10- producing CD4+ T cells or of FOXP3+ Tregs, respectively, fully restored skin inflammation, suggesting a crucial role for IL-10 and Tregs in CHS repression. Previous reports demonstrated that contact sensitization requires signaling through Toll-like receptor 2 (TLR-2) and that commensals elicit anti-inflammatory properties via the TLR2-IL-10 – axis. Therefore, we induced CHS in TLR-2 knockout (KO) – mice housed under GF and ABX conditions. Intriguingly, TLR-2 deficiency resulted in recovery of the cutaneous inflammation in CHS in both ABX and GF mice compared with CONV mice and in normalization of IL-10 levels. This indicates that TLR-2 signaling controls the production of the immunosuppressive cytokine and suppresses CHS when microbiota are depleted.

Conclusion: We found evidence that the gut microbiome mediates critical immunomodulatory properties in CHS via the TLR2-IL-10 – axis and is decisive for the outcome of this disease model.

P006 | Exploring the mechanisms during early tolerance induction in hymenoptera venom based immunotherapy

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Introduction: Tolerance can be achieved in allergic patients upon administration of increasing doses of allergen over time. This so-called immunotherapy has shown to cause long-term tolerance induction by upregulating regulatory T Cells and specific IgG against the antigen. The regulatory mechanism leading to short-term tolerance induction remains unknown by large. Knowledge of this mechanism would help in better understanding of the rush and ultrarush immunotherapy, where tolerance is achieved within hours or days. Our study aims to investigate this mechanism by checking the high affinity receptor (FcƐR1) density on the surface of basophil granulocytes and in the serum, as well as the activation of basophils in patients undergoing immunotherapy for hymenoptera venom.

Methods: We collect patient blood before and six hours after the initial doses of immunotherapy to check the reactivity of basophils using a FACS-based standardized basophil activation kit. The amount of soluble FcƐR1 in the serum is detected by ELISA and the surface density of FcƐR1 on basophils is determined by quantitative FACS analysis.

Results: Already six hours after the start of immunotherapy we could show in eight patients a median increase in the amount of IgE-unoccupied FcƐR1 on the basophils of +3.2% (IQR −3.6 to +32.0) and a median decrease in the overall surface FceR1 (whether IgE-occupied or not) of −4.9% (IQR −11.2 to +6.2). In the preliminary analysis we saw no changes of the activation of basophils within the short period of time.

Conclusions: Preliminary results suggest that fast tolerance induction in immunotherapy might be caused by a general decrease of FcƐR1 surface expression as well as an increase in IgE-free FcƐR1 on basophils. In the ongoing study, we plan to increase patient numbers and to check for changes of soluble FcƐR1 in the serum, which we hypothesize to be able to bind and inhibit IgE, just like "natural" Omalizumab.

P007 | Serological analyses minimize the need for skin tests in the diagnosis and during therapy of vespid venom allergy

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Background: In adults, allergic reactions to insect stings are among the most frequent causes of anaphylaxis, a potentially life-threatening condition. Recurrent anaphylaxis following vespid stings may be prevented by allergen immunotherapy (AIT). The aim of this study was to evaluate the benefit of measuring venom-induced wheal area in intracutaneous skin tests (ICT), in comparison to various serological and clinical parameters, for the diagnosis of severe vespid venom allergy and during AIT.

Methods: We conducted a monocentric, retrospective evaluation of 170 patients undergoing AIT against vespid venoms. We scanned ICT wheals at baseline and at three time points after AIT initiation and measured wheal area using objective data analysis software.

Results: We found that ICT histamine-induced and venom-induced wheal areas did not correlate. In addition, the venom-induced wheal area was independent from the minimal venom concentration required to elicit a wheal in an ICT and all other parameters. No correlation was found between wheal area and the severity of anaphylaxis. Wheal area standardized to the application of 0.1 µg/ml venom inversely correlated with anaphylaxis severity and positively correlated with venomspecific IgE levels. During AIT, mean areas of venom-induced wheals did not change. In contrast, venom-specific IgG and IgG4 levels, and the minimal venom concentration required to induce a positive ICT result increased, while the venom wheal area standardized to 0.1 µg/ml venom application and specific IgE levels decreased over time.

Conclusion: Wheal area evaluation did not provide additional information over specific IgE analysis. We therefore recommend that ICTs are used only as a secondary measure for confirming serological test results.

P008 | A subgroup of atopic dermatitis patients displays a disease-severity associated type-2 response against skin bacteria

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In atopic dermatitis (AD), a common chronic relapsing-remitting inflammatory skin disease, lesional skin often exhibits reduced microbial diversity as well as S. aureus colonization, both being associated with higher disease severity and connected to flares. T cells infiltrating the skin are key players in the inflammation. To some extend these react to allergens, however, for the majority the specificity remains to be elucidated. Therefore, we aimed to identify bacteria-specific T cells within lesional AD skin and investigated the role of the T cell response to bacteria in the disease.

In order to characterize T cell responses to skin bacteria in AD, we generated T cell lines by long-term stimulation of peripheral blood mononuclear cells (PBMCs) with bacterial antigens of Staphylococcus aureus, S. cohnii, S. epidermidis and Streptococcus salivarius. 60–90% of T cell lines showed significant proliferation after re-stimulation and were therefore considered as bacteria-specific T cells, which were analyzed for their cytokine secretion capacity. In parallel, we sequenced the CDR3 regions of the T cell receptor β chain (TCRB) of bacteria specific T cells of five AD patients and compared these with respective sequences of autologous lesional skin biopsies. Further on, bacteria-specific T cells in the periphery were identified by CD154 on CD4+ T cells and CD137 on CD8+ T cells after antigen stimulation and characterized by intracellular cytokine staining in combination with chemokine receptor analysis.

Bacteria-specific T cells showed a significantly increased production of IFN-γ. Further on, hierarchical clustering revealed that most patients possessed a Th17- dominated response against bacterial antigens with IL-17A, IL-17F and IL-21, while a small subgroup of AD patients exhibited a type-2 dominated response with IL-4, IL-13 and IL-5. Analyzing cytokine secretion with the objective disease

severity index (SCORAD) revealed that IL-13 secretion of S. aureus-specific as well as S. epidermidis-specific T cells correlated significantly. Sequencing of TCRB CDR3 regions of both bacteria-specific T cells and skin biopsies showed that AD lesions indeed contain these cells in clonally increased frequencies.

In conclusion, in AD lesional skin there can be clonally propagated bacteria-specific T cells, in which data from PBMCs suggest that these T cells promote the inflammation by eliciting pro-inflammatory cytokines. While a type-1/type-3 immune response to these bacteria dominates in most patients, type-2 immune responses were observed in a subgroup of patients and interestingly associated with disease severity.

Cellular Biology

P009 | Contribution of taurine, pyridoxine and pantothenic acid to the pathomechanism of pemphigus vulgaris

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Pemphigus vulgaris (PV) is an autoimmune blistering disease characterized by autoantibodies targeting desmoglein 3 and 1. Clinical hallmarks of the disease are mucocutaneous erosions and blisters. As a consequence of the barrier disruption, it is a life threatening disease. PV immunopathogenesis is attributed to autoantibodies targeting desmoglein 3 and 1. The standard of care is immunosuppression and/or anti-CD20 antibody. Yet, approximately 20% of patients do not achieve remission despite vigorous immunosuppression, and relapses are frequent. In contrast to common chronic inflammatory skin diseases, a limited number of multidimensional data is available for PV. To contribute to the molecular understanding of pemphigus, longitudinal metabolome analysis of 10 PV patients were performed: Three metabolites showed different profiles after PV remission. While pyridoxine and pantothenic acid plasma concentrations are decreased in active PV patients, taurine levels were increased and all of them normalized to those concentrations observed in healthy controls, after PV treatment. Pantothenic acid promotes wound healing through proliferation of keratinocytes and pyridoxine has anti-inflammatory properties and maintains the normal level of IL-4. Taurine also has anti-inflammatory and antioxidative properties. To test if the observed metabolomic changes in pemphigus are of functional relevance, the effects of pantothenic acid, pyridoxine and taurine on key pathogenic events in pemphigus is currently evaluated in vitro. Collectively, we show that pemphigus is associated with distinct metabolic changes.

P010 (OP03/04) | Resetting the epigenetic machinery in dysfunctional macrophages by sodium butyrate accelerates healing of diabetic wounds

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Non-healing wound disorders including diabetic ulcers, chronic venous leg ulcers and pressure ulcers still remain major unmet medical needs and pose worldwide a stern challenge to health care systems. Persistent accumulation of dysfunctional pro-inflammatory macrophages was earlier identified as a major underlying cause for chronic wounds, however, molecular details of their dysregulation are still lacking. Here we report that histone H3K27 acetylation, an epigenetic mark regulating the macrophage transcriptome, is significantly suppressed under diabetic microenvironmental conditions. We identified palmitate, a saturated fatty acid occurring at high concentrations in diabetic patients and mice, to be responsible for this. In a series of in vitro and in vivo experiments (RNA seq analysis, chromatin immunoprecipitation with DNA sequencing, assay for transposase accessible chromatin, in vivo wound monitoring, immunostaining, and shRNA silencing), we found that increased palmitate markedly suppressed the acetylation of histone by activating the HDAC3 histone deacetylase-dependent pathways. Furthermore, we observed a shift in the transcription control from STAT1 to JUN in palmitateexposed stimulated macrophages. The histone deacetylase inhibitor sodium butyrate significantly enhanced the healing of murine diabetic skin wounds via restoring histone H3K27 acetylation-dependent transcriptome and pathways in wound associated-macrophages and reinstalled their pro-regenerative STAT1 signaling. We furthermore uncovered that butyrate-mediated inhibition of the HDAC3 histone deacetylase preserved morphological features and, more importantly, improved the phagocytic and migratory activity of macrophages even under inflammatory conditions of the palmitate imprinted diabetic microenvironment. This finding is of major interest since butyrate concentrations are severely reduced in diabetic patients and mice. As butyrate is a metabolic product of gut bacteria, its decrease also hints to a compromised gut microbiome in murine and human diabetic patients. Our study highlights a novel pathogenic mechanism controlling perturbed macrophage function, which can be therapeutically exploited to improve or even cure chronic wounds dominated by macrophage dysregulation in diabetes and possibly other wound healing disorders.

P011 | Exploring neutrophil nuclear dynamics and neutrophil extracellular traps using expansion microscopy

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Neutrophils are the first responders to injury and are critical to the inflammatory process. The neutrophil nucleus is lobulated and has a structure that is highly responsive to the environment, which allows efficient migration to the site of inflammation. They possess an arsenal of antimicrobial defenses, including the expulsion of neutrophil extracellular traps (NETs), composed of DNA studded with cellular and antimicrobial proteins. However, dysregulated neutrophil activation is implicated in inflammatory diseases such as psoriasis and systemic lupus erythematosus, highlighting the need to better understand their biology. Thorough study of neutrophil nuclear morphology has been greatly limited by the resolution limits inherent in microscopy and thus often require expensive and specialized super-resolution equipment. Therefore, we have implemented expansion microscopy for imaging of neutrophils, as this technique is cost- and time-efficient and can be performed using conventional equipment. We used this to characterize the distribution of chromatin, histone H1, and nucleophosmin in both unstimulated and NETotic neutrophils. We labeled lamins B1 and B2 as nuclear envelope markers and myeloperoxidase as a cytoplasmic protein. The gain in resolution was calculated by assessing the colocalization of histone H1 and chromatin using Pearson's coefficient. Line scan analysis of NPM1 distribution and histone H1 granularity throughout the nucleus showed an increase in detail with expansion. In conclusion, expansion microscopy is a novel tool to study neutrophil nuclear dynamics and image neutrophil proteins at super-resolution using conventional microscopes. This method can be adapted to other aspects of neutrophil biology and is thus a valuable tool to analyze their morphology.

P012 (OP06/05) | Research on skin cells unravels general mechanisms of neurological degeneration

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Cockayne syndrome (CS) is a childhood disorder of developmental delay, degeneration and signs of premature aging. Neurological degeneration is one hallmark of the disease. Using patient skin fibroblasts, we could describe a novel pathomechanism in CS, ribosomal dysfunction leading to a loss of protein homeostasis. As loss of proteostasis characterizes most aging-associated neurological diseases, we asked if ribosomal dysfunction may contribute to the development of these disorders. By RNA-sequencing and analysis we defined the gene expression signature of CS patient skin cells and compared it to published gene expression signatures of neurological disorders. Amongst others, Huntington´s disease (HD) gene expression patterns matched with CS. Huntington is a dominant inherited, fatal late-onset disease of striatal neurodegeneration, characterized by choreatic movements, sarcopenia and personality disorders. The molecular pathomechanism of HD is unknown and here we propose a novel hypothesis. We can show that gene expression patterns derived from CS can discriminate HD from healthy controls to 100%. ChIP analysis revealed that huntingtin, the protein whose mutation causes HD, binds to the rDNA, encoding for the rRNA backbone of the ribosome. Pre-rRNA synthesis and processing is disturbed in cells of HD patients. The accuracy of the translation at the ribosome is reduced, leading to misfolded proteins that are carbonylated by the elevated reactive oxygen species (ROS) in HD cells. The striking similarities between a childhood disorder of premature aging and an aging-associated disease validates CS as a model for aging research and justifies research on rare diseases.

P013 (OP02/04) | Integrin-mediated cell-collagen communication is essential for robust fibroblastic mechanotransduction and development of fibrosis

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Collagens being the most abundant constituent of the extracellular matrix (ECM), play a vital role not only in maintaining the mechanical stability of the tissue but also in regulation of pathological and biological processes such as fibrosis and aging.

The major collagen receptors in skin are integrins α1β1, α2β1 and α11β1. Previous studies using single and double knockout mice lacking collagen-binding integrins have revealed their individual functions but also indicated compensatory mechanisms.

We here address directly the overall significance of cell-collagen association through integrins. For this purpose, we generated triple

knockout (tKO) mice globally lacking α1β1, α2β1 and α11β1 integrins. tKO mice live with no striking histological or ultrastructural skin alterations compared to wild type (WT) controls. The tKO dermal fibroblasts show adhesion and spreading defects on collagen I, although there is an increase in the non-integrin collagen receptor, discoidin domain receptor 2 (DDR2) in vitro as well as in vivo. This suggests that all functions required for adhesion and spreading are specifically mediated by integrins and cannot be rescued by DDR2. The tKO fibroblasts deposit an abnormal ECM as observed by proteomic analysis, which influences fibroblast properties. The tKO fibroblasts show an altered actin filament architecture and remodeling, fewer focal adhesions, lower yes-associated protein (YAP) activation and decreased traction forces. The above findings strongly indicate an incompetent mechanoperception and response by the tKO fibroblasts in vitro. Further, in vivo the tKO mice show attenuated dermal fibrotic response upon bleomycin injection with reduced myofibroblasts, less densely packed collagen fibrils and lower YAP activation.

We conclude that collagen-binding integrins play an important role in regulating tissue homeostasis by maintaining a functional cellular architecture required for establishing robust force transduction.

P014 | CREB is activated by the SCF/KIT axis in a partially ERK-dependent manner and is required for the survival and functional programs of human skin mast cells

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cAMP response element binding protein (CREB) is an evolutionarily old transcription factor (TF) highly conserved in its coding as well as regulatory regions. It functions as prototypical stimulus-inducible TF and effector initiating multiple cellular changes in response to cell activation. Despite pronounced expression in mast cells (MCs), CREB function is surprisingly ill-defined in the lineage. Skin MCs are critical effector cells in acute allergic and pseudo-allergic settings, and they contribute to various chronic dermatoses like urticaria, atopic dermatitis, allergic contact dermatitis, psoriasis, prurigo, rosacea and others. Employing in human skin MCs, we demonstrate herein that CREB is rapidly phosphorylated on serine-133 upon SCFmediated KIT dimerization, a posttranslational modification enabling recruitment of coactivators to enhance CREB-dependent transcription. Phosphorylation initiated by the SCF/KIT axis required KIT tyrosine kinase activity (inhibitable by imatinib mesylate) and partially depended on ERK1/2 activity (inhibitable by LY3214996 and SCH772984), but not on other kinases such as p38, JNK, or PI3K. We also found that CREB is constitutively present in the skin MC nucleus, where it incurs phosphorylation following KIT activation. Using the potent and selective CREB inhibitor 666-15, we demonstrate that CREB is required for SCF-facilitated MC survival (cell number determination, apoptosis). On comparison with other survival promoting modules (PI3K, MEK/ERK), CREB was the most potent by far. Knockdown of CREB by RNA interference duplicated the results achieved with 666-15 on survival. As recently reported, SCF potently induces immediate early genes in skin MCs (FOS, JUNB, NR4A2) Here, we demonstrate that CREB is an essential partaker in this induction. Interference with CREB also impacted the expression of critical MC receptors (KIT and Mas-related G-protein coupled receptor member X2 (MRGPRX2)). We finally determined that pretreatment with 666-15 reduced MC degranulability (measured by beta-hexosaminidase release), especially via MRGPRX2. Collectively, the ancient TF CREB is an indispensable component of the human cutaneous MC, where it orchestrates basic and stimulated functional programs.

P015 (OP05/02) | Mitochondrial DNA damage attenuates dermal fibrosis by inhibiting differentiation into myofibroblasts

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Post-mitotic, non-proliferative dermal fibroblasts have crucial functions in maintenance and restoration of tissue homeostasis. They are involved in essential processes such as wound healing, pigmentation and hair growth, but also tumor development and aging-associated diseases. However, it is still unclear how skin cells master these highly energetic demands metabolically.

To approach this, we created a mouse model in which accelerated cell-specific mitochondrial DNA deletions accumulate. Therefore, we crossed mice carrying a dominant-negative mutant of the mitochondrial replication helicase Twinkle (RosaSTOP system) with mice that express the fibroblast-specific Cre Recombinase (Collagen1A2 CreERT) which can be activated by Tamoxifen (TwinkleFIBRO).

Thus, we can cell-specifically induce a process which resembles the physiological aging process in humans, where these deletions accumulate in all tissues.

Upon proliferation in vitro, the Tamoxifen induced Twinkle fibroblasts deplete their mitochondrial DNA, leading to disturbed stoichiometry of the respiratory chain complexes and an anti-inflammatory and anti-fibrotic profile of the cells whereas in Sodium Azide treated, and therefore without a functioning respiratory chain, wildtype fibroblasts in vitro we observe a rather pro-inflammatory and pro-fibrotic signature.

Upon accumulation of mitochondrial DNA deletions in vivo the TwinkleFIBRO mice are protected from fibrosis development induced by intradermal Bleomycin injections. This is due to dampened differentiation of the dermal fibroblasts into α-smoothmuscle- actin positive myofibroblasts in TwinkleFIBRO mice.

Thus, these data contribute to improved understanding of mitochondrial function and dysfunction in skin and provide mechanistic insight into potential targets to treat skin fibrosis in the future.

P016 | The role of the Ca2+ channel TRPV4 ion in epidermal barrier integrity

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To form a strong and protective epidermal barrier, keratinocytes are subjected to a tight control between proliferation in the basal layer and ordered differentiation and maturation in upper layers. Ca2+ is a major regulator of this process and the Ca2+- permeable TRPV4 (transient receptor potential vanilloid 4) ion channel was shown to contribute to epidermal barrier integrity in other tissues. We directly addressed the importance of TRPV4 in the skin by creating genetic TRPV4 knockout using the CRISPR-Cas9 genome editing technology as well as siRNA mediated knockout.

We found that the expression of TRPV4 increases during 2D in vitro differentiation and that the absence of TRPV4 results in increased expression of keratin 10 and involucrin at later stages of differentiation. In 3D reconstituted epidermal models knockout of TRPV4 did not lead to obvious morphological changes. However, the epidermal barrier was compromised as Lucifer yellow penetrated into the epidermis in KO models and the expression of the tight junction marker claudin-1 was impaired. Altogether, we provide first evidence that the TRPV4 ion channel plays a role in epidermal development and barrier function probably by controlling Ca2+ influx and cell volume, which in turn regulates epidermal barrier function. Thus, the TRPV4 channel might represent an interesting target to improve epidermal barrier function. The exact mechanisms involved are currently under investigation, in particular with respect to conditions of disturbed differentiation in inflammatory skin disorders.

P017 | Altered protein synthesis in cellular senescence of human dermal fibroblasts

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Background: The accumulation of senescent cells is associated with several age-related pathologies and represents a primary driver of skin aging. Senescence cells cause tissue damage by secreting a mix of pro-inflammatory cytokines and extracellular matrix remodeling factors.

Objectives: This study elucidates how ribosomes and protein translation change in cellular senescence.

Methods: We exposed human dermal fibroblasts to doxorubicin to induce cellular senescence and measured total protein and mRNA synthesis, in vitro translation, and specific translation of senescence-associated mRNAs by polysome profiling.

Results: Global protein synthesis, ribosome biogenesis, and nucleolar size surprisingly increased in senescent compared to contact-inhibited quiescent cells. In addition, we identified several pathways specifically promoted or repressed by specific mRNA translation.

Conclusion: Protein translation by ribosomes might provide future targets for selectively eliminating senescent cells or blocking their harmful secretome.

P018 | Impact of the LRRC8 ion channel on epidermal differentiation

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Changes in cell volume are an essential regulating factor in cellular mechanisms such as proliferation and differentiation. A balance between these two processes is necessary for barrier formation in the epidermis, and dysregulation often occurs in inflammatory skin diseases. We recently showed that the volume-regulated anion channel (VRAC) LRRC8 and its essential subunit LRRC8A are necessary for the regulation of cell volume during hypotonic stress in keratinocytes.

In healthy epidermis LRRC8A is mainly located in the basal layer showing highest expression in early transient amplifying keratinocytes. This subpopulation is thought to mediate the transition from exclusively regenerative stem cells to exclusively differentiating post-mitotic cells. Interestingly, primary keratinocytes upregulate LRRC8A during the onset of differentiation in vitro while protein levels decline, when keratinocytes further differentiate, giving a bell-shaped expression pattern of LRRC8A, with a peak shortly after the onset of differentiation. Since psoriatic skin is characterized by an imbalance between proliferation and differentiation, we wondered whether LRRC8A is deregulated in inflammatory skin diseases. Interestingly, we found reduced LRRC8A immunohistological staining in lesional psoriatic skin compared to non-lesional or healthy skin. This could be confirmed in vitro, as treatment of keratinocytes with Th1 cytokines reduced the expression of LRRC8A.

Thus, we were interested whether LRRC8A is indeed necessary for the initiation of differentiation and whether dysregulation of cell volume control through loss of LRRC8A could contribute to inflammatory skin diseases such as psoriasis. siRNA-mediated knockdown in

primary keratinocytes resulted in a significant decrease of LRRC8A RNA and protein levels, leading to reduced ion channel activity. Surprisingly, reduction of LRRC8A did not affect differentiation in these cells. Since LRRC8A has a short window of elevated expression during the early differentiation phase, we targeted LRRC8A in different keratinocyte subpopulations specifically with siRNA. Strikingly, silencing LRRC8A in keratinocyte stem cells – even before LRRC8A expression increases, not only reduced LRRC8A, but greatly impaired differentiation as shown by reduced expression of involucrin, keratin 10 and filaggrin.

In summary, our work identifies LRRC8A as an essential molecule during keratinocyte differentiation, with its main function downstream of keratinocyte stem cells. LRRC8A protein could represent an important switch to control the transition from proliferation to differentiation. We are currently investigating the molecular mechanisms through which LRRC8 mediates this effect. We hypothesize that inflammation-dependent LRRC8 reduction could affect the membrane potential, which in turn impairs the activation of voltage-dependent Ca2+ channels that are important for controlled differentiation. In addition, the mTOR cascade might represent the molecular mediator as we previously described that mTORC1 also represents a switch between proliferation and differentiation and was shown to be downstream of LRR domain containing proteins in other cell types. Thus, LRRC8 might represent an attractive therapeutic target to restore proper epidermal maturation.

P019 | Beneficial effects of a plant extract mix in a psoriasis-like in vitro model

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Psoriasis vulgaris is a common immune-mediated inflammatory skin disorder with a strong negative impact on the quality of life of the patients. Psoriasis manifests in painful, itchy and dry abnormal skin areas which are characterized by skin barrier defects, epidermal hyperproliferation and inflammation. Long-term use of current topical therapeutics is a common treatment option of mild psoriasis but may have unpleasant adverse effects. Thus, additional novel topical compounds with a better long-term safety are needed. Plant extracts with their high antioxidant power and anti-inflammatory effects may therefore be an interesting source for such a compound.

To evaluate the potential anti-inflammatory effects of a standardized plant extract mix we used a 2D in vitro model to mimic a psoriasis inflammatory milieu. To this end, we used differentiated normal human keratinocytes and stimulated them with the psoriasis-associated cytokines interleukin (IL)-22, IL-17A, tumor necrosis factor (TNF)-alpha and IL-1beta. The gene expression pattern in this psoriasislike in vitro model reflected the typical conditions in psoriatic skin: inflammation was demonstrated by the enhanced gene expression of psoriasis-associated inflammation markers such as TNF-alpha, IL-17C, IL-6, IL-1 beta, S100A7 (psoriasin) and human beta-defensin (hBD)-2. The skin barrier changes were demonstrated by a downregulated gene expression of the barrier molecules filaggrin and loricrin. This in vitro 2D-model was then stimulated with the plant extract mix for 20 h. Subsequently, RNA was isolated and real-time PCR was used to measure the effects of the plant extract on the relative expression of psoriasis-relevant genes.

As a result, stimulation of the psoriasis-like in vitro 2D-model with our plant extract mix restored the gene expression pattern to the non-inflammatory state of cells without cytokine treatment. In detail, the plant extract mix downregulated the gene expression of the inflammation markers to normal levels. Accordingly, gene expression of the skin barrier molecules were upregulated to normal levels too. Together, these results show that the plant extract mix used in this study, has beneficial effects in a psoriasis-like 2D-in vitro model by inhibiting the gene expression of different inflammation markers and by restoring the gene expression of skin barrier molecules. Consequently, the plant extract mix may be a promising and safe new candidate for the topical treatment of psoriasis patients. Further studies including in vivo studies are necessary to evaluate this hypothesis.

P020 (OP01/02) | The transcription factor CEBPB drives lichenoid skin inflammation by mediating apoptosis, IFN-γ signaling and favoring the secretion of type 1- related inflammatory factors

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In chronic inflammatory skin diseases (CISD), different transcription factors have emerged as crucial players in the pathogenesis. In previous work, we have identified the CCAAT/enhancer-binding protein beta (CEBPB) to be involved in skin inflammation and have demonstrated its central role in the pathogenic epithelial response in Psoriasis. Here, we aimed at dissecting the role of CEBPB in type 1 skin inflammation.

In spatial transcriptomics, bulk RNASeq and immunohistochemistry, CEBPB was significantly upregulated in lesional skin of Lichen planus (LP) patients compared to non-lesional skin. Similarly, in vitro stimulated primary human keratinocytes showed significant CEBPB induction with Lichen-relevant cytokines, as well as with lesional T-cell supernatant derived from Lichen patients. Bulk RNASeq of CEBPB knockout (KO) keratinocytes revealed regulation of various disease-relevant pathways, such as different cell death pathways, IL-1 family signaling and IFN-γ response.

Using 3D Lichen skin models, we show that CEBPB KO inhibited apoptosis, indicating its involvement in cell death mediation under type 1 inflammatory conditions. Consistent with the described CEBPB role in acanthosis, also here the thickness of the CEBPB KO models was reduced. Moreover, CEBPB-KO significantly reduced the secreted IL-1ß levels, while increasing cell viability. The downregulation of various apoptosis markers was also confirmed in Western blot analysis.

In line, we demonstrate a positive correlation between the lesional expression of CEBPB and the clinical score of interface dermatitis in Lichen patients. Additionally, we generated an extensive CEBPB target gene signature under type 1 inflammatory conditions showing a downregulation of various disease markers, mainly IFN-γ response genes, as well as inflammatory cytokines and chemokines, in the knockout. Finally, this CEBPB gene signature could also be traced back in Lichen patients.

In summary, we assign CEBPB a critical role in regulating cell viability, promoting apoptosis under type 1 conditions and driving the expression of various diseaserelevant factors in keratinocytes, hence contributing to the clinical manifestation of interface dermatitis in lichenoid skin diseases.

P021 | Genome-scale investigation of drug-induced termination codon-readthrough in a model system of epidermolysis bullosa

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Ribosome profiling, an advanced NGS technology, measures ribosome protected footprint (RPF) abundance and identifies active translation with nucleotide resolution in annotated protein coding and non-coding regions. Ribosome profiling can therefore detect drug-induced translational readthrough (DITR) events at premature termination codons (PTCs) as a consequence of a nonsense mutation in the coding sequence (CDS) of a given gene and at normal termination codons (NTCs) as well as perturbations to the translatome in general. Using ribosome profiling, we describe the global effects of DITR by a well-known inducer, the aminoglycoside antibiotic gentamicin sulfate, in an HaCaT model system of junctional EB as well as in immortalized recessive dystrophic EB patient cells, since global investigation of DITR at NTCs has not been addressed in previous studies.

Using an adaptation of the original ribosome profiling protocol invented by Ingolia et al. (2011) and applying freely available modern software packages specifically designed for use with ribosome profiling data, we detected translation readthrough at the respective PTCs in both models studied after 24 hours of treatment with 500 µg/mL gentamicin sulfate compared to untreated control cells. However, translation readthrough at NTCs was observed in all cell lines after 24 hours of treatment with 500 µg/mL gentamicin sulfate, regardless of the model examined compared to untreated control cells. In addition, we noticed abundant translation in presumably noncoding regions of the human genome, indicating the expression of novel microproteins that have remained undetected in the past.

Our data highlight at the genomic level the effects of DITR with gentamicin sulfate in the context of EB and should inform its further development as therapeutic intervention in severe types of EB. The identification of readthrough at NTCs may explain the frequently observed side effects, as gentamicin sulfate is already used in the clinic. Observation of translation in noncoding regions may play a yet unknown critical role in skin homeostasis and will be validated by further functional studies in the near future.

Keywords: epidermolysis bullosa, readthrough, translation, ribosome profiling, translatomics

P022 | Comparison of different senolytics for treatment of cellular senescence in human skin biopsies

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Cellular senescence is a physiological mechanism by which cells enter stable cell cycle arrest while remaining metabolically active. This process is an important mechanism for preventing malignant transformation of damaged cells. While cellular senescence initially prevents cancer, the accumulation of senescent cells over time leads to chronic inflammation in tissues, promoting the development of cancer (including metastases) and other age-related diseases. Against this background, the targeted elimination of senescent cells to prevent, delay or improve age-related diseases including cancer is being discussed and investigated. Senotherapeutic polyphenols are present in many fruits and vegetables including strawberries and capers and can selectively induce apoptosis in senescent cells, potentially reducing the risk for cancer development. In a prospective intervention study including 52 volunteers, we collected two skin biopsies before and one after a 10-week food intervention. In addition to the comparison of skin tissue before and after intervention one skin sample was also treated ex vivo with the senotherapeutics

quercetin and dasatinib. Furthermore, using another collection of skin samples different senolytic drugs and combinations were compared. Preliminary analyses of senescent markers (CDKN2A, TP53BP1) revealed a reduced gene expression of these markers 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. 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.

P023 | The adaptive response of old ABCB5+ MSCs is changed upon exposure to LPS

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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 neighbourhood and endogenous tissue niche. Upon exposure of MSCs with infection mimicking lipopolysaccharide (LPS), 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 increases. Therefore, I address the question whether MSCs from old healthy donors (>65 years) unlike young healthy donors (<30 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 and an impressive increase of p-p65 in ABCB5+ MSCs from elderly adults was observed when compared to young individuals. This correlates with a higher and longer persisting expression of NF-B target genes and corresponding proteins like IL-6 in MSCs of elderly individuals compared to young individuals. Notably, ABCB5+ MSCs from young donors point out higher expression levels of IL-8 and the anti-inflammatory cytokine IL-10 compared to old donors. Furthermore, ABCB5+ MSCs from young donors express the anti-microbial peptide LL-37 in comparison to 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 LPS-concentration-dependent compared to 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. Collectively, ABCB5+ MSCs from old individuals reveal a dysregulated antibacterial response which is supported by an impressively reduced killing ability of gram negative bacteria and at least in part explained the higher susceptibility for severe bacterial infections in elderly.

P024 | SnoRNAs alter fibroblast physiology through rRNA methylation

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The epitranscriptome of ribosomal RNA changes in different cellular states and has effects on cellular behavior. Differences in the methylation pattern, guided by snoRNAs, between proliferating and non-proliferating cells might be a contributing factor to the changes in cell physiology.

This study aims to elucidate how snoRNAs can change the physiology of primary human dermal fibroblasts. Particularly we focus on alterations of the 2'-O-methylation patterns of ribosomal RNA and their effects on cells.

Using RiboMethSeq we compared the 2´-O-methylation profiles of ribosomal RNA of quiescent and senescent to proliferating fibroblasts. We then investigated the levels of snoRNAs that guide fibrillarin to differently methylated sites via qPCR.

To investigate possible differences in cellular physiology due to different methylation profiles, we changed the methylation levels of proliferating fibroblasts at these differently methylated sites by knock-down with Antisense Oligonucleotides or overexpression of the specific guiding snoRNAs.

Several differentially methylated sites, either hyper- or hypo-modified, were present in the rRNA of senescent and quiescent compared to proliferating cells. Methylation levels showed a correlation with the expression of snoRNAs. By inhibiting or overexpressing specific snoRNAs, we confirmed the causality of these modifications by analyzing cell physiology.

Our combined data suggest that even subtle modifications of the ribosomal RNA might have profound and precise effects on cellular physiology and contribute to the heterogeneity of ribosomes. By modulating the available amount of snoRNAs we could emulate changes between proliferating and non-proliferating cells.

Chemokines/Cytokines

P025 | Diagnosing hand eczema by epidermal proteomics

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Background: Skin inflammation in the palmoplantar area can often be challenging to diagnose even for experienced dermatologists if this is the only body location affected. Immunohistology is not always practical in these sensitive site nor helpful in distinguishing with certainty between psoriasiform eczema or eczematised psoriasis. Furthermore, mixed subtypes exist and eczema subtypes are difficult to distinguish by morphology alone.

Rational: Given that hand dermatitis has a severe impact on quality of life and that therapeutic options are increasingly disease specific there is an urgent need for better and easier to perform diagnostics. In a previous study, we were able to show that epidermal IL-36γ is a reliable marker to differentiate psoriasis from atopic eczema. In addition, CCL17 is recognized as a biomarker for atopic dermatitis.

Methods: We analysed 9 patients with known atopic dermatitis and a flare of hand eczema and 6 patients with known psoriasis showing palmoplantar involvement. We analysed epidermal samples and blood serum of the same patient for epidermal disease biomarkers. Tape stripping was performed on lesional and nonlesional skin (ventral forearm) and protein was extracted for ELISA analysis.

Results: Overall IL-36γ expression was 3 fold higher in the psoriasis palmoplantar lesions as compared to eczema lesions. By contrast CCL17 was 2.2 fold higher in eczema as compared to psoriasis. We observed by far the highest expression of IL-36γ in lesions with pustular psoriasis morphology. However, compared to previous results on non palmoplantar skin we observed a more mixed pattern in both groups expressing both CCL17 and IL-36γ pointing to some overlap between psoriatic and eczematous epidermal responses.

Conclusion: Our results confirm IL-36γ and CCL17 as valid biomarkers for psoriatic versus eczema lesions in the palmoplantar skin. However, our preliminary results point to the existence of overlap molecular subtypes which has previously been described. It therefore seems necessary to further define subtypes of hand dermatitis and to combine several biomarkers for accurate diagnosis.

P026 | Development of an in vitro model for atopic dermatitis and evaluation of a novel therapeutic concept

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Atopic dermatitis (AD) is one of the most common chronic inflammatory skin diseases and is associated with a high physical and psychological burden for patients. Despite progress in the elucidation of the pathogenesis and the development of novel 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. Such models are readily available using primary human keratinocytes (NHEKs) that are limited by availability, have a short life-span and display donor-specific variations. To overcome these shortcomings, we aimed at developing a three-dimensional tissue model for atopic dermatitis using immortalized cell lines to study the natural compound curcumin as an example for preclinically testing novel therapeutic concepts.

We validated immortalized cell lines that were either generated by the expression of SV40 large T antigen and hTERT or HPV E6/E7 for their use in inflammatory in vitro epidermal models. Both cell lines reconstituted a stratified epidermis, which was thinner than in models from NHEK. Nevertheless, the epidermal barrier was fully functional as Lucifer yellow was not able to penetrate and transepidermal resistance (TEER) values and expression of tight junction markers were similar to NHEKs.

By adding a cocktail of cytokines with a role in AD, such as IL-4, IL-13, IL-31 and TNF-α we were able to induce an inflammatory phenotype in the reconstituted epidermal models, as indicated by reduced TEER values and signs of spongiosis. The inflammatory state of the 3D models was also evident on the molecular level, as the transcription factor Stat6 was clearly activated. Thus, our data demonstrate that immortalized keratinocytes are suitable substitutes for primary cells and have the potential to be used as inflammatory disease models through treatment with proinflammatory cytokines.

Concerning the applicability of a photodynamic treatment of curcumin and visible light for the AD treatment we treated NHEK and hTERT 2D cultures with the above specified cocktail of AD related cytokines with and without low curcumin concentrations followed by irradiation with visible light (VIS). We observed a clear reduction of the AD cytokine related inflammatory status of curcumin/VIS treated cells. The secretion of pro-inflammatory cytokines as well as the phosphorylation of TYK2 and STAT6 were distinctly reduced. Our data suggest that the photodynamic treatment regime shows favorable properties that will be further validated in the 3D models.

P027 | IL-9 promotes a pathogenic phenotype and enhances proliferation in skintropic Th cells

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IL-9 is a pleiotropic cytokine, for which an overarching role in humans remains elusive. Both, interleukin 9 (IL-9) and its receptor, IL-9R, are predominantly expressed by skin-tropic T helper 2 (Th2) cells. This suggests that autocrine or paracrine IL-9 signals play an important role in cutaneous immunity and allergy. Yet, the mechanism of action remains incompletely understood.

Here, we aimed at deciphering the effect of IL-9 signals on Th cells in allergic skin inflammation. To this end, we isolated human Th cells from acute atopic contact dermatitis biopsies, expressing high levels of IL-9R. Transcriptional profiling showed that approx. 800 genes are differentially expressed in response to IL-9. Pathway analysis revealed that upregulated genes are associated with conventional Th2 immune response. Strikingly, we observed a strong induction of genes specifically associated with the pathogenic Th2 phenotype, such as IL9, IL17RB and HPGDS. In addition, we found that IL-9-stimulated Th cells showed a coordinated induction of genes involved in aerobic glycolysis. At the protein level, we confirmed that IL-9 prominently induced the expression of the monocarboxylate transporter 1 (MCT1), which is key for the export of lactate, the end product of aerobic glycolysis. As a functional consequence, IL-9 boosted glycolytic capacity and cell proliferation, which was abolished by MCT1 inhibition.

Together, our data delineates that IL-9 not only promotes pathogenic features, but also provides a proliferative advantage by promoting aerobic glycolysis in a subset of skin-tropic Th cells. These unrecognized roles of IL-9 might open up novel strategies for targeted manipulation of Th cells in allergic skin inflammation.

P028 | Endotypes of atopic dermatitis- Insights from a transcriptome analysis of three-dimensional skin equivalents stimulated with supernatant of lesional Tcells

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Atopic dermatitis (AD) is a highly heterogenous chronic inflammatory skin disease presenting with various clinical phenotypes. Due to the complexity of the disease, these phenotypes are not well characterized, and a deeper molecular understanding is necessary. The objective of this study is to characterize the secretome of lesional AD T-cells and to analyze the molecular response profile of 3D human skin equivalents upon stimulation with these secreted factors.

T-cells were isolated from eczematous skin lesions from patients (n = 7) with atopic dermatitis. Tcell supernatant (TCSN) was generated and analyzed via ELISA and Luminex. Primary human keratinocytes were cultured in an air liquid interface (3D) and stimulated with the pooled TCSN to mimic different endotypes of AD. Histology and RNA Sequencing was performed.

According to ELISA analysis, two endotype groups of T cell supernatants were defined, ADTCSN with (w) IFN-γ and AD-TCSN without (wo) IFN-γ. Luminex analysis revealed quantitative differences between the supernatants for IL-4, IL-2, and IL-8. Sequencing analysis revealed two distinct molecular signatures for both endotypes. Keratinocytes stimulated with AD-TCSN wo IFNy showed functional enrichment in pathways (STRING database) associated with extracellular matrix development. In contrast, top 10 functional enriched pathways of the group AD-TCSN w IFNy displayed pathways related to broad activation of the native and the adaptive immune system.

In conclusion, endotypes defined by the secretome of T-cells and individual cytokine compositions in skin lesions contribute to different barrier- and immunological hallmarks of AD. Complex cytokine interactions in the cytokine environment in skin lesions are not fully understood, and further research is needed to investigate the relevance for therapeutic options.

Clinical research

P029 | Anti-IL-23 treatment in patients with omalizumab-refractory chronic spontaneous urticaria – A case series

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Introduction: Chronic spontaneous urticaria (CSU) is a common skin disorder characterized by the occurrence of itchy wheals, angioedema or both for more than six weeks, which significantly impacts health-related quality of life. Standard guideline-recommended treatment includes antihistamines and omalizumab, an anti-IgE antibody. However, the majority of CSU patients are resistant to H1-antihistamines, and many of them do not achieve complete control with omalizumab. Thus, there is a high unmet need for better treatment options. CSU is a mast cell-mediated disease and underlying autoimmune mechanisms include IgE autoantibodies (autoallergic CSU) and IgG anti-IgE/FcεRI antibodies (autoimmune CSU). Recent data suggest that the IL-23/IL-17 axis is involved in the pathogenesis of CSU, especially autoimmune CSU, which shows poor and slow response to omalizumab treatment. Serum IL-23 levels are significantly elevated in patients with CSU

compared to healthy controls and are positively correlated with disease activity. In addition, IL-23 serum levels are higher in patients with a positive autologous serum skin test, a marker of autoimmune CSU.

Objective: To investigate the effects of tildrakizumab, an anti-IL-23 monoclonal antibody, in patients with omalizumab-refractory CSU.

Methods: Three patients with high CSU disease activity despite treatment with quadrupledosed antihistamines and omalizumab received three doses of tildrakizumab (at week 0, 4 and 12) as off-label treatment with informed consent. Treatment with antihistamines in up to 4-fold doses was continued. Assessment of Urticaria Control Test (UCT), Urticaria Activity Score 7 (UAS7) and Dermatology Life Quality Index (DLQI) was performed.

Results: All three patients had very low IgE levels (<10 U/ml), and two had elevated antithyroid peroxidase IgG antibodies, suggesting that they had autoimmune CSU. CRP levels, differential blood count, and further immunoglobulin levels were normal in all patients. Before initiation of tildrakizumab treatment, patients had moderate to severe CSU with UAS7 [0-42] scores of 16, 28, and 8; poor disease control with UCT scores of 8, 8, and 5 [0-16, higher scores indicate better control]; and poor quality of life, with DLQI scores of 5, 12, and 19. Treatment with tildrakizumab led to an improvement of disease activity and control in all three patients with UAS7 scores of 13 (−3), 12 (−16), and 2 (−6), and UCT scores of 11 (+3), 12 (+4), and 13 (+8). In addition, improvement of quality of life was observed in all three patients (DLQI: 4, 1, 0). Tildrakizumab was well tolerated, and no adverse events occurred.

Conclusion: Omalizumab-refractory CSU patients are exceptionally difficult to treat. Here, three of three patients treated with tildrakizumab showed improvement of clinical symptoms and quality of life, indicating that IL-23 is involved in the pathogenesis of CSU. Targeting IL-23 should be further explored in clinical trials with larger cohorts of CSU patients.

P030 | Kinome analysis of skin from mastocytosis patients offers new opportunities for treatment

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Mastocytosis is a group of rare neoplasias where an accumulation of mast cells (MC) in one or more organs occurs, often accompanied by mast cell activation. In the majority of patients (85%–90%), an activating somatic mutation of the tyrosine kinase receptor KIT, the KIT-D816V mutation, is found, causing ligand independent activation of mast cells. Other mutations found in mastocytosis may be related to the same gene, may be absent in KIT or may affect additional genes, underscoring the enormous pathophysiological complexity of this disease. The increase of mast cells and mediator release manifests mainly in the bone marrow and skin, but also in the gastrointestinal tract, lymph nodes, spleen or bones.

Since standard treatment regimen with mast cell stabilizators or antihistamines are often insufficient and kit-targeting drugs are either for advanced mastocytosis or show primary resistance, there is a pressing need to identify new treatment options.

Monitoring kinase activities from multiple signaling pathways simultaneously is an approach to identify appropriate targets of interest for personalized medicine. In mastocytosis, promising targets may extend from the mutated KIT gene to its downstream signaling pathways. Our strategy to infer kinase activities of interest involved a multiplex kinome analysis with samples of healthy and lesional skin from 9–10 patients with cutaneous mastocytosis using the PamGene technology.

By analyzing overall tyrosine (Tyr) as well as serine/threonine (Ser/Thr) kinase activities within protein lysates on peptide microarrays, we could show a significant increase of phosphorylation of JAK1, Pim kinases (Pim1-3) and of additional protein kinases such as CaMKIIα; PKBα, β; PKCα,δ; PKAα, PKG1,2. However, by comparing the individual kinome between patients, there was a remarkable variability in kinase activities and specificities.

Together, this multiplex kinase analysis showed an activation profiling essential for the development of targeted therapy options and furthermore highlighted individual kinome specificities of patients which underlines the importance of a patient-specific therapy for mastocytosis.

P031 | Quantification of anti-microbial peptides using a novel multi-plex assay to stratify atopic dermatitis patients

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Atopic dermatitis is a chronic inflammatory skin disease characterized by itchy, scaly, and dry skin, and has a higher onset rate at children's age compared to adulthood. Identification of biomarkers to predict therapy response requires biomaterial sampled from patients. While sampling of biomaterial from adults is feasible, biomaterial sampling from children should be limited to easy-to-access non-invasive methods. Tape stripping provides such a non-invasive method to collect skin surface samples.

Hallmarks of the pathophysiology of atopic dermatitis are elevated levels of IL-4, IL-13, and IL-31, as well as TARC and TSLP. Stimulation of keratinocytes with type 2 cytokines results in altered anti-microbial peptide expression, resulting in decreased anti-microbial peptide levels and increased risk of skin infections. Since anti-microbial peptides are directly linked to underlying immune profiles, we hypothesized that anti-microbial peptides can be used as potential biomarkers to predict treatment outcome. Therefore, we collected anti-microbial peptides using tape strips from atopic

dermatitis patients and healthy controls. For analysis we developed a multiplex assay, which quantifies hBD2, hBD3, S100A7, S100A8/9, RNAse7, LL-37, dermicidin, and GAPDH in parallel. To analyze its potential for comparative quantification at different locations, we sampled tape strips at the University-Hospital Schleswig-Holstein and at the 1st Hospital Riga. After validation of the assay, we found marked alterations in hBD2, hBD3, RNAse7, and S100A8/9 levels in skin of patients with atopic dermatitis as compared to healthy controls. Further, we correlated concentrations with severity of disease, as measured by EASI and SCORAD.

In sum, we show that is possible to quantify anti-microbial peptide concentrations on the skin of AD patients using our novel multiplex assay, potentially stratify cohorts based on anti-microbial peptide profiles and quantification might function as a potential biomarker to predict treatment response. In addition, inter-institute comparability will provide standardization of sampling and analysis. However, and most importantly, the non-invasive sampling method results in improved analysis options for children.

P032 | Electrical impedance spectroscopy is less influenced by daily routine activities than transepidermal water loss in skin barrier function assessment

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Background: Transepidermal water loss (TEWL) measurements are commonly used for clinical assessment of epidermal barrier function. An important limitation of this method is the fact that intrinsic and extrinsic factors interfere with these measurements. Electrical impedance spectroscopy (EIS) is a lesser-established method, which reflects epidermal and dermal barrier function. Effects of daily routine activities on EIS in relation to TEWL remain elusive.

Objectives: To evaluate the effect size of daily routine activities on TEWL and EIS, as well as their correlation with age and anatomical differences.

Methods: Healthy participants (n = 31) were stratified into three age groups (18–29, 30–49, and ≥50 years). In a climate-controlled room, TEWL measurements and EIS were performed on the left and right volar forearm and abdomen. The effect of body cream application, skin washing, exercise, and coffee intake on TEWL and EIS was evaluated at different time intervals.

Results: Body cream application decreased TEWL and EIS values after 15 and 90 minutes. Skin washing decreased TEWL for 15 minutes and EIS values for 15 and at least 90 minutes. TEWL was increased 5 minutes after moderate to intense exercise. Coffee intake increased TEWL on the abdomen after 60 minutes. TEWL and EIS values did not correlate with participants' age and no anatomical differences were observed.

Conclusions: Body cream application should be avoided at least 90 minutes prior to measurements of TEWL and EIS. Skin washing should also be avoided at least 90 minutes prior to EIS. Skin washing, exercise, and coffee intake should be avoided directly before TEWL measurements. EIS may be a promising tool for skin barrier function assessment and is less affected by daily routine activities than TEWL.

P033 | Microbiome characterization of hidradenitis suppurativa reveals a dysbiotic skin microbiome improved upon clindamycin/rifampicin therapy

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Hidradenitis suppurativa (HS) is a painful, chronic inflammatory skin disease characterized by deep seated painful nodules, abscesses, sinus tracts and scarring. Although HS pathogenesis is not fully understood, cutaneous microbiome dysbiosis is supposed to contribute to inflammation and disease progression. Antimicrobial therapy using clindamycin/rifampicin is one of the mainstays of HS management. More recently, the TNF-alpha blocking antibody adalimumab became the first approved HS therapy. We evaluate here the consequences of these two commonly used treatments on HS skin microbiome. Skin swabs were collected from 42 patients and 38 healthy matched controls to better characterize the disease dysbiosis. Then, we investigated in a sub-cohort of 20 patients the effects of adalimumab (n = 12) and clindamycin/rifampicin systemic therapy (n = 8) on HS microbiome. The samples were analyzed using the 16S rRNA gene sequencing approach. Our data showed that HS skin exhibits a distinct microbiome profile characterized by an expansion of pathogens from the Peptoniphilus genus, in addition to Corynebacterium urealyticus and Finegoldia magna at the cost of beneficial commensals, as Staphylococcus hominis, Cutibacterium acnes and Staphylococcus epidermidis. In contrast to adalimumab, antibiotic therapy shifted the microbiome composition of both non-lesional and lesional HS sites toward the control group and moreover increased their richness and the proportion of commensals, as Corynebacterium tuberculostearicum. We report here for the first time on the effects of adalimumab and antibiotics on HS skin microbiome demonstrating an improvement of the HS dysbiosis upon a systemic antibiotic therapy. An accurate characterization of the HS microbiome and its functional consequences would clearly improve our understanding of its role in disease pathogenesis and facilitate the development of new therapeutic strategies.

P034 | OCTOLAB – OCT optimized diagnosis and laser treatment of basal cell carcinomas in a closed-loop guided by artificial intelligence

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Introduction: In this study, basal cell carcinoma (BCC) were imaged with optical coherence tomography (OCT) assessing parameters such as subtype and tumor thickness to evaluate their possible impact on Nd:YAG laser therapy efficacy. The study aim was to optimize treatment parameters of the tumors with regard to minimal side effects. The presented findings were essential requirements in pursuit of a comprehensive approach to achieve a closed loop device with an integrated OCT as diagnostic tool into a Nd:YAG laser for BCC therapy.

Objectives: Realization of a closed-loop system for artificial intelligence (AI)-based automatic OCT diagnosis and simultaneous laser therapy of BCC.

Materials and Method: A Telesto OCT system by Thorlabs and a long-pulsed Nd:YAG laser by Hypertech Laser Systems GmbH were used as the base units. The Medical Laser Center Lübeck developed a handpiece as a fusion of OCT optic and laser handheld. Clinicians annotated OCT images regarding BCC diagnosis, subtype and tumor thickness. These informations were used to train the AI algorithm for the automatic assessment of BCC parameters in the future. Medical computer scientists calculated the confusion matrix from the test data for validation and evaluation.

Results: Until now, a Nd:YAG laser device with by a software for setting individual BCC laser parameters, was produced by HLS Hypertech Laser Systems GmbH. Depending on the tumor size, the laser spot can be varied. An OCT device with the special handpiece for the combination of OCT and Nd:YAG laser was built and is currently undergoing tests. Clinical validation and evaluation of the artificial intelligence algorithm, as well as further testing of the combined OCT-laser system will be pursued.

Conclusions: The realization of an AI-guided closed loop device for BCC diagnosis and therapy is a novel innovation and enables a comprehensive approach to optimize laser settings based upon BCC parameters obtained from OCT imaging. The fusion of both devices may allow advantages in individualized BCC treatment, eventually.

P035 | Prospective analysis of detailed body composition patterns in a cohort of patients with advanced cutaneous melanoma reveal novel predictive immunometabolic markers for survival

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Overweight and obesity are associated with increased risks of certain types of cancer and obesity-associated low-grade inflammation (metaflammation) as well as consecutively disturbed cancer-immune surveillance are suspected as important contributing mechanisms. On the other hand, metaflammation, which is particularly associated with visceral enrichment of adipose tissue, might also affect immunotherapy outcomes in established cancers as recently suggested by retrospective analyses. However, in-depth characterization of body composition and tissue distribution patterns before and during cancer immunotherapy is lacking.

In this study we aimed to identify host-derived immunometabolic biomarkers in melanoma patients with prognostic value for checkpoint inhibition immunotherapy (CI) response. We prospectively analyzed detailed body composition and immunometabolic parameters in a total of 54 consecutive patients with advanced cutaneous melanoma before and three months after CI initiation and a clinical followup of two years. Anthropometric data (weight, waist-to-height ratio (WtHR) and body mass index (BMI)) together with low-grade inflammation markers (hsCRP, leucocyte count and neutrophil-lymphocyte-ratio (NLR)) were assessed before treatment (T1) and after 3 months of immunotherapy (T2). Additionally, we determined body composition patters with regard to adipose tissue and muscle mass by bioelectrical impedance analysis (BIA) as well as routine CT scan segmentation analysis at the level of lumbar spine 3.

In our cohort, serum markers for inflammation (CRP, leucocytes, neutrophils, NLR) were significantly associated with survival and can be considered prognostically unfavorable (HR > 1).

Body composition and metabolic parameters (energy consumption, fat-free mass, muscle mass, body water) significantly influenced outcome under treatment with CI. Patients with reduced muscle mass (sarcopenia) showed shorter progression-free survival (PFS) (p-value 0.01).

Patients with lower phase angle (indicator of overall health, metabolic activity, nutritional status) presented with shorter PFS (p-value 0.013). We could observe sexspecific effects.

CT scan derived body composition analyses significantly correlated with a number of parameters that determine metabolic distress and

therefore might be of value in future analyses of immunotherapeutic studies.

Thus, we can show that BIA can function as a novel tool for measuring immunotherapeutic consequences on metabolism and to predict survival in melanoma patients and our prospective observational pilot study data suggest that metaflammation might affect anti-PD-1 immunotherapy outcomes.

P036 | An oil-in-water emulsion containing a combination of a lipohilic Zingiber officinale root extract and cannabidiol ameliorated symptoms of dry and eczema-prone skin and exhibited antipruritic properties in vivo

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After having shown anti-inflammatory (release of IL-6, IL-8, TNF-α, CCL20, and PGE2, NF-κB transcriptional activity) and antioxidative (8-isoprostane formation, ROS formation, NO production) activity in primary cells (NHEK, NHDF) and cell lines (HaCaT, RAW macrophages) in vitro, a lipophilic Zingiber officinale root extract and CBD were selected for further development. The observed effects may be mediated by modulation of the endocannabinoid system (ECS). A positive modulation of the ECS e.g. by inhibition of fatty acid amide hydrolase (FAAH) can exert antiinflammatory and antipruritic actions in vitro and in vivo. Such effects of modulating the cutaneous ECS in inflammatory and pruritic skin diseases are also known from the literature. Therefore, a newly developed oil-in-water (O/W) emulsion containing CBD and a lipophilic Zingiber officinale root extract was investigated in a clinical study.

Included subjects (number/age 24.5 20.3 years; $N = 44$ (22 adults and 22 children)) had a history of atopic eczema with itching scores in the test area of at least 1. Efficacy and tolerability of the O/W emulsion were evaluated over 6 days. Transepidermal water loss (TEWL), skin hydration, and skin color were measured at baseline and on day 6. Additionally, skin condition (i.a. erythema, dryness; 3-point score) was assessed by a dermatologist on the same days and pruritus was rated by the subjects or the subjects' parents/legal guardians on a daily basis using a Numeric Rating Scale (NRS-11; score: 0 = no itch; 10 = worst imaginable itch).

The product was very well tolerated. A statistically significant reduction of erythema (−0.67 0.72, $p < 0.0001$) and skin dryness (−1.08 0.70, $p < 0.0001$) was observed for all subjects. TEWL was decreased significantly (−4.68 7.57 g/(m2h), $p = 0.0002$) whereas skin moisture increased statistically significantly until end of the study (+7.12 9.81 a.u., $p < 0.0001$). The subjective evaluation of itch showed a continuous decrease in itching scores throughout the course of the study compared to baseline. Mean NRS-11 values for itch went down from 5.14 2.08 (day 1) to 2.30 2.14 (day 6). A statistically significant reduction of itching score has already been reached on day 2 (−0.84 1.26, $p < 0.0001$).

In summary, application of a CBD- and Zingiber officinale root extract-containing O/W emulsion to patients suffering from very dry or eczema-prone skin improved skin barrier function and skin hydration as well as the symptoms erythema and pruritus significantly while exhibiting a very good tolerability profile.

P037 | Prognostic value of coagulatory markers in cutaneous lymphoma

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Background: In cancer patients the risk of venous thrombosis or thromboembolic events is increased significantly over healthy individuals. Patients with hematologic malignancies such as lymphoma, especially non-Hodgkin lymphoma, have been described to display a significantly increased risk of thromboembolic events as well. Coagulatory markers such as von Willebrand factor (vWF), ADAMTS13 and D-dimers can be used to predict survival and prognosis of cancer patients.

The aim of this study was to assess the prognostic predictive power of blood-based coagulatory markers for disease progression of cutaneous lymphoma.

Methods: Blood-based assays including ELISA for vWF and for the activity of ADAMTS13 were used using a newly established biobank with cutaneous lymphoma patients treated at the University Skin Cancer Hamburg. The concentration of Ddimers was measured in routine laboratory. In addition, ThroLy and Wells score as well as previous thromboembolic events and anticoagulant medication were documented in all patients.

Results: Blood samples from 78 patients with cutaneous lymphoma were analyzed, including 67 with T-cell lymphoma/Sézary syndrome and 11 patients with B-cell lymphoma. Significantly higher concentrations of vWF were measured in patients with cutaneous lymphoma than in healthy controls ($p < 0.05$). No significant difference was observed in the activity measurement of ADAMTS13 between patients with cutaneous lymphoma and healthy controls. Median D-dimers blood concentration was elevated above upper normal limit in patients with cutaneous lymphoma (0.74 mg/l).

Conclusion: Elevated vWF values indicate an increased risk of thromboembolic events. Coagulatory factors such as vWF and D-dimers may be useful as predictive biomarkers for disease progression of cutaneous lymphoma patients.

P038 | Potentials of molecular differentiation between psoriasis and eczema in occupational dermatology

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The differentiation of psoriasis and eczema poses major diagnostic challenges even for experienced dermatologists. Eczematoid psoriasis or psoriasiform eczemas can make a clinical diagnosis especially difficult. These unspecific clinical pictures also complicates dermatohistopathological analysis, which does not always contribute to the reliable differentiation. With both macroscopical and microscopical diagnostics being insufficient, modern dermatology is in need of a new diagnostic tool.

Psoriasis and eczema were found to regulate the genes CCL27 and NOS2 differently in the respective diseases. These genes were used to train a classifier that showed accuracies averaging nearly 100% in test cohorts.

The newly developed Molecular Classifier (MC) offers the possibility to determine the probability of the presence of psoriasis or eczema, thus providing new insights for the diagnostic process.

Since 2021 a cohort of 282 occupational dermatology patients is being established. In 4 follow-ups over 3 years data on the course and severity of the skin disease, therapies, occupational retention and quality of life will be collected and later compared to an existing, occupational cohort where the MC was not used.

By September 2022, 256 patients (men: 136, women: 120, range_age: 21–70 years, median_age: 54 years) were included. A skin disease persisted in mean for 6.7 years. 35.8% of the patients reported a longer disease course of up to 48 years. These old cases are of special interest, since long lasting disease can affect not only the individual quality of life but also social aspects that touch society as a whole, such as high rates of sick leave, shortened working lifetime and disability pensions. A decisive factor for therapeutic success is therefore a confirmed diagnosis and a following, specific treatment.

However, for 38.2% of 233 patients the clinical diagnosis was unsure. Dermatologists diagnosed psoriasis in 24.5% and eczema in 37.3% of the cases. In the same population the MC showed a clear tendency towards psoriasis in 25.3%, towards eczema in 67.4% of the cases; only 7.3% remained unclear. In a direct comparison the congruence between dermatologists and MC results was only 36.9%. By using the MC, a clear tendency towards one of the two disease could be shown in over 98% of the cases. The results did not show relevant discrepancies between patients with longer and shorter persisting skin diseases.

Results of a histological examination were available for 121 patients, diagnosing eczema in 50.4%, psoriasis in 29.8% and being unsure in 19.8% of the cases. In 45.3% of the cases, this provided a different diagnosis compared to the clinical diagnosis of the dermatologist. Dermatohistopathology and Molecular Classifier were congruent in 36.9% cases only. Again there were no differences when stratified for disease duration.

An overall congruence between all three instances of dermatologist, pathologist and Classifier was only observed in 18.54% (n = 21) of the cases and underlines the diversity and challenges of differentiation of the two disease patterns.

One year after inclusion in the cohort, the patient's dermatologists increasingly tend to support the MCs assessment. At baseline, the proportion of congruent clinical diagnoses was 58.8% for eczema and increased up to 76.5% after 12 months. For psoriasis, dermatologists agreed on the MC at baseline in only 23% of the cases. This strongly increased up to 61.5% in the first year.

The current status shows clear benefits of the MC in the diagnostic of psoriasis and eczema. Clinically and/or pathologically unclear cases can receive a decisive impulse for the correct diagnosis through this method, even in long persisting disease courses. This poses a serious advance in the available dermatological diagnostics.

P039 | Effects of tralokinumab treatment on quantitative and qualitative measures of skin barrier function and biology in patients with moderate to severe atopic dermatitis

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Background: Atopic dermatitis (AD) is characterized by skin barrier dysfunction and inflammation with heightened T-helper-2 (Th2) signaling. One of the key Th2 cytokines is IL-13, which is overexpressed by different cell types in the skin of AD. IL-13 has a wide impact, including a downregulation of important epidermal barrier proteins and lipids and the promotion of local T cell responses. Tralokinumab is a fully human IgG4 monoclonal antibody that specifically neutralizes IL-13 and is approved for the treatment of moderate to severe AD in adults. While tralokinumab was shown to be clinically efficacious and improves the extent and severity of AD lesions as well as key symptoms such as itch in multiple trials, its specific impact on skin barrier function has not been examined so far.

Methods: We conducted an investigator-initiated phase II, single-site, open, interventional clinical pilot-study to evaluate the effects of 16 weeks of tralokinumab treatment on quantitative and qualitative measures of skin barrier function and biology. A total of 16 adult patients received 300mg of tralokinumab subcutaneously every two weeks after an initial loading dose of 600mg. Except for selected skin areas the concomitant use of topical corticosteroids or calcineurin inhibitors was allowed. The primary endpoint was the change in trans-epidermal water loss (TEWL) at one non-lesional and one lesional marker skin area at week 16 compared to baseline. Secondary endpoints comprised changes of stratum corneum (SC) hydration and biomarker (cytokine) levels of one lesional and adjacent non-lesional

marker skin area at week 16 compared to baseline. During 9 study visits demographic and clinical data comprising both physician-reported and patient-reported outcomes were collected, and skin barrier assessments including trans-epidermal water loss (TEWL) (Tewameter TM300®, Courage + Khazaka Electronic, Köln) and stratum corneum (SC) hydration (Corneometer CM 825®, Courage + Khazaka Electronic, Köln) were performed at a marker lesional and adjacent non lesional skin area. In addition, tape strips (Standard D-Squame, Monaderm, Monaco, France) were taken at baseline as well as at week 2, 12 and 16 after treatment initiation for the measurement of 21 candidate biomarker proteins using Luminex 200.

Results: All severity scores decreased significantly over time. At week 16, EASI 50, EASI 75 and EASI 90 response rates were 93.75%, 56.25% and 12.5%. The mean TEWL in AD lesions was significantly reduced from 25.44 [g/h/m2] at baseline to 17.13 [g/h/m2] at week 16, representing a 32.67% reduction towards the levels seen at non-lesional skin areas ($p = 0.01$). SC hydration was increased significantly in lesional skin from 13.81 CM-Units at baseline to 21.81 CM-Units at week 16, representing a 36.68% increase ($p = 0.004$). Multiplex ELISA analysis showed a significant decrease of several pro-inflammatory cytokines over time with the most pronounced differences seen for IL-8 (log2 foldchange −1.54 at week 16 as compared to baseline), VEGF-A (log2 foldchange −0.84) as well as IL-18 (log2 foldchange −1.01).

A total of 41 adverse events (AE) were reported in 13 patients. 28 of them were categorized as “mild”, 13 as “moderate” and none as “severe”. There were no serious adverse events (SAE) reported. The most frequently reported AE were eye disorders (including conjunctivitis and blepharitis) reported in 25.00% of all patients and local herpes simplex infections in 18.75% of all patients. There were no study drug discontinuations due to AE.

Conclusion: In this small scale open clinical trial on adult patients with moderate to severe AD, tralokinumab demonstrated favorable clinical efficacy and safety, and led to significant improvements of skin barrier function parameters as well as significant decreases of key SC AD biomarker expression.

P040 | Variable outcome of immunotherapy in advanced multiple cutaneous squamous cell carcinomas in two patients with recessive dystrophic epidermolysis bullosa

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Background: Cutaneous squamous cell carcinomas (cSCC) are the major complication of recessive dystrophic epidermolysis bullosa (RDEB) with high morbidity and mortality rates, and unmet therapeutic needs.

Objective: To evaluate the molecular pattern of cSCC, and the clinical course under immunotherapy in two RDEB patients with multiple advanced cSCC.

Methods: Clinical course and staging were retrospectively evaluated. Tumour tissue was submitted to immunohistochemical staining. DNA from blood and from cSCC was submitted to massive parallel sequencing, and somatic mutations were determined.

Results: Patient 1 survived over two years with disease control under Cemiplimab and intralesional IL-2. The target advanced cSCC demonstrated a high rate of somatic mutations and strong expression of the immune markers, IDO, PD-L1 and LAG-3. Ultimately, he succumbed because of complications related to his oesophageal carcinoma. The undifferentiated cSCC on the foot of patient 2 displayed a low mutational burden and did not express immune markers. This tumour progressed quickly under Cemiplimab.

Conclusions: These two cases underscore the challenges of the treatment of cSCC in RDEB. Multiple tumours with different molecular and immune profiles occur concomitantly or sequentially, and surgical excision is not always possible because of anatomic and tissue constraints given by the disease itself. PD-1 inhibitors seem to be an effective therapeutic choice in this particular group of patients to prevent disease progression and metastasis and stabilize advanced tumours. Somatic mutations and the immune microenvironment should be characterized to predict the therapeutic response, in particular of the aggressive undifferentiated tumours, to conduct personalized therapeutical strategies.

P041 | Subcutaneous fat abundance and density are associated with an enhanced response to immunotherapy response in metastatic melanoma: a retrospective cohort study

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Immunotherapy with checkpoint inhibitors has revolutionized the therapy of metastatic melanoma, achieving impressive responses with long duration. However, the activation of the immune system also harbors the risk for serious and intractable adverse effects, highlighting the need for novel biomarkers predicting response to therapy and risk for adverse effects.

Recently, the identification of an enhanced response to immunotherapy by patients with a body mass index (BMI) > 25 kg/m^2 has indicated that body composition might impact therapy response and could serve as a predictive biomarker. Pilot trials utilizing radiologically measurable parameters of sarcopenia and adipose tissue mass have provided initial evidence of the usability of these novel tools.

In the current work, we have analyzed the fat tissue abundance and density in a retrospective cohort of 100 patients with non-resectable stage III and IV melanoma receiving first-line immunotherapy at the University Hospital of Magdeburg since 2011. We argued that not only the mass but also the density and location of adipose tissue might impact its metabolic activity. Therefore, we measured subcutaneous, visceral and intermuscular adipose tissue via established CT-based image analyses and calculated respective "adipose tissue gauge indices" by multiplying tissue area with density and normalized for patient height. Interestingly, univariate survival analyses using Kaplan-Meier estimators highlighted a significantly longer progression-free survival (PFS) for patients with a low subcutaneous adipose tissue gauge index (SATGI) compared to patients with a high SATGI. This was confirmed in a multivariate cox regression model encompassing also S100 and LDH blood levels, BMI, height and weight. Additionally, we generated a random survival forest model with the same parameters. Permutation importance of features again revealed a predictive function of SATGI in this model. By permutation of individual parameters, we identified a sharp increase of predicted risk at a SATGI cutoff around the cohort median. Interestingly, a similar increase of predicted risk was identified for LDH and S100 at their respective laboratory upper level of norm values without any prior knowledge of these values in the model. This agnostic identification of biologically relevant thresholds validates the clinical relevance of our model.

Finally, we analyzed the occurrence of adverse events in our cohort. We detected a significant accumulation of cases of vitiligo in the SATGI low cohort. This suggests an enhanced anti-melanocytic response in patients with low SATGI values as a potential underlying mechanism of an enhanced immunotherapy treatment response.

P042 | Dynamic optical coherence tomography (D-OCT) of chronic inflammatory skin disease psoriasis vulgaris: advanced tool for treatment monitoring?

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Background: Psoriasis is a chronic, immune-mediated disease with cutaneous and systemic manifestations. Multiple biologics allow effective therapy in most patients. However, therapy monitoring is currently limited to clinical scores such as the PASI, BSA, and IGA, which are based on macroscopic examination and do not capture subclinical inflammation. There is no established objective method or biomarker for the assessment of disease activity.

Objectives: The aim of this study was to describe vascular Dynamic Optical coherence tomography (D-OCT) findings in lesional and non-lesional skin of psoriasis patients undergoing a systemic therapy with biologics.

Methods: 38 subjects with moderate to severe psoriasis receiving a biologics treatment (adalimumab, risankizumab or ixekizumab) were enrolled. OCT with dynamic features was applied to image the microvascularization of a target lesion and adjacent non-lesional skin. The density, diameter and depth of the vessels were assessed at 3 timepoints (baseline, week 2, week 14). Clinical response of therapy was measured by PASI.

Results: In total, 26% of the patients achieved PASI90 and 37% PASI100 at week 14. Our (D)-OCT results show detailed changes in vascularization of the psoriatic skin during biologic therapy. In lesional skin vessel depth increased and vessel density and diameter decreased significantly with clinical improvement determined by PASI, IGA or DLQI. Most strikingly, a significant correlation between vascular depth and PASI was seen over all samples (spearman rho −0.31, p = 1.7e−08).

In non-lesional skin D-OCT parameters tended to stay stable. The comparison between lesional and non-lesional skin showed a statistical difference in the amount of vessel density, depth, and diameter at baseline (Wilcoxon p = 0.00013, p = 2.7e-12, p = 0.00042, respectively). We demonstrated that during treatment with biologics, the vascularization parameters of lesional skin are nearly normalized to those of nonlesional skin with vessel diameter no longer significantly differing between lesional and non-lesional skin at week 2 whereas vessel density and depth did take until week 14 to equalize between lesional and non-lesional skin. Due to the small number of cases, no statistically significant differences of D-OCT parameters could be shown between the drug classes.

Conclusion: (D-)OCT features of psoriasis vulgaris correlate with overall disease activity and treatment response. (D)-OCT as a non-invasive monitoring tool offers new insights into the dynamic changes of vascularization and may represent a tool for therapy monitoring.

P043 | How to Biobank – The (LiquiMeL -) Biobank of the Fleur Hiege Center for Skin Cancer Research at the University Medical Center Hamburg-Eppendorf (UKE) presents itself

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Medical research is unthinkable without the study of biological material from cancer patients and medical data. The goal of translational research is a continuous exchange of different research foci with the aim of further processing and implementation of results in the clinic. For this reason, a biobank was established in 2018 at the University

Skin Tumor Center Hamburg under the special direction of Prof. Ch. Gebhardt and in cooperation with the Institute of Tumor Biology under the special direction of Prof. K. Pantel and includes blood, stool and tissue samples from melanoma/cutaneous squamous cell carcinoma/uveal melanoma of those patients' receiving immunotherapy in the palliative and adjuvant setting. We collaborate with other clinics across institutions and have established the LiquiMEL Biobank together with Dr. Peter Mohr, Head of the Department of Dermatology in Buxtehude. In addition, there are international collaborations with other research groups (e.g. Paul Sabatier University Toulouse) and companies (Olink, Sysmex Inostics, etc.).

The exploration of so-called biomarkers, molecules or features, for early cancer detection, real-time monitoring of therapeutic efficacy, detection of therapeutic targets and resistance mechanisms, represent our research focus (Heidrich et al, Int J Cancer 2020; Heidrich et al, Cancers 2021; Geidel et al .,NPJ Precision Oncology; Gebhardt et al, Cell Immunol. 2021; Keller and Pantel et al. Nat. Rev. Cancer 2019). This is under the principle of "liquid biopsy", which was recently highlighted in the prestigious journal NATURE as a milestone in cancer research. What makes the local (LiquiMEL) biobank special is the high frequency blood collections (every 4–6 weeks) that occur prior to therapy initiation, during therapy, and at specific additional time points, and the logistical challenges associated with them. These serial blood collections allow for tracking and identification of individual therapy trajectories and significant characteristics.

The purpose of this abstract is to shed light on the (LiquiMEL)-Biobank Hamburg in its volume of patient samples (n = >3000 samples, n = >400 patients), patient data and research potential as well as the challenge of establishing such a biobank as the key to personalized medicine and to encourage exchange.

P044 | A combination of Zingiber officinale root extract and cannabidiol alleviates clinical symptoms of dry and eczema-prone skin in adults and children

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For atopic dermatitis emollients are recommended as topical basic therapy. However, improving emollients through addition of anti-inflammatory and antioxidative substances exhibiting a very good tolerability profile is beneficial. In vitro investigations have shown anti-inflammatory and antioxidative activity of a Zingiber officinale root extract and cannabidiol (CBD). These effects are likely mediated by modulation of the endocannabinoid system which is known to regulate different aspects of the skin, e.g. proliferation, differentiation or inflammation.

Therefore, a newly developed oil-in-water (O/W) emulsion containing a lipophilic Zingiber officinale root extract and CBD was investigated in a clinical study as basic therapy for 4 weeks.

In total, 44 subjects have been included (22 adults, 22 children; age: 22.9 18.2 years). Adults exhibited a history of atopic dermatitis and dryness and redness with a score of at least 1 each on inclusion. Children suffered from atopic dermatitis with at least two acute lesions (local SCORAD ≥4).

Skin condition was assessed at baseline and on day 29. The skin condition of participants was objectively assessed by a dermatologist on a 3-point score or on the local SCORAD. In addition, adults assessed subjective parameters themselves (i.a. itching, tension, feeling of dryness; 3-point score). Skin moisture was measured by corneometry.

For adults, a statistically significant reduction of dryness (−0.73 0.69, $p < 0.0001$) was observed. Symptom intensity in children improved significantly compared to baseline (Local SCORAD sum score −1.68 2.89, $p < 0.05$). A statistically significant reduction of erythema (−0.39 0.89, $p < 0.01$; n = 44) was observed for all subjects after 4 weeks. Skin moisture increased significantly until end of the study (+8.61 10.07, $p < 0.0001$; n = 44). In addition, adult subjects documented a statistically significant reduction of itching (−0.96 0.89, $p < 0.001$), tension (−1.09 0.78, $p < 0.001$) and feeling of dryness (−1.64 0.99, $p < 0.001$) on day 29 compared to baseline. The product was very well tolerated.

In summary, application of an O/W emulsion containing Zingiber officinale root extract and CBD to patients suffering from atopic dermatitis improved clinical symptoms in adults and children while exhibiting a very good tolerability profile.

Dermato-Endocrinology

P045 | Additive and synergistic anti-inflammatory effects of cannabidiol and a lipophilic extract from Zingiber officinale root

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In a basic in vitro screening campaign, we identified cannabidiol (CBD) and a hypercritical CO2 extract obtained from Zingiber officinale root as extracts with higher anti-inflammatory activity as compared to other plant extracts. Following confirmation of the screening results both test items were selected as potential active ingredients for a topical product targeting inflammatory skin diseases.

Therefore, the test items were tested alone and in combination in two relevant assays for skin inflammation in keratinocytes to reveal additive or synergistic effects.

Effects on NF-κB transcriptional activity initially observed in HEK cells were confirmed in HaCaT keratinocytes. HaCaT NF-κB Luc cells were either stimulated with TNF-α or irradiated with UVB. Based on cytotoxicity results 6 µg/mL (CBD) and 25 µg/mL (Zingiber officinale root extract) were chosen as maximum concentrations. NF-κB activity was determined in parallel to viability. Following stimulation with TNF-α CBD and the Zingiber officinale root extract showed a significant, dosedependent inhibition of NF-κB activation (>50%) after 6 h.

Cell viability was not affected. In the case of UVB irradiation a dose-dependent, significant inhibition of NF-κB activation (>50% and >40%, respectively) was shown after 24 h. Cell viability was reduced to ~70% by irradiation with UVB without additional significant cytotoxic effect of the test items. Subsequently, we tested combinations of CBD and ginger extract. We observed additive and synergistic effects for several combinations in both, the TNF-α and the UVB assays. Next, the model of poly(I:C)-induced cytokine and chemokine secretion from normal human epidermal keratinocytes (NHEK) was used in order to assess anti-inflammatory activity. Both, CBD and Zingiber officinale root extract exhibited anti-inflammatory activity as shown by a dose-dependent reduced secretion of IL-6, IL-8, TNF-α, and CCL20 to almost basal levels.

The preliminary data suggest that the combination also shows synergistic effects on the positive modulation of the endocannabinoid tone.

Observations from a screening campaign for CBD and a Zingiber officinale root extract were confirmed in cellular in vitro models relevant for skin inflammation. Combination experiments with both test items revealed potent, dose-dependent, synergistic anti-inflammatory activity.

P046 | Induction of p53 expression and its activity in immortalized SZ95 sebocytes upon treatment of arachidonic acid and linoleic acid

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The immortalized human sebaceous gland cell line SZ95 is used worldwide in studies of sebaceous differentiation, lipogenesis as well as research of pathophysiological mechanisms and treatment of acne and other sebaceous gland-related diseases. The co-cultivation of SZ95 sebocytes in contact with tissue specimens of different skin diseases (3D-SeboSkin model) also provides a real alternative to animal trials for investigation of molecular pathogenesis and drug screening. Despite their transformation, SZ95 sebocytes are considered as primary-like cell cultures. Among others, p53, a tumor suppressor gene, controls cell proliferation and apoptosis and is dysregulated in about half of all types of cancer. In a previous work, we have shown that SZ95 cell apoptosis and autophagy coexist with lipogenesis. In this study, we focused on the expression of p53 and its posttranslational status in SZ95 sebocytes responding to two biologically relevant fatty acids, arachidonic acid (AA) and linoleic acid (LA). We detected and could induce p53 expression. Moreover, seven crucial phosphorylated or acetylated types of p53 were present under AA and LA treatment. The inducible p53 expression and its activation status in human SZ95 sebocytes provides evidence that an intact p53 cascade exists in these cells and could play a relevant role in their signal transmission under drug treatment. Blocking of p53 contribute to understanding the role of this protein in essential signaling pathways.

P047 | The immortalized human sebaceous gland cell line SZ95 exhibits no microsatellite instability

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The immortalized human sebaceous gland cell line SZ95 exhibits morphological and biochemical characteristics of normal sebaceous differentiation and lipid accumulation. Due to their previously shown normal sebocyte properties, SZ95 sebocytes have been widely used as a reliable model in sebaceous gland and developmental biology research. On the other hand, due to the immortalization, they can be maintained in culture in an undifferentiated state and be propagated without spontaneous differentiation. However, epithelial cell immortalization may lead to the development of microsatellite instability and malignant transformation indicating a defective mismatch repair been associated with genome-wide instability and carcinogenesis. The aim of our study was, therefore, to investigate the most important malignant transformation sequences and mismatch repair proteins in SZ95 sebocytes, which could possibly have been affected by immortalization. By means of fragment analysis, immunocytochemistry and immunohistochemistry we detected neither microsatellite instability nor defects in the mismatch repair protein system in contrast to sebaceous gland carcinoma cells of patient-derived tissue. Together with the facts that SZ95 sebocytes preserve their normal sebocyte characteristics for over 50 subcultures and do not induce tumor growth in nude mice, our current data corroborate the evidence that SZ95 sebocytes can be administered as normal human sebocytes in experimental research.

P048 | Dermal white adipose tissue regulates macrophage activation

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Dermal white adipose tissue (dWAT) is a unique layer of adipocytes within the skin. Recent studies showed that dWAT displays various

non-metabolic activities such as antimicrobial defense, control of hair cycling, wound healing, and thermogenesis.

In the present study we demonstrate by genome wide expression analysis that inflammatory activated dWAT from healthy mice controls macrophage activation. In detail, soluble factors of inflammatory activated dWAT induced the gene and protein expression of IL-4 and IL-13 in LPS/INF-γ stimulated M1-like macrophages. Importantly, dWAT from healthy skin did not affect IL-4 and IL-13 expression. Using pathway inhibitors we showed the involvement of Stat3, MAPK, NfkB and PI3K pathway in the induction of IL-4 and IL-13 by soluble factors of inflammatory activated dWAT. Moreover, gene expression of both cytokines are induced in CD11b + myeloid cells in a mouse model of wound healing. Next, the specific mediators responsible for the induction of IL-4 and IL-13 have to be identified.

IL-4 and IL-13 play a critical role in the polarization of monocytes into antiinflammatory M2-like macrophages as well as in the stimulation of the synthesis of extracellular matrix proteins, both processes are disturbed under obese/diabetic conditions. Interestingly, inflammatory activated dWAT from obese mice lose the ability to induce the IL-4 and IL-13 expression. Accordingly, we found a diminished expression of both cytokines in wounds of diabetic (db/db) mice compared to healthy mice.

Comparative analysis of inflammatory activated dWAT from healthy and obese mice displays strong differences in the expression of pro-inflammatory mediators like S100A9, lipocalin 2, adiponectin, leptin and IL-6 indicating that obesity induces functional changes in the dWAT.

In summary, our data demonstrate that the dWAT plays an important role in the control of macrophage activation and subsequently in the control tissue repair processes. Functional changes of dWAT might contribute to the disturbed wound healing process observed under obese/diabetic conditions.

P049 | Melatonin synergizes BRAF/MEK inhibitors-mediated mitochondria-dependent cell death and oncogenic cell signalling pathway in melanoma cells

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

P050 | Can activation of the alpha7 nicotinic acetylcholine receptor suppress endothelial to mesenchymal transition in fibrosis?

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Systemic sclerosis (SSc) is an autoimmune connective tissue disease in which microvasular injuries and excessive production of collagen result in fibrosis of skin and other organs. Although fibroblasts are key effector cells during the fibrotic response in SSc endothelial cells are likewise believed to contribute to this process via endothelial to mesenchymal transition (EMT).

Previously we reported that the alpha7 nicotinic acetylcholine receptor (alpha7nAChR) has antifibrotic activity in experimentally induced skin fibrosis presumably by acting directly on fibroblasts. Here, we wondered if activation of the alpha7nAChR can affect EMT in human dermal microvascular endothelial cells. In a first set of experiments we examined the expression of the alpha7nAChR in primary human dermal microvascular endothelial cells (HDMEC) at RNA level using semi-quantitative RT-PCR. We also confirmed the presence of this receptor in the endothelial cell line HMEC-1, a tool cell line for EMT research due to a high expression of the endothelial cell marker CD31 as shown by FACS analysis. At protein level we confirmed expression of this receptor via Western immunoblotting in HDMEC as well as HMEC-1. Immunofluorescence analysis with an alpha7nAChR antibody disclosed expression and a cell membrane-associated localisation of this receptor in both HDMEC and HMEC-1. Finally, we demonstrated the functionality of the alpha7nAChR in HDMEC by measurements of calcium influx using alpha7nAChR agonists and the antagonist alpha-bungarotoxin. Our Future research of the dermal endothelial cell system I underway to further clarify

the role of the alpha7nAChR in EMT and to get more insight into the molecular mode of action of alpha7nAChR receptor agonists in fibrotic diseases.

Dermatopathology

P051 | Characterization and pharmacological inhibition of a novel adult antibody transfer model of pemphigus vulgaris

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Pemphigus vulgaris (PV) is a severe autoimmune blistering disease, in which autoantibodies against the desmosomal adhesion molecules desmoglein (Dsg) 1 and 3 interfere with epithelial cell-cell adhesion, thereby causing intraepithelial blisters and erosions of the skin and/or mucous membranes. Despite recent advances in PV treatment, there is a high, so far unmet medical need for effective, safe, and more specific therapies. Hence, the goal of this study was to generate a mouse model of PV that reproduces key features of the human disease and that can be applied for the preclinical evaluation of novel treatment approaches in a quasi-therapeutic setting. Here, we showed that the monospecific anti-Dsg3 IgG antibody, AK1489, induced Dsg3 endocytosis and loss of cell cohesion in cultured human keratinocytes as detected by internalization and dispase-based keratinocyte dissociation assays, respectively. Following subcutaneous injection of AK1489 every other day over 10 days, adult C57BL/6J mice presented with oral and pharyngeal erosions and hair loss with suprabasal splitting and acantholysis by histopathology. Direct immunofluorescence (DIF) microscopy showed intercellular IgG deposits in mucosal epithelia and skin. Concomitant intraperitoneal injection of intravenous immunoglobulin (IVIg) significantly reduced mucosal lesions and hair loss compared to both vehicle- and methylprednisolone-treated animals. IVIg-treated mice showed significantly lower levels of circulating and, of note, also tissue-bound autoantibodies, as shown by ELISA and DIF microscopy. Application of IVIg in already clinically diseased mice will provide further information whether this mouse model will indeed be suitable to explore novel therapeutic approaches e.g., inhibitors of signaling pathways, autoantibody binding, and autoantibody circulation for pemphigus.

P052 | Anatomical expression of target antigens in autoimmune blistering dermatoses as markers for lesion formation

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Autoimmune bullous skin diseases (AIBDs) represent a group of chronic skin diseases associating with autoantibodies directed against proteins of the epidermal/epithelial desmosome (pemphigus group) and cutaneous basal membrane zone (pemphigoid diseases). Blister formation in bullous pemphigoid, the most frequent pemphigoid disorders, occurs mostly on flexural parts of the limbs and the trunk, while in pemphigus diseases mucosal areas and the upper back are mostly involved. Yet, it has not been thoroughly analyzed whether these predilection sites differ with respect to the expression of the corresponding target antigens. Here, we investigated the correlation between the expression level of target antigens and the involved sites in AIBDs. Biopsies from 13 different body sites of 10 donors without autoimmune diseases were collected and RNA was extracted from each sample to assess the expression of target antigens, i.e., BP180, BP230, Dsg1, Dsg3, LAMA3, LAMB3, LAMC2, LAMB4, and COL7 by RT-qPCR. To corroborate these results at the protein level, the strength of expression was estimated by serial dilution of antibodies against the indicated antigens by indirect immunofluorescence (IF) microscopy. The results indicate a high expression of Dsg1 on the medial upper leg, dorsal lower leg, upper arm, and forearm compared to the mucosal areas, whereas Dsg3 has a high expression in both skin and mucosal areas including buccal mucosa, cheek, and medial upper leg. Furthermore, BP180 and BP230 reveal a high expression on e.g., forearm, medial upper leg, and cheek. The highest expression of laminin 332 was found in skin biopsies of medial upper leg, foot sole, and forearm as well as buccal mucosa. The results of IF staining were well in line with RT-qPCR data. Our data demonstrate that a higher expression of AIBDs target antigens is detected in the predilection sites suggesting the anatomical expression of target antigens as a marker for lesions formation.

P053 (OP03/01) | Deciphering the pathogenesis of a psoriasis-like-phenotype in a genetically engineered mouse line

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Psoriasis, a systemic inflammatory disease, mainly affects the skin and the joints and has a worldwide prevalence of 2–3% with a well-established immunogenic basis. Although many insights into the

pathophysiology of psoriasis come from mouse models, the latter rarely reflect the human disease pattern, making suitable animal models for psoriasis urgently needed. Keratin 79 (Krt79) maintains the skin barrier function and is expressed in the hair follicle infundibulum (INF). To study the role of INF-relevant genes, we generated a driver mouse line expressing tetracycline transactivator (tTA) protein under the control of the Krt79 promoter. Unexpectedly, heterozygous mice (Krt79+/tTA) showed a psoriasiform phenotype. The phenotype was even more prominent in homozygous animals (Krt79tTA/tTA). Histologic features of psoriasis such as an increased epidermal thickness with hyperplasia and infiltration of inflammatory cells were observed in the skin of Krt79tTA/tTA mice. Skin inflammation was also verified by enhanced levels of interleukin (IL) 1A, IL1B, IL6, IL17A, and IL17F in the skin of Krt79tTA/tTA mice. The Krt79+/tTA mice heavily scratch themselves and nearly all mice develop skin lesions in prominent areas such as the neck due to the scratching. Many mast cells with histamine granules were observed in the skin of Krt79tTA/tTA mice. Besides, RNA-Seq results and further pathway analysis suggested that IL17, tumor necrosis factor (TNF), and nuclear factor kappa B (NFKB) signalling pathways, whose roles are of significant importance in psoriasis pathogenesis, were among the mostly affected signalling pathways in Krt79tTA/tTA mice. Moreover, to have a closer look into the role of various immune cell types in the pathologic phenotype of Krt79tTA/tTA mice, immune cells in different compartments such as blood, spleen, lymph nodes and bone marrow were analysed by flow cytometric approach. Interestingly, RNA-Seq results demonstrated strong down-regulation of Krt79 and Krt77. To corroborate the role of Krt77 in the observed phenotype, we are currently investigating Krt77 knockout mice. Further in silico studies revealed an unintended loss of an enhancer in intron 1 of the Krt79 gene, which could influence the expression of KRT77. In order to confirm this finding in vivo, we are characterizing a mouse line with a deletion of intron 1 of Krt79. We suspect that a combined loss of KRT79 and KRT77 triggers the observed psoriasislike- phenotype. In summary, this new mouse model reflects most clinical, histologic, and immunepathogenic features of human psoriasis and will contribute to a better understanding of the pathomechanisms involved in psoriasis and to the investigation of novel therapeutic agents.

P054 | Role of leucine-rich repeats and immunoglobulin-like domains (LRIG)-3 in skin homeostasis and cancer development

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LRIG3, the last discovered protein of the LRIG family has been consistently indicated as a tumor suppressor gene in human malignancies such as vaginal and cervical squamous cell carcinoma. LRIG proteins are best known for their essential role in the regulation of various receptor tyrosine kinases (RTKs) including the epidermal growth factor receptors and platelet derived growth factor receptors. Because of their prominent role in the regulation of RTKs they are involved in many cell signalling cascades and their expression is frequently dysregulated in many human malignancies. LRIG proteins play also an important role in embryonic development. In certain pathological tissues such as psoriatic skin the LRIG expression patterns are altered. We have revealed that dysregulation of LRIG1 and LRIG2 expression is implicated in skin homeostasis and carcinogenesis. To study the function of the LRIG3 in skin homeostasis and pathology we generated two mouse models and several human LRIG3 knockout cell lines. By using CRISPR-Cas9 technology we deleted LRIG3 in two skin cancer cell lines, A431 (squamous carcinoma cell line) and A375 (melanoma cell line) as well as in HaCaT cells. The Tet (tetracycline)-off system was utilized to generate skin-specific LRIG3 overexpressing mice under the keratin 5 (Krt5) promoter (pTRE-Lrig3/Krt5-tTA or Lrig3-TG). Skin-specific LRIG3 overexpression is neonatal lethal and double transgenic mice die on day one after birth. Transgenic fetuses (E18.5) exhibit significantly reduced body weight compared with their control littermates. In order to study embryogenesis in Lrig3-TG mice, we performed micro-computed tomography, a platform for qualitative and quantitative morphogenesis in embryos. The newborn LRIG3-TG mice showed alterations in the epidermis in several areas, which may possibly be the reason for the cause of death. To evaluate the effect of LRIG3 overexpression in skin homeostasis, we supressed LRIG3 overexpression until birth by administration of doxycycline to pregnant mice and induced overexpression directly after birth. Adult Lrig3-TG mice (6-month-old) showed significantly lower body weight compared with control littermates. Moreover, Lrig3-TG mice demonstrated alopecia and histological examination of their skin revealed epidermal and sebaceous gland hyperplasia in comparison with control animals. On the other hand, LRIG3 knockout mice with a skin-specific LRIG3 deletion showed no obvious phenotype. Furthermore, we will analyse the function of LRIG3 in cutaneous squamous skin cancer using the transgenic and knockout mouse lines by a two-step skin carcinogenesis approach using 7,12 dimethylbenz(a)anthracene (DMBA) and 12-O-tetra-decanoylphorbol-13-acetate (TPA). Using our mouse models and the knockout cell lines, we aim to elucidate the role of LRIG3 in skin homeostasis and skin cancer.

P055 | Unravel principles of the biomolecular network in pemphigus vulgaris

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Pemphigus vulgaris (PV) belongs to the group of autoimmune blistering diseases of the skin and/or mucous membranes. After the loss of tolerance, autoantibodies are produced which target either desmoglein 3 (Dsg3) or Dsg1/3. These molecules are expressed

by epidermal, hair follicle as well as mucosal keratinocytes. Autoantibodies targeting these proteins cause the disruption of cell-cell adhesion and ultimately lead to blister formation in the skin and mucous membranes. PV pathogenesis is not known in detail yet, so we want to unravel the early onset of split formation, focusing on the first steps occurring right after autoantibody binding. PV is used as a model to establish a comprehensive and integrative biomolecular and biochemical Dsg3 network. The morphological features of PV are replicated by the human skin organ culture (HSOC) model injecting different PV autoantibodies. To achieve this goal, we performed HSOCs from 9 different skin specimens from healthy donors deriving from elective plastic surgeries. The skin pieces were injected with either a single-chain variable fragment PX43 (scFv) (directed against Dsg1/3) or AK23 (directed against Dsg3) with (as a control) or without exfoliative toxin A (ETA). After 24 hours, split formation was evident in hematoxylin and eosin-stained sections of HOSCs treated with scFv and AK23 with ETA. The basal keratinocytes have been isolated by microdissection and RNA has been isolated from these cells for RNA sequencing (RNA-seq) and is currently being analysed. Next, sample collection for protein analyses by mass spectrometry isolated from the basal keratinocytes will follow. Once transcriptomic and proteomic data analysis have been completed, the epigenomic changes will be analysed by chromatin immunoprecipitation (ChIP), while kinomic changes will be analysed by PamGene technique.

We hope to define the clinically relevant, transcellular tissue communication code driving tissue remodelling during injury to shed light on the onset of disease in PV.

P056 | Elevated levels of soluble FcεRI as a biomarker of early and good response to omalizumab treatment in chronic spontaneous urticaria

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Background: Anti-IgE treatment with omalizumab is effective in most patients with chronic spontaneous urticaria (CSU). Markers of type IIb autoimmune CSU predict slow response to omalizumab treatment but predictors of fast response have not yet been identified.

Objective: Assess the value of soluble FcεRI (sFcεRI), a marker of IgE-mediated mast cell activation, as a predictor of response to omalizumab in CSU.

Methods: 67 CSU patients' serum obtained before omalizumab treatment were analysed for sFcεRI levels by ELISA and 2 ng/mL was used as cut-off for elevated sFcεRI. Treatment response during the first 4 weeks was assessed by use of the urticaria activity score (UAS7) and the urticaria control test (UCT), and speed of response by the rolling UAS7 (rUAS7).

Results: Patients with elevated pre-treatment sFcεRI levels showed higher rates of well or completely controlled disease, i.e. UCT = 12–15 (14/20) or UCT = 16 (14/19). Elevated sFcεRI levels was significantly associated with disease control (X2 = 4.94, $p < 0.05$) and lower levels were associated with poor disease control (14/64). Of the 12 and 35 patients who achieved complete and marked UAS7 response, respectively, 9 (75%) and 22 (63%) had elevated baseline sFcεRI levels. UAS7 scores were lower in patients with elevated sFcεRI levels reaching statistical significance at week 3 ($p < 0.05$). Patients with elevated sFcεRI levels achieved rUAS7≤6 and = 0 earlier than those with lower levels, at day 9 vs 13 and day 12 vs 14, respectively.

Conclusion: Elevated sFcεRI serum levels predict early response to treatment with omalizumab, which may help to better design treatment options for CSU patients.

P057 | Antibodies against integrin α6β4 are internalized in vitro but fail to induce a clinical phenotype of mucous membrane pemphigoid in vivo

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Mucous membrane pemphigoid (MMP) manifests with oral and ocular lesions in about 85% and 65% of patients, respectively. α6β4 integrin is a hemidesmosomal constituent of the basement membrane zone (BMZ) interacting with BP230, BP180 (type XVII collagen), and laminin 332. Autoantibodies against α6β4 integrin have been described in a subgroup of MMP patients with anti-α6 and anti-β4 integrin reactivity being associated with oral and ocular lesions, respectively. However, the pathogenic effect of anti-α6β4 integrin IgG has yet not been studied in vivo. Here, rabbit IgG against murine α6 (217aa-462aa) and β4 (1,489aa-1,803aa) integrin, was generated. Immune complexes of anti-α6 integrin IgG as well as β4 integrin IgG and the respective recombinant integrin induce release of reactive oxygen species (ROS) from human polymorphonuclear leukocytes (PMNs) ($n = 5$). Furthermore, incubation of cultured murine keratinocytes (C5N) with anti-α6 or anti-β4 integrin IgG led to antigen internalization. Finally, to explore the pathogenic capacity of anti-α6

and anti-β4 integrin IgG in vivo, antibodies were repetitively injected subcutaneously into adult C57BL6/J mice (n = 6/group) every other day until day 10, at a dose of 15mg per injection. Mice were probed for oral lesions by endoscopy on days 4, 8, and 12, and conjunctival biopsies taken on day 12 were analyzed for splitting at the BMZ by hematoxylin & eosin (H&E) staining. Linear deposits of IgG at the BMZ were seen in the skin close to the injection site (5/6 mice, both total α6 and β4 integrin group), buccal mucosa (4/6 mice in total α6 integrin group and 5/6 mice in total β4 integrin group), tongue (3/6 mice in total α6 integrin group and 3/5 mice in total β4 integrin group), and palpebral conjunctiva (4/6 mice in total α6 integrin group and 5/6 mice in total β4 integrin group), while C3 deposition was only detected in 2 or less mice per group and was absent in biopsies obtained from buccal mucosa. Of note, no macroscopic oral or cutaneous lesions were observed over the observation period of 12 days. In H&E stained biopsies of the investigated tissues, a mild to dense subepithelial inflammatory infiltrate were detected, however, split formation at the BMZ was not seen in any of these samples. Collectively, antibodies against α6β4 integrin showed pathogenic potential in vitro and in vivo, but failed to induce macroscopic lesions in adult mice.

P058 | Assessment of cold atmospheric pressure plasma as innovative therapy for treatment of radiation dermatitis using a mouse model

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Radiation dermatitis is a common side effect of modern radiotherapy affecting a substantial number of cancer patients and is caused by radiation damage of skin tissue. It is leading to erythema, edema, moist desquamation, and ulceration. Radiation dermatitis usually is accompanied by pain and strong pruritus, which may lead to an interruption or, in severe cases, even to an abortion of the therapy. Cold atmospheric pressure plasma (CAP), a partially ionized gas that exerts antiseptic and anti-inflammatory properties was shown to reduce pain and to support tissue regeneration as well as angiogenesis and is therefore providing an innovative therapy option. As CAP supports wound healing and regenerative processes without causing any relevant side effects, we hypothesized CAP treatment to reduce the severity of radiation dermatitis and the acute side effects of radiotherapy in cancer patients, which currently disallows an uninterrupted therapy. Hence, this study aimed to assess the clinical course and the molecular pathomechanisms of radiation dermatitis and their modulation by treatment with CAP. For this purpose, an acute radiation dermatitis was induced in nude but immunocompetent SKH-1 mice. A scoring system and noninvasive imaging techniques such as hyperspectral imaging (HSI), laser scanning microscopy (LSM), and optical coherence tomography (OCT) were used to monitor the course of the disease. An optimal radiation dose (65 Gy) was identified for inducing a moderate radiation dermatitis (score 2.5) using a gamma irradiator. Furthermore, we assessed the efficacy of CAP in treating such a moderate radiation dermatitis using an atmospheric pressure plasma jet in comparison to an untreated control group. While assessment of HSI and OCT revealed a significant difference between gamma irradiated and non irradiated mice, no difference was obsereved between CAP treated and untreated mice.

In addition to the monitoring using imaging technologies, skin biopsies were collected for immunohistochemistry and transcriptome analyses. Future molecular analyses of treated and untreated tissue may help unravel the pathomechanisms of radiation dermatitis and how this is modulated by CAP exposure.

P059 | Investigating diversity among dendritic cell subsets across age, gender and health states in mice skin

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Dendritic cells are central orchestrators of immune system that modulate functions in immunity and tolerance throughout life. Research indicates dendritic cells undergo a sequential development procedure over age and change phenotype and functions over time and disease states. These age associated changes in human dendritic cell subpopulations have impact on adaptive immune responses and often are implicated in immune dysfunction. However, further research is needed to gain insight into ontogenic diversity and environment dependent heterogeneity among DC compartment in skin which shapes the immune responses. Due to this strategic role, it would be interesting to study the ontogenetic development of dendritic cells in early life that is later outlined by environmental signals in tissues. In a latest study analyzing young murine samples it was found that early preDC (CD11cintCD45RA +) and conventional CDc CD11chighCD45RA- appear in embryonic stages in thymus and spleen and as early as day 1 in neonatal mice. Hence we hypothesize the presence and sequential development of similar subsets to be found in skin dermis and epidermis as well. CD11cint MHC-IIhigh DC population consisting mostly of CD8-CD205high can be detected at week 1 in skin and represent populations similar to epidermal Langerhans cells. Early progenitors of dendritic cells experience time dependent loss of MHC neg cells in kidneys, spleen and thymus while there is simultaneous differentiation of MHC positive cells in

skin due to tissue specific changes in cytokine secretions over time points. Multiple DC progenitors persist only weeks after birth and need to be detected at this stage as they are replaced over incoming weeks due to tissue specific changes in cytokine secretions over time points. We propose a systematic analysis of DC subset distribution across age and gender in skin tissue from different parts of body which has not yet been performed. We hope to analyze phenotypical varieties of conventional subsets like CD11c + MHC class II + DCs in skin. These include epidermal Langerhans cells, CD103+ cDC1s, CD14 + dDCs, CD1a+ dermal dendritic cells and CD11b + conventional cDC2 dendritic cells. We hypothesize that the dendritic cell repertoire differs in terms of cell number, subset distribution and marker expression across age and gender. The aim is therefore to visualize the dendritic cell compartment in week 1,3,4,5,8- week-old male and female neonatal C57BL/6 mice. Cell isolations will be performed through collagenase enzymatic digestions of C57BL6J neonatal mice skin tissue from ears, front skin and dorsal skin. Flow cytometry analysis using a preselected panel of cell surface markers using immune markers CD45, MHCII and dendritic cell associated markers SIRPa,XCR1,CD11b, CD207 and CD11c. Knowledge of the predominant dendritic cell subtype in skin at each stage of development will help determine the type of immune response associated with the antigen like th1/th2 response skewing in young mice. This data will aid in elucidating optimized dermal delivery of antigens and the corresponding the immune response in skin and helps identify factors that determine the commitment of these dendritic cells to specific lineages.

P060 | Environmental impact on S. aureus: Secretome differences between healthy and atopic eczema isolates

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Introduction: Atopic eczema (AE) is an inflammatory skin disorder affecting approximately 20% of children worldwide with early onset leading to later development of asthma and allergies. A variety of different factors have been shown to contribute to the development of AE. One such factor is the overgrowth of Staphylococcus aureus, where S. aureus is a predictive factor for severity. Although S. aureus is known as an opportunistic pathogen – present in AE and chronic wound infections – this species has also been shown to be present on healthy individuals' skin. As the environment of the skin is drastically different in AE as compared to healthy (HE), could a change in the environment result in S. aureus changing from a commensal to a pathogen?

Method: Here, we challenged 30 S. aureus isolated from 15 HE and from 15 AE participants to different environmental conditions relevant to AE, such as higher pH and oxygen presence, and observed changes in growth pattern and bacterial secretions (the secretome). More specifically, for three environments (anaerobic pH 7.0, aerobic pH 5.5 and aerobic pH 7.0) 30 isolates were tested for changes in growth pattern with a subset of 4 AE and 4 HE strains used to probe secretion differences by LC-MS2 in addition. These environments were chosen to test normal skin pH -around pH 5.5 – versus the elevated pH in AE – pH 7.0 – and to test healthy skin oxygen environment -anaerobic.

Results: Overall, all S. aureus strains grew best at pH 7.0 which is closer to AE skin pH as compared to the other two environments. Between healthy and atopic strains, the growth of AE strains in anaerobic conditions is significantly better than their healthy counterparts. In addition, location of isolation, nose (n = 8) versus skin (n = 15), appeared to be the decisive factor for improved growth within anaerobic conditions with skin isolates growing best. Beyond the growth pattern and further into the secretome, all secretions separate first by each of the three environmental conditions. Interestingly, within pH 5.5 -skin healthy pH- there is also a clear distinction between healthy and AE derived strains' secretomes.

Conclusion: The environment plays a role on both AE and HE isolates' growth and their secretions. In addition, the differential environmental response, that is expressed through secretion changes, between AE and HE isolates at pH 5.5 suggests a fundamental difference between the two types of isolates, but this must be further confirmed with more strains. Overall, this research highlights the importance of the environment on the development and progression of AE.

Epidemiology

P061 | Prevalence of self-reported and physician-diagnosed psoriasis in an adult general population sample: Results from the study of health in Pomerania

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Background: Psoriasis is a chronic inflammatory skin disease with multiple somatic and mental comorbidities causing substantial burden of disease. Until now, data about the epidemiology of psoriasis in adults are limited, and in particular data on the prevalence of psoriasis in Germany are scarce. Existing studies are mainly based on self-reported data, which are subject to bias. Studies based on physician-diagnosed psoriasis are rare. Moreover, little is known

about the validity of self-reported psoriasis in the general population. Using data from a large general population sample of German adults, the present study aimed to (i) analyse the prevalence of psoriasis based on self-report and physicians' diagnosis, and (ii) to examine the validity of self-reported psoriasis.

Methods: We analysed data from 3,048 individuals (aged 20 to 83 years, median 52 years, 51.3% female) from the population-based Study of Health in Pomerania (SHIP) Trend-0, which was conducted from 2008 to 2012 in northeast Germany. All participants underwent a standardized dermatological examination encompassing a personal interview and a clinical examination. Self-reported psoriasis was assessed using the following question: "Do you or did you ever suffer from psoriasis?". During the clinical examination, a trained dermatologist diagnosed psoriasis by visual inspection and questioning. We estimated the prevalence by calculating the frequencies with 95% confidence intervals (CIs) of self-reported and physiciandiagnosed psoriasis stratified by gender.

Results: The overall prevalence of self-reported and physician-diagnosed psoriasis was 8.0% (95%-CI: 7.0–9.0) and 3.1% (95%-CI: 2.6–3.8), respectively. Prevalence was higher in men (9.4%, 8.0–11.0%) compared to women (6.3%, 5.2–7.7%) both in self-reported and physician-diagnosed data (3.8%, 2.9–4.9% vs. 2.5%, 1.8–3.4%). In the interview, 59 participants (2%) stated not to know whether they were suffering from psoriasis; two of them were diagnosed with psoriasis during the examination and seven with atopic eczema (AE). Among participants stating to have or having had psoriasis (n = 238), 65.1% (n = 155) were not diagnosed with psoriasis during the examination but 14 were diagnosed with AE.

Analyses on the validity of self-reported psoriasis revealed a sensitivity of 0.89 (95%- CI 0.83–0.96), a specificity of 0.95 (95%-CI 0.94–0.96), a positive predictive value (PPV) of 0.35 (95%-CI 0.29–0.41), and negative predictive value (NPV) of 0.996 (95%-CI 0.99–1.00).

Conclusions: Our study is among the first to provide data on the prevalence of psoriasis in a sample of German adults from the general population. We revealed a divergence between self-reported and physician-diagnosed psoriasis prevalence with higher rates in self-reported data. This finding suggests that self-reports might overestimate the prevalence of psoriasis, and that relying on the individuals' report would lead to a relevant proportion of errors. Indeed, the probability of having psoriasis was less than 50% in case of a positive report. Clinicians and researches should be aware of this. Our data further indicate that some participants were presumably aware of having a skin disease, but did know which one. Subsequent analyses should aim to identify determinants of false positive recall considering sociodemographic (such as age, sex and educational level) and clinical (such as comorbid skin diseases and allergy) characteristics.

P062 | Relationship between periodontitis and psoriasis: A two-sample Mendelian randomization study

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Observational research suggests that periodontitis affects psoriasis. However, observational studies are prone to reverse causation and confounding, which hampers drawing causal conclusions and the effect direction. We applied the Mendelian randomization (MR) method to comprehensively assess the potential bidirectional association between periodontitis and psoriasis.

We used genetic instruments from the largest available genome-wide association study of European descent for periodontitis (17,353 cases, 28,210 controls) to investigate the relationship with psoriasis (13,229 cases, 21,543 controls), and vice versa. Causal Analysis Using Summary Effect (CAUSE) estimates and inverse variance-weighted (IVW) MR analyses were used for the primary analysis. Robust MR approaches were used for sensitivity analyses.

Both univariable methods, CAUSE and IVW MR analyses, did not reveal any impact of periodontitis on psoriasis (CAUSE odds ratio [OR] = 1.00, p = 1.00; IVW OR = 1.02, p = .6247), or vice versa (CAUSE OR = 1.01, p = .5135; IVW OR = 1.00, p = .7070). The null association was corroborated by pleiotropy-robust methods with ORs close to 1 and p-values >.59. Overall, MR analyses did not suggest any effect of periodontitis on psoriasis. Similarly, there was no evidence to support an effect of psoriasis on periodontitis.

Within the limitations of this MR study, the outcomes supported neither periodontitis affecting psoriasis nor psoriasis affecting periodontitis.

P063 | Frequencies and risk factors of atopic dermatitis and psoriasis in different regions of Germany – Results of the German National Cohort (NAKO)

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Introduction: Atopic dermatitis (AD) and psoriasis are the two most common chronic inflammatory skin diseases in western countries. Both are often experienced as stigmatizing and can be accompanied by comorbidities, resulting in a marked reduction in quality of life. Up to now, there is hardly any data on the epidemiology of AD and psoriasis in Germany with large case numbers from different regions.

Material/Methods: The NAKO has been inviting participants aged 20 to 69 in 18 study centers throughout Germany since 2014. We analyzed the data of 100.000 people from the baseline investigation from 2014 to 2019. Skin diseases were ascertained by a personal, standardized computer-assisted interview.

Results: We observed an overall prevalence in the NAKO of 7.6% (95% CI 7.4–7.8%) for AD and 6.1% (95% CI 6.0–6.3%) for psoriasis. While the prevalence for AD is higher in women (9.3% vs. 5.6% in men), it is higher for men in psoriasis (6.5% vs. 5.8% in women). Age-stratified analyses showed a decreasing frequency of AD with increasing age while the frequency of psoriasis increases with age, with a peak between 60 and 69 years.

Discussion/Conclusion: This analysis aims to better represent the prevalence of AD and psoriasis in Germany. We estimate sex and age-stratified frequencies for the whole study population and for each study center separately, and describe differences between geographical areas. In addition, possible associated comorbidities such as allergic diseases, other inflammatory diseases and psychological disorders are currently being analyzed. Further analyzes will compare the quality of life between patients and healthy participants and evaluate the association of sociodemographic parameters with both diseases.

P064 | The association of psoriasis with mental disorders in adults: Results from a cross-sectional population-based cohort study

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Background: Mental disorders including depression and suicidality are common among patients with psoriasis, but often not adequately addressed and treated. Evidence indicates that the skin might be particularly vulnerable to the development of somatization, which is defined as the tendency to experience psychological distress in the form of bodily symptoms, but studies investigating somatization in individuals with psoriasis are rare. To improve patient care and to promote screening practices, prevention and treatment, data about mental disorders in individuals with psoriasis are critical. Contributing to research in this field, the present study aimed to investigate the association of physician-diagnosed psoriasis with mental disorders (depression, suicidal tendencies, somatization) in the adult general population.

Methods: Data from 3,048 participants from the population-based Study of Health in Pomerania (SHIP) Trend-0 were analyzed (aged 20 to 83 years; 51.3% female). All participants underwent a standardized dermatological examination including a personal interview followed by a clinical examination. Psoriasis was diagnosed based on the participant's self-report in the interview and the physician's evaluation. The participants further completed a self-administered questionnaire on subjective health issues. Depressive symptoms were evaluated using the Patient-Health- Questionnaire-9 (PHQ-9). We assessed suicidal tendencies using three items which were introduced by the following question: "The following questions refer to your whole life." (1) Have you ever felt that depressed that you wanted to commit suicide?, (2) Back then, did you have concrete plans about how to commit suicide?, (3) Did you ever try to commit suicide? Participants who had responded to at least one item with 'yes' were classified as having suicidal tendencies. As an as indicator of somatization, we adminisistered the Zerssen complaint assessing 40 mental and physical complaints on a 4-point Likert scale as absent (1), mild (2), moderate (3) or severe (4). We calculated the number and the severity of mental and physical complaints. To investigate the association of psoriasis with mental comorbidities, linear and logistic regression analyses adjusted for age and sex were performed.

Results: The overall prevalence of psoriasis was 3.1% (n = 95). The prevalence was significantly higher in men than in women (3.8% vs. 2.5%, $p < .05$). Our analyses revealed that psoriasis was related to a higher level of depressive symptoms (OR = 0.90; 95% CI 0.17–1.63), but not to suicidal tendencies (OR = 1.11; 95% CI 0.51–2.41). We found a positive association of psoriasis with the severity (OR = 3.64; 95% CI 0.27–7.01), but not with the number of mental and physical complaints (OR = 1.15; 95% CI −0.17–2.48). Among participants with psoriasis, back and lower back pain (56.8%), neck and shoulder pain (53.7%), joint pain (42.1%), nervousness and rumination (36.8%, respectively) and numbness of hands or feet (34.7%) were the most commonly reported complaints.

Conclusions: In line with the existing literature, the present results indicate that depression is an important comorbidity in individuals with psoriasis. We could not substantiate evidence from previous studies demonstrating an association of psoriasis with suicidality. A major limitation of the present study is the lack of data on disease severity, which is a crucial outcome in this regard. Our analyses further showed that psoriasis is related to somatization, which needs awareness among clinicians. We found that a significant proportion of individuals with psoriasis suffered from a broad range of mental

and physical complaints, indicating the need to evaluate such symptoms in routine care. This finding might also be an indicator of the detrimental impact of psoriasis on the individuals' overall health, which requires further investigation.

P065 | Dipeptidyl-peptidase IV inhibitor-associated autoimmunity: Assessing the of risk of 24 autoimmune diseases by a global population-based study

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Dipeptidyl-peptidase IV inhibitors (DPP4i) were found to increase the risk of certain autoimmune and chronic inflammatory diseases. However, the association of DPP4i with most of these diseases remains highly controversial and poorly investigated. To evaluate the risk of wide array of autoimmune and chronic inflammatory diseases among patients with type-2 diabetes mellitus (T2DM) treated with DPP4i versus sodium-glucose cotransporter-2 inhibitor inhibitors (SGLT-2i)/thiazolidinedione. Utilizing TriNetX platform, a global population-based historic cohort study enrolled two groups of patients with T2DM managed by DPP4i (n = 328,959) and SGLT-2i/thiazolidinedione (n = 328,959). Two groups were compared regarding the risk of 24 autoimmune diseases. Propensity score matching was performed to optimize between-group comparability. DDP4i users experienced elevated risk of systemic lupus erythematosus (hazard ratio [HR], 1.22; 95% confidence interval [CI], 1.10–1.34; $p < 0.001$), systemic sclerosis (HR, 1.22; 95% CI, 1.01–1.48; $p = 0.039$), Hashimoto`s thyroiditis (HR, 1.11; 95% CI, 1.03–1.20; $p = 0.005$), Addison's disease (HR, 1.21; 95% CI, 1.06–1.37; $p = 0.005$), Crohn's disease (HR, 1.21; 95% CI, 1.10–1.33; $p < 0.001$), ulcerative colitis (HR, 1.10; 95% CI, 1.02–1.19; $p = 0.018$), multiple sclerosis (HR, 1.15; 95% CI, 1.01–1.30; $p = 0.032$), autoimmune hemolytic anemia (HR, 1.63; 95% CI, 1.40–1.91; $p < 0.001$), immune thrombocytopenic purpura (HR, 1.28; 95% CI, 1.12–1.46; $p < 0.001$), polymyalgia rheumatica (HR, 1.21; 95% CI, 1.09–1.34; $p < 0.001$), primary biliary cirrhosis (HR, 1.26; 95% CI, 1.06–1.50; $p = 0.008$), autoimmune hepatitis (HR, 1.35; 95% CI, 1.14–1.60; $p = 0.001$), and sarcoidosis (HR, 1.19; 95% CI, 1.08–1.30; $p < 0.001$). Most autoimmune diseases developed ≥3 years after commencing the drug. In conclusion, DPP4i was associated with increased risk of 13 autoimmune diseases. Physicians should be aware of these associations and refrain from prescribing this class for patients at risk for autoimmunity.

P066 | Rituximab is associated with decreased risk of long-term cardiovascular and metabolic outcomes in pemphigus – A propensity-matched global study

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The influence of different therapeutic approaches on long-term cardiovascular and metabolic outcomes in patients with pemphigus remains to be precisely evaluated. Objective: To assess the risk of long-term cardiovascular and metabolic outcomes and all-cause mortality in patients with pemphigus managed by rituximab relative to those under first-line corticosteroid-sparing agents (azathioprine and mycophenolate mofetil [MMF]).: A global population-based retrospective cohort study compared pemphigus patients managed by rituximab (n = 980) with those managed by azathioprine or MMF (n = 980) with regard to the risk of a wide array of cardiovascular and metabolic outcomes. Propensity score matching was performed to optimize comparability. Relative to those treated by azathioprine/MMF, patients under rituximab experienced lower risk of myocardial infarction (relative risk [RR], 0.59; 95% confidence interval [CI], 0.41–0.85; $p < 0.001$), stroke (RR, 0.52; 95% CI, 0.38–0.70; $p < 0.001$), peripheral vascular disease (RR, 0.59; 95% CI, 0.42–0.83; $p = 0.002$), hypertension (RR, 0.54; 95% CI, 0.43–0.66; $p < 0.001$), hyperlipidemia (RR, 0.47; 95% CI, 0.37–0.59; $p < 0.001$), type-2 diabetes mellitus (RR, 0.57; 95% CI, 0.49–0.66; $p < 0.001$), obesity (RR, 0.55; 95% CI, 0.42–0.70; $p < 0.001$), and osteoporosis (RR, 0.65; 95% CI, 0.47–0.91; $p = 0.010$). The all-cause mortality was comparable between patients in both groups (hazard ratio [HR], 1.22; 95% CI, 0.91–1.62; log rank $p = 0.183$). In conclusion, rituximab confers protection against long-term cardiovascular and metabolic outcomes. This agent might be particularly preferred in individuals with preexisting cardiovascular and metabolic risk factors.

P067 | Obesity increases the risk for chronic, non-communicable inflammatory diseases

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Background: Overweight and obesity are a global pandemic. Worldwide, their prevalence is continuously rising, and increased body-mass index is among the top 5 risks for death and disability-adjusted life-years. Hallmarked by epidemiological findings in psoriasis and systemic lupus erythematosus (SLE), obesity is recognized as major factor contributing to onset of chronic, non-communicable

inflammatory diseases (CID). Yet, for several CIDs discrepant results have been reported, sexdifferences were frequently not addressed, and for most rare CIDs, no data is available. Methods. Using TriNetX database, a global population-based cohort study compared the risk to develop 39 common and rare CIDs in individuals (i) with overweight/obesity (ICD10CM:E66, *n* = 7,209,892) to (ii) non-overweight/obese persons (*n* = 7,209,892). Propensity score matching was performed to optimize between-group comparability. Findings. Overall, overweight/obesity increased the risk of 30/39 CIDs. For 5/39 and 4/39 CIDs no impact, or a decreased risk was noted, respectively. The most prominent impact of overweight/obesity was noted for hidradenitis suppurativa (HR 3.28, CI 3.21–3.35, $p < 0.0001$). For comparison, obesity increased the risk of type 2 diabetes (HR 2.69, CI 2.68–2.71, $p < 0.0001$). The highest risk difference relating to CID risk were observed for asthma (2.12%, CI 2.09–2.14). Amongst all CIDS, the risk difference amounted to 4.51% (CI 4.40–4.84%). Additionally, we observed a sex-specific impact of obesity on CID risk. Interpretation. Overweight and obesity are a major, and sex-specific, risk factor for the development of CIDs.

P068 | Mortality in eight autoimmune bullous diseases: A global large-scale retrospective cohort study

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Our knowledge about mortality in patients with autoimmune bullous diseases (AIBDs) is scarce and confined mainly to pemphigus vulgaris (PV) and bullous pemphigoid (BP). To assess the risk of mortality in a wide array of AIBDs. A global populationbased retrospective cohort study enrolled patients with BP, mucous membrane pemphigoid (MMP), epidermolysis bullosa acquisita (EBA), dermatitis herpetiformis (DH), lichen planus pemphigoides (LPP), pemphigus vulgaris (PV), pemphigus foliaceus (PF), and paraneoplastic pemphigus (PNP). Patients with these eight AIBDs were compared with eight age-, sex-, race-, and propensity score-matched control groups regarding mortality rates. Survival analyses were conducted by the Kaplan-Meier method. Hazard ratios (HRs) for the risk of death were obtained using the Cox regression model. The current study included 17,919, 5,747, 1.503, 10,661, 170, 14,716, 1,115 and 179 patients with a diagnosis of BP, MMP, EBA, DH, LPP, PV, PF, and PNP, respectively A similar number of controls was recruited for each group. Patients with pemphigus experienced the highest risk of death (HR, 3.70; 95% CI, 3.40–4.02 for PV; HR, 4.07; 95% CI, 2.94–5.62 for PF; HR, 8.03; 95% CI, 3.46–18.66 for PNP). Risk of mortality was significantly increased also among patients with BP (HR, 2.55; 95% CI, 2.39–2.71), EBA (HR, 2.52; 95% CI, 1.81–3.50), DH (HR, 1.91; 95% CI, 1.68–2.16), and MMP (HR, 1.73; 95% CI, 1.51–1.99). In conclusion, AIBDs are associated with increased mortality. Previous studies might have underestimated the risk of death associated with the less frequent AIBDs.

Genetics

P069 | CARD14-associated papulosquamous eruption with STAT3 activation responsive to anti-IL-17 antibody treatment

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CARD14-associated papulosquamous eruption (CAPE) represents a hereditary autosomal dominant disorder caused by mutations in Caspase Recruitment Domain Family Member 14 (CARD14). Patients show skin lesions resembling psoriasis and pityriasis rubra pilaris (PRP) with facial involvement. Previous therapeutic approaches include anti-IL-12/-23 antibodies. Gain-of-function mutations in CARD14 enhance keratinocyte responses to IL-17A in mice. Treatment response to IL-17 inhibitors has not been described in CAPE, while it is effective in psoriasis and PRP. We present two siblings with CAPE carrying the same mutation (c.467T>C, p.Leu156Pro) in the CARD14 gene. Both patients showed a generalized psoriasiform skin eruption with a linear pattern and strong facial involvement. Histopathology showed aspects of psoriasiform dermatitis and immunohistochemical analysis revealed an immune cell infiltrate consisting of dendritic cells and T cells. Immunophenotyping of the blood showed upregulation of phosphorylated STAT3 in CD4+ and CD8+ T-cells in both patients. Clinical and immunological response to treatment with Ixekizumab (IL17A-inhibitor) were investigated in one patient. He showed significant improvement under treatment with IL-17A antibody Ixekizumab accompanied by reduced STAT3-phosphorylation in CD4+ and CD8+ T cells. Together, our data highlight the pathogenic role of IL-17A in CARD14-associated skin disorders, which might have consequences for future treatment decisions in this rare condition.

P070 (OP06/02) | Genetic knockout of hyaluronan synthesis in mice results in increased skin adipogenesis, a pro-inflammatory gene expression signature and exacerbated acute skin inflammation

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Purpose: Hyaluronan (HA) represents a high molecular weight, linear polymer of repeating disaccharides of glucuronic acid and N-Acetylglucosamine. HA is a major part of the extracellular matrix of the skin. HA is synthesized by three HA-synthases (Has1-3) through cell membrane and many of HA functions depend on the individual synthase and the length of the HA-chain. HA content in skin changes during ageing, inflammation and repair. We analyse the functional impact of individual HA-synthases and strongly reduced HA content in skin with respect to homeostasis, inflammation and tissue repair.
Methods: We crossed constitutive Has1,3 double KO mice with an inducible Has2-knockout strain under the control of the UbiquitinC-promoter (Has2loxp/loxp-UBC-creERT +/-). The resulting Has1,3-/- Has2loxp/loxp-UBC-creERT+/- mice lack activities of all known HA synthases upon induced recombination. These mice were analysed in resting state by immunohistochemistry and ELISA, as well as by microarray gene expression analysis of whole skin. Acute skin inflammation was induced by topical application of 2-chloro-1,3,5-trinitrobenzene (TNCB, 5%) on shaved back skin. Inflamed skin was analysed by single cell RNAseq, immunohistochemistry and isolation of inflammatory cell subsets.
Results: Knockout of all HA synthases leads to a 90% reduction of skin HA, but the mice are viable with normal weight, motility and behaviour. The loss of skin HA results in epidermal thickening, a thinner dermis and a significant increase of the dermal white adipose tissue. Microarray analysis showed that the absence of HA-synthesis leads to differently expressed pathways, like glycolysis and gluconeogenesis, TCA cycle, fatty acid beta-oxidation confirming increased adipogenesis and epidermal dysregulation. Moreover, changes in metabolic pathways and activation of cytokine receptor-mediated signalling pathways were significant.
In inflamed skin of Has-knockout mice, an exacerbated influx of granulocytes and a enhanced M1-macrophage polarization was detected leading leads to more severe inflammation.
Discussion: Our data indicate that HA loss is compensated by the organism resulting in a normal phenotype. However, the abrogation of HA synthesis leads to modification of metabolic pathways, matrix rearrangement with enhanced adipogenesis in the skin as well as a pro-inflammatory signature of the skin. Acute inflammatory reactions are exacerbated suggesting a connection between changes of ECM composition and the inflammatory status of the skin.
Conclusion: The novel mouse model enables comprehensive investigations of HA metabolism and its impact on various physiological and pathophysiological processes in the organism.

P071 (OP06/01) | A gene variant of AKR1C3 contributes to interindividual susceptibilities to atopic dermatitis triggered by particulate air pollution

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Background: The pathogenesis of atopic dermatitis (AD) involves an impairment of the skin barrier by an interplay of genetic and environmental factors. The resulting inappropriate defense against allergens, microbes, and pollutants results in a chronic, mainly T helper (Th) 2 cell-driven skin inflammation. Environmental factors which may increase the risk for AD are airborne particulate matter (PM) and commonly associated polycyclic aromatic hydrocarbons (PAHs). However, the available epidemiological data provide a heterogeneous picture. While some studies found a significant association between PM exposure and AD symptoms, particularly in children, other studies reported null associations. This data inconsistency in airborne PM exposure-related AD may depend on interindividual genetic susceptibilities.
In lesional AD skin, activated mast cells release prostaglandin D2 (PGD2) which subsequently stimulates Th2 cell-driven inflammation. PGD2 is unstable and spontaneously dehydrates to anti-inflammatory 15Δ-PGJ2 or is reduced by aldo-keto reductase (AKR) 1C3, an enzyme that is highly expressed in lesional AD skin, to 9α,11β-PGF2. Like parental PGD2, 9α,11β-PGF2 has a chemotactic effect on Th2 cells and maintains allergic inflammatory responses through downstream stimulation of Th2 cells.
By assessing the functional and clinical relevance of a gene variant of AKR1C3 (rs12529), i.e. a C > G transversion causing an His > Gln exchange in codon 5, we aimed to investigate the role of AKR1C3 in AD while considering airborne PM exposure.
Materials & methods: Epidemiological analysis, mechanistic in vitro and ex vivo experiments, qPCR analysis, SDS-PAGE/Western blot analysis, site-directed mutagenesis, LC-MS analysis, CRISPR/Cas-based gene knockouts.
Results: Treatment of human keratinocytes with benzo(a)pyrene (BaP), an exposurerelevant PAH present in PM, induces the expression of AKR1C3 via non-canonical aryl hydrocarbon receptor signaling. The BaP-induced upregulation of AKR1C3 results in an enhanced reduction of PGD2 into the Th2 stimulatory metabolite 9α,11β-PGF2. Importantly, a topical application of PAH-rich airborne PM, namely diesel exhaust particles, to human ex vivo skin also resulted in an upregulation of AKR1C3. Next, we assessed the clinical relevance of AKR1C3 for AD development by conducting an epidemiological study focusing on the AKR1C3 gene variant rs12529. Specifically, an analysis of the GINIplus/LISA birth cohort, enrolling 457 participants with available AD diagnosis at the 15-year follow-up examination, air pollution and genetic data, revealed that under constant chronic PM exposure the risk for developing

AD increases by 38 % per one effect allele. Subsequent mechanistic analyses showed that the rs12529-related amino acid exchange affects AKR1C3 enzyme activity indirectly, by enhancing protein stability.

Conclusion: This study revealed that carriers of the AKR1C3 SNP rs12529 are at an enhanced risk to develop airborne PM-induced AD. PM exposure induces AKR1C3 expression in human skin, therefore this observation might be explained by an enhanced protein level and corresponding catalytic activity of AKR1C3. Given that the effect allele frequency of rs12529 varies across continental populations, we speculate that due to a higher effect allele frequency, some populations might be more susceptible to PM/PAH exposure-induced or -exacerbated AD than others.

P072 | Relationship between genetically proxied vitamin D and psoriasis: A Mendelian randomization study

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Background: Observational research suggests that vitamin D levels affect psoriasis. However, observational studies are prone to potential confounding or reverse causation, which complicates interpreting the data and drawing causal conclusions.

Objectives: We applied Mendelian randomization (MR) methods to comprehensively assess a potential association between vitamin D and psoriasis.

Methods: Genetic variants strongly associated with 25-hydroxyvitamin D (25OHD) in a genome-wide association study (GWAS) of 417,580 individuals of European ancestry served as instrumental variables. As outcome variable, we used GWAS data of psoriasis (13,229 cases, 21,543 controls). We used a biologically validated genetic instrument and a polygenic genetic instrument to assess the relation of genetically proxied vitamin D to psoriasis. We used inverse variance weighted (IVW) MR analyses for the primary analysis. In sensitivity analyses, we used robust MR approaches.

Results: MR analyses did not show an effect of 25OHD on psoriasis. Neither the IVW MR analysis of the polygenic instrument (OR = 0.997; 95 % CI = 0.81 – 1.22; p = 0.973) nor that of the focused instrument (OR = 0.990; 95 % CI = 0.88 – 1.12; p = 0.873) revealed an impact of 25OHD on psoriasis.

Conclusions: The present MR study did not support the hypothesis that vitamin D levels measured by 25OHD affect psoriasis.

P073 | Evolutionary diversification of epidermal barrier genes in vertebrates

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The epidermal differentiation complex (EDC) is a cluster of genes encoding essential components of the skin barrier in terrestrial vertebrates. The human EDC is comprised of S100A genes, S100 Fused-Type Protein (SFTP) genes such as filaggrin, and Single-coding-exon EDC (SEDC) genes such as loricrin and involucrin. The evolution of the EDC can be reconstructed by comparative analysis of genome sequences of phylogenetically diverse vertebrates. Here, we screened determined the presence or absence of S100A, SFTP and SEDC genes in the latest releases of genome sequences from the main clades of amniotes (mammals, reptiles, birds), amphibians (frogs, salamanders, caecilians) and lungfish. S100A gene clusters were identified in all species investigated, and SFTP genes were found in amniotes and amphibians but not in fish. SEDC genes are present in high numbers (20–100) in mammals, reptiles and birds, whereas no SEDC genes exist in frogs, salamanders and lungfish. We show that 2–4 SEDCs are present in the EDC of caecilians. Comparative analysis of tissue transcriptomes indicated predominant expression of SEDC genes in the skin of these legless amphibians. The proteins encoded by caecilian SEDC genes resemble human small proline-rich proteins and involucrin with regard to high abundance of proline and potential sites of transglutamination, i.e. glutamine and lysine residues. The results of this study demonstrate that SEDCtype skin barrier genes are not restricted to amniotes and, therefore, appear to have originated during the evolutionary transition from fully aquatic to terrestrial life in a common ancestor of modern tetrapods.

P074 | Amino acid substitution in the cysteine-rich region of the integrin beta 4 subunit causes mild late onset junctional epidermolysis bullosa without extracutaneous involvement

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Introduction: Integrin alpha 6 beta 4, which is encoded by ITGA6 and ITGB4, is a transmembrane component of hemidesmosomes and plays an important role in connecting keratinocytes with extracellular matrix proteins. ITGB4 or ITGA6 biallelic pathogenic variants cause junctional epidermolysis bullosa (JEB) with pyloric atresia, which is associated with high lethality. Patients that survive usually develop JEB of intermediate severity and uro-renal manifestations. Here we report on a previously unreported missense mutation in the integrin beta 4 subunit that causes localized JEB without any

extracutaneous manifestations and unveil the possible molecular mechanism.

Methods: We performed immunofluorescence mapping and genetic testing by Sanger sequencing of candidate genes or targeted next generation sequencing (NGS) in four women (2 of them siblings) aged between 18 and 43 years, with localized skin blistering and nail dystrophy. Analysis in silico was conducted and three immortalized cell lines were generated with the keratinocytes from normal human, patients with the new variant and ITGB4 nonsense mutation, respectively. We used Immunoblotting, fluorescence activated cell sorting and immunofluorescence staining to detect the impact of the novel variant on keratinocytes on the protein level, as well as the functional detachment assay. RNA sequencing was employed to uncover the dysregulated gene expression files and signaling pathways.

Results: In all cases, manifestations started in childhood (age 4–12 years) and consisted of blistering on acral, mechanically exposed areas and progressive nail dystrophy. The immunoreactivity for hemidesmosomal proteins including plectin, BPAG1e, integrin alpha 6 beta 4 and type XVII collagen was reduced, and skin cleavage was either absent or junctional.

The previously unreported homozygous ITGB4 variant c.1642G>A, p.Gly548Arg was identified in all cases by NGS. It has a minor allele frequency of 3.98e-6 and is predicted to be disease causing (Polyphen2 is 0.987, Mutation Taster 0.999, CADD 28.6). Gly548 is located in the third cysteine-rich tandem repeat of the beta 4 subunit, where two other amino acid substitutions, p.Cys562Arg and p.Cys590Tyr have been described in patients with JEB and pyloric atresia. The arginine for glycine substitution may affect the structure of beta turn among aa546-549 in the predictive models.

Experiments on keratinocytes showed that ITGB4, p.Gly548Arg mutant did not affect the expression amount of protein integrin beta 4 subunit and other hemidesmosomal proteins. However, the distribution of beta 4 integrin subunit and the combination with other proteins may alter.

RNA sequencing results presented numerous dysregulated genes in ITGB4, p.Gly548Arg group and ITGB4 negative group, compared with NHK, especially those involved in collagen organization and inflammation process.

Conclusion: Taken together, we report on a very rare subtype of late onset nonsyndromic JEB associated with a recurrent amino acid substitution in the cysteine-rich tandem repeat of integrin beta 4. The diagnosis may be missed, or delayed, due to confounding immunofluorescence and clinical findings.

Health Services Research

P075 | Longitudinal validity of the Hyperhidrosis Quality of Life Index (HidroQoL ∧): responsiveness and MID values over time using data from a phase III b clinical trial

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Background: The Hyperhidrosis Quality of Life Index (HidroQoL ∧) is a welldeveloped patient-reported outcome measure assessing the quality of life impacts in hyperhidrosis, which shows very good measurement properties regarding structural validity, internal consistency and other measurement properties. Our aim was to substantiate and extend the results of previous longitudinal validation studies in patients with primary axillary hyperhidrosis. For this purpose, responsiveness or rather sensitivity to change was assessed over time and additionally, minimal (clinically) important difference (MID/MCID) values were estimated for different measurement time points (up to 72 weeks).

Methods: Data (from a phase III b clinical trial) was collected at baseline, week 4, week 8, week 12, week 28, week 52, and week 72. For the assessment of responsiveness, HidroQoL change scores were correlated with corresponding change scores of the Hyperhidrosis Disease Severity Scale (HDSS), the Dermatology Life Quality Index (DLQI), and the gravimetric sweat production. Furthermore, it was tested whether the different HDSS change score groups differed significantly from each other over time. This was extended by the calculation of matched-pair tests and effect sizes to test significance for each change group separately. For the estimation of MID values, different anchor-based and integrated approaches were used.

Results: In total, the sample was composed of 357 patients. For the assessment of responsiveness, 14 out of 42 a-priori hypotheses regarding the correlation of the change scores could be confirmed. Furthermore, the HidroQoL showed responsiveness or rather sensitivity to change towards improvement at every measurement time point. Effect sizes were large as expected ($r \geq 0.916$). MID values were proposed for every measurement time point (MID values of 6 to 11 from week 4 to week 72, respectively). An increasing MID value over time was observed.

Conclusion: Assessing responsiveness and estimating MID values over time, this study provided further evidence for the longitudinal validity (up to 72 weeks) of the HidroQoL and proposed MID values for different time intervals after baseline. These results support the excellent measurement properties of the HidroQoL and are in line with previous validation studies.

Immunology

P076 | How neutrophils influence skin colonization and persistence of Staphylococcus aureus

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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 studied the role of neutrophils in S. aureus skin colonization. We focused on the interaction of neutrophils and keratinocytes to unravel the mechanism underlying the neutrophil-mediated enhanced S. aureus colonization. Moreover, we analysed the role of neutrophils on the persistence of S. aureus on inflamed and not inflamed skin.

Material and Methods: Using an in vitro co-culture model we investigated the interaction of neutrophils and primary human keratinocytes by studying the induction of pro-inflammatory mediators via LEGENDPLEX analysis and qPCR and by analyzing activated signaling pathways by western blot and blockade studies. We further investigated the mechanism underlying the neutrophil-mediated enhanced S. aureus skin colonization by analyzing neutrophil recruitment and S. aureus skin colonization in inflamed and not inflamed skin of different knock-out mice using an epicutaneous mouse skin infection model. Moreover, we analyzed the role of neutrophils in the persistence of S. aureus on inflamed and not inflamed skin by neutrophil depletion in vivo.

Results: We show that the crosstalk between keratinocytes and neutrophils during the co-incubation primes the neutrophils for enhanced NET formation upon S. aureus infection which positively correlates with enhanced S. aureus colonization. These effects are mediated by soluble factors which are released during the co-culture following activation of distinct signaling pathways in keratinocytes. Moreover, we found a possible role of neutrophils in the persistence of S. aureus on inflamed skin as observed by neutrophil depletion in vivo and by assessing S. aureus colonization up to day 7 after infection.

Conclusion: Our data indicate that in inflamed skin, the interaction of neutrophils and keratinocytes induces the secretion of pro-inflammatory mediators that prime neutrophils for enhanced NET formation thus enhancing S. aureus skin colonization. Furthermore, S. aureus induced NET formation fuels further neutrophil recruitment to the skin thereby promoting persistence of S. aureus on inflamed skin.

[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

P077 (OP01/05) | Antigen-presenting cells from HCA2-KO mice are defective in inducing regulatory T cells

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There is evidence that gut commensal microbes affect the mucosal immune system via expansion of regulatory T cells (Treg) in the colon. This is mediated via short-chain fatty acids (SCFA), bacterial metabolites generated during fiber fermentation. SCFA are also produced by commensal skin bacteria and may activate resident cutaneous Treg, the activity of which is diminished in certain inflammatory dermatoses. SCFA exert their anti-inflammatory effects upon binding to G-protein coupled receptors (GPR). One of the most relevant receptors for SCFA is GPR109a/HCA2. Since ligation of HCA2 causes suppression of intestinal inflammation, we asked whether HCA2 signaling promotes also anti-inflammatory properties of immune cells in the skin. Thus, we studied whether absence of HCA2 might be responsible for the impaired activity of Treg utilizing HCA2-knock out (KO) mice. Adoptive transfer of Treg isolated from lymph nodes and spleens of sensitized wild type (WT) or HCA2-KO mice revealed that WT-Treg significantly reduced contact hypersensitivity (CHS) in recipient animals, whereas this effect was not observed in recipients injected with Treg obtained from HCA2-KO, suggesting that the suppressive potency of the latter cells was impaired. To study whether the impaired suppressive activity of Treg in the absence of HCA2 can be observed also in the another skin inflammatory model, we used the psoriasis-like imiquimod (IMQ)- induced skin inflammation model in mice. Application of IMQ resulted in psoriasiform skin (thickening, erythema, scales) with a significantly stronger response in HCA2-KO mice, indicating that HCA2-KO Treg are reduced in their suppressive activity in both models. Major players in priming Treg are antigen-presenting cells (APC). To study whether HCA2-deficiency affects also APC, bone marrow-derived dendritic cells (BMDC) obtained from WT and HCA2-KO mice were examined by flow-analysis. This revealed that BMDC from HCA2-KO mice failed to release IL-10 and aldehyde dehydrogenase 1A (Aldh1a). Aldh1a production by APC is involved in the generation of forkhead box P3 (Foxp3) positive Treg. This phenotype might be responsible for losing the capacity to induce Treg. To prove the potency of APC in priming T cells, co-culture experiments were performed. CD4+ T cells isolated from the sensitized WT mice were mixed with BMDC in the presence of the relevant hapten for 48 h. BMDC obtained from WT or HCA2-KO animals were removed after this time period and flow analysis of co-cultured CD4+ cells was conducted. T cells coincubated with HCA2-KO APC expressed lower levels of the Treg markers Foxp3, glycoprotein A repetitions predominant (GARP),

cytotoxic T-lymphocyte-associated protein 4 (CTLA4) than cells cocultured with WT APC. These results suggest that deficiency of HCA2 impairs APC in their capacity to induce Treg. This may contribute to the proinflammatory phenotype of HCA2-KO mice.

P078 | Deciphering the role of immunogenic cell death in extracorporeal photopheresis

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Extracorporeal Photopheresis (ECP) is a photoimmunotherapy providing both immunity against cancer cells and suppression of immune reactions in transplant patients. The molecular mechanism behind ECPs bidirectional manner remains unclear. Indirect evidence supports the hypothesis that ECP triggers immunogenic cell death (ICD), a regulated type of cell death that induces adaptive immunity against dying cells. ICD has not been directly investigated in primary human ECP treated samples.

We established a human in vitro model for ECP applied to lymphocytes of healthy donors and cutaneous T cell lymphoma (CTCL) cell lines, in which ICD inducing stressors were separately examined (e.g.: physical stressors (centrifugation force and UV-A) and chemotherapeutic stressors (8-methoxypsoralen)). Additionally, we studied blood from CTCL patients before, during and after ECP treatment. Samples were stained with a panel of immune cell markers, apoptosis (DAPI) and the ICD cell membrane marker Calreticulin for analysis with flow cytometry. We used qPCR to analyse several markers of ICD (e.g.: HMGB1, CXCL10). Additionally, proliferation and cytotoxicity of the samples was assessed using EZ4U Cell Proliferation and Cytotoxicity Assay (Cat. No.: BI-5000).

Upon in vitro ECP treatment, human lymphocytes upregulate ICD markers. Remarkably, first results show evidence for upregulation of ICD associated danger signals in ECP-treated patient blood, too. ECP-treated cells (ex vivo and in vitro) demonstrate impaired proliferation and an increase in danger signals on protein and RNA level in specific lymphocyte subsets compared to controls, starting as early as 24 hours post ECP treatment.

Proving the induction of ICD in ECP grants a new perspective for elucidating the mode of action of ECP. Since results are pointing towards an ICD response of specific lymphocyte subsets, we will analyse those subsets and decipher their role in ECP in CTCL, which will also pave the way towards better understanding of the pathogenesis of the diseases.

P080 (OP05/01) | Imiquimod perturbs amino acid metabolism in human CD8+ T cells

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Imiquimod (IMQ), a TLR7 agonist, is a standard local treatment for nonmelanoma skin cancers (NMSC). IMQ triggers inflammation, ultimately resulting in immunological tumor destruction. Albeit IMQ promotes the recruitment of effector T (TE) cells, IMQ triggers anergy in CD4+ T cells. Meanwhile, how IMQ affects human CD8+ TE cells, pivotal to immunological cancer control, remains unclear. To address this, we studied CD8+ TE cell lines from NMSC and circulating, skin-homing CLA +CD8+ TE cells. In both models, IMQ-treated TE cells showed significantly reduced proliferation and IFN-γ production. Because metabolism fundamentally underlies the function of T cells, we hypothesized that metabolic changes underlie the suppressive effects of IMQ on TE cells. Along this idea, we found that IMQ-treated TE cells have reduced mTOR activity. To gain broader insight into metabolic adaptations of IMQtreated T cells, we performed proteomics, revealing dysregulation of amino acid (AA) transporters, especially the SLC1A5 transporter in IMQ-treated CD8+ TE cells. Meanwhile, IMQ reduced intracellular levels of AA transported by SLC1A5, including glutamine, asparagine, aspartic acid. Consistent with a known supportive role of several AA, including glutamine and asparagine, in TE responses, adding these AA to IMQ-treated TE cells restored effector functions. Our finding that SLC1A5- dependent AA rescue IMQ-induced hypo-responsiveness of CD8+ TE cells provides a rational for studying if exogenous AA can improve effectiveness of IMQ-based destructive NMSC therapies.

P081 | Role of CD4+ T cells in occurrence of irAEs in patients with stage III/IV melanoma during treatment with immune checkpoint inhibitors

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Immune checkpoint Inhibitors (ICIs), more specifically the PD(L)-1 and CTLA-4 inhibitors have improved the outcome of patients with advanced melanoma and other skin cancer types 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 have severity from low-grade to potentially life threatening. Most of the irAEs are mediated by abnormal T cell reactions, although the exact pathogenic mechanisms are not fully understood. We aim to follow changes in CD4+ T cell subsets during ICIs to better understand immunological relations to the risk for irAEs.

In this prospective observational study, we studied peripheral blood mononuclear cells (PBMCs) from patients with stage III and IV melanoma from baseline and up to twelve months of ICI treatment or development of irAEs. All studied patients recruited were immunotherapy naive. T cells were analyzed by flow cytometry based on the expression of surface receptors including chemokine markers. We analysed T helper (Th) and T follicular helper (Tfh) cell subtypes, based on their CXCR5 expression. We were also interested in T regulatory (Treg) and T follicular regulatory (Tfr) cells, which were analyzed based on their expression of CD25 and CD127.

In total, 64 patients were enrolled in the study (male n = 41, female n = 23). The median age was 71a. The majority of patients received nivolumab monotherapy (n = 39), followed by combination treatment of nivolumab with ipilimumab (n = 19) and pembrolizumab alone (n = 6). Out of the 64 patients, 26 patients developed any kind of irAEs (CTCAE I-IV). Most common immune-induced side effects were pancreatitis (n = 7), colitis (n = 6) and skin-related side effects (n = 5). As expected, most of the irAEs occurred in the initial three months of treatment. We initially compared baseline T cell composition of patients who did not develop irAEs under treatment versus patients who developed irAEs under our immunological monitoring. We could not identify differences in the investigated Th/Tfh cell subsets, which could be useful to predict a risk for irAEs. Interestingly, prospective analysis showed that at time of irAE occurrence, patients developed marked increase in total CD4+ T lymphocytes and in certain T cell subsets, especially in the Th2 compartment.

P082 | The immune landscape of psoriasis based on single-cell transcriptomic analysis implies prominent roles of IL-18 and IL-32 in the disease pathogenesis

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Psoriasis is a chronic inflammatory skin disease whose pathogenesis is characterized by multiple cytokines and chemokines such as TNF-α, IL-8, IL-23, and IL-17. This knowledge has led to the development of highly efficient treatment modalities targeting these cytokines or associated pathways. However, a detailed understanding of the different cell types and cytokines involved in its pathogenesis is still missing. Here we used single-cell transcriptomic analyses to identify relevant immune cell and non-immune cell populations for an in-depth characterization of cell types and inflammatory mediators that may contribute to psoriasis pathogenesis. Psoriasis skin lesions were analyzed using the 10x Genomics single-cell technology and were compared to healthy control samples. Data were validated further by in situ hybridization, serum analyses of human samples and in a murine model of psoriasis. Several different immune-activated cell types with particular cytokine patterns were identified, such as keratinocytes, T helper cells, Th17 cells, dendritic cells, macrophages, fibroblasts, and endothelial cells. The expression of well-known pathogenic factors such as TNF-α, IL-8, L-23 and IL-17 was confirmed in different inflammatory cells. Furthermore, IL-18 and IL-32 were identified as less well-known and putative new pathogenic factors. While prominent expression of IL-18 was found in keratinocytes and Langerhans cells, the expression of IL-32 was found in T helper and regulatory T cells. This expression pattern could be validated by in situ hybridization. In a murine model of psoriasis, IL-18 was significantly higher expressed in psoriasis-like skin lesions than in normal skin. In an analysis of serum samples from psoriasis patients, higher protein levels of IL-18 were observed in serum from psoriasis compared to healthy controls, but did not correlate with psoriasis severity score and also not with treatment response to biologic treatment. IL-32 expression in serum samples correlated with the presence of additional joint involvement. Taken together, we provide an in-depth view of psoriasis immune-cell and non-immune cell phenotypes that might help to guide treatment decisions and disease monitoring in clinical trials.

P083 | Virus-infected mast cells directly activate virus-specific CD8+ T Cells

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Efficient anti-viral responses of CD8+ T cells require signals that promote their effector cell differentiation, for example by dendritic cells (DCs). Mast cells (MCs) are key drivers of DC maturation, but also influence their migration and antigen presenting properties and therefore indirectly mediate CD8+ T cell activation. MCs initiate innate immune responses at pathogen entry sites, promote the development of adaptive immune responses after infection, and release mediators including chemokines that recruit and activate immune cells including T cells during viral infections. However, whether MCs can directly activate virus-specific CD8+ T cells remains largely unknown. Here, we used an in vitro viral infection model with lymphocytic choriomeningitis virus (LCMV)-infected MCs or DCs co-cultured with LCMV-specific CD8+ T cells or with unspecific CD8+ T cells. Similar to LCMVinfected DCs, LCMV-infected MCs clustered with virus-specific but not unspecific CD8+ T cells and induced their activation and production of antiviral cytokines. The co-stimulatory molecules CD86 and OX40L, but not CD80, were upregulated on MCs after LCMV infection. Our findings suggest that MCs support DCs to directly prime CD8+ T cells during viral infections. Direct MC-mediated CD8+ T cell activation might be especially important within infected tissues where direct cellular interaction can take place. A better understanding of anti-viral functions of MCs may help developing new strategies to better treat viral infections.

P084 | The transcription factor ATF3 distinguished between innate and adpative immunity

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The signals deciding whether innate immunity causes autoinflammation or whether innate immune responses initiate adaptive, T cell mediated responses remain elusive. The transcription factor ATF3 is among the first signals activated in response to innate signalling, and suppresses key molecules of innate immune responses. As this decision occurs very early during the process of immune activation, we studied the effects ATF3 on the initiation of innate and of T cell mediated response in vivo.

Starting with TPA-induced skin inflammation, we found that Atf3ko mice had significantly higher levels of Il1b than controls; in sharp contrast, TPA failed to induce Il2 or Il4 electively in Atf3ko mice. Ifng was similarly induced in Atf3ko mice and in wt mice, suggesting that Atf3 may have opposing effects on either innate or adaptive immune responses.

To determine whether this effect is restricted to TPA or whether this is of relevance for T cell mediated immunity, the role of ATF3 in the context of contact hypersensitivity was elucidated. Atf3ko or wt mice were sensitized with TNCB and challenged on day 7 at one ear. After 24 hours the ear swelling response was measured and ear samples were taken for histology and molecular analysis. Following the challenge, Wt and Atf3ko mice showed similar ear swelling responses. Yet, histology was different between Atf3ko and wt mice. Atf3ko showed a stronger oedematous swelling, but a reduced infiltrate of mononuclear cells and less pronounced acanthosis. As expected, in Atf3ko mice Il6 was strongly induced, as was Cxcl1, while the mRNA levels of the IL-1 cytokine family members were similar in Atf3ko and wt mice. In line with this, ears from Atf3ko mice showed a stronger infiltrate of neutrophils.

In contrast, the T cell associated cytokines Il2 and Il4 were again exclusively induced in the wt mice, and almost at background in Atf3ko mice. Ear tissue from Atf3ko mice displayed reduced levels of Ifn and Ifn-induced Cxcl10. To determine, whether ATF3 affected the adaptive immune response, we analysed CD4+ cells isolated from draining lymph nodes. Indeed, Il2 and Il4 mRNA was only induced in CD4+ T cells from wt mice but not from Atf3-/- mice, and CD4+ T cells from Atf3-/- mice also showed significantly reduced levels of Ifng mRNA as compared to controls.

These early data suggest that ATF3 differentially regulates innate and adaptive immunity. In contrast to many other known activators of innate immunity, suppression of ATF3 does not stimulate both, innate and adaptive immunity, but promotes a state of autoinflammation with enhanced neutrophil recruitment and suppressed T cell activation.

P085 | Expression and function of the endocytic receptor DEC205 in endothelial cells

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The antigen uptake receptor DEC205 was originally described in dendritic cells (DCs). Within the intracellular domain we characterized three defined sequences, mediating (a) the uptake of ligands, (b) the targeting to deeper endosomal compartments and (c) the recycling back to the surface of DCs. This makes DEC205 a highly specialized receptor for absorptive endocytosis. When testing other cell types for DEC205 expression, we found that among all non-leukocyte populations only brain endothelial cells (ECs), as indicated by colabeling PECAM-1 (CD31), react with the DEC205 antibody. We therefore further tested a cellular model for ECs, the endothelial cell line bEnd.3 that is derived from mouse brain. Here, similar to brain sections, we found expression of DEC205 by PCR and FACS staining. Moreover, these results are further supported by in vivo analysis, as FACS staining of ex vivo isolated brain ECs showed

substantial expression of DEC205 too. Thus, DEC205 is expressed by brain ECs and bEnd.3 cells are a suitable model to investigate the functions of DEC205 receptors in ECs. According to our results obtained in DCs, showing that DEC205 facilitates receptor mediated endocytosis, we hypothesize that DEC205 analogously serves as transporter for nutrients and/or antigens in ECs. Therefore, we started experiments by incubating monolayers of the DEC205+ EC line bEnd.3 with graded doses of fluorescent latex beads for 1h. The beads were either coated with IgG or with anti-DEC205 antibodies. Thereafter, beads were washed away and the cultures were stained with Hoechst 49126 to mark cell nuclei, followed by automatic analysis for cells and endocytosed latex beads by live cell image analysis. Here we could show an increased uptake of αDEC-coated beads by bEnd.3 cells in comparison to IgG coated controls, indicating a function for DEC205 receptors in mediating endocytosis in bEnd.3 cells. Thus, DEC205 may serve as "linker" molecule connecting the function of ECs with those of the immune system.

P086 | Analysis of DEC205 expression by neutrophils in naive mice and under inflammatory conditions

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DEC205 is an endocytic receptor, which is highly expressed on dendritic cells (DCs). Previous studies have revealed that other cells, such as B cells, T cells and macrophages, also express DEC205 at different levels. For DCs, it has been demonstrated that DEC205 has the properties of promoting antigen uptake and recognizing apoptotic and necrotic cells. However, the function(s) of DEC205 in cells other then DCs remains unclear and the exact tissue distribution and function of DEC205 on neutrophils have not been studied yet. Here we show that neutrophils in murine bone marrow, blood and lymph nodes can express DEC205 by comparing wildtype with DEC205-knock out mice. In a TNCB-driven contact hypersensitivity model, neutrophils in the inflamed ear down-regulated DEC205 expression and became fully-activated, while in bone marrow, blood and lymph nodes, neutrophils still maintained DEC205 expression and a non-activated state. Upon in vitro stimulation with phorbol 12-myristate 13-acetate, neutrophils isolated from the bone marrow became activated and down-regulated expression of DEC205 too. When injecting freshly isolated neutrophils from EGFP-transgenic mice into syngeneic hosts, followed by sensitization at the ears, we identified tissue-homing neutrophils by their EGFP expression in the ears. Here, expression of DEC205 by neutrophils was downregulated after transmigrating from blood to ear, suggesting that DEC205 may function in neutrophilic tissue accumulation. Overall, DEC205 is expressed by neutrophils and is down-regulated when neutrophils enter tissues or have been fully activated.

P087 | The role of the NLRP3 inflammasome in Langerhans cells for pathogenesis of vitiligo

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Vitiligo is an autoimmune disease that leads to the progressive destruction of melanocytes by autoreactive CD8+ T cells. Elevated ROS levels and an inability to deal with cellular stress in melanocytes are major causes for vitiligo. Stressed melanocytes release damage-associated molecular pattern (DAMP) and express receptors for cellular stress, like NLRP1 and NLRP3. These molecules were found to be upregulated and are involved in vitiligo pathogenesis. Despite that, the role of the innate immune system, especially of dendritic cells (DC) in the induction of vitiligo is still unclear.

Due to the fact that epidermal Langerhans cells (LC) 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 through NOD-like receptors and present melanocyte-antigens to induce autoreactive T cell responses. Furthermore, we hypothesize that targeting skin DC by antigen-coupled antibodies could be an effective vitiligo therapy.

In order to investigate the importance of LC and NLRP1/3 for vitiligo pathology, we will establish chemically-induced vitiligo models in mouse and human skin. Melanocyte stress and early activation of the innate immunity, with a focus on DC, will be determined by using multicolor flow cytometry, microscopy and quantitative PCR. The role of NLRP1/3 in LC will be investigated by using a LC-depletion mouse model, the huLangDTR mice, bone marrow chimera models and human skin explants. Finally, we will evaluate if DC-based immunotherapy in combination with NLRP1/3 inhibitors has the potential to stop the progression of vitiligo.

Taken together, this project will shed light on the role of NLRP1/3 in LC for vitiligo pathogenesis. Furthermore, we will evaluate novel therapeutic strategies that might be beneficial for vitiligo patients.

P088 | Association of polymorphous light eruption with NOD-2 and 1 TLR-5 gene polymorphisms and GvHD

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Background: Polymorphous light eruption (PLE) is a common, immunologically mediated, photosensitive skin disease. After ultraviolet-B (UV-B) irradiation, patients with PLE show reduced Langerhans cell (LC) depletion in the epidermis, which results in a non-suppressive microenvironment in the skin. Interestingly, severe

acute graft-versus-host disease (aGvHD) occurred in stem cell transplanted patients that showed no or incomplete depletion of LCs after UVB irradiation. Genetic variation in nucleotide-binding oligomerization domain 2 (NOD-2) and toll-like receptor 5 (TLR-5) genes also confers susceptibility to aGvHD.

Objectives: We hypothesized that PLE is associated with genetic variation in the NOD-2 and TLR-5 genes.

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

Results: The frequency of NOD-2 alleles with the SNP R702W was significantly higher in PLE than in controls (31.8% vs. 6.3%; $p < 0.0001$), and homozygous carriers of this mutation were more common in PLE (27.9% vs. 0%; $p < 0.0001$). For SNP 3020Cins, the allele frequency (7.3% vs. 0.7%; $p = 0.0025$) and the number of heterozygotes (14.7% vs. 1.3%; $p = 0.0019$) were higher in PLE. The frequency of alleles with the N392STOP SNP of the TLR5 gene, which is associated with a truncated, non-functional receptor, was significantly higher in PLE (21% vs. 5%; 7% vs. 1% homozygotes, 28% vs. 8% heterozygotes; $p < 0.0001$). The other SNPs did not differ significantly.

Conclusions: This study yielded a high frequency of functional SNPs in the NOD-2 and TLR-5 genes in PLE. The same SNPs are associated with aGvHD and there are similarities in the reaction of LCs after UVB irradiation between aGvHD and PLE. This leads to the hypothesis that patients with PLE may be more susceptible to developing GvHD after stem cell transplantation, an assumption that needs to be investigated further.

P089 (OP04/01) | IL-32 prevents keratinocyte necroptosis in lichen planus via induction of death associated protein kinase 1

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Lichen planus (LP) is a Th1- dominant inflammatory skin disease of unknown etiology. Histologically, it is characterized by an interface dermatitis with a dense lymphocytic infiltrate at the epidermodermal junction and cell death in the stratum basale. IFN-y and TNF-a play a pivotal role in regulating inflammation and two mechanisms of keratinocyte cell death: apoptosis and necroptosis. However, contributing factors might favor one mechanism of cell death over the other. Here, we sought to investigate the role of IL-32 and its downstream target death associated protein kinase 1 (DAPK1) in LP.

Using spatial transcriptomics and bulk sequencing data, IL-32 was found to be highly upregulated within the inflammatory infiltrate of LP skin biopsies. The number of IL32-transcripts was 1000 times higher in LP compared to other inflammatory skin diseases, 100 times higher than IFNG and correlated significantly with the strength of interface dermatitis. Stimulation of primary human keratinocytes with recombinant IL-32y led to enhanced expression of death associated kinase 1 (DAPK1) protein, a kinase known to be involved in cell death regulation. Of note, DAPK1 expression was potentiated by combining IL-32 with Th1 cytokines IFN-γ and TNF-α indicating that Th1 cytokines and IL-32 synergistically regulate DAPK1 induction. Immunohistochemistry showed DAPK1 positive staining at the basal keratinocytes in LP tissue sections. CRISPR/Cas mediated DAPK1 knock-down in primary keratinocytes resulted in an increase of necroptosis and a decrease of apoptosis upon stimulation with the supernatant of T cells isolated from LP skin biopsies. Furthermore, DAPK1 knock-down critically enhanced the release of IL-1β upon stimulation with Th1 cytokines, indicating that DAPK1 mediates antiinflammatory and anti-necroptotic functions in keratinocytes.

In summary we demonstrate a pro-apoptotic immune axis mediated by IL-32 and DAPK1 counteracting inflammatory cell death in LP, a mechanism potentially contributing to the maintenance and chronification of skin inflammation in LP.

P090 (OP01/04) | Neo-adjuvant immune checkpoint inhibition further boosts therapy response and prevents therapy resistance mechanisms

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Melanoma therapy was revolutionized by the approval of both targeted-therapies (TT) and immune checkpoint inhibition (ICI). Now patients with locally advanced stage III melanomas are even treated with adjuvant TT or ICI to prevent metastatic spread. However, still around two thirds to half of stage IV melanoma patients develop therapy resistances and a third of stage III melanoma patients suffers from recurrent melanoma or disease progression. Especially, patients with liver metastases show reduced response to TT or ICI. Therefore, there is need for novel therapeutic concepts, such as neo-adjuvant ICI, to further improve therapy responses and to circumvent resistance mechanisms. With this project we analyze the influence of palliative, adjuvant and neo-adjuvant ICI on hepatic melanoma metastasis.

To this end, we developed a mouse model simulating primary melanomas by intradermal injection of WT31 or B16F10 luc2 melanoma

cells. Hepatic metastases were induced by spleen injection of melanoma cells. Mice received three doses of anti-PD-1 and anti-CTLA-4 or isotype antibodies as palliative, adjuvant or neo-adjuvant treatment. Melanoma liver metastasis was quantified. Immune cell composition in the hepatic immune microenvironment, blood and intradermal melanomas was comparatively analyzed by immunofluorescence stainings or flow cytometry. Besides, cytokines were measured by bead-based multiplex cytokine assays or in situ hybridization.

The number of hepatic melanoma metastases was significantly decreased when palliative ICI was initiated on day 6 instead of day 9. Adjuvant ICI led to an even stronger clinical response as 60% of mice showed a complete response. Significantly increased numbers of CD3+ CD8+ T cells and decreased numbers of CD3+ CD4+ T cells were found in the adjuvant setting as compared to the palliative one. Besides, less CD11b+ F4/80+ hepatic macrophages that mediate therapy resistances were found when mice received adjuvant ICI. When neo-adjuvant ICI was applied only a few mice developed hepatic metastases and both the clinical response and the reduction in hepatic metastasis were further improved in relation to adjuvant ICI. Analyses of the hepatic immune cells revealed higher numbers of CD3+ CD8+ or CD3+ CD4+ T cells in the liver, peripheral blood and even the intradermal melanoma when mice received neo-adjuvant ICI as compared to adjuvant ICI. Analysis of hepatic cytokine levels showed lower levels of IL-4 and IL-15 when the intra-dermal tumor was present during ICI. Besides, the numbers of CD3+ IFNg+ T cells and CD4+ Tbet+ T cells were increased while CD4+ Gata3+ T cells were decreased in the neo-adjuvant setting in comparison to the adjuvant treatment.

Our data demonstrate that development of therapy resistance mechanisms can be overcome by adjuvant ICI and confirm its potential in protection from hepatic melanoma metastasis. However, neo-adjuvant ICI was even more superior and protected best from hepatic metastasis by driving an even stronger systemic antitumoral Th1 immune response. This indicates that neo-adjuvant ICI might be an highly appealing therapeutic option for CM to further boost therapy response to ICI and to circumvent therapy resistance mechanisms, especially for melanoma liver metastasis.

P091 | Unraveling the role of an Inflammatory-induced CD64+ DC population in melanoma

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Dendritic cells (DC) are potent antigen-presenting cells, initiating immune responses and bridging the gap between innate and adaptive immunity. The conventional DC (cDC) subsets cDC1 and cDC2 contribute to cancer-related immunity by inducing T-cell-mediated anti-tumor responses. While investigating the role of cDC in transplantable melanoma mouse models, our lab identified an inflammatoryinduced DC population that is distinct from cDC1 and cDC2 as it expresses CD64, a marker used to distinguish monocytes and macrophages from cDC. Despite their CD64 expression, these cells were identified as DC based on their expression of Zbtb46, a transcription factor restricted to the cDC lineage. While a similar DC subset has already been described in other models of inflammation, its existence in transplantable melanoma mouse models has not yet been described.

So far, we observed that CD64+ DC represent the dominant DC population in tumordraining lymph nodes of B16.OVA and D4M.3A tumor-bearing mice. For this reason, we aimed to characterize them phenotypically to gain a deeper understanding of their origin and possible function. By utilizing a multiplex 26-marker flow cytometry panel which allows us to discriminate the DC compartment from other myeloid cells, we learned that CD64+ DC share phenotypic characteristics with cDC2. Further, we observed that migratory (MHC-II-high CCR7+) CD64+ DC express the DC activation marker CD40, indicating that CD64+ DC are likely to be involved in the induction of effector T cell responses. In addition, we investigated the expression of the coinhibitory receptors PD-L1 and PD-L2, whose upregulation on myeloid cells has been reported in inflammatory conditions. We could demonstrate that CD64+ DC showed similar levels of PD-L1 and PD-L2 as cDC2.

The next step in this project will be to understand if CD64+ DC are of real cDC origin or whether they derive from the monocytic lineage. Moreover, we wanted to understand their specific role in the induction of T cell responses for tumor immunity. Hence gaining more profound knowledge of this cell population's biology might identify them as possible targets for DC-based personalized melanoma therapy.

P092 | Laminin beta 4 is a constituent of the cutaneous basement membrane zone and autoantigen of anti-p200 pemphigoid

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Anti-p200 pemphigoid is a subepidermal autoimmune blistering disease characterized by autoantibodies against a 200 kDa protein. Laminin γ1 (LAMC1) has been proposed as target antigen; however, its pathogenic involvement in the disease could not be demonstrated. Here, we identified laminin β4 (LAMB4) as a structural component of the dermal-epidermal junction and autoantigen of antip200 pemphigoid by immunoprecipitation using anti-p200 pemphigoid antibodies depleted of anti-LAMC1 IgG reactivity and extracts of human skin followed by mass spectrometry. Epitope mapping revealed that all analyzed anti-p200 pemphigoid sera (20 of 20) reacted with the C-terminus of LAMB4. LAMB4 is located at the level of the cutaneous basement membrane zone (BMZ) with predominant expression in epidermal keratinocytes. Independent experimental approaches suggested laminin α3 (LAMA3) and laminin γ2 (LAMC2) as binding partners of LAMB4. Invitro assays demonstrated the ability of anti-LAMB4, but not anti-LAMC1 IgG to exert tissue damage and subepidermal split formation. The identification of LAMB4 as autoantigen of anti-p200 pemphigoid extends our current knowledge on the cutaneous BMZ and will improve diagnostic and therapeutic management of pemphigoid diseases.

P093 | The impact of glycosylation on IgG2-induced signaling in neutrophils

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Pemphigoid diseases (PDs) are a group of prototypical organ-specific, antibodydriven autoimmune diseases, characterized by autoantibodies targeting the hemidesmosomal adhesion proteins of the dermal-epidermal junction (DEJ). Multiple lines of evidence indicate that neutrophils are crucial for the antibody-induced tissue destruction. Furthermore, IgG subclasses also have an impact on disease severity in PDs; in particular IgG1 and IgG3 are pathogenically relevant, whereas data regarding IgG2 and IgG4 is sparse.

Post-translational changes in antibody glycosylation may influence the exertion of pro- or anti-inflammatory potentials of the different IgG isoforms. Specifically, sialylation has been linked to anti-inflammatory effects whereas agalactosylated IgG autoantibodies are linked to aggressive, pro-inflammatory responses. We analyzed the impact of different glycosylation patterns on the activation of neutrophils by immune complexes (IC) generated from recombinant hCOL7 and monoclonal antihCOL7 IgG2 glycoforms, such as: endoS-digested (deglycosylated IgG), desialylated and degalactosylated (G0), galactosylated (G2), and galactosylated plus sialylated (G2 S1/S2).

We observed significant differences between the different IgG2 glycoforms in matters of ROS release, spreading, granule release, and expression of activation markers and apoptosis. In addition, we validated the strikingly different kinase signaling activity in neutrophils after stimulation with IgG2-IC by multiplex kinase assays as well as Western Blot analysis.

Based on these data, we obtained detailed insights into the molecular (kinome) and functional impact of different glycosylation patterns of IgG2 ICs on neutrophil functions. These data provide new insights into neutrophil biology, and may also be of clinical relevance in PDs, i.e. for targeted therapies based on the isotypes or glycoforms of the IgGs present.

P094 | C5aR2 contributes to neutrophil activation and function during the pathogenesis of epidermolysis bullosa acquisita

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Epidermolysis bullosa acquisita is a rare autoimmune skin blistering disease caused by autoantibodies against type VII collagen (COL7) for which no targeted therapy is yet available. Passive transfer of anti- mouse COL7 antibodies into mice mimics the effector phase of EBA, which is characterized by infiltration of neutrophils and destruction of the dermal-epidermal junction (DEJ) zone by reactive oxygen species (ROS) and proteases, leading to subepidermal blistering. Activation of the complement system by COL7/anti-COL7 antibody immune complexes (ICs) at the DEJ has been identified as a crucial step in EBA pathogenesis, leading to excessive generation of complement component 5a (C5a). The anaphylatoxin C5a is a potent chemoattractant and binds to the seven-transmembrane receptors C5aR1 and C5aR2, which are broadly expressed on myeloid cells and especially on neutrophils. The pro-inflammatory contribution of the C5a/C5aR1-axis to the EBA pathogenesis through neutrophil recruitment and activation has been demonstrated by experiments using mice deficient in C5aR1. However, the contribution of C5aR2 to the pathogenesis of EBA was still elusive. Therefore, here we investigated the functional role of C5aR2 in the disease development of EBA by using mice deficient in C5aR2 (C5ar2-/- mice) in the antibody transfer model of EBA. C5ar2-/- mice showed an ameliorated disease phenotype compared with wild-type (WT) mice, suggesting delayed pathogenesis. Moreover, neutrophils from C5ar2-/- mice showed significantly reduced C5a-induced activation and migration in vitro, an effect that was dependent on the presence of C5aR1, as both the absence of C5aR1 and simultaneous blockade of both C5a receptors completely abolished C5a-induced activation and migration. Interestingly, not only C5a-induced neutrophil effector functions were impaired by C5aR2 deficiency, but also IC-induced ROS release. When stimulated with COL7/anti-COL7 antibody ICs in vitro, neutrophils from C5ar2-/- mice showed significantly reduced ROS release compared with WT cells. We found that this may be due to lower expression of activating Fcγ receptors (especially FcγRIV) on neutrophils from diseased C5ar2-/- mice, which have been shown to be important players in the pathogenesis of EBA by possibly contributing to the sustainment of tissue inflammation. Taken together, this suggested that the effects of C5aR2 deficiency on EBA pathogenesis in the antibody transfer model are mainly due to deficiency of C5aR2 on neutrophils, the major effector cells in this model. We confirmed this by showing that mice with neutrophil/monocyte-specific C5aR2 deficiency exhibited a similarly ameliorated disease phenotype as global knock-out mice (C5ar2-/- mice). Finally, single-cell RNA-sequencing (scRNA-seq) confirmed that both C5a receptors are required for optimal C5a signaling. In addition, scRNA-seq revealed slightly different transcriptome profiles between neutrophils from WT and C5ar2-/- mice after C5a stimulation, providing some insights into possible underlying molecular mechanisms. Deciphering these molecular mechanisms may be key to the development of much-needed novel targeted therapies for the treatment of C5a-dependent neutrophil-driven (autoimmune) diseases like EBA.

P095 (OP03/05) | SERPINB3/B4 is an autoantigen driving the clinical phenotype of eczematous psoriasis

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Psoriasis is a heterogeneous t helper type (Th) 17 driven chronic inflammatory skin disease (ISD) with a prevalence of 2–3% worldwide. Previous studies have suggested an autoimmune basis for psoriasis which was corroborated by the identification of autoantigens in human skin such as LL37 and ADAMTSL5. Using a hypothesis-free approach based on mass spectrometry of immunoaffinity purified HLA-ligands in the psoriatic skin we identified SERPINB3/B4 as novel putative autoantigen in a subcohort of psoriasis showing eczematous features (EczPso) but not in healthy controls or typical psoriasis.

Transcriptome data and immunohistochemistry revealed indeed higher abundance of SERPINB3/B4 in the skin of EczPso compared to psoriasis and Th2 driven ISD atopic dermatitis (AD). In line, SERPINB3/B4 expression showed stronger upregulation in primary human keratinocytes upon stimulation with a combination of Th2 (IL-4) and Th17 (IL-17, TNF-α) cytokines, than with Th2 or Th17 cytokines alone, respectively. To next assess the immunogenic role of SERPINB3/4 as putative autoantigen, we stimulated lesional T cells in a co-culture with SERPINB3-stimulated autologous monocyte derived dendritic cells and observed significant proliferation upon

SERPINB3 in EczPso, but not in psoriasis or AD. Supernatants of cultures from SERPINB3 proliferating T cells were characterized by a mixture of Th17 and Th2 cytokines which induced both eczema-typical spongiosis and psoriasiform acanthosis in reconstructed human epidermis skin equivalents. Finally, injection of Serpinb3b into wildtype mice resulted in both local inflammation and sensitization of T cells as demonstrated by T cell proliferation assays.
Taken together, this data not only confirms SERPINB3/4 abundance in EczPso but also highlights its proinflammatory and auto-antigenic role for EczPso which may open novel therapeutic options for this psoriasis subtype.

P096 | Environmental antigens may cross-activate the psoriatic autoimmune response against melanocytes in psoriasis

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Psoriasis is an HLA-C*06:02-associated T-cell-mediated autoimmune disease. Using a pathogenic psoriatic Vα3S1/Vβ13S1 T-cell receptor (TCR), we had demonstrated that HLA-C*06:02 mediates an autoimmune response of CD8+ T cells against melanocytes by presenting an autoantigenic peptide from ADAMTS-like protein 5 (ADAMTSL5). Psoriasis is not a congenital disease, but develops during life under the influence of environmental conditions, lifestyle and infections. We used the peptide recognition motif of the Vα3S1/Vβ13S1 TCR to search for environmental antigens that can cross-activate the pathogenic psoriatic autoimmune response. Through this, we identified peptides from the microbiota, infectious agents, yeast, tobacco and wheat that stimulate the Vα3S1/Vβ13S1 TCR and CD8+ T cells of psoriatic patients. Using peptide-loaded HLA-C*06:02 tetramers, we demonstrated that psoriasis patients have increased circulation of CD8+ T cells that recognize both the autoantigen and wheat peptides. A gluten-free diet improved previously refractory psoriasis in several patients. These results show that environmental antigens can activate and maintain the psoriatic autoimmune response. Autoantigen- and wheat peptide-loaded HLA-C*06:02 tetramers could be used as biomarkers in psoriasis.

P097 | Uncovering neutrophil behavior during pathological ECM remodeling

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Since their discovery, neutrophils have been seen as short-lived 'unsophisticated thugs'. However, in the past decades, this view has been challenged. Neutrophils are in fact sophisticated modulators of immune responses. As a consequence, the role of neutrophils in pathophysiological conditions has also been reconsidered. Recent insights indicate an underestimated role in chronic inflammation, cancer, and fibrosis. One important aspect uniting these diseases is extensive mechanical remodeling of the involved tissue. While neutrophils have been reported to sense and respond to mechanical cues, their reaction to altered tissue mechanics is largely unexplored. This includes the impact of pathological extracellular matrix (ECM) remodeling on neutrophil behavior. This project aims to shed light on this aspect, particularly in the context of breast cancer. The main goal is to unravel how such biophysical cues affect neutrophil recruitment to diseased tissue and modulate their function towards a disease-supporting phenotype. Therefore, neutrophils and differentiated HL-60 cells are examined at the single-cell level in collagen I-rich 3D cancer models combined with dedicated high-throughput imaging techniques. The results of this study will provide comprehensive insight into the impact of disease-associated ECM remodeling on neutrophils, including a detailed analysis of their migratory behavior in 3D matrices.

P098 (OP02/03) | IL-13 is constitutively produced by a unique population of innate lymphoid cells in healthy skin leading to pro-Th2 imprinting

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Innate Lymphoid cells (ILC) are resident immune cells found in most barrier tissues. They control immune responses through the production of cytokines and respond in an antigen-independent manner to tissue damage or dysregulation.
Type 2 ILC (ILC2) primarily respond to the alarmins IL-25, IL-33 and TSLP produced by keratinocytes or epithelial cells and secrete large amounts of IL-13 upon activation, thus playing an important role in anti-parasite immunity, wound healing but also allergic responses.
While tissue-resident ILC2 typically express alarmin receptors and have the potential to expand quickly after activation, their cytokine

production is tightly controlled and usually not observed at the steady state.

We found however that a unique population of tissue-resident ILC in healthy skin produced constitutive levels of IL-13. While these ILC expressed IL-7R, CD90.2 and ICOS and were negative for the typical lineage markers associated with other innate and adaptive immune cells, they did not express alarmin-receptors or KLRG1 in contrast to resident ILC2 in other tissues. Furthermore GATA3, which is a key transcription factor of ILC2, was expressed at much lower levels compared to conventional ILC2, suggesting that despite the constitutive production of IL-13 this population of skin ILC did not belong to the conventional ILC2 lineage. Levels of IL-13 remained stable in naïve TSLPR-KO, ST2-KO and germ-free mice indicating that homeostatic IL-13 production is not controlled by alarmins or the microbiota. However, less IL-13+ skin ILC were found in male mice, a phenomenon also reported for conventional ILC2 in other tissues and linked to androgen-receptor signaling.

IL-13-KO mice showed a normal distribution of innate and adaptive immune cells in healthy skin, but lacked a skin-specific population of Type 2 dendritic cells (DC2), which expressed low levels of CD11b. CD11b-low DC2 were also absent from STAT6-KO, IL4Ra-KO and IL13Ra1-KO mice, indicating that they directly respond to IL-13 signaling and require STAT6 activation for their differentiation. In the absence of IL-13 signaling, dermal DC2 were stable in number but remained CD11b-hi, while DC2 populations in other tissues were unaffected by STAT6 or IL-13 deficiency. IL-13 was also sufficient to rescue CD11b-low DC2 differentiation in IL-13-deficient mice in vivo and resulted in a concentration-dependent differentiation of CD11b-low DC2 in vitro, which expressed a CD11b-low DC2-associated transcriptomic signature.

In the absence of IL-13 signaling, dermal DC2 continued to take up antigen but showed a diminished capacity to support IL-4+ GATA3+ Th2 cell development against parasite antigens and allergens, while anti-fungal IL-17+ RORγt+ responses were increased.

Thus, our work identified that a unique population of ILC in the skin produce homeostatic levels of IL-13, which mediates the differentiation of a unique DC2 population responsible for regulating a pro-Th2 environment, which limits inflammation but might also increase the risk of allergic sensitization.

P099 | Glucocorticoids contribute to the resolution of inflammation by altering monocytes phagocytosis

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Glucocorticoids (GC) are still the drugs of choice for the treatment of many chronic inflammatory diseases. Our previous studies have shown that GC treatment does not suppress monocyte functions but induces a distinct anti-inflammatory phenotype in these cells. Also the treatment of LPS-stimulated monocytes with GC leads to re-programming of the cells towards a specific population involved in resolution of inflammation. Here we show that the ability of monocytes for phagocytosis is significantly improve by GC treatment. We stimulated human monocytes for 48h with GC and subsequently analyzed their phagocytic activity for 4 and 20h. We could demonstrate that GCs exhibit pro-phagocytic effects in monocytes resulting in enhanced uptake of latex beads as well as pathogens like Leishmania major and Bacteria. Interestingly, in addition to pathogens and latex beads we could also observe that carboxylate modified latex beads (mimicking apoptotic cells) or granulocytes are increasingly taken up by monocytes stimulated with GCs. These data indicate that GC-treated monocytes are important for the resolution of inflammation but at the same time are still capable to neutralize pathogens. Thus, we reveal a new strategy of GCs -possibly as cell therapy- to fight inflammation without increasing the risk of infection.

P100 (OP04/05) | EBI2 marks CD8+ tissue-resident memory T cells of the skin

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Background: Allergic contact dermatitis (ACD) is an inflammatory skin disease and in many industrialized countries the most prevalent occupational disease. ACD and many other inflammatory skin diseases like psoriasis and atopic dermatitis are characterized by site-specific recurring lesions, that are maintained for years. Tissue-resident memory T cells (TRM) remain sessile at the site of inflammation for decades and have been linked to the recurring pathology of chronic inflammatory skin diseases and ACD, but their tissue tropism makes it difficult to pharmacologically address them. Epstein-Barr Virus-induced gene 2 (EBI2) is a G protein-coupled receptor (GPR183) that mediates chemotaxis towards its ligand 7α,25-dihydroxychlesterol (7α,25-OHC) and is involved in generating lymphocyte responses. CH25H and CYP7B1 are the two enzymes that synthesize 7α,25-OHC. These enzymes, together with EBI2, have been reported to be involved in a plethora of disease pathologies, such as rheumatoid arthritis, multiple sclerosis, chronic obstructive pulmonary disease and inflammatory bowel disease. EBI2 has not been characterized in the context of TRM cells as of today.

Methods: We made use of the TNCB-induced contact hypersensitivity (CHS) reaction, the murine ACD model. Using qRT-PCR, we could

assess mRNA-expression of relevant genes of the EBI2-oxysterol-axis in the ear skin of CHS mice. Utilizing an EGFPreporter mouse strain for EBI2, we were able to identify TRM cells in murine CHS skin and characterize their EBI2 expression. Using high-parameter flow cytometry, we could demonstrate the induction and the phenotype of murine TRM cells in the context of CHS. Additionally, we re-analyzed publicly available raw transcriptome bulk-RNAseq data from the NCBI GEO data base, to compare the mRNA expression of relevant genes of the EBI2-oxysterol-axis in different human skin diseases. Also using high-parameter flow cytometry and clustering algorithms, we were able to characterize human TRM cells from healthy human skin samples regarding markers for the classical immune response types, functional state markers and direct effector molecules, such as cytokines, as well as EBI2.

Results: Rapidly after antigen-challenge of the ear, EBI2-ligand-producing enzymes were upregulated in the murine skin. The same genes were also highly significantly upregulated in human skin samples, comparing lesional vs. non-lesional skin of atopic dermatitis and psoriasis patients. TRM cells are strongly induced in the skin of CHS mice after antigen-challenge and they reside locally for months, where they mediate the memory recall response after a second challenge. About 80 % of CD4+ TRM cells are EBI2+, and strikingly, ~95 % of CD8+ TRM cells are EBI2+. In human healthy skin we find 60–80% of CD4+ TRM cells to express EBI2, whereas again ~80 % of CD8+ TRM cells were EBI2+. Peripheral tissues like blood and, for the mouse also lymph nodes, showed that most CD8+ T cells were EBI2-.

Conclusion: Upregulation of genes of the EBI2-oxysterol-axis in lesional murine and human skin is a strong indicator, that the EBI2-oxysterol-axis could be involved in the pathology of skin diseases, especially atopic dermatitis and psoriasis. TRM cells are described to be present in ex-lesional skin and to mediate chronic flare ups. We find those cells expressing EBI2 in the mouse as well as in human. The striking contrast in EBI2- expression between peripheral CD8+ T cells and skin CD8+ TRM cells implicates a functional role for EBI2 on TRM cells that is yet to be uncovered.

P101 | AIBD-related specific B cells develop under the absence of functional regulatory T cells leading to a pathogenic autoantibody triggering a noninflammatory form of EBA

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The dysfunction in regulatory T cells (Treg) triggers the development of different severe autoimmune diseases. A missense mutation in the transcription factor foxp3 leads to the absence of functional Treg in scurfy mice. In these mice, high titers of autoantibodies cause blister formation in the skin and the development of autoimmune blistering diseases (AIBD).

To further understand the production of pathogenic autoantibodies and antigenspecific B cell responses in the context of AIBD, we analyzed the development and frequency of antigen-specific B cells in scurfy mice. Performing Enzyme-linkedimmuno- spot (ELISpot) assays with scurfy lymphocytes and different known AIBD antigens, we found elevated frequencies of specific B cells reactive against AIBDrelated proteins. Further studying the AIBD development in the absence of functional Treg, we found an altered B cell compartment in skin-draining lymph nodes and spleen of sick scurfy mice compared to WT littermates. In addition, we could identify significantly higher frequencies and cell numbers of B cells in the skin of scurfy mice.

Taking a closer look at the development of AIBD in these Treg-deficient mice, we could previously isolate a spontaneously developed scurfy autoantibody (H510) which is pathogenic in vivo after injection in neonatal C57BL/6 mice (WT) by targeting the murine von-Willebrand-Factor-A-like domain 2 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 further understand the mechanism of blister formation, we injected the anti-Col7 antibody in Fc-gamma receptor knock-out (KO) mice and found subepidermal blisters in most injected KO mice. To confirm a pathomechanism independent of inflammatory immune cells, we plan to silence the Fc-part of H510 to completely inhibit the interaction of immune cells and inject the silent antibody in neonatal WT mice for examination of blister formation. Finally, we aim to get more insights into the autoimmune reactions leading to the onset of AIBD.

P102 | The impact of glycosylation on IgG1-induced signalling in neutrophils

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Epidermolysis bullosa acquisita (EBA) is an autoimmune skin blistering disease which is characterized by binding of autoantibodies against human collagen VII (hCOL7) to Fc receptors, and the subsequent activation of neutrophils. Consequently, this leads to the release of reactive oxygen species (ROS) and proteases that ultimately cause characteristic neutrophil-driven tissue damage.

Analyses have revealed that IgG1 is among the pathogenically relevant IgG subclasses in EBA. To date, studies analysing its function in EBA pathology are scarce. Interestingly, differentially glycosylated IgGs have already been shown to have either pro- or anti-inflammatory effects. While galactosylation and sialylation are correlated with anti-inflammatory effects, afucosylated and agalactosylated (auto)antibodies were observed to contribute to pro-inflammatory responses.

We investigated the influence of different IgG1 glycoforms on the function of human neutrophils activated by immune complexes (ICs). The ICs were composed of recombinantly produced hCOL7 and IgG1 treated with the deglycosylating enzyme EndoS, degalactosylated and desialylated IgG1 (G0), galactosylated IgG1 (G2) as well as galactosylated and sialylated IgG1 (G2S1/S2). We could demonstrate significant differences between the distinct IgG1 glycoforms regarding ROS release, adhesion, and expression of surface markers for activation. Furthermore, we performed a multiplex kinome analysis revealing different activity levels for certain kinases. Additionally, these results were validated in a Western Blot analysis.

Thereby, we gained new information on the function of neutrophils in IgG1 signalling and the complex pathogenicity of EBA. Eventually, our results might pave the way for novel treatments on the basis of IgG isotypes and subclasses in autoimmune bullous diseases including EBA.

P103 | Cystine/glutamate antiporter (Slc7a11/xCT) and fibrosis: ferroptosis as a potential therapeutic target in systemic sclerosis?

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Systemic sclerosis (SSc) is a chronic disease of the connective tissue with three main features: vasculopathy, immune activation and excessive collagen production leading to tissue fibrosis. A major player in this pathogenesis is oxidative stress in form of reactive oxygen species (ROS). It is already known, that ROS levels are highly elevated in SSc fibroblasts compared to healthy fibroblasts. Through RNA sequencing analysis of skin sections from SSc patients vs healthy donors, we found an overexpression of the Slc7a11/xCT antiporter. Slc7a11 imports cystine in exchange for glutamate to synthesize glutathione (GSH), which inhibits ROS formation by the enzyme GSH peroxidase 4 (GPX4).

In line with increased Slc7a11 upregulation in SSc skin, we observed, that profibrotic TGF-β and ROS increase Slc7a11 activity and cystine uptake in human fibroblasts. In order to analyze the functional role of Slc7a11, we performed an inducible SSc mouse model by i.d. injection of hypochlorous acid (HOCl) in Slc7a11 KO mice. Importantly, absence of the antiporter led to a significantly reduced fibrosis development in form of impaired collagen accumulation and myofibroblast activation compared to control mice. In addition, Slc7a11 KO skin grafts even kept their fibrosis resistance upon HOCl injections when transplanted onto WT recipients. Fibroblasts of Slc7a11 KO origin failed to proliferate and thrive in vitro and Prime Flow analysis revealed significantly elevated Slc7a11 mRNA transcripts in CD45-CD90+ SSc fibroblasts, confirming our data in human SSc skin and suggesting an important fibroblast related Slc7a11 function in SSc.

Further analysis of the underlying molecular mechanisms showed an upregulation of GSH in WT skin, whereas the Slc7a11 KO mouse displayed no regulation of oxidative stress induced ROS. GSH and Slc7a11 are also known as regulators of iron-dependent ferroptotic cell death. Since iron deficiency is an unexplained clinical symptom in SSc patients, we addressed ferroptosis and Slc7a11 function in SSc fibroblasts. Treatment of SSc mice with the Slc7a11 inhibitor imidazole keton erastin (IKE) in vivo resulted in a significantly impaired fibrosis development, highlighting Slc7a11 as a therapeutic target for SSc.

To conclude, Slc7a11 antiporter deficiency reduces dermal fibrosis and Slc7a11 inhibition is effective to prevent dermal fibrosis.

P104 | CD4+ effector T cells orchestrate indirect inflammatory killing of IFNunresponsive tumors

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The discoveries of the molecular interaction between tumor cells and the immune system have revolutionized cancer treatment over the last decade. CD8+ cytotoxic T cells functions are taken advantage of in the clinic to directly target and kill cancer cells. However, CD8+ T cell focused therapy strategies are limited by the emergence of immune evading IFN unresponsive or MHCI deficient tumors. On the contrary CD8+ T cells, CD4+ effector T cells not only exert cytotoxic functions, but also target cancer cells indirectly, which has the potential to eradicate tumors, that would normally escape direct T cell killing. The mechanism behind the CD4+ T cell mediated

anti-tumor response are still incompletely understood. Here we show that CD4+ T cells can eradicate the tumor directly through the release of effector molecules like TNFa and IFNg. However, CD4+ T cells can also kill cancer cells indirectly by recruitment and activation of inflammatory monocytes via IFNg. Activated inflammatory monocytes are upregulating iNOS, which leads to the release of nitric oxide (NO). In order to further investigate CD4+ T cell effector mechanisms against immune evasive tumors, we utilized the CRISPR/Cas9 genome editing tool to generate IFN unresponsive (Jak1 KO) and MHC deficient (CIITA KO) cancer cells. Next we explored previously described inflammatory killing mechanism of CD4+ T cells and monocytes in vitro in order to see how the effector molecules act on genetically IFNg and MHCI deficient murine cell lines. We found that IFN unresponsive cells are more likely to be killed by a combination of TNFa and NO then their IFN responsive counterpart, which are mostly killed by a combination of IFNg and TNFa. This result was also reproduced in various human melanoma cell lines. Lastly, we investigated whether the blockade of iNOS in our CD4 ACT would have an effect on tumor growth and therapy outcome in vivo. Indeed it was shown that the inhibition of iNOS in our ACT setting leads to reduced survival of mice with an IFN unresponsive tumor, while therapy efficacy against IFN responsive tumors was not altered by iNOS-inhibition. These findings highlight the diversity and strength of CD4+ T cell effector mechanisms in a therapeutic setting.

In our future work we want to explore the mechanism on how CD4+ T cells are directly and indirectly killing cancer cells. In order to reach this goal we are generating a fluorescent apoptosis reporter cell line. The reporter cell line changes its fluorescence when effector caspases like caspase 3 are activated and cleaved. Furthermore, we are generating a Caspase 8 KO cell line via CRISPR/Cas9 genome editing, which will reveal the role of the intrinsic apoptosis pathway in tumor cells after CD4+ adoptive T cell transfer.

To summarize, our findings show how CD4+ effector T cells orchestrate indirect inflammatory killing of immune evasive tumors by unleashing inflammatory monocytes that, in response to IFNg, release the effector molecule NO. In order to exploit this pathway in a clinical setting, future experiments will investigate the killing mechanism of the cancer cells.

P105 | A standardization of the DNCB-induced atopic dermatitis mouse model in BALB/c mice to study novel therapeutic compounds

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In dermatology, animal models are still a common strategy to study novel therapeutic compounds. According to the EU Animal Welfare Directive 2010/63/EU of 2010, the 3R ("reduce", "replace" and "refine") principle intents to limit the number and the burden of animals used in laboratory experiments. The 2,4-dinitrochlorbenzene (DNCB)-induced atopic dermatitis in BALB/c mice is a popular model in dermatological research, as it displays many disease-associated characteristics of human atopic dermatitis. However, according to published studies, reproducibility of the model is challenging, due to the lack of vital information on handling of the mice, scoring parameters, or housing conditions. Moreover, studies used highly variable DNCB concentrations and applied volumes as well as application frequencies and time regimes. In accordance to the "3R" principle, we assume that a comprehensive and detailed description of the methodology would reduce the number of animals, which are initially used for reconstruction of the model.

The DNCB-induced atopic dermatitis mouse model is conducted in two phases, a short-time sensitization and a prolonged challenging phase. In our study, BALB/c mice (female, 8 weeks old) housed under specific and opportunistic pathogenfree conditions were used. We examined the effect in mice treated for three weeks with either 0.2, 0.3 or 0.5 % DNCB in the challenging phase, after one week of sensitization with 1 % DNCB. Clinical scoring included severity of skin dryness, erythema, edema and excoriation/crust formation. Disease characteristics such as hyperplasia, mast cell infiltration, loss of skin structure proteins (filaggrin, involucrin) using immunohistochemical staining were investigated. Pro-inflammatory cytokines, such as IL-6, TNF-α, T-helper cell derived cytokines (INFγ, IL-4, IL-13, IL-5, IL-10) and IgE-levels in serum were analysed by fluorescence cytometry. Activation of mitogen-activated protein kinases in skin was analysed using western blot, and prostaglandins as wells as specialized pro-resolving mediators were determined using high-pressure liquid chromatography. Real-time polymerase chain reaction was used to investigate expression levels of 45 transcripts in skin.

With our study, we provide a comprehensive spectrum of standard humanlike disease parameters of the DNCB-induced atopic dermatitis BALB/c mouse model, combined with a detailed description of the methodology. Based on our study, scientist will be able to decide without time-consuming literature reviews whether this model is suitable for their research question. Moreover, in line with our comprehensive description of methodology, researchers are able to work immediately with this model, ideally without the need of additional animals which must be used for establishing the model.

P106 | Alternative C5 – convertases promote skin inflammation in experimental epidermolysis bullosa acquisita

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Pemphigoid diseases (PDs) comprise a group of blistering skin conditions in which autoantibodies against basal keratinocyte antigens cause loss of cell adhesion to the dermal-epidermal junction (DEJ). The deposition of linear IgG and C3 deposits are diagnostic hallmarks of PD. Blockade of the C5/C5aR1 axis has previously been identified as a key driver of skin inflammation in experimental murine PD. However, the functional importance of C3 in PD has not been conclusively addressed. We here, using antibody transfer-induced PD models in C3-deficient mice, demonstrate that in three pre-clinical PD models, namely epidermolysis bullosa acquisita (EBA), bullous pemphigoid (BP), and mucous membrane pemphigoid (MMP), experimental PD develops independently of C3. This indicates that C5a in these model conditions can be proteolytically generated independently of C3. Thus, we next addressed the contribution of alternative C5-convertases using the antibody transfer-induced EBA mouse model. EBA was induced in neutrophil-elastase (NE) deficient mice and in (WT) mice treated with the thrombin inhibitor argatroban, both considered alternative C5 convertases. While in NE-deficient mice, EBA developed like in wildtype littermate controls, blockade of thrombin led to a significant reduction of clinical disease severity in antibody transfer-induced EBA. However, the degree of reduction in clinical disease activity was not as pronounced when compared to mice deficient in the C5/C5aR1-axis. Thus, additional alternative pathways for C5a generation are most likely operative. Experiments addressing the contribution of elastase and thrombin in established MMP and BP mouse models are ongoing. Preliminary data suggest that thrombin is also required for MMP induction. In summary, we here provide evidence that skin inflammation in experimental PD can develop independent of C3, and that C5a is, at least partially, generated by thrombin.

P107 (OP02/05) | Adoptively transferred CD4+ T cells control established melanomas through indirect antigen-specific activation in the tumour microenvironment

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The cytotoxic effector functions of CD8+ T cells to eradicate tumours are well known and used in standard immunotherapies such as checkpoint blockade and adoptive T cell therapy. However, tumours can acquire resistance to CD8+ T cell-mediated control through mechanisms like the loss of MHC molecules or IFN-signalling deficiencies. The contribution of CD4+ T cells to control tumours remains incompletely understood, as anti-tumour CD4+ T cells can facilitate tumour regression not only directly, but also through several indirect effector mechanisms. Here, we developed an adoptive cell therapy (ACT) protocol that allows us to investigate the requirements for a successful immunotherapy mediated by CD4+ T cells in direct comparison to the better-understood cytotoxic CD8+ T cells. Our ACT therapy protocol consists of chemotherapeutic preconditioning using cyclophosphamide one day prior to adoptive cell transfer, adenoviral vaccination to prime the adoptively transferred tumour-specific CD8+ and CD4+ T cells and innate immune stimulation with the nucleic acids poly I:C and CpG. In a transplantable tumour model, we found that adoptively transferred CD4+ T cells are able to control B16 and HCmel12 melanomas as efficiently as adoptively CD8+ T cells. Strikingly, genetically engineered knockout variants of HCmel12 lacking MHC-I, MHC-II or Jak1 expression were still able to be controlled by adoptive CD4+ T cell transfer, while CD8+ T cells failed to control MHC-I and Jak1-KO tumours. To be able to investigate the dynamics of CD4+ and CD8+ T cells in the tumour microenvironment via intravital 2-photon microscopy, we crossed TCR-transgenic donor mice to eGFP and eYFPexpressing reporter strains. We found that CD4+ T cells have fundamentally different spatial and temporal properties when compared to CD8+ T cells. While CD8+ T cells preferentially decelerate in proximity to tumour cells, CD4+ T cells form local clusters with CD11c-YFP+ cells at the invasive tumour margin in an antigen-dependent manner. More so, the spatio-temporal behaviour of both CD4+ and CD8+ T cells was different in IFN-unresponsive and MHC-deficient tumours when compared to their wild-type counterparts. These data suggest that localisation of anti-tumour T cells within the tumour microenvironment and co-localisation with potential antigenpresenting cells can predict therapy success.

In summary, our results show fundamental differences in the spatial and temporal behaviour of anti-tumour CD8+ and CD4+ T cells.

While direct tumour recognition is essential for elimination of tumour cells by cytolytic CD8+ T cells, CD4+ T cells can recognise antigen indirectly and eradicate tumours that evade CD8+ T cell based therapies. Exploiting anti-tumour CD4+ T cell functions could be used to target MHCdeficient tumours that evade CD8+ T cell-focused therapies and complement current clinically applied immunotherapy strategies.

P108 | Tumour-specific CD4+ T cells recruit inflammatory monocytes that acquire IFNactivated functions to control immune evasive tumours

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Unleashing CD8+ cytotylic T cell immunity is the predominant focus of current clinically applied cancer therapies. These strategies, however, are impaired by the emergence MHC-I-deficient or IFN-unresponsive tumour cells and the development of an immunosuppressive tumour microenvironment. CD4+ effector T cells can contribute to tumour immune defence independent of CD8+ T cells. However, the potential and the mechanisms of CD4+ T cell-mediated anti-tumour immunity are incompletely understood. Using an adoptive cell therapy (ACT) model, we showed that relatively few CD4+ T cells controlled MHC-deficient and IFN-unresponsive tumours that resist CD8+ T cell-mediated therapy.

To better understand how a comparatively small number of CD4+ effector T cells cause the eradication of established tumours, we immune-profiled treatmentinduced alterations of the tumour immune microenvironment. A comprehensive characterisation of tumour-infiltrating cells in wild type mice confirmed the dynamic recruitment of inflammatory CD11b+CCR2+Ly6C-hi monocytes following CD4 ACT. Our ACT treatment protocol combined the in vivo activation of CD4+ T cell effector functions with additional innate immune stimulation using TLR agonists. To separate the contribution of both interventions for the recruitment and activation of monocytes, we omitted either the innate stimuli or the CD4+ T cell transfer from our combined ACT therapy scheme and performed single-cell RNA-seq analyses of sorted tumourinfiltrating myeloid immune cells. Dimensionality reduction and visualisation showed a separate clustering of myeloid cells between untreated and all treated conditions, with the most pronounced effects occurring after combined activation of innate and adaptive immunity. Differential gene expression and gene set enrichment analyses between myeloid cells from untreated and CD4 ACT-treated tumours revealed a strong activation of IFN-response genes upon therapy. Importantly, both innate immune stimulation and CD4+ effector T cells independently induced the expression of IFN-response genes. Pseudotime inference and subsequent graph abstraction using PAGA identified three distinct trajectories in CD4 ACT-treated tumours, corresponding to differentiation pathways towards phenotypes of monocyte-derived dendritic cells, monocyte-macrophage effectors and Ly6C-lo mature monocytes. The endpoint cellular states of these three trajectories in CD4 ACT-treated mice represent IFN-activated counterparts of the intratumoural monocyte-macrophage network found in untreated controls.

Our data reveal that CD4+ T cells and innate immune stimuli synergistically reprogrammed the myeloid network in treated tumours. This network is characterised by the recruitment of inflammatory monocytes which acquire IFN-activated cellular states and dynamically shift towards MHC-II antigen-presenting and tumouricidal effector phenotypes. Our work suggests a great potential for new treatment possibilities that target CD4+ effector T cells and simultaneous activation of nonspecific innate inflammatory defence mechanisms against tumours.

P109 | Imiquimod induces psoriatic skin inflammation in previously affected human skin

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Imiquimod (IMQ), a toll-like receptor (TLR)7/8 agonist, was shown to induce a selflimited Th17-dominated contact dermatitis in human healthy skin, but not the full picture of psoriasis. Whilst such an innate “first strike” of IMQ is needed for inducing inflammation, a “second strike” might perpetuate inflammation and induce the characteristic phenotype of psoriatic plaques. Here, IMQ was tested on clinically healed plaques of 4 psoriasis patients for inducing a “second strike”, namely the activation of CD103+ tissue-resident memory T cells (TRM) that recognize specific cutaneous antigens and thereby maintain the inflammatory response in the skin. Former psoriatic lesion (FL) and never-affected area as control (NL) were treated with IMQ 5% cream, 0.2 g/cm2, twice a week for 4 weeks.

Skin biopsies (n = 2) were collected at baseline and day 28 from each site and processed for histology, FACS and bulk RNA-seq analysis. Only 1 patient showed the full clinical and histological picture of psoriasis in activated FL. Results were also confirmed by transcriptome analysis using an existing dataset on psoriasis. Moreover, an increased number of CD4+CD103+KI67+ cells was detected in the skin of this patient compared to the others. Interestingly, preliminary data hints at triggering of T cell proliferation by autoantigens from the skin in this patient. We validated for the first time a "two-strike" model for psoriasis, potentially leading to "treat hard and early" concepts to avoid the accumulation of TRM in the skin and thus prevent relapse-remitting affection of the same sites.

P110 | Generation of human suppressive Treg cells from primary memory T cells by a small molecule compound

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Introduction: Present treatments of allergies are mainly aimed at the prevention or amelioration of signs and symptoms rather than curing patients. Specificimmunotherapy can achieve this, but comes with many limitations including low compliance and local or systemic side effects such as asthma attacks and lifethreatening anaphylactic shock. Small molecule compounds that restore tolerance against allergens by inducing functional regulatory T cells may overcome these limitations, but are not available yet.

Methods: A library of 40,000 small molecules was screened for compounds that significantly upregulated the expression of Foxp3, the master transcription factor of Tregs. One candidate molecule (hereafter referred to as C5) was selected and further validated using primary human memory CD4+T cells. Dose response studies were performed and long-term toxicity, cell proliferation, and the production of proinflammatory cytokines was analyzed. Furthermore, the TSDR-methylation status and the suppressive capacity of C5-induced T cells (C5-iTregs) towards effector cells was investigated.

Results: The selected compound C5 significantly and dose dependently increased Foxp3 expression within five days in primary human memory CD4+T cells without impairing cell proliferation and viability. Even though, C5 did not induce a stable Treg phenotype (i.e. no demethylation of the TSDR), C5 treatment resulted in generation of CD4+Tcells with significant suppressive capacity towards responder T cells. In addition, C5 limited the secretion of the allergy-promoting Th2 cytokines IL-4 and IL-13.

Conclusion: Immunomodulatory, i.e. Foxp3-expressing T cells, can be generated in vitro from human memory CD4+T cells using a small molecule compound. Our identified molecule C5 might be a promising candidate for restoring immune tolerance e.g. in allergies or chronic inflammation.

P111 | IL-17-producing cells in oral mucosal on patients with oral lichen planus

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Lichen planus (LP) is a chronic inflammatory disease of mucous membranes and skin, with a T lymphocyte-mediated immune pathogenesis. CD4+ T helper (Th) cells appear to play a central role, and are present in early LP lesions, mainly in the superficial connective tissue. These cells show mainly a Th1 profile and activate CD8+ T cytotoxic (Tc) cells, which may trigger keratinocytes apoptosis and aggravate the inflammation. The presence of IL-17-producing cells was also demonstrated in LP lesions, yet it is unclear which type of lymphocyte they belong to and where they are localized.

We hypothesize that different populations of IL-17-producing cells, such as Th17 and Tc17 cells, contribute to OLP and that they are localized within different regions of the oral mucosa to perform distinct pathological roles.

We have obtained samples of oral mucosa from patients with OLP. We have developed methods to isolate immune cells from skin biopsies and analyze them by surface and intracellular staining by flow cytometry. Per biopsy from lesional mucosa, we can collect 40.000 live cells, of which 30% are immune cells. The CD45+ immune cell compartment consists of 85% T cells, which can be divided into CD8+ T cells (40%) and CD8- T cells (55%). From perilesional mucosa, we can collect 30.000 live cells, of which 30% are immune cells. The CD45+ immune cell compartment consists of 60% T cells, which can be divided into CD8+ T cells (30%) and CD8- T cells (65%). This gives us enough cells to interrogate their cytokine profile and we have currently developed analysis techniques to study IFN-gamma, IL-17A, IL-17F, and IL-22 production by infiltrating CD4 and CD8 Cells. In parallel, we are developing protocols to visualize the immune infiltrate in Formalin-fixed paraffin-embedded sections, collected from the same patient's biopsy. Challenges to overcome included the optimization of the antigen retrieval to identify the epitopes of interest, and the protocol development to the intracellular cytokine staining. This allowed us to investigate the localization and frequency of Th17 and T cytotoxic 17 (Tc17) cells in the different oral mucosa layers.

These outcomes will give us an indication of the different IL-17-producing cells' frequency and location in the oral mucosa of patients with oral lichen planus. It will also allow us to study this cell's capacity of producing cytokines in the oral mucosa.

P112 | Type I interferon activation in dermatomyositis

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Background: Dermatomyositis (DM) is a rare autoimmune disease, which manifests in patients' skin and muscle tissue, causing violaceous erythema in sun exposed areas, dermal atrophy, ulcerations and proximal muscle weakness. DM is characterized by an elevated type I interferon (IFN) signature in patients' blood, but 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 solar irradiation.
Methods: Fibroblasts were isolated from patients' skin tissue and cultivated in vitro. The ISG expression of blood samples and cultivated fibroblasts were analyzed using RTPCR. The Patients' symptoms were assessed using the Cutaneous Dermatomyositis Disease Area and Severity Index (CDASI). RNA Sequencing was performed in native as well as irradiated fibroblasts. Solar simulated radiation (SSR) was used to stimulate the cells. The Sequencing data was evaluated using Gene Set Enrichment Analysis (GSEA). Cells were stained using antibodies for DNA, γH2AX and 53BP1.
Results: After isolation and cultivation of the fibroblasts we observed that most of the patient cell lines had a higher expression of type I interferon stimulated genes (ISGs) than healthy controls (26 patients, 7 controls). The height of the ISG expression correlated positively with the severity of patients' skin symptoms. 48% of significantly upregulated genes in native DM patient cells were type I IFN stimulated genes. SSR increased the upregulation of genes involved in the pathway of antiviral mechanism through IFN stimulated genes. Further GSEA of the RNA Sequencing data displayed significant downregulation in the pathway of nuclear envelope (NE) sealing by ESCORT III, in native fibroblasts. After UV light exposure, pathways involved in NE breakdown and reassembly, as well as responses to increased DNA damage were strongly upregulated. At the microscopic level, a significantly increased number of micronuclei were observed in DM patients cells. UV light irradiation further induced micronuclei formation and demonstrated to have a stronger effect on patients. The number of micronuclei with impaired nuclear envelopes was significantly higher in DM patient cells. While we did not observe a significant increase in DNA double strand breaks (DNA-DSB) in patients following irradiation, a slower repair process became evident.
Conclusion: Here we demonstrate the elevated expression of ISGs in DM patients' fibroblasts and it's preservation in cell culture. The disease trigger UV-irradiation led to an increased ISG upregulation. Following irradiation, patient cells showed slower DNADSB repair. These unrepaired DNA breaks could subsequently cause missegregation of the chromosomes and the heightened formation of micronuclei containing resulting fragments. Compared to the nucleus, micronuclei seem to be more prone to experience nuclear envelope ruptures. Our observations of an elevated level of micronuclei in DM patient cells, as well as a higher number of micronuclei whose DNA was accessible to a DNA antibody align with this hypothesis. If the NE integrity is lost, the contained DNA could be exposed to the cytosol and act as a damage associated molecular pattern triggering an immune response and type I interferon induction. In conclusion, the slower DNA-DSB repair and enhanced micronuclei formation in DM patients could therefore be a possible cause of the IFN activation and inflammatory manifestation in DM. The increased formation of micronuclei after irradiation can be relevant for the explanation of UV induced flares.

P113 (OP02/02) | Chronic ER stress promotes cGAS/mtDNA-induced autoimmunity via ATF6 in myotonic dystrophy type 2

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Background: Myotonic Dystrophy (DM) type 2 is characterized by autosomal dominant progressiv 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 and the potential innate immune response to the nucleic acid accumulation.
Methods: Fibroblasts isolated from skin of DM2 patients were analysed by RNA FISH for detection of repeat expansions and the expression of interferon stimulated genes (ISG) were determined by RT PCR. 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, Seahorse Assay and measurement of reactive oxygen species (ROS) production were used to determine mitochondrial stress. The downregulation of different nucleic acid sensors by siRNA was used to understand their influence in the upregulation of ISGs in DM2 patients.
Results: Using RNA FISH technique we demonstrated that fibroblasts of DM2 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 DM2 patients. Repeat accumulation was associated with a chronically elevated ER stress response on mRNA and protein level, which is characterised by an activation of the Activating transcription factor 6 (ATF6) signalling pathway. Patient cells formed elevated levels of ROS that correlated with the intensity of repeat expansions in the cell and mitochondrial stress indicated by a reduced membrane potential and enhanced oxygen consumption rate (OCR). This chronic stress response was associated with an enhanced type I interferon response in blood and fibroblasts of DM2 patients. Interestingly, downregulation of DNA sensor cyclic GMPAMP synthase (cGAS) and the stimulator for interferon genes (STING) lead to a reduction of the type I interferon signature in DM2 patients. Along with that, a release of mitochondrial DNA (mtDNA), which colocalized with the DNA sensor cGAS, could be detected in DM2 fibroblasts. Thus chronic ER and mitochondrial stress within DM2 patients triggered by the RNA repeats leads to the release of mtDNA, which can be recognised by cGAS and thereby activates the cGAS-STING signalling pathway, resulting in increased type I interferon production.

Conclusion: Altogether, our study demonstrates a novel mechanism by which large repeat expansions cause chronic ER and mitochondrial stress and induce a type I interferon response that predisposes to autoimmunity.

P114 | Increased allergic skin inflammation in mice lacking the metabolite sensing HCA2/Gpr109a receptor

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The hydroxycarbolic acid receptor HCA2 is highly expressed in adipose tissue but also in keratinocytes, macrophages, monocytes, neutrophils, dermal dendritic cells,enterocytes, and microglia. Endogenous agonists of this G-protein coupled receptor are the short chain fatty acid (SCFA) butyrate, produced by anaerobic fermentation of dietary fiber and the hydroxycarbolic acid β-hydroxybutyrate, produced in the liver through beta-oxidation of fatty acids (ketoacidosis). HCA2 also is a target for niacin (nicotinic acid). In an experimental mouse model of psoriasis, topical application of sodium butyrate attenuated the inflammatory immune response by inducing regulatory T-cells and IL-10. In the mouse model of bullous pemphigoidlike epidermolysis bullosa acquisita the recruitment of neutrophils was regulated through HCA2 receptors. These findings indicate an important role of HCA2 signaling in cutaneous physiology.

In our work, we comparatively examined contact allergic immune responses to the obligate contact sensitizer DNFB in Hcar2-/- and WT C57BL/6 mice. The increase in ear thickness after repetitive DNFB challenges was significantly higher in sensitized Hcar2-/- mice compared to WT mice. H&E staining and weighing of inflamed ear tissue revealed prominent tissue edema and an increased infiltration of neutrophils. Pro-inflammatory markers like Ccl8, IL-4 and TNFα significantly were increased in Hcar2-/- mice during contact allergic inflammation. We subsequently performed experiments with wild type and Hcar2-/- mice involving adoptively transferred bulk lymphocytes isolated from spleen and lymph nodes as well as bone marrow chimeric mice. We found that the adoptive transfer of sensitized lymphocytes derived from wildtype mice induced significantly stronger allergic inflammation in recipient Hcar2-/- mice when compared to an adoptive transfer of sensitized lymphocytes derived from Hcar2-/- mice into recipient wildtype mice. Furthermore, Hcar2-/- mice reconstituted with wildtype bone marrow developed stronger allergic inflammation than wildtype mice reconstituted with Hcar2-/- mice bone marrow. This provides evidence that Hca2 receptors on radio resistant resident skin cells in the challenge phase of CHS have an important role in the regulation of allergic inflammation in the skin. Based on our observations we hypothesize that the HCA2 receptor and its metabolite ligands plays an important role in limiting excessive cellular immune activation in the skin. To further characterize the impact of Hca2 receptor signaling in single cell subtypes we will perform in vitro assays with primary keratinocytes, dendritic cells and lymphocytes and measure metabolic intermediates in inflamed ear tissue to define potential Hca2 ligands.

P115 | Impact of the metabolite sensing receptor Hcar2 on keratinocytes and lymphocytes

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The hydroxycarbolic acid receptor HCA2/Gpr109a is highly expressed in adipose tissue but also in keratinocytes, macrophages, monocytes, neutrophils and dermal dendritic cells. In the skin, the role of the HCA2 receptor and its ligands butyrate, beta-hydroxybutyrate (BHB), and niacin (nicotinic acid, vitamin B3) primarily was investigated in psoriasis. Monomethyl fumarate, a metabolite of dimethyl fumarate (DMF) and HCA2 ligand, used for the treatment of psoriasis, elicits a cutaneous reaction called flushing as a side effect after binding to HCA2 receptors on keratinocytes and epidermal Langerhans cells. In our work, we found that contact allergic immune responses to the obligate contact sensitizer DNFB are significantly enhanced in Hcar2-/- in comparison to WT mice. FACS analyses showed an increased infiltration of neutrophils and using RT-PCR we found a differential expression of the pro-inflammatory markers Ccl8, IL-4 and TNFa in inflamed Hcar2-/- ear tissue. Here, we further characterized the impact of Hca2 signaling in primary keratinocytes (KC) and lymphocytes (LC) as well as in co-stimulation assays with haptenized DCs and lymphocytes isolated from DNFB sensitized mice.

We first compared primary cultures of Hcar2-/- and WT keratinocytes isolated from tail skin of adult mice. All cultured KCs were

morphologically indistinguishable and showed the same differentiation pattern with strong expression of keratin 5. There were no differences in proliferation rates of KCs in crystal violet assays. We found no differences in basal levels of constitutively secreted Ccl2 and Cxcl2 in the supernatant of near-confluent primary KCs. Upon stimulation with IFNy Ccl8, an important chemoattractant significantly increased in Hcar2-/- KCs. Upon unspecific stimulation with a cell activation cocktail CFSE-labeled Hcar2-/- LC displayed higher proliferation rates over 72h in comparison to wild type LC. The antigen-specific proliferation in co-culture experiments with LC from DNFB-sensitized wild type and Hcar2-/- mice and DCs haptenized with DNBS also revealed increased proliferation rates of knockout LC. Hcar2-/- LC released higher IL-17 levels upon antigen-specific activation. Based on our observations we propose that Hca2 receptors modulate the secretion of pro-inflammatory mediators in keratinocytes and lymphocytes as well as proliferation in lymphocytes.

P116 | How skin inflammation shapes behavior – role of the metabolite sensing Hca2/Gpr109a receptor

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The crosstalk between inflammatory pathways in the periphery and neurocircuits in the brain can lead to behavioral responses. In this process, key stress response systems like the HPA axis, cellular routes and soluble mediators are involved. Several studies highlighted the connection between chronic inflammatory skin diseases and depression, anxiety disorders or addictive behavior. However, the underlying molecular mechanisms connecting skin disease, stress and psychiatric illness are only insufficiently understood. Currently, the metabolite sensing hydroxycarbolic acid receptor HCA2 and its ligands butyrate and betahydroxybutyrate have been discussed as a potential link between the microbiome, host metabolism, inflammation and behavior. We examined its impact on cutaneous and CNS inflammation as well as behavioral responses.

Hcar2 is expressed on adipocytes, keratinocytes, immune cells and microglia. We previously demonstrated that Hcar2-/- mice display significantly increased allergic immune responses in the experimental model of DNFB-induced contact hypersensitivity in comparison to wild type mice. Here, pro-inflammatory markers like Ccl8, IL-4 and TNFα significantly were elevated in inflamed skin of Hcar2-/- mice. To evaluate the impact of Hcar2 signaling on the CNS during chronic inflammation, Hcar2 WT and Hcar2-/- mice were exposed to repetitive DNFB applications. The expression of inflammatory mediators and neuronal markers then was evaluated in the prefrontal cortex, important for cognitive control functions as well as in the dorsal and ventral hippocampus, important for learning, memory and anxiety. IFNy and Ccl8 already were increased in the CNS of naïve Hcar2-/- mice. Upon chronic DNFB treatment IFNy, Ccl8 and IL-4 significantly increased in the prefrontal cortex and hippocampus of Hcar2-/- mice in comparison to WT animals. Iba1 and CD68, neuronal markers for activated microglia where not changed. To evaluate the effect of Hcar2 signaling on behavior in naïve and chronic inflamed mice, we performed different behavioral tests to assess anxiety, learning and memory. Interestingly, naïve Hcar2-/- mice already showed increased anxiety and reduced contextual fear memory, pointing to hippocampal dysfunction. These initial observations point towards an important role of HCA2 receptors in the "skin-brain-axis" and may link peripheral inflammation and behavioral responses. Experiments on the impact of Hcar2 signaling on behavior during chronic inflammation are currently ongoing.

P117 | Tregs of mice with experimental epidermolysis bullosa acquisita show a loss of function but therapy by an adoptive Treg transfer ameliorates autoantibodyinduced inflammation

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Pemphigoid diseases (PDs) are a group of prototypical organ-specific, and antibodydriven autoimmune diseases, characterized by a severe blistering phenotype, and the presence of autoantibodies targeting adhesion proteins of the dermalepidermal junction (DEJ). Multiple lines of evidence indicate that myeloid cells like neutrophils are the main effector cells for tissue destruction in PD. Intriguingly, different lymphocyte populations like regulatory T cells (Tregs) orchestrate the recruitment and activation of these myeloid cells. 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). To study the role and potential therapeutic impact of Tregs in PDs, freshly isolated Tregs labelled with CFSE from healthy mice were adoptively transferred intravenously one day before disease induction in the antibody transfer-induced mouse model of EBA. In this group, reduction of clinical disease manifestation of up to 50 % compared to an untreated mice group could be observed at the last day of experiment. Additionally, CFSE-labeled transferred Tregs could be found in all investigated lymphatic organs, but especially in the inflamed skin. Also, the number of Tregs in the skin after adoptive transfer was significantly higher than in the control group and the number of neutrophils was significantly reduced.

To further study the potential role of Tregs in PDs, additional in vitro experiments were performed. Tregs from untreated and EBA-diseased mice were compared on their effect on neutrophils and other T effector cells. Here, Tregs from lymph nodes from untreated or mice with antibody transfer-induced or immunized EBA were

isolated and co-cultured with either neutrophils or T effector cells. For neutrophils, the effect on their migration marker CD18, and for T effector cells their proliferation by CFSE labelling was assessed by flow cytometry. Interestingly, isolated Tregs from untreated mice showed the expected and already described results with the downregulation of CD18 on neutrophils and the proliferation of T effector cells. On the other hand, Tregs isolated from the lymph nodes of EBA-diseased mice showed a significantly reduced effect in their function to downregulate these two cell types.

Taken together, these findings suggest that adoptive Treg transfer can modulate antibody-induced inflammation in EBA. The reduced effect of the Tregs from EBA mice on neutrophils and T effector cells points to a loss of function of Tregs in PD, which will be further explored and could serve as a potential therapeutic target. To do so, the gene expression of these Tregs will also be analysed to further deepen our understanding of the function of Tregs in PDs.

P118 | Treatment of experimental epidermolysis bullosa acquisita with an IL-4/anti- IL-4 antibody complex ameliorates the inflammation by altering T cell and macrophage populations in the skin

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Pemphigoid diseases (PD) represent a group of severe blistering autoimmune dermatoses. The mostly elderly patients suffer from chronic inflammation of the skin and/or mucous membranes. One form of PD is epidermolysis bullosa acquisita (EBA), which is characterized by autoantibodies against type VII collagen (COL7). However, therapy of EBA patients remains unsatisfactory due to insufficient efficacy and/or side effects. Therefore, the development of safe and effective therapeutic alternatives for the treatment of EBA represents an unresolved medical problem. Moreover, the pathogenic mechanisms involved in disease progression are incompletely understood, and may be key to the development of new therapeutic strategies.

In recent studies focusing on the involvement of T cells and monocytes/macrophages to skin inflammation in experimental PD, an increase in the expression of the classical CD4+T helper type (Th) 2 cytokines IL-4/IL-13 and the proinflammatory Th1 cytokine IFN-gamma was observed. In addition, these Th1/Th2 cytokines are known to have an effect on macrophage differentiation: IFN-γ promotes the proinflammatory M1 phenotype via expression of TLR agonists, whereas IL-4/IL-13 stimulate the more anti-inflammatory M2 macrophage type. Although neutrophils are previously thought to be the main effector cells of the disease, we hypothesize that macrophage differentiation (via the cytokines IFN-γ and IL-4/IL-13) may have a significant impact on disease progression, and in particular on healing. Therefore, we tested a possible therapeutic option in an antibody transfer-induced EBA mouse model by administration of an IL-4/anti-IL-4 antibody complex (IL-4c).

As hypothesized, we could show that treatment with IL-4c significantly improved antibody transfer-induced EBA and that in total, a sustained administration of the complex over the time of the disease model at a concentration of 25 µg IL-4/5 µg anti-IL-4 significantly reduced EBA permanently compared to the control group.

Further flow cytometric and RT-PCR analysis showed that treatment with IL-4c indeed resulted in a shift of the M1 macrophage population to an M2 macrophage population within lesional skin. In addition, IL-4c treatment significantly decreased the Th1 cell population in the spleen. To confirm these findings, we performed single cell RNAseq of inflamed skin, and showed several significant alterations in different markers of T cell and macrophage populations in lesional skin in the treatment group compared to the control group. Both indicate a shift from a pro-inflammatory to an anti-inflammatory milieu in IL-4c-treated mice.

Taken together, treatment with IL-4c ameliorated the inflammation in an antibody transfer-induced EBA mouse model. Especially, effects on macrophages and T cells could be observed which in the end, resulted in a shift of a pro-inflammatory milieu to a more anti-inflammatory one. To further understand the effect and mechanism of IL-4 in EBA, flow cytometric experiments are planned to deeper analyse the observed alterations of the single cell RNA Seq in the T cell and macrophage population.

P119 | Anti-CD73 antibodies suppress activation of highly activated and proliferative CD73+CD4+ T cells

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Regulatory T cells almost ubiquitously express the 5'ectonucleotidase CD73, which contributes to their suppressive function by production of adenosine. But so far unappreciated is the fact, that nearly 50% of conventional (CD4+FoxP3-) T cells express CD73 as well. FACS sorted

naïve CD73-CD4+ T cells gained CD73 expression upon activation by anti-CD3/CD28 antibodies, along with the bona-fide activation markers CD69 and CD25. Of note, CD73+ T cells did not lose CD73 and the mean fluorescence increased. As CD73, beyond being an ectoenzyme, may also function in cell signaling, we used an anti-CD73 antibody(clone TY23) in vitro. We found that anti-CD73 antibodies inhibited the proliferation and activation of T cells induced by anti-CD3/CD28 stimulation. It also suppressed release of IFN-gamma, TNF-alpha, IL-17A and GMCSF. But the expression of other costimulatory and coinhibitory molecules on T cells were not influenced by TY23. Furthermore, to analyze the underlying mechanisms, phosphorylation of Lck, Erk and Src family members in T cells upon

activation was assessed. Here, T cells derived from CD73 knock out animals displayed reduced phosphorylation in those kinases, which was parallel by observations that TY23 treatment caused reduced phosphorylation of Lck in wildtype T cells. In summary these data suggest a role for CD73 as activation marker for CD4+ T cells and engagement thereof may exert inhibitory effects. Therefore, up-regulation of CD73 may be a counterregulatory effect to avoid over boarding inflammation during T cell activation. This has to be considered when using anti-CD73 antibodies for anti-tumor therapies in the future.

P120 | Cell-derived nanoparticles regulate the pro-inflammatory cytokine secretion in the murine macrophage cell line J774A.1

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Cell-derived nanoparticles (CDNPs) are a mixture of intracellular proteins (e.g., annexins, peroxiredoxins, and histones) isolated from eukaryotic cells. CDNPs are defined as immunomodulators in sepsis, infections, and autoimmune diseases. Specifically, CDNPs are found to participate in the re-epithelization of human skin wounds in vitro and the healing of cutaneous inflammation in vivo. It is shown that CDNP treatment can significantly reduce the affected body surface area in the murine model of the skin blistering disease epidermolysis bullosa acquisita (EBA) by increasing the secretion of anti-inflammatory cytokine IL-4. Yet, the underlying mechanism of CDNPs is still unknown. Herein, we investigated if CDNPs would modulate the activity of antigen-presenting cells (APCs) by measuring the secretion of proinflammatory cytokines in-vitro.

To determine the influence of CDNPs, the murine macrophage cells J774A.1 were treated for 5 and 24 hours with different concentrations (5, 2.5, 1, and 0.5 µg/ml) of porcine-derived CDNPs and activated with the Toll-like receptor 2 ligand Pam3csk4. mRNA expression and secretion of cytokines were measured by quantitative RTPCR and ELISA.

Our results indicate that CDNPs effectively reduce the gene expression and secretion of proinflammatory cytokines in J774A.1 cell in a concentration-dependent manner while other proteins such as Ovalbumin or Annexins have no effect.

In conclusion, our data identify the regulatory effects of CDNPs on APCs as one potential mechanism for their immunomodulatory activity in the autoimmune disease EBA. Hence, CDNPs can be considered a therapeutic agent to intervene in the transition from a pre-disease state to a disease manifestation in EBA.

P121 (OP06/03) | Dysfunctional CD19^{+}CD24hiCD38hi transitional B cells in patients with pemphigus

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Pemphigus vulgaris (PV) is a rare autoimmune disease clinically characterized by the presence of blisters and erosions on the skin and mucous membranes. Pathophysiologically, PV is mediated by the close interaction of B cells and autoreactive T cells, inducing proliferation, differentiation, and eventually production of autoantibodies by B cells targeting desmosomal adhesion proteins, namely desmoglein (Dsg)3 and/or Dsg1. To elucidate potential B cell alterations at different clinical phases of PV, canonical B cell populations, like naïve, memory, transitional, non-switched, and switched B cells as well as plasmablasts, were both monitored longitudinally in peripheral blood of patients with PV and functionally analyzed by multiparametric flow cytometry.

Transitional B cells, characterized by the expression of CD27-IgM/IgD +CD24hiCD38hi, are known to represent a crucial link between immature B cells in the bone marrow and mature peripheral B cells. Our results revealed a significant decrease in the frequency of this B cell subset in active PV patients (n = 32), i.e. in newly diagnosed and chronic patients, as compared to remittent PV patients (n = 27) and healthy control individuals (n = 23). Moreover, following PV patients for up to 30 months after B cell depletion therapy with anti-CD20 antibodies, we observed that frequencies of transitional B cells in active patients recovered to levels of healthy individuals, suggesting a deficit of this population may contribute to the pathophysiology of PV. As a regulatory role of transitional B cells under inflammatory conditions was suggested in other autoimmune diseases, we additionally determined their regulatory capacity via the secretion of IL-10, TGF-β, and IL-35, but also proinflammatory IL-6. Enriched B cells of both PV patients and healthy control subjects were subjected to a combination of stimuli, such as CpG acting through Toll-like receptor 9, CD40 ligand co-stimulating B cells through CD40 receptor, and/or Dsg3 antigen. Interestingly, CD24hiCD38hi transitional B cells from active patients (n = 15) were refractory to further stimulation by CpG and CD40L, resulting in diminished production of regulatory cytokines. Moreover, cytokine co-expression patterns of CD24hiCD38hi transitional B cells revealed the presence of distinct regulatory profiles in healthy individuals (n = 11). These patterns were missing in PV patients, which were rather characterized by a pro-inflammatory cytokine signature upon activation with CD40L, IL-21, and Dsg3.

Therefore, our findings indicate that both the diminished frequency as well as the decreased regulatory capacity of transitional B cells

in active stages of the disease render them unable to effectively inhibit autoimmune processes in PV. Consequently, these cells might be considered both as a potential biomarker for clinical efficacy and a target for immunomodulatory therapeutic approaches.

P122 (OP05/04) | Selective inhibition of ERK5 as a novel treatment option in epidermolysis bullosa acquisita

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Pemphigoid diseases (PD) are a group of severe and difficult to treat autoimmune blistering diseases lacking therapeutic options that specifically target molecular structures and pathways involved in the disease pathogenesis. Thus, treatment of PD is still based on high-dose corticosteroids which show severe and lifethreatening side-effects. As one representative of PD, epidermolysis bullosa acquisita (EBA) is notoriously difficult to treat and clinical remission is almost exclusively achieved by long-term therapy with corticosteroids in combination with other immunosuppressants. EBA is caused by autoantibodies targeting type VII collagen, and formed immune complexes (IC) initiate activation of neutrophils, the key players in the effector phase of EBA. By binding the skin-bound IC via activating Fc gamma receptors, signal transduction pathways in neutrophils are triggered resulting in the release of reactive oxygen species (ROS) and proteases that mediate tissue destruction. Thus, neutrophils and IC-induced signaling molecules are promising targets in the development of new therapeutic strategies.

In a multiplex kinome analysis of IC-activated neutrophils, screening the activity of 156 kinases at different time points revealed an increased activity of the extracellular signal-regulated kinase (ERK) 5. To further validate the potential of ERK5 as therapeutic target in EBA, XMD8-92 was chosen as suitable inhibitor showing a favorable IC50 value and a good specificity for ERK5. Inhibiting ERK5 in vitro reduced the production of ROS in IC-activated neutrophils in a dose-dependent manner and also decreased the shedding of CD62L from the cell surface, which indicates decreased neutrophil activation. In contrast, blocking ERK5 had no impact on the IL-8-dependent chemotaxis or CD18 expression on the cell surface of neutrophils as well as on the cell survival when used below a concentration of 10 µM. In a next step, the possible in vivo effect and the potential therapeutic use of ERK5- targeted treatment in EBA were investigated in the local murine model of antibodytransfer induced EBA. Here, systemic treatment with XMD8-92 significantly reduced disease severity characterized by a lower percentage of the ear affected by EBA lesions and by less infiltrating immune cells into the skin.

In summary, blocking ERK5 by XMD8-92 impairs neutrophil functions and effectively treats experimental EBA. Collectively, our results identify ERK5 as potential therapeutic target in the treatment of EBA and related autoantibody-mediated, neutrophil-driven diseases.

P123 (OP03/02) | Noncanonical NF-B signaling is inevitable for development of chronic contact hypersensitivity reaction (CHS)

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Introduction: Autoimmune diseases, as well as CHS, are classified as delayed-type hypersensitivity reactions (DTHR) and are orchestrated by interferon-γ-producing CD8+ (Tc1) and CD4+ (Th1) T cells. Although NF-B plays an important role in inflammatory diseases, the role of noncanonical NF-B activation during T cell mediated immune responses remains largely unclear. Aim of this study was to uncover the impact of noncanonical NF-B activation during acute and chronic cutaneous DTHR using NF-B2.p100-/-/p52-/- (NF-B2-/-) mice.

Methods: NF-B2$^{-/-}$ and wild type (WT) mice were sensitized with 5% TNCB at the abdomen and challenged with 1% TNCB at the right ear 7 days later to elicit acute CHR. To induce chronic CHR we challenged mice every 48 h up to 5 times. We determined the temporal dynamics of CD8+ T cell homing in vivo by non-invasive simultaneous positron emission tomography (PET)/MRI imaging using 89 zirconium (Zr) labelled CD8 minibody. Additionally, we conducted extensive ex vivo analyses of inflamed ears focusing on impairments in immune cell homing and differences in the inflammatory microenvironment (adhesion molecules, angiogenesis, chemokines, cytokines).

Results: Disruption of noncanonical NF-B signaling did not affect the sensitization phase and acute CHS, as we observed no differences in ear swelling response, severity of inflammation and immune cell infiltration between the NF-B2-/- and WT mice. However, inflamed ears of NF-B2-/- mice with chronic CHS revealed strongly reduced ear swelling response, lack of edema, hyperkeratosis and acanthosis, accompanied by a reduced infiltration of neutrophils, T cells and macrophages when compared to WT mice. In line with this, in vivo PET/MRI imaging revealed a strongly reduced migration of CD8+ T cells into the inflamed ears and draining lymph nodes (dLNs) of NF-B2-/- with chronic CHS. Flow cytometry analysis of the dLNs

from NFB2-/- mice with chronic CHS displayed an increased T cell activation compared to WT mice, indicating an impaired immune cell homing into the inflamed ear tissue. Therefore, we conducted extensive proteome analysis of the inflamed ear tissue with acute and chronic CHS from both experimental groups. We observed a very similar proteome expression profile between the NF-B2-/- and WT mice during acute CHS, confirming an adequate sensitization and effector phase. In inflamed ears of NF-B2-/- mice with chronic CHS we determined a heavily reduced expression of numerous chemokines e.g. CXCL2, CXCL5, CXCL9, CXCL10, CCL11 and CCL12 as well as the angiogenesis promoting mediators MMP9, HGF, FGF and AREG. Furthermore, immunofluorescence microscopy of the inflamed ear tissue of NF-B2-/- mice with chronic CHS exhibited a reduced expression of the vascular endothelial adhesion molecules ICAM-1 and E-selectin when compared to WT mice.

Conclusions: Disruption of the noncanonical NF-B-signaling alleviates the chronic CHS by impairing chemotaxis, expression of endothelial adhesion molecules and angiogenesis resulting in a decreased recruitment of the immune cells into the inflamed ear tissue and thus strongly reduced inflammation.

P124 | Direct binding of complement components to the bullous pemphigoid antigen BP180 may precipitate autoimmune skin disease

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Bullous pemphigoid (BP), the most frequent autoimmune blistering skin condition, is characterized by autoantibodies that bind to collagen XVII (COL17, BP180) and typically promote complement fixation, inflammation and 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. Incubating recombinant COL17-NC16A antigen with a complement source (in the presence or absence of complement-inhibitory amounts of tinzaparin sodium) we performed immunoprecipitation and reducing SDS-PAGE. This resulted in the appearance of novel bands that were subjected to tandem mass spectrometry analysis, identifying the presence of C3b alpha' chains and the C3b beta chains in complement-activating conditions. Our results point toward direct opsonization of the immunodominant COL17-NC16A antigen with activated complement C3 fragments, in the absence of COL17-directed autoantibodies. To further investigate conditions that foster this spontaneous activation and deposition of C3 onto adhesions proteins, we incubated 3D cultured keratinocytes with various single or combined stimuli (TNF alpha, IL-6, S. aureus) and assessed gene expression of proteins of the three complement pathways by qPCR. We found an increase in mRNA expression of most investigated genes, mainly in MASP1, C1r, C1s, C2, C3 and most importantly, Factor B of the alternative pathway, which drives auto-hydrolysis, activation and opsonization of antigens by C3. 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. During local inflammatory or infective episodes, or for genetic or age-related reasons, these processes that are normally kept in check by complement regulatory or inhibitory mechanisms, may lose their protective capacity, leading to C3 activation and direct covalent binding of C3b, the first opsonizing fragment of activated complement component C3, to autoantigens. This may increase the normally low immunogenic potential of COL17-NC16A self-antigen, possibly to a point where tolerance cannot be maintained, leading to the emergence of autoantibodies. We propose that the binding of complement factors to skin autoantigens may constitute one of the earliest processes in the pathogenesis of autoimmune skin blistering diseases such as BP, mucous membrane pemphigoid or epidermolysis bullosa acquisita. Our evidence warrants further exploration of its relevance and contribution in developing skin autoimmunity.

P125 | Circulating Th17.1 cells and skin IL-4/IFN-g expression as potential targets to assess risk for bullous pemphigoid

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Background: Bullous pemphigoid (BP) is a rare autoimmune, blistering skin disorder mostly affecting the elderly. Affected patients develop skin-targeting autoantibodies, produced by B cells with the help of T cells. Previous studies have described T cell involvement in other blistering skin diseases, but the exact role of T cell subsets in BP pathogenesis needs further investigation.

Objective: We aim to examine the distribution of Th and Tfh populations from peripheral blood mononuclear cells (PBMCs), their cytokine profiles and tissue cytokine gene expression in BP patients and non-BP controls in an age-dependent manner.

Methods: Multiparametric flow cytometry was utilized to study the frequencies of Th and Tfh cell subsets. Real-time quantitative PCR was performed for transcriptome analyses of BP lesional versus healthy skin. Patient medical histories were examined to record comorbidities and autoantibody titers against BP antigens 180 (BP180) and 230 (BP230).

Results: There are currently 62 BP patients and 39 non-BP controls enrolled in this study. Preliminary results show increased numbers of circulating Th17.1 cells in the peripheral blood of patients with active disease versus those in remission. In addition, IL-4 and IFN-g gene expressions in skin were elevated in acute BP patients compared to non-BP controls. No major difference was found in our first analysis on other Th and Tfh cell subsets.

Conclusion: Patients with active BP show elevated frequencies of Th17.1 cells in peripheral blood. Acute BP patients show increased expression of IL-4 and IFNg. Thus, like pemphigus disease, BP immunopathogenesis is more complex than originally thought. Our next analysis will help find relevant disease-associated immunological patterns to assess risk for BP.

P126 | Structural characterization of the pathogenic anti-Desmoglein 3/1 autoantibody clone PX43 obtained from a patient with mucocutaneous pemphigus vulgaris

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Structural characterization of proteins, including antibodies (ab) and antigens (ag), is invaluable for understanding the relationship between structural properties and biological function, allowing for design of new drug molecules and elucidation of pathophysiological mechanisms. It has been suggested that the bullous pemphigoid immunodominant epitope NC16A undergoes significant structural changes when anti-BP180 abs bind, potentially leading to a much better exposure of the autoag towards the adaptive immune system in an early phase of disease initiation and potentially activating intracellular signaling cascades. Likewise, little is known about structural changes induced by anti-Desmoglein (Dsg)3/Dsg1 abs during binding to their autoags in pemphigus, and their effect on eliciting an aberrant immune response.

To address these questions, we here aimed to structurally characterize the monoclonal ab clone PX43 that was previously obtained from an active pemphigus vulgaris (PV) patient using X-ray diffraction and by artificial intelligence (AI)-based approaches. Despite efficient expression, large-scale screening of crystallization conditions and obtaining crystals; X-ray diffraction data was unconclusive so far. However, with the use of Alphafold2/ABodyBuilder, we were able to predict the structures of Dsg3/1 and PX43 and obtained the docked ag-ab complex structure. To validate the in silico modelled complex structure, we tested five point mutations each in Dsg3/1 by wet-lab experiments. Results partially confirmed AI predictions, with incongruent results being used for further refinement and training of AI-based approaches.

We predict that AI-based structure prediction may serve as a valid substitute for crystallography-based structure prediction, especially in cases where crystals cannot be obtained or analyzed easily.

P127 | Gene expression pattern of anti-PD1 skin lesions shows pronounced similarities to toxic epidermal necrolysis

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Immune checkpoint inhibitors (ICI) including anti-PD1 therapies have revolutionized the management of many cancers, particularly of malignant melanoma (MM). Yet, under anti-PD1 treatment, patients frequently develop lichenoid skin reactions, ranging from mild rashes to severe mucocutaneous reactions.

We aim to characterize the gene expression pattern and cell composition of anti-PD1 skin lesions and compare them to healthy skin and different types of adverse drug reactions using RNA sequencing (RNAseq) and multiplex immunohistochemistry stainings.

RNAseq analysis revealed a clear separation of anti-PD1 lesional from healthy skin with over 3600 differentially expressed genes. Cytotoxicity-related and proinflammatory genes were significantly upregulated in anti-PD1 lesional skin, while biological pathway analysis revealed neutrophil degranulation as a prominent pathway in anti-PD skin lesion development. The comparison of anti-PD1 skin lesions and mild macular papular rashes (MPR), as well as toxic epidermal necrolysis (TEN) showed strong similarities of anti-PD1 lesions with TEN rather than MPR skin. These findings could be related to clinical severeness. Transcriptomic results could be further extended to the level of spatial resolution using multiplex imaging which displayed high numbers of neutrophils in anti-PD1 lesions, thus confirming transcriptomic results.

Collectively, anti-PD1 skin lesions differ clearly from healthy skin on the transcriptomic and protein level, yet show close similarities to severe cutaneous adverse drug reactions such as TEN.

P128 | Asthmatic lungs reveal decreased expression of regulatory cytokines in skin barrier-deficient FlgHrnr-deficient mice

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The risk to develop atopic diseases is strongly influenced by both genetic and environmental factors. Skin barrier dysfunction (e.g. by the loss of filaggrin (Flg) and hornerin (Hrnr)) is one of the most prominent predisposing factors for the development of allergic skin diseases, but also for posterior development of asthma (atopic march). Allergic asthma is a chronic inflammatory disease of the airways dominated by Th2 immune response.

Here, we analyze the influence of skin-barrier disruption on the outcome of asthma using skin barrier-disrupted FlgHrnr-/- mice (Balb/c background) in an experimental model of atopic march. This model combines the induction of an AD-like phenotype by the topical application of MC903 (calcipotriol) together with allergen sensitization either by systemic or epicutaneous application of OVA, which is then followed by an allergen airway challenge with OVA-aerosol.

In general, skin barrier-disrupted FlgHrnr-/- compared to wildtype mice showed a higher susceptibility to the proceeding of the atopic march. In detail, MC903- treated FlgHrnr-/- mice showed worsened signs of AD-like dermatitis (stronger ear swelling response), a facilitated antigen-sensitization (higher levels of Th2- like immunoglobulins), and exacerbated asthma-like features (increased BAL cell counts, especially eosinophils, higher histologic inflammation score of the lungs) when compared to wildtype mice. However, we found more pronounced differences between strains when using epicutaneous sensitization compared to systemic sensitization.

To have a closer look onto the type of airway inflammation, we analyzed the expression of different cytokine markers for T cell driven immune response in mouse lung tissue 24 h after the last challenge, including Th1, Th2, Th17 and Treg profile using RT-qPCR. In general, we found that MC903 treatment on the skin during sensitization influenced expression levels of several cytokines, such as IL-33 or IL-1β, in the lungs, compared to mice treated with EtOH. Hereby, systemically and epicutaneously sensitized mice portrayed similar trends of cytokine expression. Most interestingly, the data revealed a highly decreased expression of the regulatory cytokines IL-10 and TGFβ in the lungs of MC903-treated skin barrier-deficient FlgHrnr-deficient mice compared to wildtype mice. Again, trends in systemically and epicutaneously sensitized mice were similar.

Since both IL-10 and TGFβ are two of the most important immunosuppressive cytokines and are mainly expressed by induced Treg cells, the lack of their expression therefore hints at diminished or disrupted regulatory mechanisms in mice with a skin barrier deficiency. This is sustained by the strength of inflammation, since immune cell infiltration in BAL inversely correlates with the expression level of these regulatory cytokines in the lung. However, the phenotype of these pulmonary Treg cells and their detailed function has to be elucidated in the future.

Together, our results further the understanding of the pathomechanism involved in the worsened asthmatic phenotype due to cutaneous inflammation in a model of primary skin barrier-disruption, namely the loss of filaggrin and hornerin. A potential role of modulated regulatory T cell function in preceding phases of the atopic march, like during atopic dermatitis, their correlation to their role in allergic asthma, as well as the exact pathomechanism remains to be elucidated.

P129 | The role of the IL-1/IL-36 inflammatory axis in Pyoderma gangraenosum

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Pyoderma gangrenosum (PG) is rare neutrophilic dermatoses, which presents with painful irregular ulceration and undermined violaceous edges. The ulcer typically develop after mechanical irritation or trauma on the lower legs, but can affect any body site and can grow rapidly. PG is a rare disease and as such the pathomechanism are not fully explored yet. Experimental studies showed a Th1/Th17 involvement with cytokine expression including IL1A, IL1B, TNF, as well as IL6, IL8, IL17, IL23 and IL36. In the current project we were particularly interested in the expression of IL36 family members in PG, the three pro-inflammatory agonists IL36A, IL36B, IL36G, and the IL-36 receptor (IL36R) antagonist, IL36RN. We also studied the expression IL1A, IL1B, IL8, IL17A, IL22 and IL23, which are characteristic factors in psoriasis, an inflammatory skin disorder with IL36 expression. We analyzed FFPE tissue samples from patients with PG (N = 11), patients with pustular psoriasis (PP, N = 17) and patients with plaque-type psoriasis (PV, N = 15) in comparison to skin from control patients (8) by using qRT-PCR. Our results show a strong involvement of IL36A, IL36B and IL36G in all three inflammatory skin diseases compared to healthy skin samples. The highest expression of IL36A, B, G was observed in PP skin followed by PV and PG. By far the highest expression was seen in the gene IL36A (mean ΔΔCt of PP = 215, PV = 65, PG = 9) followed by IL36G (mean ΔΔCt of PP = 23, PV = 15, PG = 1.8) and IL36B (mean ΔΔCt of PP = 2.8, PV = 3.3, PG = 1.4). In contrast, we observed the highest expression of IL1A, IL1B and IL8 in the samples of patients with a PG followed by PP and PV. In addition we performed IHC staining of IL-36α, β, γ and the IL-36 receptor in the same tissues. Our preliminary results on IHC protein staining are in agreement with our mRNA expression data. In addition, the expression of the psoriasis-typical genes IL17, IL22 and IL23 was determined in the project. In all three cases, a significantly lower expression was observed in the patients with PG than in the patients with PP or PV. Taken together, the IL-1/IL-36 inflammatory axis appears to be a key player of disease pathology

in PG. This might be of clinical interest since the IL-36 receptor antibody Spesolimab has shown significant impact in the treatment of patients with generalized pustular psoriasis and could serve as a treatment option in PG.

P130 | Gene flow from archaic hominins causes HLA-class I associated diseases, psoriasis, ankylosing spondylitis, and Behcet disease

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Ancient genome sequencing has revealed gene flow from Neanderthals or Denisovans into our direct ancestors. Recent studies have shown that gene alleles originally derived from archaic hominins are associated with several traits, providing a dominant strategy to validate the utility of the current notion. Going beyond this strategy and based on the cutting-edge knowledge in human evolution and medical genetics, we show how we can advance our understanding of genetic complexity in common diseases.

Endoplasmic reticulum aminopeptidase 1 (ERAP1) variants, which form 10 haplotypes (Hap1-Hap10) with different enzymatic activities, control the risk for psoriasis, ankylosing spondylitis (AS) and Behçet's disease (BD) mediated by the disease-specific risk genes, HLA-C*06:02, HLA-B*27 and HLA-B*51. Through this statistical epistasis, Hap1-Hap3 increase psoriasis or AS risk in carriers of HLAC* 06:02 or HLA-B*27, while Hap10 protects against these diseases but enhances BD risk in HLA-B*51 carriers. In psoriasis, this corresponds to different autoantigen amounts generated by different ERAP1 haplotypes for HLA-C*06:02 presentation to activate CD8+ T cells (Arakawa et al. 2021). The origin of this pathogenic epistasis is unknown. We analyze the strongest epistasis in the human pathology, for which we could know the pathological function. Though the importance of epistasis is well established in genetics of model organisms, epistasis is hardly identified in human diseases.

Here we show that ERAP1 Hap1 has Neanderthal origin, while Hap10 and Denisovan ERAP1 share the evolutionary pathway and weaker activities. Hap2 and Hap3 likely evolved in Homo sapiens. We identified inferred Denisovan ancestry of HLA-C*06:02 and HLA-B*27, and Neanderthal introgression of HLAB* 51. Assuming these evolutionary pathways, ERAP1 haplotypes of different origins to the HLA increase the HLA-mediated disease risk, probably because the uncoordinated evolution in different lineages causes disproportionate ERAP1- dependent autoantigen supplies for HLA-class I presentation. Our analyses demonstrate that gene flow from archaic hominins may directly and dynamically regulate the emergence of genetically complex human diseases.

P131 | Determining mechanisms of melanoma dedifferentiation in response to innate immune receptor activation

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Intratumoral application of immune-stimulatory agents are currently tested in clinical trials in combination with anti-PD-1 immune checkpoint blocking antibodies as a treatment option for unresectable melanoma. Such agents comprises synthetic agonists of the innate immune receptor RIG-I mimicing the effects of a viral infection. Previously, we demonstrated that RIG-I activation in melanoma cells by viral RNA mimetics (3pRNA) increases their immunogenicity leading to enhanced killing by CD8 T cells. Besides, we recognized a strong dedifferentiation of melanoma cells upon RIG-I activation. Important to note, acquired resistance to different therapies has repeatedly been associated with a dedifferentiated melanoma cell phenotype, indicating the importance to unravel the molecular mechanism(s) underlying RIG-linduced dedifferentiation. Combining studies with signaling pathway inhibitors and conditioned medium, we demonstrated involvement of distinct mechanisms: a JAKdependent process triggered upon direct RIG-I activation and a JAK-independent paracrine mechanism driven by soluble factors released from 3pRNA-treated tumor cells. Currently combined transcriptome and conditioned medium analyses are performed to unravel the nature of the soluble factor(s). Knockledge of the factor(s) could be exploited to prevent melanoma dedifferentiation and resistance development.

P132 (OP06/04) | Cutaneous adverse events induced by Tebentafusp, an anti-melanocytic bispecific molecule

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Tebentafusp (tebe) is a novel bispecific molecule redirecting T cells against cells presenting a gp100-derived peptide on HLA-A*02:01 and is approved for metastatic uveal melanoma. Skin adverse events (AE) were shown to correlate with gp100+ epidermal melanocytes. Thus, the analysis of tebe-induced cellular and molecular processes in patient skin samples may allow a better mechanistic understanding of this off-tumor/on-target effect.

Skin biopsies from 11 patients treated with tebe were collected at baseline and upon development of skin AE (n = 9) or within the first 3 weeks on treatment for patients without skin AE (n = 2), as well as from depigmented skin which developed at a later stage (n = 5).

We analyzed retrospective clinical data and performed histology, TUNEL staining (detection of DNA breaks) as well as multiplex immunohistochemistry (IHC) with simultaneous detection of melanocytes (Melan-A, SOX10), T cells (CD4/CD8), macrophages (CD68) and keratinocytes (PanCK). Furthermore, we conducted single cell RNA-sequencing (scRNAseq) on paired baseline and rash samples from 3 patients.

Most cutaneous AE developed within the first 3 weekly infusions. Diffuse erythema (n = 8, 73%) and pruritus (n = 5, 45%) co-occurred frequently. Facial edema developed in 2 patients (18%), while maculopapular and vesicular exanthema were observed in 1 patient, respectively. Later on treatment, depigmentation was reported in 6 patients (55%).

Rash showed increased lymphocytic infiltrates in a lichenoid distribution (p = 0.012). A higher fraction of TUNEL+ nuclei suggested epidermal cell death (OR = 12.2, $p < 10^{-16}$). Multiplex IHC proved increased proportions of CD4+ and CD8+ Tcells and macrophages as well as decreased keratinocytes in rash (p-adj < 0.05). Melanocytes did not change in rash skin biopsies but were reduced in 4 of 5 depigmented lesions. scRNA-seq revealed upregulation of interferon type I/II responses and a downregulation of pigment synthesis in melanocytes, resulting in an increased fraction of gp100-negative melanocytes. Keratinocytes overexpressed inflammation-associated keratins 6A/B and 16 and downregulated differentiation factors such as MYC, RORA and AP-1 transcription factors. T cells subclustered in tissue resident (CD69), CCR4+, regulatory (FOXP3, CTLA4), cytotoxic (IFNG, GZMB), activated (IL2RA) and proliferating (MKI67) T cells. Numbers of proliferating T cells were strongly increased in rash. In several T cell subclusters, markers of cytotoxic activity were upregulated. In the myeloid compartment, an increased ratio of M1:M2 macrophages (OR = 2.9, p < 0.01) and recruitment of plasmacytoid (pDC) and immunoregulatory dendritic cells (mregDC) was observed.

Collectively, the skin microenvironment on tebe treatment is characterized by T cell proliferation and cytotoxic activity, recruitment of myeloid cells, as well as downregulation of the drug target gp100 in melanocytes and their partial loss in depigmented skin areas. Longitudinal skin biopsies of skin AE are therefore promising as a minimally-invasive tool to study the pharmacodynamics of anti-melanocytic bispecifics.

P133 | Tyrosine kinase 2 inhibition with deucravacitinib attenuates Th1-mediated immune response in preclinical models of type 1 chronic inflammatory skin diseases

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Background: Non-communicable inflammatory skin diseases (ncISD) are a heterogeneous group of diseases leading to non-infectious inflammation of the skin and thereby severely affecting patients' quality of life. Based on the immunological signature, ncISD can be divided into four major patterns. Of those, type 1 ncISD (also known as lichenoid diseases) are characterized by a strong IFN-α and Th1 immune response causing apoptosis and necroptosis of epidermal cells. There is a high unmet medical need for the treatment of type 1 ncISD as no targeted therapies are approved up to date.

Tyrosine kinase 2 (TYK2) regulates downstream effects of IL-12, IL-23 and type I interferon receptors in human cells. Hence, it is a central player in the mediation of type 1 ncISD. Deucravacitinib selectively binds TYK2 pseudokinase in human cells and inhibits receptor-mediated downstream effects. Recently, it gained approval for the treatment of plaque psoriasis by the U.S. Food and Drug Administration (FDA). It is therefore a promising therapeutical agent for patients suffering from type 1 ncISD.

Methods: To investigate the impact of TYK2 inhibition in Th1-mediated ncISD we stimulated primary human keratinocytes and three-dimensional skin models comprised of human suction blister-derived keratinocytes with IFN-α and mixed supernatant of lesional T cells derived from cutaneous lupus erythematosus and lichen planus under the presence or absence of deucravacitinib. Gene expression of central Th1-derived cytokines was measured by qRT-PCR and RNA sequencing. Differentially expressed genes (DEG) and pathway analysis were conducted to identify potential TYK2-specific biomarkers.

Results: Deucravacitinib treatment of two- and three-dimensionally cultured keratinocytes that were stimulated with IFN-α and mixed supernatant of lesional T cells derived from cutaneous lupus erythematosus and lichen planus prevented upregulation of proinflammatory Th1-associated mRNA levels. Genes strongly upregulated by IFN-α and by mixed Th1 cell supernatant were CXCL9, CXCL10, CXCL11, CCL2, CCL5, IL32, and ICAM1, amongst others. TYK2 inhibition attenuated the effect of Th1 stimulation: significant upregulation of these genes in the Tyk2-preincubated, Th1-stimulated condition versus unstimulated was not observed. Further, pathway analysis revealed enrichment among the upregulated genes of Th1-related pathways like interferon- or JAK/STAT-mediated immune response in IFN-α- and mixed Th1 cell supernatant-stimulated keratinocytes. This enrichment was not observed among the genes that were upregulated in the deucravacitinib-treated Th1- stimulated condition.

Conclusions: The TYK2 inhibitor deucravacitinib is capable of attenuating Th1- mediated immune response in preclinical models of type 1-related ncISD. Thus, it is a potential therapeutic agent in treating lupus erythematosus, lichen planus, and other Th1-mediated ncISD in the future.

P134 | Macrophage dysregulation drives granuloma formation in sarcoidosis

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Sarcoidal granulomas are characterized by an accumulation of macrophages and T cells inside the tissue, which remain in a hyperactivated immune state causing tissue destruction. We aim to study cellular and molecular patterns leading to pathological, disease-driving immune cell populations within the granuloma environment. Therefore, we generated monocyte-derived macrophages (MoMac) as well as induced pluripotent stem cell-derived macrophages (iPSMac) from healthy controls and sarcoidosis patients and showed that they have similar characteristics as granuloma-associated macrophages. When we incorporated these macrophages into a dermal skin equivalent we observed stronger migration of patient cells into one direction, indicating cell aggregation similar to what is seen during granuloma formation. To identify potential disease-driving pathways, we established an in vitro granuloma-formation assay and treated macrophages with GM-CSF, IFN-γ and lymphotoxin alpha/beta (LTα/β), cytokines which are produced by inflamed T cells in skin lesions of sarcoidosis. We observed increased and more compact clustering in macrophage cultures from sarcoidosis patients compared with healthy controls upon stimulation with IFN-γ and LTα/β. Patient macrophages showed higher levels of CXCL16 and IL-23 expression important for T cell recruitment and Th17 polarization, the predominating disease-driving T-helper cell phenotype. Additionally, we found increased levels of macrophage proliferation and STAT3 phosphorylation, a known factor contributing to granuloma formation. Furthermore, PD-L1 and PD-L2 were higher expressed in macrophages derived from diseased individuals, while T cells showed an exhausted phenotype with PD1 upregulation within granulomas. These data confirmed what we observed in our transcriptomic dataset of sarcoidal skin lesions, revealing a complex crosstalk between macrophages and exhausted Th17 cells that ultimately lead to tissue granulomas. In the next steps, we will apply 3D co-cultures of macrophages and T cells to study their interaction in the process of granuloma formation in more detail.

P135 | Novel isolation and identification protocols for Innate Lymphoid Cells in human skin, and their role in Dendritic cells development

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As the largest organ and the major immune barrier, the skin protects against external noxious stimuli. Throughout the skin layers, and among epithelial cells, fibroblasts, adipocytes, neurons, and a wide array of immune cells can be found dispersedly, creating an interacting barrier of networked cells. Among immune cells found in the skin, a population of innate lymphoid cells (ILC) can be isolated, although they represent a small percentage of this immune population, about 5%. These cells have been demonstrated to be fundamental to ensuring barrier immunity, by regulating microbiome balance, aiding tissue repair, promoting allergic inflammation, and playing a role in the expulsion of intestinal parasites. Despite ILCs extensive characterization in the lung and intestine, the role of these cells under pathological conditions is yet to be clarified in the skin and current isolation methods fail to guarantee the ideal isolation of these cells. Previous works in our group have demonstrated that ILC are indeed found in mouse skin and that these cells constitutively express IL-13. IL-13 has a direct immunological function, influencing the development and differentiation of type 2 dendritic cell precursors, mainly the development of CD11b(low) DC2. In the absence of ILC-derived IL-13, CD11b(low) DC2 did not develop, leading to a reduction in Th2 response. Our hypothesis is that ILC are found in healthy skin during homeostasis and play a fundamental role in DC development and IL-13 production is regulated in a similar manner in healthy human skin. Henceforth, the aim of this study is to establish a novel skin digestion protocol for the isolation of ILC and DC, investigate other cellular populations that can also be producing IL-4/IL-13, and investigate cellular markers that allow better identification of ILC. Furthermore, we will compare these cellular populations in human healthy skin and atopic dermatitis skin and other diseases. The results of our study will allow a better understanding of the intricate function of ILC in the skin, their interaction with other cells, especially in DC development, and how IL-13 production can impact Th2 response. Not only that, but this knowledge can be detrimental to the development of new approaches and treatments for skin conditions such as Atopic Dermatitis.

P136 | Circadian rhythmicity of immune cells in systemic lupus erythematosus

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The autoimmune disease systemic lupus erythematosus (SLE) is characterized by a variety of symptoms such as the butterfly rash on the face or kidney inflammation, which appear in recurring flares. The disease development and its triggers are mostly uncharacterized. The circadian rhythm is a vital aspect, guiding all of the body's functions. The interactions of various genes and proteins form interlocked feedback-loops, coordinating their transcription and repression in a timeframe of about 24 hours (h). Therefore, the master clock, called suprachiasmatic nucleus, is located in the brain and its signals synchronize peripheral clocks throughout all organs and cells

in the body. Additionally, extrinsic factors such as light and food-intake are able to support or disturb this rhythm. It is known that such a disturbance can cause or worsen diseases. Shift work is one of the main life-style factors that force people to act against their natural timings. Its deleterious effects have been studied with regard to cancer, overweight and much more. However, it is not clear how shift work is able to trigger autoimmunity on a cellular level. We compared the 24 h rhythms of different immune cell populations of blood and four organs of wild type mice with those of SLE-prone mice. It turned out the SLE-prone mice differ in their blood composition, as well as in cell-trafficking markers on immune cells in different organs. Future experiments will simulate shift work in SLE-prone mice to disturb their circadian rhythm and thereby find out how this is dysregulating the cellular mechanisms and drives them towards autoimmunity. Moreover, we will hand out sleep- and chronotype questionnaires to shift workers and SLE patients to identify potential circadian disruptions in their lifestyles and compare this to several blood parameters. This work especially aims to identify the role of the circadian rhythm on the development of SLE, the so-called pre-disease state.

P137 | High-yield production and cellular implementation of a novel Desmoglein 3 EC5 binding antibody into a keratinocyte based in-vitro skin model

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Pemphigus vulgaris (PV) is a severe autoimmune blistering disease characterized by pathogenic autoantibodies (auto-ab) against the desmosomal adhesion molecules desmoglein3 (Dsg3) and Dsg1. Underlying mechanisms inducing 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 auto-ab response in PV patients is polyclonal, including auto-ab directed against both amino- and membrane proximal epitopes.

In this study, the pathogenicity of a novel murine monoclonal antibody directed against the membrane-proximal region (EC5) of the Dsg3 ectodomain was analysed. This Dsg3-specific antibody was isolated from the supernatant of a Dsg3-specific Bcell hybridoma. After production optimization, it was tested in various specificity and functional assays including a novel in-vitro skin model.

Results clearly demonstrate that this murine auto-ab specifically binds human Dsg3 and is capable of inhibiting intercellular keratinocyte adhesion accompanied by the activation of the p38 MAPK signal transduction pathway. A full skin model, comprised of a dermal part with collagen matrix and human fibroblast and a differentiated epidermis formed by human keratinocytes was developed containing the respective antibody secreting cells to establish a pathogenic in-vitro patient situation. We found that this novel technique allows characterization and additionally modification of antibody-based cellular dissociation characteristic for PV. Our results deliver new aspects of a more defined understanding of auto-ab-induced blister formation in PV.

P138 | Ion channel inhibitors as modulators of neutrophil activation

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The autoimmune skin blistering disease epidermolysis bullosa acquisita (EBA) is characterized by pathogenic autoantibodies directed against type VII collagen (COL7) located at the dermal-epidermal junction. The binding of autoantibodies to COL7 results in the recruitment of neutrophil granulocytes to the dermis and their subsequent activation. These cells display changes in ion currents across their membranes, as well as changes in their cytoplasmic ion concentrations. Activated neutrophils induce the formation of blisters in the skin by the release of reactive oxygen species (ROS), inflammatory cytokines and proteases. The development of new drugs for treatment of bullous autoimmune diseases, such as EBA, is necessary as most patients nowadays are treated with a systemic suppression of the immune system by corticosteroids combined with other immuno-modulating agents. Screening for immuno-modulating effects of neutrophil granulocytes from healthy donors using in vitro and ex vivo models of EBA are beneficial to identify new lead compounds in drug discovery. To identify substances which influence ion currents with a potential effect on neutrophil activity, a library of substances affecting the transport of ions across cellular membranes was screened using a luminescencebased neutrophil ROS release assay. Therefore, human neutrophils were isolated from fresh peripheral blood of volunteers and ROS production was induced by immune complexes consisting of the E-F fragment of human COL7 and anti-human COL7 antibodies. Substances which caused an average reduction of ROS production exceeding 50% as compared to vehicle control at a concentration of 1 µM were considered as candidates for further in vitro validation. A total of 16 substances were identified from the library, mainly targeting calcium, potassium and chloride channels. We further validated these substances in a dose-dependent manner for toxicity, their effect on ROS release, as well as the expression of L-selectin and CD18 as markers of neutrophil activation. In the ROS release assay, 14 of the substances caused a significant reduction in ROS production at 10 µM, while 5 substances had significant effects at 10 and 1 µM. Flow cytometric experiments showed no significant cytotoxic effects for 15 of the substances at any tested concentration. The same experiment showed that while having no significant effect on L-selectin shedding, several substances reduced the increase in cell surface CD18 expression upon cell activation. To further characterize the effects

of these substances, experiments measuring cellular kinase activities, cytoplasmic calcium concentration and pH changes are planned.

P139 | HLA class II haplotype loss in melanoma and its association with patient resistance to immune checkpoint blockade

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Effective anti-tumor immunity depends on cytotoxic CD8+ T cells, killing tumor cells upon recognition of cognate tumor antigen-HLA class I (HLA I) surface complexes. Accordingly, haplotype loss of HLA I genes can evolve in the course of tumor progression and prevent an efficient elimination of corresponding tumor lesions by CD8+ T cells. Aside from CD8+ T cells, there is accumulating evidence for a role of CD4+ T cells in HLA II-dependent killing of tumor cells. So far, knowledge about HLA II gene alterations and their role in reshaping anti-melanoma CD4+ T cell response is lacking. Here, we studied alterations in both HLA I and HLA II genes during melanoma progression and addressed their significance for evasion from T cell surveillance. Among 34 analyzed short-term cultured consecutive melanoma cell lines (<20 passages) from 12 patients, loss of HLA haplotype was detected in 9 cell lines (26.5%) from 3 patients (25%). Strikingly, all cell lines showing HLA haplotype loss in our cohort exhibited deletion of both HLA I and HLA II genes. Furthermore, the 3 patients with combined HLA gene loss were either primary resistant to immune checkpoint blockade or developed resistance along with HLA haplotype loss. With autologous mixed lymphocyte/tumor cultures, we focused on interaction between CD4 and tumor cells and demonstrated that re-expression of lost HLA alleles resensitized melanoma cells to CD4 T cell-mediated tumor killing. Taken together, melanoma cells with HLA II haplotype loss can evade CD4 T cell surveillance, suggesting the contribution of predominant anti-tumor CD4 T cell responses in selecting immune escape variants lacking 'key' HLA molecules.

P140 | Development of an immunocompetent 3D-bioprinted skin-on-chip model

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Due to the complexity of the skin, the development of in vitro models to study skin biology is challenging. Commonly used human skin equivalents consist of collagen with fibroblasts topped with epidermal layers composed of keratinocytes. We are interested in developing a 3D-bioprinted skin-on-chip model that will be complemented with immune cells. For a start we tested how dendritic cells (DC) can be incorporated into the dermal compartment.

For this purpose, we embedded monocytes or monocyte-derived DC (moDC) together with fibroblasts into a gelatin-methacrylate mix that upon blue light exposure polymerizes forming stable hydrogels. With flow cytometry analysis we investigated viability, differentiation and maturation of DC in hydrogels.

First of all we observed that moDC stayed viable in hydrogels and displayed an immature phenotype when analyzed by flow cytometry. Moreover, these moDC could be activated within hydrogels by addition of a maturation cocktail consisting of TNF-alpha, IL-1beta, prostaglandine E2 and IL-6. When CD14+ monocytes were embedded into hydrogels and differentiated by GM-CSF and IL-4, they upregulated HLA-DR but still showed expression of CD14, resembling an inflammatory monocyte phenotype. Interestingly, these differentiated cells also expressed the maturation marker CD83 indicating spontaneous maturation. These preliminary results suggest that monocytes or moDC are viable and can be further differentiated in hydrogels. In the next step we will now implement these cells into our 3D-bioprinted skin-on-chip model to establish an immunocompetent skin model. In future we hope to use the immunocompetent human skin model for drug testing and vaccine developments.

P141 | mTOR inhibition as treatment for persistent cutaneous sarcoidosis

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Background: Sarcoidosis is an inflammatory disease of unknown etiology that belongs to the group of multisystem granulomatous disorders. Cutaneous sarcoidosis lesions can appear as an initial manifestation of the disease or develop later in the clinical course. The treatment for cutaneous sarcoidosis can be challenging and current therapeutic options are limited. Recent evidence from a mouse model for sarcoidosis indicates an important role of mTOR for granuloma formation. We conducted a clinical study of persistent cutaneous sarcoidosis with the mTOR inhibitor sirolimus as topical and systemic treatment.

Methods: We included patients of full age presenting persistent, histologically-proven, cutaneous sarcoidosis. Topical treatment.12 patients applied 0.1% sirolimus in vaseline, or vaseline only, in a placebo controlled, double-blinded study design. Each treatment phase lasted two months and was followed by a one-month wash-out period. Systemic treatment. All patients received Sirolimus 1 mg/ml, once daily for 4 months. 12 patients completed systemic treatment. Treatment efficacy was assessed before and after each treatment phase using clinical scores, histologic and laboratory evaluation, and close patient monitoring following routine clinical practice.

Results: While topical treatment did not alter cutaneous lesions, systemic treatment resulted in clinical and histologic remission of skin in 70% of patients. We identified papular, nodular, plaques, scar and tattoo sarcoidosis as responding morphologies. Improvement of cutaneous lesions appeared long lasting up to 1.5 years after end of treatment. Systemic treatment showed improvement in additionally affected organs, including sarcoidosis of the lung, eyes and spleen.

Conclusion: MTOR inhibition with sirolimus presents a safe and efficient new treatment option for persistent cutaneous sarcoidosis.

P142 | Diet impacts T cell receptor repertoire diversity in murine lupus model

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Background: Autoimmune diseases are one of the major medical burdens worlwide. They affect around 9% of the population and lead to increased disability and mortality rates. Pathogenesis of autoimmune disorders is multifactorial, and includes not only genetic factors but also many environmental factors, such as infections, diet, microbiome, etc. Systemic lupus erythematosus is a prototypic autoimmune disease caused by broad spectrum of autoantibodies and autoreactive lymphocytes. T-cell mediated stimulation of autoimmune response is indispensable in induction and maintenance of lupus. However, exact mechanisms of lupus pathogenesis are not completely understood.

Methods: In order to decipher T-cell role in systemic lupus erythematodes and changes of T-cell receptor (TCR) repertoire, we exposed NZM2410 mouse strain to 3 different diets over 28 weeks (control, caloric restriction and "western" diet). After sampling of murine splenocytes we performed high-throughput sequencing of variable regions in α and β TCR chains.

Findings: Overall, diet had severe impact on incidence of systemic lupus erythematodes in NZM2410 mouse model. TCRα and TCRβ immunosequencing demonstrated statistically significant differences in clonality and diversity between diseased and non-diseased mice. Diseased animals demonstrated lower diversity of TCR compared to healthy mice. At the same time, mice on caloric restriction had higher TCR diversity in comparison to control and "western" diet group. Taken together, our data demonstrate correlation of TCR repertoire with disease status and diet, providing additional insights into the pathogenesis of lupus and autoimmune diseases in general.

P143 | Identification of novel activators of keratinocyte CARD/BCL10/MALT1 complexes

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Psoriasis is a chronic inflammatory skin disease the pathogenesis of which is driven by pathological interaction between the innate and adaptive immune system and keratinocytes. Even though this disease affects 2-3% of the general population, the underlying mechanisms that initiate the pathology have been insufficiently defined. CARD14 is a protein of the CARD-coiled-coil family and is expressed mainly in keratinocytes. It forms together with adaptor molecule BCL10 and paracaspase MALT1 the "CBM"-complex, which activates

pro-inflammatory cell responses via the NF-B-pathway. Not only have highly penetrant gain-of-function mutations of CARD14 been causally linked to familial psoriasis, aberrant BCL10/MALT1 activity is also frequently detected in skin samples of sporadic psoriasis. Nevertheless, what triggers CBM complexes in keratinocytes in the absence of activating CARD14 mutations is largely unknown. Therefore, we set out to investigate which upstream signals can activate keratinocyte CBM complexes.

To this aim, we knocked out BCL10 in HaCat human keratinocyte cell line using CRISPR-Cas9 and reconstituted BCL10 expression in a doxycycline-inducible manner. NF-B activity upon different stimuli was measured using immunoblotting of phosphorylated members of the NF-B pathway, fluorescent imaging of p65 nuclear translocation and real-time PCR of NF-B target genes.

Stimulation with the double stranded RNA analogue poly(I:C) either extracellularly, i.e. via the Toll-like receptor 3 or by transfection i.e. via the RIG-I receptor induced comparable activation of NF-B in BCL10-deficient and proficient HaCat cells. Similarly, upon stimulation of the intracellular receptor NOD2 with muramyl dipeptide, a cell wall component of most gram-positive and gram-negative bacteria, the NFB pathway was activated both in the presence and absence of BCL10 expression. However, activation of the Dectin-1 receptor with β-glucan, a fungal cell wall component, led to p65 nuclear translocation only in the presence of BCL10. Interestingly, etoposide-induced DNA damage signaling, which was previously reported to be mediated by STING, also resulted in greatly reduced p65 nuclear translocation in the absence of BCL10. Moreover, the transcriptional upregulation of CXCL10, INFB and CCL20 upon etoposide treatment was also found to be dependent on the presence of BCL10. These results indicate potential new mechanisms through which external triggers such as fungal overgrowth on the skin or drugs inducing DNA damage may induce keratinocyte CBM signaling and thus may contribute to disease flare-ups in psoriasis.

P144 | NK cells are activated and possess immunoregulatory functions in patients with pemphigus vulgaris

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Natural killer (NK) cells are innate lymphocytes, which possess the capacity to kill target cells and secrete various cytokines. In pemphigus vulgaris (PV), an autoimmune bullous disease, which results from loss of desmosomal cell-cell adhesion caused by IgG antibodies against desmoglein (Dsg) 1 and Dsg3, the role of the adaptive immune system is well-established. However, little is known about the immunoregulatory function of NK cells in autoimmune diseases, including PV. Our aim was to explore the leukocyte compartment and comprehensively characterise NK cells regarding their phenotype and functionality using peripheral blood samples from PV patients as compared to healthy controls using multicolour flow cytometry. Our results revealed changes in the immune profile of PV patients, particularly in subsets of the innate immune system. Moreover, NK cell frequencies in healthy controls negatively correlated with CD4+ T cells, a correlation which is lost in PV patients, potentially indicating a loss of a regulatory mechanism for autoreactive T cells. Moreover, a detailed phenotypic analysis showed that both the CD56dim and the CD56bright NK cells are activated (CD69) and the CD56bright NK cell subset is proliferating (Ki67). NK cell maturity was not affected in PV patients, whereas CXCR3 and PD-1 expression was decreased on both CD56bright and CD56dim NK cells. Furthermore, a higher expression of CD39 was detected CD56bright NK cells, and granzyme B was increased on CD56dim NK cells, indicating a more regulatory function of CD56bright NK cells and a highly cytotoxic potential of CD56dim NK cells. Skin-resident NK cells were predominantly CD56bright and expressed CLA, CXCR3, CCR5 and varying levels of CCR8, a phenotype that could be recapitulated on proliferating or responding (Ki67+) CD56bright NK cells from PV patients. Peripheral blood NK cells from PV patients retained their functional capacity and produce high amounts of cytokines upon stimulation with target cells or upon cytokine priming. The immunoregulatory role of NK cells in skin will be investigated in future experiments in which lesional NK cells from PV patients will be comprehensively characterized by scRNA sequencing. Taken together, our results indicate a potentially immunoregulatory role of NK cells in PV.

P145 | Non-canonical role of complement C3 in Epidermolysis Bullosa Acquisita

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In this study we set out to access the impact of complement C3 on experimental, murine epidermolysis bullosa acquisita (EBA), an autoimmune blistering disease (AIBD) from the pemphigoid spectrum, which is hallmarked by linear deposition of IgG and complement C3 at the dermal-epidermal junction (DEJ). To do so, an active murine disease model for experimental EBA was applied to complement factor C3 deficient (C3 KO) and wildtype (Wt) mice.

The active EBA mouse model is based on animals maintained on the genetically susceptible B6.SJL-H2s C3c/1CyJ (B6.S) background. Wt and C3 KO littermates on this background were immunized with a single dose of recombinant von Willebrand factor type A-like domain 2 of murine collagen VII (mCOL7^{vWFA2}), the immunodominant epitope in EBA, in emulsion with the adjuvant TiterMax classic, to

drive the induction of mCOL7^{vWFA2}-specific autoantibodies and associated autoimmune disease. A total of 154 mice were immunized, weekly scored for their disease affected body area, and sacrificed at pre-defined timepoints Day 0, 2 and week 1, 2, 3, 4, 6, 8, 10 post-immunization (p.I.). C3 KO mice developed active disease, characterized by clinical blistering as early as week 4 p.I., whereas increased disease development in Wt animals could be observed starting at week 6 p.I. The observed effect of early aggravated disease in C3 KO continued until week 9, after which the disease scores in these mice dropped sharply, whereas Wt mice scores kept increasing. None of the control mice (C3 KO and Wt) that were immunized with adjuvant only developed any signs of disease.

On the level of autoantibodies C3 KO and Wt mice exhibited no significant differences in anti-mCOL7^{vWFA2} serum IgG, IgG1, IgG2b, IgG2c or IgG3 titers at any tested timepoint (W1-W10). Also, avidity testing of pooled sera by ELISA revealed no differences in binding strength of specific IgG between C3 KO and Wt mice at the tested timepoints. We will further expand our analysis of the humoral immune response by analyzing total serum IgG and antigen specific IgM at early time-points of disease.

We hypothesized that C3 serves a non-canonical function during local skin inflammation in experimental EBA, providing tissue protection through its crucial role in tissue homeostasis by aiding the effective removal of apoptotic material during early stages of disease, thus protecting from aggravated inflammation. According to our data, the turning point from regulatory complement activation to exacerbated inflammation is reached approx. at week 6 p.I.

We aim to further characterize the observed phenotype by analyzing flow cytometric datasets, generated from EBA skin specimens at the time of sacrifice, using two adjacent immunostaining panels for cellular composition, immune infiltrate, and possible local sources of C3.

P146 | Characterization of autoantibody response and previously identified monoclonal antibodies, derived from mice with actively induced Epidermolysis Bullosa Acquisita

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Current antibody transfer models of pemphigoid disease are hampered by the xenogenic nature of the transferred (auto)antibodies. To establish a wholly murine antibody transfer model for epidermolysis bullosa acquisita (EBA), we used phage display selection to identify and generate a panel of autoantibodies targeting the second von Willebrand factor type A-like domain of murine collagen VII (mCOL7^{vWFA2}), the immunodominant epitope in EBA.

We identified a defined panel of antibody clones binding mCOL7^{vWFA2}, from two single-chain fragment variable (scFv) immune libraries, generated from the spleen (S) and inguinal lymph nodes (LN) of a mCOL7^{vWFA2} immunized mouse with active EBA, using two consecutive rounds of phage display selection with recombinant mCOL7^{vWFA2}.

The initial library sizes, as reflected by colony forming units (CFU) (2.35 × 10E7 (S); 3 × 10E7 (LN) CFU), dropped by a factor of 10E4 (1.24 × 10E3 (S); 1.9 × 10E2 (LN) CFU) indicating scFv-phage selection and suggesting a narrow B-cell response against the applied recombinant autoantigen. The massive enrichment after the second panning, yielding library sizes of 2.04 × 10E7 (S) and 1.8 × 10E7 (LN) CFU respectively, strengthened the further.

Sanger sequencing of 33 scFv clones revealed 14 individual scFv antibody clones that grouped into four variable heavy chain (VH) clonotypes, all incorporating the IGHV1 39 V gene and IGHJ3 J gene, which differed in individual point mutations, CDRH3 sequence and length. Interestingly one clonotype was unique to the lymph nodes, two to the spleen, and another one occurred in both tissues. ScFv antibodies from all VH clonotypes exhibited comparable mCOL7^{vWFA2} specific binding, as measured by ELISA.

We extracted DNA from the initial immune libraries and after each panning, which currently undergo next generation sequencing (NGS) to (a) estimate the frequency of our identified clonotypes within the originating immune libraries, (b) characterize the diversity of the B-cell response against mCOL7^{vWFA2} in our model and (c) identify potential differences in the B-cell immune response between two secondary lymphoid organs from the same mouse.

Complement activation has been shown to be crucial in EBA, which is in line with elevated titers of complement fixing IgG2b antibodies in sera from mice immunized with mCOL7^{vWFA2}. Thus we chose the six most frequent VH-VL combinations from sanger sequencing for recombinant expression as mIgG2b mAbs.

We are currently characterizing these novel six antibodies *in-vitro* regarding antigen binding, complement fixation and effector cell activation, which we will thereafter extend to *in-vivo* pathogenicity testing.

Overall, our data will provide detailed insights into autoantibody maturation in experimental EBA, as well as provide murine tools allowing to better study pathogenesis and treatment responses in this still difficult-to-treat disease.

Infectious Diseases

P147 | Ex vivo wound models to study skin colonization and invasion of *Candida auris*

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Fungi are omnipresent in the environment and colonize countless ecological niches including mammalian mucosal surfaces as well as skin. Even though the incidence of lethal fungal infections in humans is relatively low, invasive fungal diseases are responsible for an accountable number of deaths per year worldwide. The genus Candida represents the most prevalent fungal pathogens that can cause life-threatening infections in immunocompromised humans. Candida (C.) auris is a newly emerging fungal pathogen showing dramatic resistance to all clinically used antifungal drugs and has been classified as an urgent threat by the Centers for Disease Control and Prevention and by the World Health Organization. Even though the spread of C. auris is facilitated through its easy transmission by skin-toskin contacts in hospital environments, yet nothing is known about if and how C. auris penetrates epithelial barriers. Consequently, there is a high demand for robust methods that allow to study host-fungal interactions. We established several ex vivo wound infection models with C. auris using ex vivo human and mouse skin aiming to investigate its attachment, colonization as well as invasion potency and depths and included the well-studied C. albicans as a control. Skin was wounded using different approaches (tape-stripping, micro-needling, removing of the epidermis with a milling cutter). Subsequently, fungal suspensions were applied topically or via intra-dermal injections. Periodic Acid-Schiff staining and immunofluorescence analysis of infected and uninfected skin biopsies revealed that C. auris cells only invaded human skin when its barrier function was disrupted, while C. albicans easily penetrated even unwounded human skin. In mouse skin, a barrier disruption was not necessary for successful penetration, however invasion depth and intra-tissue distribution differed between C. auris and C. albicans. Importantly, we identified that C. auris was able to acquire pseudohyphae phenotypes in the dermis of both human and mouse skin. Our standardized Candida infection models revealed first insights on fungal skin colonization and invasion and further forms a crucial basis for future studies to fully understand the molecular mechanisms of skin invasion by C. auris.

P148 (OP05/03) | A single nucleotide polymorphism associated with eczema herpeticum in patients with atopic dermatitis suggests that collagen XXIII alpha 1 protects macrophages from HSV-1 infection

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Background: Eczema herpeticum (EH) is a disseminated skin infection caused by herpes simplex virus-1 (HSV-1), which occurs in around 3% of atopic dermatitis (AD) patients, and if left untreated is associated with a significant risk of hospitalization and mortality. Several genetic variants are associated with higher EH risk, but only a few unbiased sequencing approaches have tried to identify genetic risk factors for this uncontrolled spread of HSV-1.

Methods & Results: We performed next-generation whole-exome sequencing of representative patients suffering from AD and a history of EH (ADEH+). Reassessment of potential genetic variants in a larger cohort comprising 117 ADEH+, 117 AD patients without a history of EH (ADEH-), and 188 healthy controls showed that the heterozygous single nucleotide polymorphism rs2973744, affecting the gene encoding collagen XXIII alpha 1 chain (COL23A1), was significantly associated with the occurrence of EH in AD patients. While other transmembrane collagens are known to possess antiviral properties, COL23A1 is a marker of M2-polarization of macrophages, a hallmark cell type in AD.

Since myeloid cells are important to control HSV-1 infection, we characterized the influence of COL23A1 and the newly associated gene variant rs2973744 on the susceptibility of M2 macrophages to HSV-1. We generated monocyte-derived M2 macrophages from peripheral blood mononuclear cells of healthy donors as well as ADEH+ and ADEH- patients. Macrophages carrying the rs2973744 variant were more susceptible to HSV-1 infection than those of an ADEH- patient or a healthy donor.

Interestingly, single-cell labeling revealed that M2 macrophages expressing high levels of COL23A1 were less susceptible to HSV-1 than low-expressing cells. COL23A1 is processed by host protease furin to release its soluble ectodomain from cells. We further studied the effects of the selective Furin inhibitor 1 (FI) as well as externally added recombinant WT human COL23A1 on HSV-1 gene expression. Treating cells with the furin inhibitor FI, full-length COL23A1, or the ectodomain for one hour prior to infection significantly decreased HSV-1 infection of M2 macrophages derived from healthy donors or carrying the susceptibility variant rs2973744.

Conclusion: The variant rs2973744 was associated with the occurrence of EH in AD patients, which was also reflected by an increased susceptibility of M2 macrophages to HSV-1 infection. Our data suggest that cell surface COL23A1 may protect M2 macrophages from HSV-1 infection.

P149 | Potential role of keratinocyte-derived collagen XXIII alpha 1 in eczema herpeticum

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Introduction: Atopic dermatitis (AD) is a common chronic inflammatory skin disease, which increases susceptibility to bacterial and viral infections such as the potentially life-threatening, generalized HSV infection termed eczema herpeticum (EH). Although several single nucleotide polymorphisms have been associated with EH, the genetic and immunological background is still not clear. By next-generation whole exome sequencing, we have identified the new variant rs2973744 as being significantly associated with EH. rs2973744 is located in COL23A1 which encodes the type II transmembrane collagen XXIII alpha 1 chain (COL23A1). COL23A1 is either transported as a full-length protein to the cell surface, or cleaved, mainly by the host protease furin to be released as a soluble ectodomain.

Aims: We aim to elucidate the risk factors and the mechanisms involved in the pathogenesis of severe HSV infections in AD patients with special emphasis on COL23A1.

Materials & Methods: We analyzed COL23A1 gene expression of hair follicle-derived human keratinocytes of AD patients without (ADEH-) or with (ADEH+) a history of EH by qPCR and Western blot and determined their susceptibility to HSV-1 infection. To investigate whether COL23A1 had any impact on HSV-1 infection of primary keratinocytes, we measured viral gene expression after silencing or overexpression of COL23A1 or blocking its cleavage and release by inhibiting furin.

Results: Primary human keratinocytes from an ADEH+ donor with the variant rs2973744 had lower COL23A1 mRNA levels and were more susceptible to HSV-1 when compared to those of ADEH- individuals. Furthermore, treating the keratinocytes with a furin inhibitor raised the surface expression of COL23A1 and increased their susceptibility to HSV-1 as monitored by GFP reporter expression. Interestingly, overexpression of COL23A1 in the human keratinocyte cell line HaCaT decreased HSV-1 gene expression.

Conclusion: ADEH+ patient-derived primary keratinocytes displayed a higher susceptibilty to HSV-1 infection, which supports the concept of a genetic predisposition for EH. Our data suggest a potentially protective role of soluble COL23A1 against HSV-1 infections of keratinocytes. Impaired COL23A1 gene expression or COL23A1 protein processing may contribute to the increased risk for EH observed in the subgroup of AD patients.

P150 | Long-term intraanal HPV16 infection in HIV-infected men who have sex with men: Persistence or reinfection?

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Introduction: Persistent infections with high-risk HPV can cause anal intraepithelial neoplasia and anal cancer. The aim of this retrospective study was to analyse whether the repeated and long-lasting detection of HPV16 in intraanal swabs of HIVinfected men who have sex with men (HIV+MSM) reflects persistent infection with the same isolate or repetitive new infections with different HPV16 isolates in sexually active individuals.

Methods: Of more than 600 HIV+MSM participating in anal cancer screening between 2003 and 2019, 54 patients with repetitive intraanal HPV16 detection were further analysed. Both HIV+MSM with continuously HPV16-positive intraanal swabs (group-1, $n = 26$) and HIV+MSM with HPV16-negative intervals (group-2, $n = 28$) were included. Median follow-up time was 6.8 years. Two variable HPV16 non-coding regions (long-control region, LCR, and non-coding region between E5 and L2, NCR) were analysed by Sanger sequencing in 156 HPV16-positive intraanal swabs of the 54 patients. The mean number of analysed swabs per patient was 3 (range 2-6).

Results: 150 LCR and 151 NCR sequences of 54 HPV16-positive HIV+MSM were obtained. The vast majority of patients, 92% (24/26) of group-1 and 75% (21/28) of group-2, had absolutely identical NCR and LCR sequences over a period of several years, respectively. Few patients (8% in group-1, 25% in group-2) had probable de novo infections with different HPV16 isolates in consecutive swabs. To corroborate our findings, whole-genome sequencing analyses using NGS techniques are currently being performed.

Conclusions: The repeated detection of intraanal HPV16 in HIV+MSM most likely represents long-term persistence of the same HPV16 isolate. HIV+MSM are exposed to a large number of HPV-types shortly after initiation of sexual activity and probably fail to permanently clear these infections which is associated with a high risk for anal precancer and cancer. Our results also explain the poor efficacy of prophylactic HPV vaccination in this patient group.

P151 | The *Staphylococcus epidermidis* transcriptional profile on healthy and atopic skin

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Staphylococcus epidermidis is an important component of the human skin microbiota, where it contributes to skin barrier homeostasis and the prevention of colonization by pathogens. To date, only limited information is available on the factors that allow the bacterium to colonize its two major ecological niches: the nose and the skin. Recently, it has been reported that expression of the protease EcpA leads to an exacerbation of clinical symptoms in patients with atopic dermatitis. Thus, the colonization of human skin by the bacterium may also have some harmful effects depending on the external conditions such as a disturbed skin barrier.

To gain insights into the adaptation mechanisms of S. epidermidis, we performed in vivo transcriptional analysis (using qRT-PCR of a total of 22 genes) during human colonization and compared the expression patterns of healthy individuals with those of patients with atopic dermatitis.

While heterogeneity in expression levels was observed for some genes, in several cases the expression level was the same in the nose or skin or both niches in all individual donors (regardless of whether the skin was healthy or diseased [atopic]). For example, expression of the S. epidermidis regulator sarA was found similarly in the nose and on the skin of all individuals. Also, genes encoding colonization and immune evasion factors (sdrG and capC), as well as the sphingomyelinase encoding gene sph, were expressed. Despite some heterogeneity between individuals, a site-specific expression profile for the putative chitinase-encoding SE0760 was identified in healthy individuals. Interestingly, in vivo expression of SE0760 was also significantly increased on non-lesional atopic skin, whereas expression on lesional skin reached maximal expression in vitro in only 5 patients (out of 11).

In summary, our analysis identifies site-specific gene expression patterns of S. epidermidis during colonization. In addition, the observed expression signature was significantly different from growth in vitro. Interestingly, gene expression on healthy skin is very similar to the expression pattern on atopic skin.

The differential gene expression patterns related to defined body sites provide important novel insights into the tremendous flexibility S. epidermidis employs to cope with varying environmental conditions. These insights could provide crucial information necessary to define novel therapeutic approaches to maintain human skin homeostasis.

P152 (OP01/03) | In cutaneous leishmaniasis, IL-1 release, and protective immunity depend on receptor-interacting serine/threonine-protein kinase (RIPK) 1/3 and Mixed Lineage Kinase Domain Like Pseudokinase (MLKL)-dependent cell death of DC

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Infected, skin-derived dendritic cells (DC) are critical for the development of protection against L. major infection via induction of IFNγ-producing Th1/Tc1 cells. The role of DC cell death in this process remains unclear, even though it is well established that MyD88-dependent signalling in and release of IL-1α/β from infected DC is very important for protective immunity. Since it is well known that IL-1 production/release depends on a loss of cell integrity, we now assessed the role of various cell death forms (necroptosis, pyroptosis, apoptosis) in cutaneous leishmaniasis. To this aim, using GM-CSF and IL-4, bone marrow-derived DC were generated from wild type C57BL/6 mice or mice genetically devoid of MLKL, RIPK3 or inactive RIPK1. DC were infected with L. major amastigotes ($1 \times 10E6$ cells/ml, MOI = 5) isolated from BALB/c ear lesions with or without stimulation with the following cell death inhibitors/inducers: Emricasan (inhibits Caspase-8 resulting in loss of necrosome inhibition thus increasing necroptosis), Birinapant (restores sensitivity to cell death stimuli by inhibiting cIAP1/2), and Necrostatin-1 (blocks necroptosis by inhibition of RIPK1). In necroptosis, RIPK1 and RIPK3 are responsible for necrosome formation due to auto- and transphosphorylation in absence of active caspase-8. The phosphorylation of MLKL by the necrosome then leads to plasma membrane pore formation resulting in cell death and cytokine release. When assessing parasite uptake and cytokine release after 18 hrs, similar infection rates of DC independent of the stimulation were observed. In line with previous studies, we found significant release of IL-1α, IL-1β, IL-12p40 and TNF-α from DC upon infection ($p \leq 0.05$). While treatment of infected samples with Emricasan had little effect on IL-12p40 release, TNF-α release was increased 1.7-fold and IL-1β release 5-fold ($p \leq 0.0001$). Interestingly, however, IL-1α production was increased 13.6-fold ($p \leq 0.0001$). The Emricasan effect was strongly attenuated by adding necrostatin-1 (reduction to 26% [IL-1α], 29% [IL-1β], and 65% [TNF-α]) compared to Emricasan alone, while the addition of Birinapant massively amplified the Emricasan effect (2.9- fold [IL-1α], 3.7-fold [IL-1β], and 3.1-fold [TNF-α]). Next, cell viability was studied using flow cytometry and propidium iodide/Annexin V (PI/AnV) staining as well as transmission electron microscopy. Infection of DC led to a reduction of PIneg/AnVneg cells (50% compared to 63% in uninfected samples, $p = 0.0001$) and an increase of PI+/AnV+ CD11c+/MHCII+ cells. This effect was strengthened upon stimulation with Emricasan. Interestingly, the effect of Emricasan on IL-1α/β release and proportion of PIneg/AnVneg CD11c+/MHCII+ cells

was stronger in infected compared to uninfected cells. A distinction between different cell death pathways based on the distribution of PI+/AnVneg or PIneg/AnV+ cells has proven less useful; recent studies showed that not only in early apoptosis phosphatidylserine (binding AnV) is flipped on the outside of the plasma membrane, but also in early necroptosis. Nevertheless, infection led to increase of the portions PI+/AnVneg and PIneg/AnV + CD11c+/MHCII+ cells. Treatment with Emricasan did not have a clear effect on the frequency of PIsingle+ CD11c+/MHCII+ cells, but enhanced the numbers of AnVsingle+ cells, more so in infected samples (2.8-fold) than in uninfected ones (1.8-fold). Morphologically, using transmission electron microscopy, we observed that cell death alterations were already visible starting after 6 hours with swelling of L. major infected DC, continuing in infected DC with first losses of cell membrane integrity after 12 hours, low cytoplasmic density and loss of chromatin culminating in a peak after 18 hours with more frequent cellular collapse. These observations were confirmed using DC from MLKLKO/KO, RIPK3KO/KO and RIP1D138N/D138N (inactive mutation) mice. Here, in all genetic models, IL-1α/β release from DC after infection with L. major was reduced compared to wild type DC, while IL-12p40 and TNF-α release was not affected. In addition, in RIPK3- and RIPK1-deficient cells, the effect of Emricasan was attenuated compared to wild type mice. In MLKLKO/KO cells the enhancement of IL-1α and IL-1β release upon Emricasan treatment was also detectable, but a difference between cytokine release from uninfected and infected DC (like with inactive RIPK1/3) was not detectable. In summary, we establish that the release of IL-1α and IL-1β from L. major infected DC is dependent on RIPK1 and RIPK3 followed by necrosome formation and phosphorylation of MLKL leading to plasma membrane pore formation, which results in necroptosis-like cell death. Amplification of IL-1α/β by blocking caspase-8 and consequently a reduction of the inactivating cleavage of RIPK1 and RIPK3 upon Emricasan treatment underlines this conclusion. In contrast, IL-12p40 and TNF-α release is regulated independently of this cell death pathway. All in all, our data shows that myeloid cell death, most likely DC necroptosis, is critical for the generation of protective immunity against L. major. Improved understanding of the mechanism behind IL-1 cytokine release and the underlying cell death mechanism involved in the immune response against L. major (and possible active interferences of the parasite with these processes) will help us understand the resulting inflammation and allow targeting this important human pathogen.

P153 | Reliable and rapid identification of terbinafine resistance in fungal skin infections

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Background: Fungal infections are the most frequent dermatological disease. The gold standard antifungal treatment for dermatophytosis is the squalene epoxidase (SQLE) inhibitor terbinafine. Clinically relevant dermatophytes no longer responding to terbinafine are an emerging global threat. Here, we determine the proportion of fungal skin infections resistant to treatment, analyze the molecular mechanisms of terbinafine resistance, and validate a method for its rapid and reliable identification.

Methods: We screened 5634 consecutively isolated Trichophyton for antifungal drug resistance determined by hyphal growth on Sabouraud dextrose agar medium containing 02 µg/ml terbinafine. All Trichophyton isolates with preserved growth capacity in the presence of terbinafine underwent SQLE sequencing. Minimum inhibitory concentrations (MICs) were then determined by the broth microdilution method.

Findings: Over an 8-year period, the proportion of fungal skin infections resistant to terbinafine increased from 063% in 2013 to 13% in 2021. Altogether, our routinely performed phenotypic in vitro screening analysis identified 083% (n = 47/5634) of Trichophyton strains as having in vitro terbinafine resistance. Molecular screening detected an underlying mutation in the SQLE in all of these cases (L393F, L393S, F397L, F397I, F397V, Q408K, F415I, F415S, F415V, H440Y, or A398A399G400 deletion). All 47 strains featured significantly higher minimum inhibitory concentrations (MICs) than terbinafine-sensitive controls. The mutationrelated range of MICs varied between 0004 and 160 µg/ml, with MIC as low as 0015 µg/ml already conferring clinical resistance to terbinafine.

Interpretation: Based on our in vitro studies and clinical data, we propose MIC of 0015 µg/ml as a minimum breakpoint for predicting clinically relevant terbinafine treatment failure. We further propose growth on Sabouraud dextrose agar medium containing 02 µg/ml terbinafine and SQLE sequencing as fungal sporulationindependent methods for rapid and reliable detection of terbinafine resistance and optimized antifungal therapy.

P154 | Investigation of the humoral immune response after COVID-19 vaccination in psoriasis patients on biological therapy

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After SARS-CoV-2 outbreak in 2019, vaccines were developed to counteract the pandemic. A competent immune system is a prerequisite for the development of appropriate immune protection after vaccination. Modern therapy with biologics for psoriasis patients, however, aims to suppress certain immune responses in the skin and thus possibly interferes with the immune response to vaccination.
The purpose of this study is to investigate how the measurable immune response of psoriasis patients receiving therapy with biologics differs from healthy controls not receiving therapy with biologics.
We examined titers of IgG and IgA antibodies directed against SARS-CoV-2 spike protein by ELISA analysis in 55 psoriatic patients under biologics treatment and in 63 control persons. The blood sampling was performed in a fixed time frame (4 to 8 weeks) after complete vaccination against COVID19.
The psoriatic patient group comprised 28 persons under treatment with IL17 inhibitors, 24 persons treated with IL23 inhibitors and 3 persons treated with TNF alpha inhibitors. ELISA analysis revealed that anti-SARS-CoV-2 spike protein IgG antibody titers are lower in patients under biologic medication compared to the healthy control group without treatment. Similar results were gained for anti-SARSCoV- 2 spike protein IgA antibodies.
These results suggest a possibly impaired immune response in the investigated group of psoriasis patients, probably due to their biologic medication.

P155 | Identification of dermatophyte and non-dermatophyte agents in onychomycosis by PCR and DNA sequencing–A retrospective comparison of diagnostic tools

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Introduction: Rapid and reliable fungal identification is crucial to delineate infectious diseases, and to establish appropriate treatment for onychomycosis. Compared to conventional diagnostic methods, molecular techniques are faster and feature higher accuracy in fungal identification. However, in current clinical practice, molecular mycology is not widely available, and its practical applicability is still under discussion.
Methods: This study summarizes the results of 16,094 consecutive nail specimens with clinical suspicion of onychomycosis. We performed PCR/sequencing on all primary nail specimens for which conventional mycological diagnostics remained inconclusive.
Results: In specimens with a positive direct microscopy but negative or contaminated culture, molecular mycology proved superior and specified a fungal agent in 65% (587/898). In 75% (443/587), the identified pathogen was a dermatophyte. Positive cultures for dermatophytes, yeasts and non-dermatophyte molds (NDMs) were concordant with primary-specimen-DNA PCR/sequencing in 83% (10/12), 34% (22/65) and 45% (76/169), respectively. Among NDMs, agreement was high for Fusarium spp. (32/40; 80%), but low for Penicillium spp. (5/25; 20%) and Alternaria spp. (1/20; 5%).
Conclusion: This study underlines the improvement in diagnostic yield by fungal primary-specimen-DNA PCR/sequencing in the event of a negative or contaminated culture, as well as its significance for the diagnosis of dermatophyte and nondermatophyte onychomycosis. Molecular mycology methods like PCR and DNA sequencing should complement conventional diagnostics in cases of equivocal findings, suspected NDM onychomycosis or treatment-resistant nail pathologies.

P156 | Testing drug sensitivity of currently circulating monkeypox viruses after infecting dermal and epidermal cells

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On September 9th 2022, the ongoing global monkeypox outbreak has been classed as 'Public Health Emergency of International Concern' by the WHO. According to the CDC 56,609 cases in 103 countries and 18 deaths were recorded. About 9%-13% of these patients require hospital treatment.
Tecovirimat (ST-246), brencidofovir (CMX001), and cidofovir are considered for antiviral monkeypox treatment based on their efficacy in preclinical models. The symptoms of the current monkeypox outbreak differ from the previous ones e.g. shorter incubation periods, milder rashes, different rash morphology, fewer manifestations affecting face, trunk, and extremities, and a higher frequency

of anogenital lesions. It is unknown whether the underlying changes may affect virus sensitivity to antiviral drugs.

We tested monkeypox virus isolates derived from 12 patients during the current outbreak for sensitivity to cidofovir, brincidofovir, and tecovirimat in primary cultures of pathologically relevant cell types (normal human fibroblasts (NHF), normal human keratinocytes (NHK)) and commonly used cell lines.

All isolates replicated in both NHF and NHK indicated by orthopoxvirus immunofluorescence staining and the detection of virus DNA in cell supernatants 72h post infection. Cell specific differences concerning cytopathogenic effects could be observed.

Tecovirimat, cidofovir, and brincidofovir inhibited monkeypox virus infection in a dosedependent manner. The drug concentrations that inhibited virus infection by 50% (IC50) ranged from 4 to 20nM for tecovirimat, from 5 to 32µM for cidofovir, and from 9 to 152nM for brincidofovir. Notably, IC50 values determined in continuous cell lines significantly differed from those obtained in primary cultures, in particular for cidofovir and brincidofovir, stressing the importance of using physiologically relevant models.

The tecovirimat, cidofovir, and brincidofovir IC50s are at the lower end of values reported for previous monkeypox virus strains and within the range of therapeutic plasma concentrations. Notably, tecovirimat displayed the highest difference between therapeutic plasma levels and IC50s (200 to 1,000-fold) followed by brincidofovir (3.9 to 67-fold) and cidofovir (2.5 to 16-fold).

Hence, our data show that the currently circulating monkeypox viruses remain sensitivity to the currently available antiviral drugs.

P157 | Earliest possible prediction of COVID-19 severity correlated with potent Th1 immune response in asymptomatic SARS-CoV-2 infection

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Background and objectives: COVID-19 patients present a versatile range of severity from asymptomatic to debilitating symptoms and critical conditions requiring hospitalization. It is still not understood why some patients have a severe or highly symptomatic COVID-19 disease course while others remain asymptomatic. To understand the viral and immune correlates of severity we investigated multiple dimensions of the immune response, including single cell proteomics, in asymptomatic versus symptomatic versus hospitalized patients early after a SARSCoV- 2 positive test.

Materials and Methods: Patients (*N* = 174) were recruited early (at the day of PCR testing or within seven days post symptom onset) after SARS-CoV-2 infection (wildtype and alpha variants) and four samples were taken within the first month. Serum cytokine levels by ultra-sensitive ELISA (Simoa, Quanterix) and SARS-CoV-2 viral load (RNA and Nucleocapsid-antigen) were measured. PBMCs were analyzed by singe-cell intra-cellular-staining (ICS) cytometry and single-cell CyTOF, before and after stimulation with Spike and Nucleocapsid peptide pools. Bioinformatics analysis (PCA, UMAP and ML) was performed to cluster the patients' groups and identify the cellular immune correlates.

Results: A cytokine combination, based on the ratio of inflammatory cytokines to type-I-interferons, was identified as a highly accurate (>95%) early predictor of both the likelihood for hospitalization and symptoms´ severity, already at the day of PCR testing. Hospitalized patients have significantly higher levels and longer duration of inflammatory cytokines.

Moreover, asymptomatic patients present with significantly lower viral loads and cytokines, in correlation with higher frequencies and counts of SARS-CoV-2 specific activated CD4 and CD8 T-cells. In particular, asymptomatics show a significantly higher frequency of Th1 related cytokine expression, and of note a higher level of polyfunctional CD4 and CD8 T-cells expressing multiple cytokines. Interestingly, asymptomatic status is more significantly associated with a potent response against Nucleocapsid-antigen, rather than against Spike-antigen.

Conversely, anti-SARS-CoV-2 antibody levels (IgG and IgA) are higher in highly symptomatic patients, in correlation with higher counts of Th2-like T-cells.

Furthermore, inflammatory cytokine (e.g., Interleukin-6) levels best correlate with a higher count of classical monocytes, rather than counts of activated, or cytokine expressing, CD4 and/or CD8 T-cells specific for SARS-CoV-2. Interestingly, IL-10 levels, early after infection, do not correlate with Treg counts.

Conclusions: COVID-19 hospitalization and symptoms´ severity can be accurately predicted already within one week of symptoms onset and at the time of PCR-testing. This early predictor, using cytokines levels that are feasibly measurable at pointof- test setting, can guide personalized medicine with anti-viral or cytokine-inhibitor therapy. Furthermore, extensive single cell analysis allowed us to identify the immune correlates behind this predictor. Asymptomatic disease course is characterized by a potent anti-Nucleocapsid CD4 and CD8 Th1 response, in association with lower viral loads and lower inflammatory cytokine levels. Conversely, more severe patients have strong antibody and Th2 response, associated with higher viral loads and levels of inflammatory cytokines. These results have important clinical relevance for the development of both personalized therapy and vaccines aimed at reducing severity, by indicating the correlates of asymptomatic disease course.

Pharmacology

P158 | Identification of a Zingiber officinale root extract and CBD as potential ingredients with anti-inflammatory and antioxidative activity for use in cosmetic products applying a dedicated screening platform

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Plants are an attractive source of active ingredients for topical products, fulfilling consumer demands for natural active ingredients and excipients for this product class. Not only do plant-derived extracts contain numerous functional bioactive compounds, plants are also a sustainable source for raw materials.

Due to the high number of available plants/drugs in combination with various possible extraction methods there exists an even higher number of potential extracts for application as active ingredients. Therefore, a screening algorithm is needed which allows to discriminate between these extracts. Preferably, anti-inflammatory and antioxidative activity are tested in parallel and results are evaluated in a multivariate way.

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, HEK293t, RAW 264.1 macrophages). Antioxidative effects (8-isoprostane formation, ROS formation, NO production) and anti-inflammatory activity (release of IL-6, IL-8, and PGE2, NF-κB transcriptional activity) were assessed at three non-toxic concentrations. A principal component analysis was applied for overall evaluation. Based on the evaluation, CBD and a hypercritical CO2 extract obtained from Zingiber officinale root were selected for confirmation of the screening results (5 concentrations, $n = 3$). A high reproducibility of the screening results was shown.

Application of a screening algorithm for anti-inflammatory and antioxidative activity to different plant extracts followed by confirmation of the results lead to the identification of CBD and a hypercritical CO2 extract obtained from Zingiber officinale root as potential active ingredients for topical products targeting inflammatory skin diseases.

Photobiology

P159 | Interactions of different wavelengths present in sunlight: Impact on apoptosis, DNA-damage and skin carcinogenesis

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Our skin as the external barrier of our body is permanently exposed to physical and chemical stress factors and a particular challenge is the ultraviolet (UV) radiation. UVC radiation thereby is absorbed by the atmosphere, while UVA and UVB radiation can penetrate the skin to different depths. In principal, UV radiation results in the generation of reactive oxygen species, DNA-damage and apoptosis. It is now well established that not only UVB and UVA, but also visible light, and infrared A (IRA) radiation contribute to skin aging. Historically, the vast majority of studies has exclusively used irradiation protocols, in which one specific wavelength region (e.g. only UVA or only UVB) was used. However, as our skin is simultaneously exposed to the complete spectrum of solar light, such studies might have missed interactions between different wavelengths. Thus, the aim of our study was to compare single irradiation wavelength regions with simultaneous ones and a sequential exposure as well. Interestingly, our results indicate that a simultaneous exposure with UVA (at a dose not causing skin damage) and UVB (at a skin damaging dose) radiation dampens the UVB induced apoptosis, independently of visible light and infrared light, in vitro. In addition, this decreased elimination of UVB damaged cells was not detectable after a sequential irradiation with UVA and a subsequent exposure with UVB radiation and the other way around. Moreover, we found a correlation between increased heme oxygenase-1 expression and a decreased amount of gamma H2AX positive cells, which is an established marker for DNA-double strand breaks. Our results so far indicate that under simultaneous exposure conditions, UVA radiation dampens UVB-induced DNA damage and associated keratinocyte apoptosis. Given that an elevation of keratinocyte apoptosis is known to protect against carcinogenesis, we wondered whether a simultaneous exposure with UVA and UVB radiation increases skin carcinogenesis. Therefore, we performed a photocarcinogenesis study in SKH1 hairless mice simultaneously exposed to UVA and UVB radiation using a UVA dose that is not carcinogenic. In point of fact, the results showed that simultaneously exposed mice developed more skin tumors than animals only exposed to UVB radiation alone. Thus, these data so far emphasize the importance of apoptosis in the elimination of UVB-damaged cells and support the urgent need for integrating UVA filters in sunscreen, and thus are of direct clinical and regulatory relevance.

P160 | One filter many possibilities – New insights on protective and therapeutic applications of HelioVital Filter Foil

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From the effective solar UV radiation reaching the Earth's surface, UVA, (320 nm – 400 nm) is the major component with 95% prevalence. This makes UVA a leading player in photo-induced skin damage such as accelerated photoaging and skin cancer. The main path, where UVA exerts its negative effects on the skin is the induction of reactive oxygen species (ROS), which can cause oxidative damage to

DNA like base modifications (8-oxoguanosine, 8-HdG), DNA double-strand breaks, and lipid and protein oxidation.

However, UV radiation can be more than just harmful to human health. Rather, it can bring therapeutic benefits, with a continuously increasing spectrum of medical UV-applications in the sphere of healthcare disinfection, light- and heliotherapy. The spectrum of diseases that can be treated with UV-containing therapies is also broad and ranges from seasonal diseases, depression and burn-out to psoriasis, neurodermatitis and osteoporosis. However, as mentioned above, one major disadvantage of many forms of irradiation therapy is its mutagenic effect in cells. This damaging effect of UV irradiation can have delayed long-term consequences, manifesting years after treatment. Therefore, new UV protective strategies need to be tested for their efficiency to shield against UV induced damage without reducing its therapeutic potential.

Amongst the fields that can benefit from improved UVA protection strategies is the one of Photodynamic Therapy (PDT). PDT is a treatment usually prescribed in cases of actinic keratosis (AK), Bowen disease, and certain types of basal cell carcinoma. In recent years, an alternative to the classical in-hospital PDT has been gaining popularity, namely Daylight-Mediated Photodynamic Therapy (daylight PDT). Daylight PDT has been successfully implemented in the treatment of AK independent of weather conditions. Even with irradiation reduction by 83%, a successful treatment could be achieved as long as a minimal irradiation dose of 3.5-8J/cm2 was reached. One important side effect from the therapy is the pain reported by the patients during treatment. The pain score varies greatly depending on weather conditions, with the lowest pain-levels recorded on rainy or cloudy days. However, 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 show that during treatments with UVA HelioVital Filter Foil has protective effects against changes in the expression of MMP1, MMP2, and MMP15. These three matrix metalloproteinases are crucial for tumor progression and metastasis and MMP1 and MMP3 are involved in the process of skin aging. In addition, the use of the HelioVital filter film during irradiation mitigated UVA-induced DNA damage in primary human fibroblasts. These results could pave the way for clinical studies with HelioVital filter foil shielding during phototherapy and other forms of irradiation therapy, thereby increasing the treatment safety.

The HelioVital filter foil absorbs approximately 60% of solar UV irradiation, a range mimicking cloudy or partially cloudy weather conditions. This feature, together with the filter's observed protective effects against DNA-damage and increased MMP expression, could be used during daylight PDT in the form of special greenhousegazebos covered with HelioVital Filter Foil. This would give the patients the benefit of reduced pain load even on sunny days without the variable of cloud formation.

P161 | UVA and pyruvate – An unexpected combination

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One of the major inducers of photoaging and skin cancer is the exposure to UV radiation and especially UVA (320–400 nm). UVA irradiation is prone to inducing reactive oxygen species (ROS), which can induce DNA mutations, such as 8- oxoguanosine. Furthermore, UVA triggers the expression of photoaging associated matrix metalloproteinases (MMP) especially MMP1 and MMP3. In addition to this, it was recently shown that UVA and UVA-induced ROS also can increase glucose metabolism of melanoma cells, leading to increased invasiveness in vitro.

However, the influence of UVA on glucose metabolism non-malignant cells of murine and human skin is not investigated in detail. In the current work we investigated the UVA-induced changes in glucose metabolism and the functional relevance of these changes in normal non-malignant cells of the skin, namely primary human fibroblasts. These cells showed an UVA-induced enhanced glucose consumption and lactate production, as well as changes in pyruvate metabolism. Notably, pyruvate has been proposed to have antioxidant properties, therefore, we also tested the functional relevance of pyruvate as protector against UVA-generated ROS. In the current work, we show that pyruvate treated with H2O2 or UVA is non-enzymatically transformed to acetate. We also demonstrate that in fibroblasts pyruvate indeed has antioxidant properties as enhanced levels of pyruvate reduced UVA-induced ROS and partially protected the cells from the DNA mutation 8-oxoguanosine. Furthermore, UVA in combination with pyruvate downregulated UVA-induced gene expression of MMP1 and MMP3.

These findings indicate that UVA-induced metabolic remodeling, especially in the case of glucose consumption and lactate production, is a general phenomenon in normal skin and is not limited to tumor cells. Furthermore, we show that pyruvate, a product of glycolysis, can effectively reduce UVA-derived ROS production and ameliorates the effects of downregulates the expression of UVA-induced, photoaging associated matrix metalloproteinases.

P162 | Efficacy of curcumin-visible light irradiation is wavelength and intensity specific

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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. metabolic activity, cell viability, proliferation and apoptosis.

Human keratinocyte cell lines (HaCaT and A431) were treated with 0.2 to 0.6µg/ml curcumin for 1h prior to irradiation with 3-35J/cm2 at 420nm, or were irradiated with a commonly used PDT regime, subsequently irradiating with 420nm, 585nm and 635nm, using a daylight PDT device (MultiLite®, GME). After 24hrs proliferation, cytotoxicity and apoptosis were monitored.

None of the applied wavelengths influenced the cell integrity. Monitoring the influence on proliferation and metabolic activity showed curcumin concentration and wavelength specific effects. The most prominent curcumin concentration dependent anti-proliferative impact was observed after irradiation with 420nm. Combination of the different wavelengths showed a less prominent anti-proliferative effect. Monitoring caspase3/7 activity as well as DNA fragmentation also showed that irradiation with 3-15J/cm2 with 420nm induced the most pronounced pro-apoptotic effect.

These results indicate that curcumin treatment and irradiation with 420nm as single wavelength treatment is the most effective treatment regiment. Further investigations of wavelength specific effects related to the application of curcumin during a potential photodynamic therapy are advisable.

P163 | A combination of photodynamic therapy and cold atmospheric plasma shows synergistic treatment effects on squamous cell carcinoma cells

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Actinic keratosis (AK) is defined as reddish, sometimes skin-coloured, roughness in sun-damaged skin, which is considered a precursor to squamous cell carcinoma (SCC). Photodynamic therapy (PDT) with the photosensitizer 5-aminolevulinic acid (ALA) in combination with red light is an established therapy for the treatment of AK, which is limited by the depth of penetration of the photosensitizer into the skin. The effectiveness of PDT can be increased by enhancing the skin penetration depth of ALA. Cold atmospheric plasma (CAP) is a partially ionized gas with penetrationenhancing and anti-cancer properties. For this reason, we speculated about a synergistic effect of PDT and CAP as combination therapy.

In the present study, we investigated the ALA penetration ability of the combination therapy ex vivo on human skin excidates and the anti-cancer properties in vitro on human SCC cells.

Our results showed an increased uptake and penetration of ALA into the skin after the combination of PDT and CAP. Viability, migration, invasion and apoptosis studies on SCC cells further supported an improved anti-cancer behaviour after PDT and CAP combination treatment compared to conventional PDT.

In summary, our ex vivo and in vitro approaches suggest that PDT/CAP combination therapy could be a highly effective new modality for AK treatment and provides the basis for clinical trials in patients with AK.

Pruritus

P164 | Medical care of patients with chronic pruritus: Possibilities and limits of medical care process optimization

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The medical care of patients with chronic pruritus is complex as it comprises care on several levels including diagnostics, therapy, adverse event management, interdisciplinary collaboration and systems for questionnaire assessment and easy accessibility for treated patients. As this requires an excellent organization, controlling costs without reducing the quality of medical care is the current and future challenge. The Lean Management approach has established itself in business administration as a method for process cost control with constant quality control. The focus is on detecting process costs that do not contribute to value creation and are not necessary for performance. This system offers an approach to check medical care processes.

Using interviews and process description, the medical care processes in the outpatient clinic of the Center for Chronic Pruritus were analyzed.

In particular, personnel costs, less material or equipment costs were identified to be necessary for medical care in the outpatient clinic. A reduction of the waiting and throughput times of the patients and optimal patient timing supports the targeted use of staff. Patient self-history reporting via tablets and transmitting this into the physician documents in the electronic health record further reduces the administrative load on the team. In addition, establishment of telemedicine services with patients reduces the time load at the side of patients to visit the center. In order to objectify the effects of the developed measures, a one-year patient survey was launched in

August 2022. Inclusion criteria are all patients who are treated for clinical treatment in the KCP outpatient clinic.
With the help of the structured Lean Management approach, systematic recommendations for improved medical care which improves in addition economic efficience and maintains patient satisfaction through continuous process optimization is possible.

P165 | Interaction of Substance P and IL4/13 pathway – another link in the neuroimmune crosstalk

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Typical clinical signs of atopic dermatitis (AD) are eczema and pruritus. There is a common consensus that Th2 cells and ILC2 characterized by the production of cytokines such as IL4, IL13, IL5 and IL31 play a crucial role in pathogenesis of AD. In particular IL4/13 appear to be of major importance as targeting these cytokines by an antibody directed against the IL4Ralpha chain which is chaired by both the IL4R and the IL13R turned out to be highly effective in the treatment of patients with AD. However, there is accumulating evidence that targeting the neuropeptide substance P (SP) is an effective treatment of pruritus, too. In addition, IL4 was found to directly interact with the SP pathway by upregulation of the neurokinin 1 receptor (NK1R) on immune cells. Based on these observations we hypothesize an interaction between both the IL4/13 and the SP pathway in mediating itch as well as inflammation in patients with AD. Therefore, we modified the gene expression of IL4/13 by plasmid-mediated overexpression and analyzed the effect on SP gene expression. Furthermore, we investigated the effect of blocking NK1R by treatment with the antagonists casopitant and aprepitant on gene expression of inflammation markers such as IL1beta, but also IL4 and IL13. We investigated the influence of IL4Ralpha on the expression of SP, IL4, and IL13 in both human primary keratinocytes and human mast cells by treatment with the monoclonal antibody dupilumab (anti-IL4Ralpha). In addition, we analyzed the effect of anti-IL4Ralpha treatment on NK1R downstream signaling via activation of ERK1/2 by western blot. Our results show that overexpression of both IL4 and IL13 leads to increased gene expression of SP, demonstrating a direct link. Blocking of NK1R showed an increase in gene expression of IL1beta, IL8, SP, IL4, and IL13 in a dose-dependent manner. Blocking of IL4Ralpha showed only a small effect on IL4/13 expression in mast cells, but a marked increase in gene expression of SP. In keratinocytes, targeting of IL4alpha showed an inhibitory effect on gene expression of IL4. Furthermore, blocking of IL-4Ralpha induced a reduced phosphorylation and thus activation of ERK1/2. In sum, our results strongly suggest an interaction of the IL4/IL13 signaling cascade and the SP pathway that may affect both, inflammation and pruritus in AD patients.

P166 | Upregulation of IL13 and IL13 receptors in atopic prurigo

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Aim of the study was to investigate the expression of members of the interleukin 13 pathway in in atopic dermatitis (AD). IL13 is an emerging target in the treatment of AD. It exerts its effects by binding to the signaling receptor IL13Ra1, through the IL4Ra/IL13Ra1 receptor system Furthermore, IL13 can also bind to the decoy receptor IL13Ra2 which may attenuate the IL13-mediated responses. Knowledge on the role of IL13 and its receptors in itch signaling in AD including different clinical phenotypes is missing, but is important for a better understanding of itch response to an anti-IL13 therapy. For that, patients with either chronic AD (AD-C) or prurigo nodularis in AD (AD-PN) were recruited, as well as sex and age matched healthy controls (HC). Patients were given clinical questionnaires and punch biopsies on the arms were used to analyze the gene expression in the skin of IL13, IL4, IL31, IL4R, and IL13Rα1, IL13Rα2, and IL31R (qPCR). Expression on protein level of IL13Rα1 and IL13Rα2 was analyzed by immunofluorescence staining on cryosections of the biopsies. The results showed increased expression of IL4 and IL13 and their receptors IL4R and IL13Rα1 in AD-C and AD-PN compared to HC. Interestingly, the expression of IL13Ra2 was only increased in AD-C (vs. HC and ADPN). No differences in gene expression of IL31RA could be seen across the cohorts. Analysis of the expression of IL13Rα1 and IL13Rα2 at the protein level showed no significant difference between the groups. Both, AD-C and AD-PN, showed a positive correlation between the gene expression levels of IL13 and IL13Rα2. In sum, we mapped the expression of relevant markers for chronic pruritus in AD-C and AD-PN. The upregulation of IL4, IL13 and their receptors IL4R and IL13Ra1 could support the chronification in both of them. The lack of overexpression of the decoy receptor IL13Ra2 in AD-PN, which is upregulated in AD-C, may worsen the disease in AD-PN and lead to prolonged scratching behavior and multiple pruriginous lesions.

P167 | Bad vibrations? The prevalence of itch and erythema induced by whole body vibration training: A questionnaire-based study in users and therapists

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Background: Itch and erythema can be induced by whole body vibration (WBV) training, yet the underlying mechanisms for this phenomenon are poorly understood.

Objectives: To describe the prevalence of itch/erythema and their dependency on the technical settings (e.g. device, frequency, amplitude, intervals) in users of whole body vibration (WBV) devices.

Methods: Information on the occurrence of itch and/or erythema during WBV training were gathered from WBV users (musculoskeletal rehabilitation or fitness training) and therapists in four fitness studios in Germany and Switzerland using questionnaires especially designed for this study.

Results: In WBV-users, the prevalence of either symptom was 22% (15/67). More than half of the subjects experiencing itch reported concurrent erythema. Symptoms appeared in the majority of individuals already during the first session and the pattern remained consistent during consecutive sessions. No permanent discontinuation of WBVtreatment due to symptoms was reported. While symptoms were more prevalent for side-to-side alternating vibration devices with a pooled prevalence of 31.4%, no symptoms were reported for the tri-planar device ($p < 0.001$).

Conclusions: The results of our study indicate that itch and erythema are underreported, unintended side effects frequently experienced by WBV- users, both athletes and patients. These symptoms are common, unpleasant, but usually not limiting side effects of WBV exercise and depend on the device used. Moreover, we assume that the device type and technical settings are decisive for the development of itch and erythema. However, individual factors that have not been identified yet are likely to contribute.

P168 | Identification and characterization of lead compounds against chronic itch

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Itch can be divided into acute and chronic as well as histamine-dependent and - independent forms. Generally, chronic itch is closer related to histamine-independent forms. Importantly, 5-hydroxytryptamine (5-HT, Serotonin) has been identified as a potent inducer of serotonergic itch that plays an important role in acute and chronic itch signaling. Concomitantly, the serotonin receptor (HTR) and transient receptor potential channel (TRP) families were established as key mediators of chronic itch in mouse models of atopic dermatitis.

Epidermolysis bullosa (EB) designates a highly diverse group of inherited skin disorders, resulting from mutations in genes encoding structural proteins of the skin. Based on patients' feedback, itch (pruritus) is one of the most prominent symptoms across all EB subtypes. Since in EB patients, itch can either develop as a generalized phenomenon or predominantly occur in wounded and blistered sites, it usually affects both intact skin and healing wounds. Currently, the mechanisms for development of pruritus in EB are often unknown.

Due to its emerging role in the development of chronic itch, targeting HTR signaling could represent a promising opportunity to alleviate itch in EB. In this context, we tested several novel HTR-binding molecules for their potential to abrogate itch-related signaling. To interrogate the HTR-signaling cascade and activation/deactivation of downstream TRP channels, model cells were transiently transfected with HTR family members and the associated TRP channel. We identified compounds that selectively bound to HTR family members with IC50s in the low nanomolar range (cell membrane binding assay). Patch clamp whole-cell recordings and a cAMP Glo Assay were performed to further investigate the effect of novel compounds on the HTR-TRP-axis. Using the same techniques, the behavior of these compounds was contrasted to known agonists and antagonists of 5-HT receptors and TRP channels, e.g. LP-44 (agonist receptor), gallein (inhibitor of G-protein signaling), 2´,5´-dideoxyadenosine (inhibitor of AC), allylisothiocyanat (agonist channel), A-967079 (channel-blocker).

In summary, we identified and characterized novel HTR-binders that could represent promising candidates for modulating signaling cascades involved in the development of chronic itch, particularly in the context of EB.

Keywords: Chronic itch, pruritus, epidermolysis bullosa, serotonin signaling, agonists, antagonists, patch clamp, cAMP Glo Assay

Tumor Biology

P169 | Modified microbiota by skin disinfection through topical triple antibiotic treatment delays tumor growth and increases survival in a cutaneous T-cell lymphoma mouse model

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Introduction: Cutaneous T-cell lymphomas (CTCL) are a heterogeneous group of lymphoproliferative disorders of skin-homing mature T-cells causing chronic inflammation, with an impairment of immune environment leading to severe infections and/or sepsis due to

dysbiosis. Together, this promotes progression of disease, resulting in poor quality of life and high mortality. We were thus interested in microbiome characteristics of CTCL and if modulation of the skin microbiota could beneficially affect the course of CTCL.

Methodology: Here we established a CTCL murine model by intradermally grafting murine EL4 T-cell lymphoma cells in C57BL/6 mice and treated them with conventional therapeutics such as psoralen plus UVA (PUVA) or UVB in the presence of normal microbiota or diminished microbiota achieved by disinfection with a topical triple antibiotic cream, containing neomycin, bacitracin and polymyxin B sulfate (Neosporin).

Results: Our in vivo results indicated that skin disinfection significantly delayed tumor appearance and growth and prolonged survival of mice irrespective of allocation to therapeutic agents (PUVA, UVB or none). The effect of triple antibiotic cream on skin microbiota reduced Shannon diversity index and bacterial richness that correlated with diminished tumor growth. Moreover, it induced the growth of certain presumably beneficial staphylococcal species compared to vehicle treatment. Moreover, the effect of triple antibiotic cream on tumor growth was similar to the targeted therapy drugs, such as STAT3/5 blocker or multi-kinase inhibitor.

Conclusion: In summary, we conclude that modifying the microbiota of the skin by disinfection through topical triple antibiotic treatment delays tumor growth and increases survival in a murine CTCL model. This observation opens up the avenues for the investigation of new therapeutic approaches in CTCL focusing on modification of the microbiota.

P170 | In vitro transition of SCC to an MCC-like phenotype

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Merkel cell carcinoma (MCC) is an extremely aggressive neuroendocrine skin cancer with a poor prognosis. Approximately, 20% of MCC tumors are due to somatic mutations with typical UV signatures, which lead to inactivation of critical pathways including p53 and RB1. Although etiologic factors and pathways involved in the transformation are known, one of the remaining critical questions for MCC research is: What is the cell of origin of these tumors?

Some insight into potential MCC progenitor cells was obtained from analysing combined tumors. In this regard, our previous data on Squamous Cell Carcinoma (SCC)/MCC combined tumors demonstrate a high number of shared mutations in both compartments suggest that an epithelial cell can be origin of UV-associated MCCs. While the frequency of RB1 mutations in pure SCC is clearly lower than in virus-associated MCC, we observed in all our analysed SCC/MCC combined tumors in both parts RB1 aberrations. Hence, RB1 inactivation occurred before SCC-to- MCC transformation, and might be an essential step to promote SCC/MCC transition. Notably, a set of oncogenic drivers, including cMYC, constitutive active AKT, and BCL-2 has recently been demonstrated as contributing factors to the epithelial/neuroendocrine transition.

Consequently, we investigate whether the manipulation of the specified target molecules in primary SCC cell lines promote neuroendocrine transformation. We exhibited increased levels of MCC markers following shRNA-mediated RB1 knockdown in SCC cell lines. In addition, overexpression of oncogenic drivers individually achieved upregulation of key keratins characteristic for Merkel cells hinting to a possible involvement of this molecular set in SCC/MCC transition.

P171 (OP03/03) | Cell polarity signals inhibit field cancerization and SCC formation

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Many cancers including squamous cell carcinoma of skin (SCC) are preceded by premalignant lesions often converging to form areas of field cancerization. Moreover, oncogenic mutations are already present in sun-exposed but physiologically healthy skin. The barriers that prevent field cancerization and subsequent progression into invasive SCC are not known. The aPKCλ polarity kinase was previously shown to serve as a boundary for epithelial-mesenchymal transition and invasion in breast cancer. Using epidermal loss of p53 that in mice drives local SCC formation, we addressed the role of epidermal aPKCλ polarity in epidermal-dermal crosstalk, regulation of inflammation and SCC formation.

Epidermal deletion of both aPKCλ and p53 (eDKO) resulted in field cancerization and early SCC onset compared to p53 single knockouts. These eDKO mice showed early signs of chronic inflammation particularly dermal infiltration with macrophages, that was accompanied by epidermal activation of Stat3 signaling and increase in the hypoxia mediator Hif1α. Depletion of macrophages using liposomal clodronate significantly improved epidermal hyperthickening and hyperproliferation. Interestingly, epidermal deletion of Stat3 not only inhibited field cancerization and SCC formation but also macrophages recruitment and Hif1α activation, thus indicating that epidermal activation of Stat3 function upstream of Hif1α to stimulate macrophages recruitment in the edKO.

In human SCCs both high and low aPKC expression have a worse prognosis, but only low aPKC-SCCs show upregulation of Hif1α and Stat3. Together, our data identify aPKC polarity signaling as an important boundary for field cancerization and the formation of SCCs. Using eDKO mice as a model that recapitulates human skin carcinogenesis, we show that the macrophage inhibitor

Pexidartinib (PLX3397) is a promising candidate for treatment of field cancerization.

P172 | Sensitivity of cutaneous T-cell lymphoma cells to the Mcl-1 inhibitor S63845 correlates with the lack of Bcl-w expression

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Long-term, curative treatment of cutaneous T-cell lymphomas (CTCL) remains a major challenge. Therapy resistance is often based on apoptosis deficiency and may depend on antiapoptotic Bcl-2 proteins as Bcl-2, Bcl-xL, Bcl-w and Mcl-1. For their targeting, several antagonists have been generated, which mimic the Bcl-2 homology domain 3 (BH3 mimetics). As dysregulation and overexpression of Mcl-1 was reported in CTCL, the use of Mcl-1 inhibitors appears as an attractive strategy. Here, we investigated the effects of the selective Mcl-1 inhibitor S63845 in a series of four CTCL cell lines, in comparison to ABT-263 and ABT-737 (inhibitors of Bcl-2, Bcl-xL and Bcl-w). In two cell lines (HH, HuT-78), S63845 resulted in significant apoptosis induction, decrease of cell viability, loss of mitochondrial membrane potential and caspase activation, while two other cell lines (MyLa, SeAx) remained completely resistant. An inverse correlation was found, as S63845-resistant cells were highly sensitive for ABT-263/-737, and S63845-sensitive cells showed only moderate sensitivity for ABTs. Combinations of S63845 and ABT-263 partially yielded synergistic effects. As concerning Bcl-2 protein expression, weaker Mcl-1 expression was found in S63845-resistant MyLa and SeAx, while for Bcl-2 and BclxL, the lowest expression was found in the highly sensitive cell line HH. The most striking difference between S63845-resistant and sensitive cells was identified for Bcl-w, which was exclusively expressed in S63845-resistant cells. Thus, CTCL can be efficiently targeted by BH3 mimetics, only the right target needs to be preselected, and Bcl-w expression may serve as a suitable marker.

P173 | Intratumoral infiltration with neutrophils and NETs is associated with necrosis and size in human melanoma metastases

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Introduction: Neutrophil granulocytes are able to release their DNA equipped with lysosomal proteins into the extracellular space, a process called NETosis. These neutrophil extracellular traps (NETs) can kill pathogens, but may also act as pro- or antitumorigenic factors in cancer. In addition, murine studies indicate an impact of NETs on the response to immune checkpoint inhibitors in gastrointestinal cancers.

In human malignant melanoma neutrophil infiltration in primary tumors and metastases is associated with poor prognosis.

Although NETs have been documented in association with neutrophils in primary melanoma, NETs show anti-tumor effects in vitro. However, their occurrence in metastases and their local contribution to the immune response have remained unclear so far.

Therefore, the aim of this study was to further characterize the distribution of neutrophils and NETs within metastases of melanoma to better understand their relevance for the immune response to melanoma.

Methods: Paraffin embedded metastases (n = 81) of 61 patients were stained for neutrophils and NETs, targeted by CD15 and citrullinated histone 3 (H3Cit). Infiltration was assessed by immunofluorescence microscopy and size measured with ImageJ on H&E and/or IHC staining that was used for necrosis evaluation.

Results: Almost half of the metastases contained neutrophils (n = 40) and NETs could be seen in every third metastasis (n = 25).

We could find a significant association of neutrophil infiltration and necrosis with 83.3% of 36 necrotic metastases containing neutrophils (n = 30) and 66.6% showing NETs (n = 24). This association was particularly distinct for metastases with “medium” and “massive” neutrophil and NET infiltration.

The median cross-sectional area of metastases that contained neutrophils (148.7mm2) or NETs (151.0 mm^2) was greater than in those without neutrophils (N: 37.8 mm^2; NET: 58.04 mm^2). Between the metastases showing both, NETs and neutrophils, and those just being positive for neutrophils there was no significant difference in size. Interestingly, all metastases greater than 201.4 mm^2 showed neutrophil infiltration (n = 13).

Metastases of skin, lymph nodes, liver and lungs were analyzed. No significant difference was seen for infiltration depending on metastasis' site, except for a greater number of neutrophils in liver metastases compared to lung metastases. However, liver metastases showed a greater median size which might explain the observation.

In the overall survival, defined as time after removal of the metastases until death or censorship, no significant difference between metastases with or without neutrophils and NETs could be detected, possibly due to the limited number of observed patients. Further investigation is needed to elucidate whether NETs behave as proor antitumorigenic in patients with metastatic melanoma.

Discussion: Taken together, our study was able to provide first evidence of NETs in melanoma metastases. They are associated with neutrophil infiltration, necrosis and size of the metastasis whereas the site of metastasis seems to play a minor role. The strong association between necrosis and infiltrate may be due to necrosis attracting neutrophils and initiating NETosis. Taking into account the high proportion of neutrophil (49.3%) and NET (31.2%) positive metastases, the effects of neutrophils and NETs regarding the tumors, their microenvironment and response to therapies may be a profound subject for future studies.

L.W. and F.S. contributed equally to this work.

P174 | Combination of PARP inhibitors and MAPK inhibitors as an effective treatment strategy for malignant melanoma

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Melanoma patients with hyperactivation of the MAPK signaling pathway profit from MAPK inhibitors (MAPKi). However, the rapid onset of drug resistance limits the utility of this therapy. This study focuses on novel treatment strategies for both MAPKi sensitive and resistant melanoma patients.

We demonstrate that melanoma cells can effectively be targeted with PARP inhibitors (PARPi) and that MAPKi resistant cells show particularly high sensitivity. We found that MAPKi resistant melanoma cells have impaired initiation of the double-strand break repair by homologous recombination repair (HRR) and that this confers sensitivity towards PARPi treatment. In addition, we show that a combination of PARPi and MAPKi has a synergistic effect on the cell viability of melanoma cells. We discovered, that MAPKi induce a HRR deficient phenotype in melanoma cells and thereby cause synthetic lethality in combination with PARPi. We found that even melanoma cells that are resistant towards BRAF inhibitors and MEK inhibitors can be effectively treated with a combination of PARPi and ERK inhibitors or pan RAF inhibitors.

This research broadens the spectrum of PARPi therapy to melanoma patients, that do not harbor somatic or germline mutations in the HRR pathway by inducing a HRR deficient phenotype with MAPKi treatment.

P175 | Thromboembolism in patients treated with cancer immunotherapy

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Question: Immune-checkpoint inhibition has become a gold standard in the treatment of highrisk or metastatic malignant melanoma. Venous and arterial thromboembolic events (TEE) are known to have negative effects on overall survival of cancer patients. While an increased incidence of TEE has been observed in patients treated with immune checkpoint inhibition (ICI), results differ between different treatment regimes. Therefore, we aim at examining the incidence of TEE in patients treated with different ICI regimens and at identifying potential risk factors.

Methods: We conducted a prospective cohort study of 211 patients with stage III or IV Melanoma who received treatment with ICI between April 2018 and September 2022. Blood was drawn before the first therapy cycle and examined for various cell counts and serum markers. Khorana score was calculated using platelet count, leukocyte count, hemoglobin level and body mass index. TEE was defined as thrombosis, stroke, pulmonary embolism or transient ischemic attack.

Results: Out of 203 patients, 87 were stage IV and 116 were stage III at the beginning of therapy.

129 were treated with anti-PD-1 antibodies only. 68 patients started with combination therapy consisting of anti-PD-1 plus anti-CTLA-4 antibodies. 15 patients switched to combination therapy after progression while under monotherapy. Median age at the first cycle was 70 years and median follow up was 32 months. 3.1% of all patients under monotherapy experienced TEE. 12% of patients experienced any form of TEE when treated with combination therapy. In patients with stage IV melanoma who received only anti-PD-1 monotherapy, the TEE rate was significantly lower (5%, OR: 0.42).

Discussion: Patients treated with combination ICI therapy seem to be at significantly higher risk of TEE. When deciding for a therapy in melanoma patients, TEE risk factors should be considered.

P176 | Neutrophils - The cellular phoenix in melanoma

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The cure of cancer patients is still hampered by the resistance of tumor cells against drugs and the immune system. Tumor-associated neutrophil granulocytes (TAN) form an important component of the immunological infiltrate of solid tumors, such as melanoma. Nevertheless, the contribution of tumor-associated neutrophils to cancer progression and tumor immunity has been a matter of debate for decades. Due to their ability to actively migrate, TAN are recruited to the vicinity and center of melanomas. In doing so, these cells show enormous functional heterogeneity. On the one hand, TAN may contribute to enhanced tumor growth, more aggressive metastasis, and development of resistance to tumor therapies through a variety of mechanisms. On the other hand, TAN have been described to exert marked tumor control. These phenomena point to a new understanding of cancer as a highly plastic system, in which cell populations switch into each other depending on the current therapeutic, microenvironmental, and immunological context. Due to the lack of suitable preclinical models to date, the underlying mechanisms of this differential functionality (pro- vs. anti-tumor) are not well elucidated. Thus, it has also not been possible to selectively target TAN to improve response to targeted tumor therapy. As part of the Clinical Research Unit 337 "PhenoTIme" (https://www.uni-due.de/phenotime/) the generation of novel innovative mouse models with transplanted or spontaneously developing melanomas

clearly indicate a reciprocal phenotypic impact between cancer cells and neutrophils in the context of PD-1 targeted immunotherapy in melanoma. Data of tumor-induced proteomic changes in neutrophils in general, but also the impact of the tumor cell phenotype on neutrophil functions open the discussion on the use of neutrophil diversification in cancer therapy.

P177 | Communication between melanoma cells and astrocytes promotes behavioural changes that may influence the brain microenvironment during metastatic development

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Malignant melanoma is the most deadly of all skin cancers. The main cause of mortality in melanoma is the high rate of metastasis to distant organs, including the brain. Melanoma brain metastases (MBM) are incurable and are associated with a poor survival rate. The incidence of brain metastases has increased in recent years due to improved diagnostic imaging that allows the diagnosis of metastases that are not clinically manifest.

Tumor cells have been shown to trigger the reprogramming of cells in the brain microenvironment to form a hospitable metastatic niche through paracrine and systemic secretion of soluble factors, which affects the behaviour of astrocytes and microglia. Astrocytes are glial cells that perform many functions in maintaining brain homeostasis and play an important role in tissue repair processes. In response to brain damage, activated astrocytes regulate the production of proinflammatory cytokines and chemokines and impair the permeability of the blood-brain barrier, which allows the invasion of leukocytes and cancer cells. Astrocytes are thought to promote the growth of brain metastatic tumor cells through proinflammatory signalling, but the underlying mechanisms need further investigation.

Melanoma cell lines derived from various metastatic tissues were established to obtain brain-specific melanoma cells that may have a homing signature. Melanoma cells that metastasise to the brain have been shown to have important genetic alterations for migration and differentiation. These multiple genetic changes, advantageous during the metastatic process, could determine the behaviour of the cell in the brain compared to extracranial tumor cells. By co-culturing these melanoma cells and astrocytes, we observed a deregulation of inflammatory cytokines (IL-1β, IL-17, TNFα) in astrocytes and a favoured migration and invasion process by melanoma cells giving us hints about the interplay of both cell types.

In conclusion, the use of our established melanoma cell lines derived from brain metastases allows the study of the interplay between melanoma and astrocytes, which may promote the remodelling of the brain microenvironment that favours the development of melanoma brain metastases.

P178 | IFN-α therapy of peritoneal carcinosis induces senescence in metastatic melanoma cells

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Immune responses can control the development of tumors by eliminating tumor cells. Sufficient immigration of immune cells into the tumor microenvironment and tumor cell killing are required to control tumor growth. In addition to tumor cell killing, cellular immune responses may control tumors via soluble factors namely IFN-γ and TNF. Tumor control by IFN-γ and TNF induces the features of senescence in tumor cells and has been coined as cytokine-induced senescence (CIS). In vitro, we found that not only the combined action of IFN-γ, that signals through STAT1, and TNF, but also the combined action of IFN-α, that signals through STAT1 and STAT3, and TNF can induce senescence in melanoma cells. We therefore treated two patients who developed ascites from metastatic melanoma when all therapies had failed, by intraperitoneal IFN-α. As an ascites contains TNF, melanoma cells were exposed to IFN-α and TNF in vivo. Subsequently melanoma cells obtained from ascites puncture, were analyzed for markers of cell death and for markers of senescence. IFN-α therapy was associated with a strong reduction of the ascites and the melanoma cells. Following IFN-α therapy, melanoma cells expressed either the cell cycle inhibitors p16Ink4a or nuclear phospho-p21. Moreover, they remained growth arrested when cultured in vitro, while melanoma cells that were isolated prior to therapy proliferated strongly. To experimentally address whether CIS really can control ascites, either IFN-α- and TNF-resistant WM115 tumor cells or IFN-α- and TNF-susceptible A204 tumor cells were intraperitoneally injected into mice. IFN-α therapy of the respective mice only controlled the growth of intraperitoneal A204 cells whereas intraperitoneal WM115 cells grew exponentially also in the presence of IFN-α and TNF. Taken together, the data show that the combined action of IFN and TNF contributes to the control of metastatic cancer cells, but only if the IFN-induced senescence signaling pathway is present.

P179 | Circulating tumor DNA (ctDNA) as clinical marker in advanced melanoma patients

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Introduction: Circulating tumor DNA (ctDNA) or liquid biopsy is a promising blood-based tool for monitoring disease status of patients with advanced tumors. Especially in melanoma the hotspot mutations in the tyrosine kinase BRAF are an excellent tumor-specific surrogate marker to monitor the disease by measuring the circulating tumor DNA burden over the course of treatment. Therefore, we aimed to standardize the detection of BRAF hotspot mutations by digital droplet PCR (ddPCR). Our assay focuses on the most common BRAF mutations V600E (c.1799T>A) and V600K (c.1798_1799GT>AA) in melanoma.

Methods: To determine the specificity and sensitivity of the assay, serial dilutions of cell lines SK-Mel-28 (homozygous for BRAF V600E), Ma-Mel-69 (heterozygous for BRAF V600K) and MeWo (BRAF V600 wildtype) were prepared by creating triplicates of five dilutions of mutant DNA in wildtype DNA background (1:2; 1:10; 1:100; 1:1000; 1:10000). This approach was repeated for different DNA concentrations (30, 20, 10, 5, 3, 2, 1 ng/µl). In each assay a probe specific for the respective mutation (FAM) and for the wildtype (HEX) were used. Next, we applied this assay to plasma samples from advanced melanoma patients with either BRAF V600E or V600K mutations detected in tumor tissue. The cut-off for a positive testing result was set to 3 positive droplets.

Results: For the BRAF V600E mutation we performed temperature gradients to identify an optimal annealing temperature. Best clustering of positive and negative populations was achieved at 61°C. For BRAF V600K we used a validated assay with an annealing temperature of 55° according to the manufacturer's protocol. In serial dilutions the sensitivity and specificity of assays were determined. Both assays showed no mutant copies in any control. The sensitivity for the BRAF V600E assay was at 1:1000 mutant DNA molecules until a DNA concentration of 20ng/µl, 1:100 between 3-10ng/µl and 1:10 for 1ng/µl. An even higher sensitivity was observed for the BRAF V600K assay with 1:1000 mutant DNA molecules until a DNA concentration of 20ng/µl and 1:100 between 1-10ng/µl. Further, we obtained blood samples from 29 patients, (age range 38-90 years, mean 63 years, 66% male, 33% female) of which 13 were diagnosed with melanoma stage III and 16 with stage IV. CtDNA was isolated with a median concentration of 1,72 ng/µl, resulting in a DNA input of 2ng-30ng per ddPCR reaction. R0-resected patients showed no ctDNA at baseline compared to non-curatively resected patients [0/12 (0%) vs 10/17 (58,82%)]. The detection of ctDNA positive versus ctDNA negative patients was associated with increased S100 (100% vs 10,53%) and LDH values (70,00% vs 31,58%).

Conclusion: Our assay can detect low burden mutations in peripheral blood at low DNA concentrations. Detection of ctDNA seems to correlate with the tumor burden as no mutations were detected in an adjuvant setting compared to 10 positive cases in a non-adjuvant setting. Detection of hotspot mutations in ctDNA could serve as a predictive marker and for monitoring in addition to S100 and LDH in melanoma patients. Further investigations with a larger cohort, follow-up samples and correlation with clinical parameters are needed to evaluate the clinical relevance of this promising biomarker.

P180 | Expression of proton-sensitive TRPC4 in common skin tumors

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Transient receptor potential classical or canonical channels TRPC4 are protonsensing sensors in the plasma membrane. They can be activated or inhibited by low extracellular pH (pHe), which is a hallmark of the tumor microenvironment in solid tumors. However, the role of TRPC4 in the development of skin tumors is still unclear.

In this study, we investigated the expression profiles of TRPC4 in squamous cell carcinomas (SCCs), basal cell carcinomas (BCCs), nevus cell nevi (NCN), and malignant melanomas (MMs).

We performed immunohistochemistry using paraffin-embedded tissue samples from patients and found that most skin tumors express TRPC4.

The results show that BCCs are often negative for TRPC4, while nearly all SCCs express these markers. MMs and NCN show similar expression patterns. The lower frequency of TRPC4 expression in BCCs when compared to SCCs is a novel histological feature distinguishing these two entities. Moreover, BCCs also show lower expression of TRPC4 as compared to NCN and MMs.

P181 (OP04/02) | Inside the tumor microenvironment of malignant melanoma: Identifying regulatory players of early malignant transformation with single-cell multiomics

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Analyzing the heterogeneity of the tumor microenvironment and understanding the complexity of malignant transformation are two essential factors for identifying novel therapeutic targets in malignant melanoma. In this study, we used singlecell transcriptomics

and epigenomics to get insights into the inter-tumor and intratumor heterogeneity of 10 primary melanoma lesions and one nevus. Therefore, a total of 11 high-resolution datasets consisting of 116.000 cells were analyzed using a broad spectrum of bioinformatics tools. First, we explored the tumor microenvironment of malignant melanoma by applying self-organizing maps (SOM) machine learning, which transforms multidimensional gene expression patterns into two-dimensional data landscapes. These landscapes provide an intuitive view on the tumor heterogeneity across cell types and decipher the tumor phenotypes as well as their molecular hallmarks with individual resolution. By mapping skin-specific cell clusters identified by Reynolds et al. (2021) onto our datasets, we investigated the cell type composition of each melanoma lesion and found that it differs strongly in terms of melanoma cell number, immune cell invasion, and fibroblast occupancy. In addition to that, we implemented a differentiation analysis of the melanoma cells based on literature gene sets. This analysis classified the melanoma subtypes according to their de-differentiation stages, showing that the cells progress from a more melanocytic stage, through transitory and neural crest-like stages to the dedifferentiated state. This analysis of melanoma subtypes has not been done on a single-cell level before and helps to understand the development of treatment resistance. Further, we apply so-called pseudotemporal methods to deduce dedifferentiation trajectories on a cellular level and to identify molecular branching events that form the basis of progressing malignancy. Additionally, by applying a ligand-receptor interaction framework (LIANA) to our datasets, we evaluated the cellular crosstalk between different cell types within our tumor specimens. Herein, we focused on the interaction of the diversely differentiated melanoma cell clusters and lymphocytes, to identify novel ligand-receptor (LR) pairings that might play a role in tumor development, tumor growth, and immune escape. The list of LR-interactions identified includes known pairings like MIF and CD74, but also novel interactions that have not been investigated up until now. By looking at both the transcriptomic and epigenetic components, the project will provide a comprehensive picture of the described processes with presumptive consequences for new therapeutic targets.

P182 | Lyve-1 deficiency enhances the hepatic immune microenvironment entailing altered susceptibility to liver metastasis

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Background: Hyaluronan receptor LYVE-1 is expressed by liver sinusoidal endothelial cells (LSEC), lymphatic endothelial cells and specialized macrophages. Besides binding to hyaluronan, LYVE-1 can mediate adhesion of leukocytes and cancer cells to endothelial cells. Here, we assessed the impact of LYVE-1 on physiological liver functions and metastasis.

Methods: Mice with deficiency of Lyve-1 (Lyve-1-KO) were analyzed using histology, immunofluorescence, microarray analysis, plasma proteomics and flow cytometry. Liver metastasis was studied by intrasplenic/intravenous injection of melanoma (B16F10 luc2, WT31) or colorectal carcinoma (MC38).

Results: Hepatic architecture, liver size, endothelial differentiation and angiocrine functions were unaltered in Lyve-1-KO. Hyaluronan plasma levels were significantly increased in Lyve 1-KO. Besides, plasma proteomics revealed increased carbonic anhydrase-2 and decreased FXIIIA. Furthermore, gene expression analysis of LSEC indicated regulation of immunological pathways. Therefore, liver metastasis of highly and weakly immunogenic tumors, i.e. melanoma and colorectal carcinoma (CRC), was analyzed. Hepatic metastasis of B16F10 luc2 and WT31 melanoma cells, but not MC38 CRC cells, was significantly reduced in Lyve-1-KO mice. In vivo adhesion assays with B16F10 luc2 cells were unaltered between Lyve 1 KO and wild-type mice. However, in premetastatic Lyve-1-KO livers numbers of hepatic CD4+, CD8+ and regulatory T cells were increased. In addition, iron deposition was found in F4/80+ liver macrophages known to exert pro-inflammatory effects.

Conclusion: LYVE-1 deficiency controlled hepatic metastasis in a tumor cell-specific manner leading to reduced growth of hepatic metastases of melanoma, but not CRC. Anti-tumorigenic effects are

likely due to enhancement of the premetastatic hepatic immune microenvironment influencing early liver metastasis formation.

P183 | Loss of CDKN2A affects melanoma immunogenicity

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Immunotherapy has greatly improved the outcome of advanced melanoma but a substantial number of patients still has limited benefit from this approach. Insufficient tumor-specific T cell activation in these patients is one of the major causes of nonresponse to immunotherapeutic agents for which the mutational landscape of the tumor is a determining factor. One of the most frequently altered loci in metastatic melanoma is the CDKN2A locus. Loss of CDKN2A is seen in more than 75 % of melanoma metastases and is accompanied by a decreased T cell infiltration. We thus hypothesized that melanoma immunogenicity is affected by alterations of CDKN2A.

Recognition of tumor antigens is a crucial step in the development of anti-tumor immunity. In this perspective, we created CD8+ T cells from healthy donors transduced with antigen-specific T cell receptors targeting melanoma antigens. These receptors are functional since the IFN gamma is produced by the transduced T cells (TCR T cells) upon exposure to melanoma cells presenting the cognate antigens. These antigen-specific T cells are used as a tool for measuring the immunogenicity of target cells.

To test the influence of CDKN2A loss on the immunogenicity of melanoma cell lines, we created tetracycline-inducible CDKN2A knockout melanoma cells and cocultured them with TCR T cells. We found that the loss of CDKN2A massively decreased TCR T cell recognition of target cells in a HLA-I dependent manner accompanied by an improvement of target cell viability.

Our results show that a major event in melanoma metastases, the loss of CDKN2A, is resulting in a decreased T cell recognition, which might have implications for the effectiveness of immunotherapy in these patients. In future studies we want to elucidate the underlying mechanism behind these findings.

P184 | Does aberrant glutamate metabolism in melanoma affect dendritic cell function?

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Tumor immunity can be negatively regulated by metabolites secreted in the tumor tissue. Metabolic reprogramming impacts the activation and maturation of dendritic cells (DC) and DC fate. We are working on the transgenic melanoma mouse model tg(Grm1)EPv, which spontaneously develops melanoma due to an overexpression of the metabotropic glutamate receptor 1 (Grm1) in melanocytes. This aberrant glutamate metabolism might also affect immune cell function in the tumor microenvironment. The goal of this PhD thesis is to investigate the metabolic changes in progressing melanoma with a focus on glutamate metabolism and its possible effects on tumor-infiltrating DC and T cell responses.

A first screening of the tg(Grm1)EPv mice for metabolic changes at different stages of the disease with a glutamate assay showed a decrease in glutamate levels with tumor progression.

To confirm these findings and extend analyses to other metabolites, we are currently screening tg(Grm1)EPv mice for metabolic changes with LC/MS technology. There appears to be an initial decrease in both TCA cycle and glycolysis metabolites during tumor-early stages that recover at the advanced stage. Moreover, we are planning to analyze the amino acid metabolism during tumor development with LC/MS.

We are currently performing detailed analyses of myeloid subsets in tumors and draining lymph nodes during tumor progression with multi-color flow cytometry. An initial investigation of DC precursors showed possible alterations in the bone marrow of tumor-bearing mice.

Further investigations will focus on alterations in the DC function as determined by in vitro co-cultures of Grm1-overexpressing melanoma cell lines and DC.

Acquired knowledge can benefit 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 improve response rates in cancer patients.

P185 | Enhanced apoptosis and loss of cell viability in melanoma cells by combined inhibition of pERK and Mcl-1 is related to loss of mitochondrial membrane potential, caspase activation and upregulation of proapoptotic Bcl-2 proteins

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Inhibition of MAP kinase pathways by selective BRAF inhibitors as vemurafenib and dabrafenib has evolved as key therapy for BRAF-mutated

melanoma, whereas the strategy does not apply for BRAF-WT or NRAS-mutated melanoma. Furthermore, also in BRAF-mutated melanoma, tumor relapse and therapy resistance often follow after an initial phase of tumor regression. Inhibition of the MAP kinase pathways downstream at the level of ERK1/2 as well as inhibitors for antiapoptotic Bcl-2 proteins as for Mcl-1 may serve as alternative strategies to overcome therapy resistance. As shown here, the BRAF inhibitor vemurafenib (10-30 μM) and the ERK inhibitor Sch772984 (0.1-10 μM) showed only limited efficacy, both in BRAFmutated (A-375, Mel-HO) and in BRAF-WT melanoma cell lines (MeWo, SKMel- 23), when applied alone. However, the effects of vemurafenib were strongly enhanced in BRAF-mutated cells, and the effects of Sch772984 were strongly enhanced in BRAF-mutated and WT cells in combinations with the selective Mcl-1 inhibitor S63845 (1 μM). This resulted in up to 90% loss of cell viability and loss of cell proliferation. Apoptosis was induced in up to 70% of cells, as determined by quantification of sub-G1 cells (cell cycle analysis) and by Annexin V/propidium iodide staining and flow cytometry. As concerning the proapoptotic pathways, combination of Sch772984 and S63845 in A-375 and MeWo cells resulted in activation of caspases cascades (caspase-3, -8 and -9, shown by Western blotting) and loss of mitochondrial membrane potential (MMP), as determined by TMRM + staining and flow cytometry as well as by JC1 staining of cells and microscopic analysis. Proving the critical role of caspases, a pan-caspase inhibitor (QVD-Oph) prevented apoptosis induction and loss of cell viability, and it inhibited loss of MMP in MeWo. We further aimed to unravel the roles of the family of Bcl-2 proteins, which represent critical regulators in apoptosis control in melanoma cells. Thus in relation with the inhibition of ERK, Sch772984 alone activated proapoptotic Bad, seen by its decreased phosphorylation, and it enhanced the expression of proapoptotic Bim and Puma. The combination finally resulted in downregulation of antiapoptotic Bcl-2 and enhanced expression of proapoptotic Noxa. In conclusion, combined treatment with ERK and Mcl-1 inhibitors showed impressive efficiency both in BRAF-mutated and WT melanoma cells, and may thus indicating a new strategy for overcoming drug resistance. The effects are accompanied by several changes of proapoptotic Bcl-2 proteins, thus again indicating this level in apoptosis control as a particular target in melanoma therapy.

P186 | RECQL4 drives genomic instability and reduced survival in patients with malignant melanoma

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The DNA helicase RECQL4 is an important genome caretaker due to its role in DNA replication, recombination, transcription and damage repair. Loss of function mutations in this gene are associated with several autosomal recessive and cancerprone syndromes. Besides, high RECQL4 expression is correlated with poor clinical outcome in several tumor entities. However, its impact in melanoma has not yet been elucidated. This prompted us to investigate the role of RECQL4 in disease progression, but also in response to therapy in patients with malignant melanoma.

To address the association between RECQL4 expression and patient's prognosis, data from 684 melanoma patients (including 213 patients treated with immune checkpoint inhibitors) of five independent cohorts were analysed by using the PhenoTImE database and the cBioPortal platform. The CoxPH model adjusted for different risk factors and Kaplan Meier analyses were performed to test the independence of RECQL4 as a prognostic factor. In addition, we addressed the impact of RECQL4 copy number variations in a pan-cancer cohort (15632 primaries and 10143 metastases from 25775 cancer patients) by multivariate analyses. Finally, we explored the association between expression level or copy number alterations of RECQL4 and numbers of intratumoral immune cells.

Our data show that high RECQL4 expression correlates with both, poor progressionfree and overall survival and increased genomic instability in melanoma patients. In addition, we observed that high copy number amplifications of RECQL4 are mainly presented in metastatic sites of multiple cancer entities when compared to primary tumours and predict worse overall survival. Besides, increased RECQL4 copy number correlate with a higher fraction of genomic alterations and increased tumour purity, but decreased tumour mutational burden in melanoma. Interestingly, high RECQL4 amplification is paralleled with downregulation of immune-related pathways and reduced immune cell infiltrate in tumours on the basis of mRNA expression level, creating an immune-evasive phenotype, which could argue the worse disease progression observed in the patients' treated with immunotherapy. In summary, RECQL4 expression but also copy number load could serve as a potential biomarker of patients' prognosis. Although the connection between RECQL4 and the immune system needs to be explored in more detail, targeting RECQL4 could present a promising strategy for the improvement of immunotherapy response in cancer patients.

P187 | Extracellular vesicles derived from melanoma cells induce carcinomaassociated fibroblasts by the transport of miR-92b-3p

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Background: Extracellular vesicles (EVs) are crucial mediators of intercellular communication, by interacting with various cell-types (e.g. immune cells and stromal cells) in the tumor microenvironment. EVs transport whole packages of functional molecules such

as proteins, and otherwise easily degradable mRNAs and microRNAs (miRNAs) safely through the extracellular space. MiRNAs are small non-coding RNAs that posttranscriptionally regulate protein expression. In melanoma, carcinoma-associated fibroblasts (CAFs) are important components in the tumor microenvironment, promoting tumour growth and facilitate the metastatic spread of malignant cells. We have evidence that EVs from melanoma cell lines decisively influence the formation of CAFs by virtue of miRNAs.

Aim: We investigated the molecular mechanisms how miRNAs delivered in EVs from melanoma cell lines contribute to formation of CAFs.

Methods: We isolated EVs from melanoma cell lines and normal melanocytes by ultracentrifugation and size exclusion chromatography, according to MISEV guidelines. We incubated primary normal human dermal fibroblasts (NHDFs) with these EVs and analysed the biological functions and gene expression profiles of induced CAFs. Additionally, we comparatively analysed miRNAs of EVs from melanoma cell lines and EVs from normal melanocytes by next generation sequencing.

Results: Uptake of melanoma cell-derived EVs in vitro by NHDFs induces a CAF-like phenotype, defined by increased expression of CAF marker genes (e.g. IL-8, αSMA and FAP). This was associated with an increase in cell viability, proliferation and migration. By next generation sequencing, we identified a strong enrichment of miR-92b-3p in melanoma cell-derived EVs compared to EVs derived from normal melanocytes (NHEMs). Correspondingly, we found an accumulation of miR-92b-3p in NHDFs after incubation with EVs derived from melanoma cell lines. We subsequently overexpressed miR-92b-3p mimic in NHDFs. It resulted in a similar CAF-like phenotype as in NHDFs incubated with EVs from melanoma cells.

We then blocked incorporation of miR-92b-3p into melanoma EVs by locked nucleic acids (LNAs). This procedure resulted in prevented formation of the previously observed CAF-like phenotype.

To search for possible targets of miR-92b-3p we used databases and identified the tumour suppressor PTEN. Correspondingly, treatment with melanoma cell-derived EVs or overexpressing miR-92b-3p leads to decreased expression of PTEN protein in NHDFs.

Conclusion: In summary, we have shown that miR-92b-3p is enriched in melanoma cell-derived EVs and is transported into fibroblasts, where it triggers proliferation and migration. So, miR-92b-3p contributes to the induction of a CAF-like phenotype by targeting PTEN.

P188 (OP04/04) | Th1/Tc1 tissue resident memory T cells mediate checkpoint blockade-induced dermatitis and colitis

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Background: Immune checkpoint blockade (ICB) has been immensely successful in treating a variety of cancers including melanoma or cutaneous squamous cell carcinoma. Off-target effects of ICB are called immune-related adverse events (irAE). IrAEs can cause inflammation of all organ systems but most frequently affect the skin or gastrointestinal tract. To prevent treatment pauses or cessation it is detrimental to investigate the immunopathology of irAEs.

Methods: We leveraged multiparameter immunofluorescence, spatial transcriptomics, and RNA in situ hybridization (RISH) on FFPE tissue of irAE dermatitis (22 cases) and irAE colitis (7 cases). IrAE dermatitis was compared to psoriasis, which is a Th17-driven disease and healthy skin samples. IrAE colitis was compared to healthy colon samples.

Results: Multiparameter IF could show an expansion of CD4+ and CD8+ tissueresident memory T (TRM) cells in the lymphocyte-rich portion of skin in cutaneous irAEs compared to healthy skin controls. Spots containing TRM cells were then analyzed with spatial transcriptomics to explore the functional phenotype of TRMs. Expression of Th1-associated genes was upregulated in irAE and Th17- asociated genes in psoriasis. In addition, increased expression of the inhibitory receptors PD-1, CTLA-4, LAG-3, TIM-3 and TIGIT was observed in irAE cases. A combined approach of IF and RISH technology confirmed expression of IFNγ, TNFα, CXCL9, and CXCL10 in other irAE dermatitis samples. IFNγ transcripts were found specifically within TRM cells. The Th1-centered phenotype was also observed in irAE colitis cases compared to healthy colon.

Conclusion: TRMs with upregulated inhibitory checkpoints are likely re-invigorated by ICB. The Th1/Tc1-based immune response with increased production of IFNγ and TNFα argues that severe steroid-refractory irAE dermatitis could potentially be targeted with systemic TNFα-blockade and topical JAK inhibitors could be considered for low-grade irAE dermatitis as corticosteroid sparing reagents.

P189 | Immune checkpoint inhibitor-based activation of tumor infiltrating cells is related to an increase in NFB activity within the tumor microenvironment (TME) monitored by bioluminescence imaging (BLI)

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Introduction: Since decades, cancer research is highly engaged in understanding the underlying mechanisms of tumor cell immune escape. We have recently established a combined cancer immunotherapy (COMBO) consisting of an initial 2 Gy whole body radiation, tumor-antigen (TA) specific IFN-y producing CD4+ T cells (Th1) as well as immune checkpoint blockade (ICB: anti-PD-L1 and anti-LAG-3 mAbs). In this study we aimed to visualize the effect of COMBO treatment on the activation of tumor infiltrating cells and to monitor their related sites of immune cell activation.

Methods: Initially, we s.c. inoculated ovalbumin expressing (OVA)-MC38 adenocarcinoma or (OVA)-B16 melanoma tumor cells in NFB-luc reporter mice which express luciferase exclusively upon NFB activation to follow the effect of our COMBOtreatment approach on the tumor-microenvironment (TME) in an ICB sensitive and non-sensitive tumor model. Our COMBO-treatment consisting of an initial 2 Gy total body radiation, one OVA-Th1 cell administration (OT-II) and three ICB injections was initiated four days after tumor cell inoculation and in vivo BLI was performed daily to monitor NFB activation in the tumor until the end of the experiment. For ex vivo characterization of the activation state of the tumor infiltrating cells and immune cells we additionally performed multicolor flow cytometry (MFC) analysis of the immune cell infiltrate within the tumors.

Results: COMBO treatment significantly decreased the relative tumor growth of OVA-MC38 tumor-bearing NFB-luc reporter mice whereas the relative tumor growth of OVA-B16 melanomas was not significantly affected when compared to sham-treated mice. Exclusively the endogenous immune cells within COMBO-sensitive OVA-MC38 adenocarcinomas exhibited an enhanced NFB activity-related signal intensity (SI: 5015 p/s/cm2/sr) when compared to the tumors of sham-treated mice (SI: 3432 p/s/cm2/sr) indicating a COMBO-induced immune cell activation within the TME. Moreover, we determined a lower NFB activity-related SI in OVA-B16-tumors of NFBluc reporter mice when compared to OVA-MC38-tumors. In line with the increased NFB activity-related SI In OVA-MC38 tumors of COMBO-treated NFB-luc reporter mice continuative MFC analysis of OVA-MC38 tumors revealed a pronounced infiltration of CD69+ expressing activated immune cells.

Conclusion: COMBO treatment of OVA-MC38 and OVA-B16 bearing NFB-luc reporter mice effectively inhibited tumor growth of OVA-MC38 adenocarcinomas but not of OVAB16 melanomas. Treatment efficacy was associated with an increased NFB activity of endogenous immune cell within the MC38 tumors and the presence of activated CD4+, CD8+ T cells, CD19+ B cells, and CD11+ within the TME. Thus, longitudinal noninvasive in vivo monitoring of immune cell derived NFB activation within the TME might exhibit a novel innovative tool to identify immune therapy response or failure.

P190 | Extracellular vesicles derived from melanoma cells transport miR-183-5p into macrophages thereby inducing CD163 expression

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Introduction: The tumor microenvironment in malignant melanoma has been shown to promote tumor growth and to facilitate metastatic spread of malignant cells. Tumor associated macrophages (TAMs) are crucial mediators in the tumor microenvironment. We have evidence that extracellular vesicles (EVs) derived by melanoma cells contribute to the polarization of TAMs. EVs, including microvesicles and exosomes, are small (<200 nm) membrane nanovesicles, which safely transport functional molecules as proteins, mRNAs and noncoding RNAs. MiRNAs are small noncoding RNAs which posttranscriptionaly regulate protein expression by guiding the RNA induced silencing complex (RISC) to the 3' untranslated region (UTR) of mRNAs. We and others showed that melanoma cell-derived EVs (MEVs) contribute to the formation of tumor-associated macrophages (TAMs) by the transport of miRNAs. But, the molecular mechanisms how macrophages are polarized into TAMs in melanoma are not completely understood yet.

Methods: MEVs were isolated by ultracentrifugation and size exclusion chromatography and analyzed accoding to internationationally consented protocol (MISEV 2018). Macrophages derived from cell line THP1 or from peripheral blood mononuclear cells were treated with MEVs and analyzed by flow cytometry and qRT-PCR. By next generation sequencing, we identified miRNAs enriched in MEVs and analyzed their transport in macrophages. For functional analyses miRNAs were overexpressed by mimics or inhibited by locked nucleic acids (LNAs) in macrophages.

Results: We showed that MEVs induce a pro-inflammatory and pro-angiogenic phenotype in macrophages, which was characterized by increased expression for pro-inflammatory cytokines (IL-1β, IL6, IL-8), immune checkpoint receptor ligands (PD-L1, PD-L2) and cell

surface marker (CD163). In comprehensive analyses we identified miR-92b-3p, miR-182-5p and miR-183-5p as significantly enriched miRNAs in MEVs compared with EVs from normal melanocytes. Moreover, we found these miRNAs accumulated in macrophages after treatment with MEVs. In functional analysis we revealed that overexpression of miR-183-5p alone resulted in an enforced expression of CD163, a cell surface marker associated with either immunosuppressive M2 macrophages or pro-tumorigenic TAMs.

Conclusion: MEVs transfer melanoma-derived miR-92b-3p, miR-182-5p and miR-183-5p into macrophages contributing to the polarization of a particular pro-inflammatory, but tumorigenic TAMs. Functional analysis of the identified miRNAs will reveal the molecular mechanisms mediating this education of TAMs.

P191 | Granzyme B and granulysin expressing human DCs in melanoma

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Advanced stage malignant melanoma is still a therapeutic challenge. Despite impressive treatment results with immune checkpoint inhibitors (ICI) or targeted therapies, therapy resistance (primary or acquired) is an existing problem. A successful immune response requires the presence of antigen presenting cells. Dendritic cells are the most effective cells for the initiation of an effective immune response against tumor cells, as they have the unique ability to cross-present exogenous antigens. Therefore we are studying DC function in effective tumor cell eradication.

In this present study, human monocytes from healthy donors were differentiated to DCs, activated with or without lipopolysaccharide (LPS) before we performed co-culture experiments with either a melanoma cell line (MeWo) or immortalized keratinocytes (HaCaT). After 24 hours we tested the viability of target cells by flow cytometry. Furthermore, we investigated whether co-culture with either cell line led to changes in the maturation of the DCs. Here we studied the DC surface markers CD83, CD86, CD40, CD80, and HLA-DR. We also examined the expression of the cytotoxic factors granzyme B and granulysin in DC and manipulated their function by siRNA.

Human DCs could induce cell death/apoptosis in melanoma cells but not in keratinocytes during co-culture. The cytotoxic capacity of DC was increased by LPS pre-treatment. LPS pre-activation of DCs also resulted in killing of HaCaT cells compared to the non-activated DCs. In addition, the costimulatory molecules CD83, CD86, CD40, CD80, and HLA-DR were upregulated on DCs upon co-culture with MeWo but not/to a lesser extent by HaCaT co-culture. Interestingly, we found that the expression of granzyme B but not granulysin was elevated in DCs following coculture with MeWo. To identify the functional role of these cytotoxic proteins, we manipulated the expression of these cytotoxic factors in DC by siRNA targeting granzyme B or granulysin. DC treated with granzyme B siRNA showed less cytotoxic capacity compared to none-treated DC. Co-culture with HaCaT cells did not induce the expression of cytotoxic factors in DCs.

In summary, our results demonstrate a specific cytotoxic activity of DC against melanoma cells, which is mediated by granzyme B but not by granulysin. DCs with cytotoxic factors could be important players in the induction and/or maintenance of anti-melanoma immune responses.

P192 | Melanoma cell-produced heparan sulfate regulates the tumor microenvironment

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Heparan sulfate is ubiquitously present on the surface of mammalian cells and involved in the regulation of various processes such as cell migration and cell adhesion. Recently, we have shown that loss of heparan sulfate at the surface of melanoma cells promotes vascular adhesion and thus hematogeneous metastasis. Next to its ability to regulate cell adhesion, heparan sulfate is known to control the activity and function of growth factors and cytokines. However, the impact of melanoma cell-produced heparan sulfate on the tumor microenvironment is unknown. In the present study, we therefore aimed to investigate the impact of heparan sulfate on angiogenesis and immune cell recruitment into melanoma tissue. We generated murine melanoma cells (B16F10) lacking heparan sulfate by genetic depletion of exostosin 1, a key enzyme of heparan sulfate biosynthesis. Upon intradermal or intravenous injection of the B16F10 cells, cutaneous tumors or lung metastasis are formed. In comparison to the control, the growth of the primary tumor was not significantly affected by the lack of heparan sulfate. However, tissue expression of the blood vessel marker CD31 using fluorescence microscopy revealed that lack of heparan sulfate was associated with significantly fewer blood vessels. In correlation with the reduced angiogenesis, we found that the lack of heparan sulfate decreased also the numbers of tumor-infiltrating CD8+ T cells and Ly6G+ neutrophils. Moreover, we measured an attenuated infiltration of immune cells into lungs bearing metastasis of heparan sulfate deficient melanoma cells. Taken together, these findings suggest an impaired immune response towards heparan sulfate deficient melanomas. In line with this notion, analysis of exostosin 1 expression in tumor tissues of melanoma patients indicate that reduced mRNA levels of exostosin 1 correlate with reduced patients' survival. In conclusion, our study provides the first insights into the fundamental role of heparan sulfate-mediated regulation of the tumor microenvironment. Further analysis will reveal whether the impact of heparan sulfate

on the tumor microenvironment is also associated with the response to immune modulatory drugs such as immune checkpoint inhibitors.

P193 | Transport of melanoma cell-derived miR-1246 between subpopulations of melanoma cells via extracellular vesicles potentiates invasiveness by targeting CCNG2

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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 cells resulted in a higher invasive capacity in a 3D spheroid model. Based on these results, we wondered if EVs isolated from the supernatant of the BLM-HI subpopulation would mediate this invasive quality. We found that these EVs indeed increased the invasive ability of parental BLM cells. By next generation sequencing (NGS) we observed a differential gene expression in BLM cells treated with BLM HI-EVs compared to untreated cells. Transcriptome analysis revealed significant upregulations of pathways related to extracellular matrix organization and pathways connected to the hallmark of epithelial-mesenchymal transition (EMT) - a key process for metastasis. These findings support our thesis that EVs and their contents are able to promote invasion and phenotype switching within tumor cell subpopulations.

Since one of the main functions of EVs is the intercellular transport of miRNAs, we analysed and identified differential enrichment for specific miRNAs in EVs derived from the BLM-HI subpopulation compared with parental BLM cells by small RNA sequencing. We found miR-1246 significantly enriched in EVs from BLM-HI cells. Treatment of parental BLM cells with EVs released by BLM-HI resulted in increased intracellular levels of miR-1246. Inhibition of miR-1246 by locked nucleic acids (LNAs) decreases invasion of BLM-HI cells. By a database screen, we found Cyclin G2 (CCNG2), which is known to inhibit EMT, as a putative target of miR-1246. Treatment of BLM cells with EVs derived from BLM-HI cells strongly reduces CCNG2 protein expression. In contrast to this, the block of miR-1246 by LNAs increases CCNG2 protein level in BLM cells.

Conclusion: Transfer of EVs from melanoma cells is a key process regarding intercellular cooperation and transformation of cellular phenotypes, including increasing invasive potential. We have evidence that miR-1246 contributes to a pro-invasive phenotype of melanoma cells by targeting CCNG2.

P194 | Resistance to BRAF inhibitors: EZH2 and its downstream target PLK1 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-MEK-MAPK 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 contributes to the emergence of resistance of melanoma cells to vemurafenib, cell viability assays were carried out and apoptosis and the cell cycle were analyzed using flow cytometry. EZH2 expression and H3K27me3 were detected by western blot. To investigate downstream targets of EZH2, next-generation sequencing analyzes (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 BRAFi susceptible 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 analyzes, 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. In contrast to these results, A375 cells did not show these differences when comparing vemurafenib monotherapy and the combination of vemurafenib and tazemetostat. In our NGS analyses, we identified PLK1, a known cell cycle regulator, as a downstream target of EZH2. Inhibition of BRAF signaling and EZH2 function decrease PLK1 expression in A375R cells. In addition, the inhibition of PLK1 in A375R cells induces cell death.

Conclusion: EZH2 contributes to the development of BRAFi resistance in melanoma. Therefore, combining tazemetostat with vemurafenib or affecting the expression of downstream targets of EZH2, e.g. PLK1, may represent new therapeutic options for melanoma.

P195 | Aberrant glycosylation patterns on melanoma cell surface mediate local complement activation

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Growing body of evidence indicates that the complement system is essentially involved in cancer progression and cancer cells can directly trigger the lectin pathway due to an altered glycosylation of proteins at their surface. Aberrant glycosylation is considered a hallmark of cancer and occurs in the majority of tumor types, including melanoma. Our own previous work indicated that neutrophils activated by complement-derived membrane attack complex destabilize the adjacent endothelium, thus increasing vascular permeability. Melanoma cells exposed to the complement factors after intravasation into the circulation. However, the role of plasmatic complement on these disseminated tumor cells is still scarce. Here, we report that the aberrant glycosylation patterns on melanoma cell membrane mediate local surface complement activation. First, we analyzed the recruitment of plasma proteins to blood flowing melanoma cells by mass spectrometry. Interestingly, we found a plethora of complement factors bound to human melanoma cells, including C3 and mannose-binding lectin (MBL). C3b fragment deposition (a marker for complement activation) on melanoma cells was further confirmed by flow cytometry and immunofluorescence staining. Decreased binding of complement factor H to the surface of melanoma cells was associated with increased complement activation. Factor H is stored at the cellular surface by the interaction with different glycan structures. Therefore, experimental alteration of melanoma cell exposed glycans promoted complement activation. To further investigate the role of complement in melanoma metastasis, we performed lung metastasis experiments by intravenous injection of melanoma into WT and complement C3 and C5 deficient animals. In line with the supposed pro-metastatic properties of complement, we found significantly less lung metastasis in complement deficient mice. Our data suggest that inhibition of complement activation is a potent strategy to abolish hematogenous dissemination in melanoma.

P196 (OP05/05) | A potential role of neutrophils in resistance to MAP-kinase inhibition in melanoma

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Dual MAP-kinase inhibition (BRAF- plus MEK-inhibition) is an established standard care treatment for patients with metastatic BRAF-mutated melanoma. The initial response to targeted therapy by induced inhibition of the MAP-kinase pathway induced dramatic tumor regression and improved patient survival. However, despite impressive and frequent responses, almost all tumors become resistant to targeted therapy. Intrinsic and acquired resistances to this form of therapy have been described. Some data indicating the involvement of myeloid cells mediating resistance to therapy. In addition, clinical studies highlight the relevance of peripheral eosinophils and neutrophils as predictive marker in melanoma patients, with neutrophils being associated with poor prognosis. However, the interaction of granulocytes with melanoma cells and their direct influence on the course of the disease is still unclear.

In this study, we functionally determined the effect of neutrophils on cultured melanoma cells under the influence of BRAF- plus MEK-inhibition. CFSE-labeled melanoma cells were co-cultured with freshly isolated human peripheral neutrophils in the presence of absence of the inhibitors. The viability of melanoma cells was determined by flow cytometry by Annexin V/7-AAD staining. Transwell experiments and cultures under different adherence conditions were carried out to determine contact dependence of the investigated effects. The latter was visualized by HE staining of cytospins. In blockade and rescue experiments, further insights could be achieved to understand the cell interaction and the effectiveness of targeted therapy. Propidium iodide and ki67 stainings were performed to elucidate the effect of granulocytes on cell cycle and proliferation of melanoma cells. Interestingly, screening for differentiation markers, we could show the reduction of MITF and induction of c-JUN expression in melanoma cells upon co-cultures. When performing immunohistochemistry and immunofluorescence staining of MITF, we observed a shift of MITF localization in melanoma cells. MITF was detectable in both the nucleus and cytoplasm in control and treated cells. Strikingly, the intensity of MITF was diminished and limited to the cell nucleus in melanoma cells when co-cultured with neutrophils.

Our study shows a survival promoting ability of neutrophils on melanoma cells in vitro. In addition, the induction of apoptosis in melanoma cells by combined MAPK inhibition is reversed by neutrophils. Our preliminary data suggest a MITF/c-JUNmediated resistance mechanism to MAP-kinase inhibitors mediated by neutrophils. Elucidation of this mechanism could lead to the development of therapeutic strategies to inhibit the resistance in melanoma patients to existing therapies.

P197 | 4-(5-Methyl-2H-pyrazole-3-ylamino)phenyl-2H-phthalazin-1-one inhibits MCPyV T antigen expression in Merkel cell carcinoma independent of Aurora kinase A

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Merkel cell carcinoma is a highly lethal skin cancer frequently caused by the Merkel cell polyomavirus (MCPyV) and proliferation of MCPyV-positive MCC tumor cells depends on expression of the virus-encoded T antigens (TA). Using a cell based assay to identify compounds which specifically inhibit growth of MCC cells by repressing TA expression we identify 4-[(5-methyl-1H-pyrazol-3-yl) amino]-2Hphenyl- 1-phthalazinone (PHT) a reported inhibitor of Aurora kinase A. PHT effectively inhibits TA expression, but we demonstrate that this outcome is not caused by inhibition of Aurora kinase A. However, we demonstrate that β-catenin a transcription factor repressed by active glycogen synthase kinase 3 (GSK3) is activated by PHT, suggesting that PHT bears a hitherto unreported inhibitory activity against GSK3, a kinase known to function in promoting TA transcription. Finally we demonstrate that PHT exhibits in vivo antitumor activity in an MCC xenograft mouse model suggesting a potential use in future therapeutic settings for MCC.

P198 | Increased chlormethine induced DNA double stranded breaks in malignant T cells from mycosis fungoides skin lesions

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

P199 | Cytometry and machine learning based approaches for diagnosis of malignant cells in Sézary syndrome

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Background: In cutaneous T-cell lymphoma (CTCL), the lack of early diagnostic biomarkers is a challenge. This results in delayed diagnosis,

and can seriously affect treatment and prognosis. Therefore, defining accurate methods for early identification of rare malignant cells is of pivotal importance. We explore the potential of artificial intelligence (AI) to uncover tumor-defining cells in Sézary syndrome (SS), a leukemic type of CTCL.

Methods: We established a mass-imaging approach and a masscytometry panel to acquire large-scale single-cell data from peripheral blood, and following the datasets were analysed by a trained AI model, called CellCNN. CellCNN is a supervised machine learning algorithm that trains a convolutional neural network with a single layer using labelled single-cell data or labelled datasets as inputs. In the mass-imaging approach, we enrolled 4 healthy individuals (HDs) and 5 patients with SS. The prediction and classification were done by data-driven analysis. As for the masscytometry study, we included a discovery cohort of 60 individuals (20 patients with SS, 20 patients with atopic dermatitis (AD), and 20 HDs) and a validation cohort of 33 individuals (11 individuals of each group). Algorithm performance was assessed with area under the curve (AUC), specificity and sensitivity.

Results: We successfully developed the first machine learning method for morphology based diagnosis for SS samples. The CellCNN approach delivered the best separation of Sezary (84.6% abnormality) and healthy specimens (13.9% abnormality) in comparison with other machine learning models. This is also the first label-free imaging cytometry and weakly-supervised machine learning method to discriminate healthy and diseased samples in general. For a more bench-to-bedside translational approach, we combined mass-cytometry based techniques with AI. Our algorithm can sensitively and specifically identify tumor-defining SS cells in blood according to the pattern of cell surface markers. The result achieved zero false positives and only one false negative prediction with a high accuracy (AUC of 0.98, sensitivity of 0.91, specificity of 1.0). Our findings pave the way for an easy-to-implement and sensitive diagnostic approach to facilitate early diagnosis of Sézary syndrome and other tumors with blood involvement.

P200 | The role of dendritic cells in tumor-targeted therapy mediated anti-tumor immunity

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Melanoma has a high mutational load with driver mutations affecting genes regulating critical signaling pathways involved in proliferation and cell growth. Half of the melanoma patients carry a specific point mutation affecting BRAF, 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 due to the development of therapy resistance.

Tumor-targeted therapy modulates the tumor immune microenvironment. However, the functional role of dendritic cells (DC) in anti-tumor responses by BRAFi 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 deep insights into the different DC subsets. Using this optimized panel, we can show that treatment with BRAFi decreases immunosuppressive myeloid cells in the tumor microenvironment. Furthermore, this targeted therapy recruits activated DC to the inflammatory tumor milieu, which subsequently migrate in an increased frequency to the tumor-draining lymph nodes. In addition, by using Zbtb46-GFP mice, a DC-specific reporter mouse strain, we identified a CD64+ DC population in tumor-draining lymph nodes. We are currently investigating the function of this cell population in more detail to gain insights about the relevance in anti-tumor immunity, e.g. antigen presentation and cytokine production.

The detailed phenotypcial and functional characterization of tumor-infiltrating DC 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. With this knowledge novel combinatorial therapies can be developed.

P201 | Crosstalk between MET-dependent receptor tyrosine kinase signaling and oncogenic Gαq mutations in melanoma

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G-protein-coupled receptors (GPCRs) transduce their signals through interaction with heterotrimeric G-protein subunits (Gα, Gβ, and Gy). The important role of GPCR-Gαq/11 signaling in melanocyte neoplasia became apparent with the discovery of somatic Gnaq mutations in blue melanocytic nevi in the skin and in uveal melanomas. Oncogenic driver mutations in the G protein α subunits Gαq and Gα11 lead to a constitutively active signaling in the Gαq pathway and its downstream effectors. Our group has established the genetic Hgf-Cdk4R24C melanoma model, in which mice develop spontaneous skin melanomas that metastasize to the lymph

nodes and lungs. Melanomagenesis can be accelerated by epicutaneous application of the carcinogen 7,12-dimethylbenzanthracene (DMBA). Using next generation sequencing, we found that the genetic environment in Hgf-Cdk4R24C mice favors the acquisition of mutations in Gαq209 and Gα11209 proteins in spontaneous and DMBA-induced melanomas. BRAF mutations were not detected. In vivo, the onset and progressive growth of melan-a GnaqQ209L tumors was significantly increased in Hgf-Cdk4R24C mice when compared to Cdk4R24C mice. To better characterize the impact of strong Met signaling on tumors carrying different oncogenic mutations, we compared melan-a GnaqQ209L cells with melan-a BrafV600E cells in vitro and in vivo. Melan-a GnaqQ209L but not melan-a BrafV600E cells already constitutively showed p-Met expression. Hgf treatment induced p-Met in both cell lines, but the specific Gαq inhibitor FR-900359 only inhibited p-Met in melan-a GnaqQ209L. Both cell lines formed progressively growing melanomas in immunodeficient NOD/SCID mice, whereas melan-a GnaqQ209L tumors were highly pigmented, melana BrafV600E tumors were amelanotic with strong NGFR expression. In contrast to melan-a GnaqQ209L cells, melan-a BrafV600E only developed progressively growing amelanotic tumors in 2 out of 7 mice Hgf-Cdk4 mice and did not grow at all in Cdk4 animals. Our results indicate a crosstalk between receptor tyrosine kinase signaling and Gq signaling. Strong Met signaling in the microenvironment of Hgf- Cdk4 mice mainly favors the growth of Gαq- but not BrafV600E mutated tumors. Further experiments have to elucidate the molecular basis for the crosstalk between Gq and RTK signaling.

P202 | Cutaneous SCC tumor cells reveal pro-tumorigenic expression patterns upon fibroblast-populated collagen matrix interaction

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The tumor microenvironment of epithelial tumors such as cutaneous squamous cell carcinoma (cSCC) is of major importance for disease progression and therapeutic responses. Particularly, tumor stroma associated fibroblasts regulate central tumorigenic properties due to their ability to build a tumor scaffold and contract upon activation, similar to wound-associated myofibroblasts. However, interaction mechanisms of neoplastic keratinocytes and tumor surrounding dermal fibroblasts are largely uncharted, especially in pre-invasive tumor stages.

Therefore, a 3D co-cultivation model using human neoplastic keratinocyte cell line A431 and fibroblast-populated collagen matrices (FPCM) was established simulating an organotypic tumor environment. Both were separated by 3 µm poresized transwell inserts for a 72h interaction period. Subsequently, gene expression of central pro-fibrotic und pro-tumorigenic markers of both collagenase-dissolved fibroblasts and of tumor keratinocytes were analyzed.

Of note, tumor keratinocytes showed increased tumorigenic and fibrotic marker expression such as alpha smooth muscle actin (αSMA) and plasminogen activator inhibitor 1 (PAI-1) following interaction with FPCM, compared to tumor cells alone or in contact with an acellular collagen matrix. Dermal fibroblasts, however, showed constant pro-fibrotic and pro-tumorigenic expression levels following interaction with tumor keratinocytes.

Altogether, this points at tumor promoting effects of FPCM on tumor keratinocytes by yet unknown mechanisms and suggests new target genes potentially involved in tumor-tumor stromal fibroblast interaction. Ongoing organotypic co-cultivation of fibroblasts and tumor keratinocytes from cSCC patients will gain deeper insights into tumor cell - tumor stromal fibroblast interaction mechanisms with potential for early therapeutic interference strategies.

P203 | The role of von Willebrand factor in coagulability and progression of various malignant tumor entities

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Patients with cancer-associated thrombosis (CAT) have a worse prognosis than tumor patients without venous thromboembolism (VTE). We hypothesized that von Willebrand factor (VWF), a marker of endothelial dysfunction, increases the risk for the development of CAT and metastasis-related death. The aim of this study was to evaluate the predictive value of biomarkers of endothelial dysfunction, including VWF, the VWF-degrading enzyme a disintegrinlike and me-talloproteinase with thrombospondin type I repeats 13 (ADAMTS-13) as prognostic parameters in monitoring various solid tumor diseases.

We performed an exploratory analysis in patients with malignant tumors of the pancreas, stomach, esophagus, colorectum, lung (n = 155), basal cell carcinoma (BCC; n = 39) and in healthy volunteers (n = 56). Clinical features, incidence of cancer-associated VTE, disease stage, and overall survival (OS) were recorded and correlated with VWF and ADAMTS-13 activity. Immunofluorescence staining

for VWF and intraluminal platelets were performed in tumor samples and compared with the corresponding peritumoral regions.
VWF levels were significantly increased in patients with colorectal ($p \leq 0.01$), gastric ($p \leq 0.0001$), esophageal ($p \leq 0.0001$), and pancreatic ($p \leq 0.0001$) carcinoma, which was associated with significantly decreased ADAMTS-13 activity (Spearman's $\rho = -0.41$, $p \leq 0.001$). During the 6-year follow-up period, 26.5% of patients developed VTE, which was associated with a higher risk of tumor-related death. Multivariable analyses identified the VWF/ADAMTS-13 ratio as a predictor of VTE and mortality in cancer patients. Complimentary, we demonstrated the formation of ultra large VWF fibers in the vascular lumen of tumor tissue compared to peritumoral tissue and the conferring binding and aggregation of platelets. The relative increase of intratumoral vessel with ULVWF in relation to peritumoral was significant and increased levels of VWF fibers in the tumor tissue were associated with a reduced OS
The data confirm that increased plasmatic and local VWF antigen levels in combination with a systemic reduction in ADAMTS-13 activity are associated with a higher risk of thromboembolic events and worse survival of cancer patients and should therefore be further investigated in prospective large-scale studies.

P204 | Uncovering the moonlighting function of nuclear GARP in melanoma

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Skin cancer is one of the most common types of cancer, and malignant melanoma is its deadliest form. Despite recent therapeutic advances, especially using immune checkpoint inhibitors, late-stage patients are faced with limited treatment options - highlighting the 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 activated regulatory T cells (Treg) and platelets, and it is released into the tumor microenvironment as a suppressive factor. Acting as a regulator and activator of latent-TGF-beta, GARP is well known to exhibit various suppressive functions in the tumor microenvironment, including inducing peripheral Treg and M2 macrophages as well as inhibiting effector T cell function.
Recently, it was discovered that GARP is highly expressed in the nuclei of cancer cells - even more so than on the cell surface. Intriguingly, we could also demonstrate that a high nuclear GARP expression correlates with poor patient outcomes in glioblastoma. These factors compelling support the potential of GARP acting as a moonlighting protein in nucleus, where it may exhibit an alternative and uncharacterized function. However, little to nothing is known regarding the nuclear localization mechanism and the potential role of GARP inside the nucleus.
This study aimed to predict in silico a possible nuclear localization sequence (NLS) hidden in the GARP protein and to identify potential interaction partners of nuclear GARP. Furthermore, it also aimed to determine whether nuclear GARP is expressed in the tumors of melanoma patients and to examine its implications on patient outcomes. Expression of GARP in the nuclei of human melanoma cells was confirmed and validated in vitro by confocal microscopy, flow cytometry, and western blot. To predict a potential NLS, a multiple sequence alignment of GARP homologs was performed to identify highly conserved regions in the GARP protein with a special focus on clusters of basic amino acids, that typically compose NLSs. A possible bipartite NLS, rich in leucine and arginine, could be identified on the surface of the GARP protein, notably not located in the TGF-beta-1 binding cleft. Literature search and coupled with in silico protein interaction predictions could identify with high confidence two possible interaction partners of nuclear GARP. Lastly, nuclear GARP expression was confirmed in both primary and metastatic human melanoma tissues via immunohistochemistry.
Future studies will test these predictions in vitro by performing site directed mutagenesis on the identified NLS candidate in the GARP protein as well as perform co-immunoprecipitation of GARP to pull down potential binding partners. They will also focus on analyzing nuclear GARP as both a prognostic and predictive biomarker for melanoma patients.
In summary, this study showed that GARP is intranuclearly localized in both human melanoma cells and tumor tissue. It also predicted a previously uncharacterized NLS of the GARP protein as well as possible interaction partners of nuclear GARP. The findings of this work provide us with insights on novel but poorly understood molecular mechanisms in cancer cells, and they may also offer ways to target nuclear GARP, which could be applied to improve the clinical outcomes of cancer patients.

P205 | Establishment of CRISPR-Cas9-based prime editing in melanoma cells to introduce human-relevant oncogenic mutations

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Oncogenic mutations promote cancer growth by activation or inhibition of protooncogenes or tumor suppressor genes, respectively. Activating mutations in components of the MAPK signaling pathway are particularly frequent and play a central role in the development of melanoma. In addition, it has become more evident that these molecular alterations, but also the differentiation state of tumor cells, affect the tumor microenvironment, which in turn also influences responses to therapeutic approaches, such as cancer immunotherapies.

CRISPR-Cas9 has revolutionized the field of genome editing and has been adapted and developed more and more in recent years. The newly established CRISPRCas9- based "prime-editing" technology enables targeted insertions, deletions and all classes of point mutations without DNA double-strand breaks and donor DNA template. By using this technique, this project aims at establishing human-relevant syngeneic melanoma cells carrying oncogenic point mutations, typically found in melanoma patients.

We have developed two strategies that enable enrichment of successfully primeedited cells. These were validated by Next generation sequencing, functionally characterized, e.g. by qRT-PCR to determine differentiation cell state, and tested for their sensitivity to MAPK signaling pathway inhibitors. Using this methods, we have been able to establish novel syngeneic melanoma cell lines that represent differentiated and dedifferentiated cell states and also harbour human-relevant mutations. Studies in experimental mouse models will now help to determine aspects of tumorigenesis as well as effects on immune regulation, and will enable the identification of novel and personalised treatment strategies in the future.

P206 | Estrogen receptor beta stimulation as a possible novel therapeutic target to precisely treat 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 have been shown. Mycosis fungoides is histopathologically characterized by epidermotropism of malignant T cells and is therefore predestined for topical treatments. We could recently demonstrate a distinct tumor suppressive effect of ERβ agonists in different established CTCL cells However, ERβs role on CTCL malignat cells isolated from different patients is yet unknown. Further the penetration of ERβ agonists in CTCL cells and its impact on human skin cells is uninvestigated.

Methods: Here, we analyzed the impact of LY500307, a synthetic, highly selective and potent ERβ agonist in CTCL malignant cells isolated from 8 CTCL patients. Blood samples were obtained from CTCL patients at the University Hospital Frankfurt. CD4+ T cells were selected with a negative selection kit and supplemented with antibodies to select for CD7- or CD26- cells depending on the known aberrant phenotype of the patient. We further analyzed the impact of LY500307 on HaCaT cells and fibroblasts from healthy donors in comparison to CTCL cells lines (SeAx, MyLa, Hut, HH). We performed MTS proliferation assays and lactate dehydrogenase (LDH) assays. To analyze the metabolism of LY500307 in CTCL cells (SeAx, MyLa), HaCat cells and primary human fibroblasts we measured the concentration of LY500307 in the cell pellet and supernatant at different time points using liquid chromatography-electrospray ionization tandem mass spectrometry (LC-ESI-MS/MS).

Results: We could show that the treatment with LY500307 significantly reduced cell proliferation in all 4 investigated CTCL cell lines. Proliferation of most of the malignant cell isolates was impaired through treatment with LY500307. Yet, isolates from some patients were resistant to treament with LY500307. HaCat cells, primary keratinocytes and fibroblasts were resistant to treatment with LY500307. LY500307 concentration was significantly higher in CTCL cells compared to the concentarion measured in HaCat keratinocytes and fibroblasts.

Conclusion: Altogether, LY500307 might be a novel well-tolerated drug for the (topical) treatment of CTCL.

P207 (OP04/03) | Dissecting and targeting HLA class II-positive melanoma cell states to overcome therapy resistance

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Melanoma cells can switch between phenotypes characterised by distinct transcriptional and epigenetic states. These phenotypes range from melanocytic, proliferative states to more de-differentiated phenotypes characterised by invasive cells, less sensitive to targeted therapies with combined BRAF and MEK inhibitors (MAPKi). Thus, melanoma cells can acquire resistance to MAPKi therapy by switching from a differentiated towards a more de-differentiated phenotype.

We noted that acquired MAPKi resistance of melanoma cells is frequently associated with de novo expression of major histocompatibility complex (MHC) class II molecules. Surface MHC class II molecules present antigens to CD4+ T cells, identified recently as cytolytic anti-tumour effectors. Transcription of MHC class II is driven by the class II transactivator CIITA; interestingly some melanoma cells have been reported to express CIITA constitutively (constCIITA).

This study aims to unravel the signalling and epigenetic mechanisms leading to constCIITA expression and to exploit this potential vulnerability.

We found that in melanoma cells, constCIITA is associated with distinct dedifferentiated states with neural crest characteristics, with these cells being less sensitive to targeted therapy when compared to cells not expressing CIITA. Notably, melanoma cells under MAPKi acquire de novo constCIITA expression only when they develop resistance by switching to these specific phenotypes. Finally, using autologous patient-derived tumour-T cell models, we demonstrate that MAPKiresistant melanoma cells could be recognised by autologous CD4+ T cells, leading to pro-inflammatory cytokines release and direct cytotoxicity.
Our data suggest that tumour cell-intrinsic constitutive CIITA/MHC class II expression could be exploited to target a phenotype identified as highly aggressive and therapyresistant.

P208 | Biobanking of Melanoma Tissue for Identification and Validation of Prognostic and Predictive Biomarkers

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Tissue-based analysis of tumors is a hallmark of cellular and molecular diagnostics and allows for a multiomics spatial approach that addresses the complexity of tumorand host-specific mechanisms within the tumor micromilieu. When accompanied by blood-based analysis i.e. liquid biopsy this becomes an even more wholesome approach allowing for temporal and dynamic monitoring of cellular and molecular mechanisms and repetitive routine use. Setting-up a translational tissue biobank for specimens of cancer patients in the clinical routine presents a challenge. Here, we present a translational tissue biobanking approach, the Hamburg Melanoma Tissue Biobank, at the University Skin Cancer Center Hamburg and the Department of Pathology of the University Medical Center Hamburg-Eppendorf (UKE). Retrospectively and prospectively, we have included 455 patients with a history of malignant melanoma that received a sentinel lymph node (SLN) biopsy at our center since 2018. SLN positivity reached 20.2%. Clinicopathological factors of the associated primary melanoma such as ulceration, tumor depth or angiolymphatic invasion were shown to be of significant prognostic value for a positive SLN in our cohort. After acquiring consent from patients, the formalin-fixed paraffin embedded tissue blocks of primary tumor and associated sentinel lymph node are collected. Most of the primary resections have been performed outside of our center requiring the collaboration with over 50 different histo-pathology centers. 253 of the 351 externally excised primary tumors were collected.
In addition to an extensive follow-up data and the already established blood-based Melanoma Biobank this Tissue Biobank will allow for the validation of liquid biopsy findings on melanoma tissue samples thereby facilitating the identification and validation of prognostic and predictive biomarkers.

P209 | Keratinocytes inhibit migration of melanoma cells with low metastatic potential

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Metastatic potential of tumor cells is strongly influenced by the tumor microenvironment. Keratinocytes (KC) are the largest cell population in the tumor microenvironment of primary melanoma. They are crucial for preventing uncontrolled proliferation and dispersion of melanocytes under physiological conditions. Surprisingly, little is known about the influence of KC on melanoma cells. Here we investigate the influence of secreted factors derived from naïve KC on initiation of metastasis and migration on melanoma cells with different metastatic potential.
We found that A375P melanoma cells, derived from a primary tumor, migrated significantly slower in KC conditioned medium compared to A375P cultured in unconditioned medium. In contrast to this, for A375M2, a sub-clone of A375P that was specifically selected for its higher metastatic potential, KC conditioned medium had no effect on migration. A375P conditioned medium or heat inactivated KC conditioned medium did not reduce migration of A375P cells. We could show that KC secrete factors that adhere to the culture surface and form a layer on which cell -attachment and -spreading of A375P cells are enhanced and migration is reduced. This is most likely not mediated through transcriptional or post-transcriptional regulatory processes, since the full effect of KC conditioned medium on cell spreading occurs inside 30 minutes and is reversible within the same period of time.
Interestingly, we could show that co-culture with KC also significantly reduced migration of cells isolated from a primary tumor (A375P), while migration of cells originally isolated from metastasis (MV3 and WM9) was not altered in the presence of KC.
It is therefore possible that in order to initiate metastasis and migration melanoma cells first need to overcome the anti-migratory effect of keratinocytes in the tumor microenvironment.

P210 | Targeting melanoma plasticity to improve both targeted and immune therapy

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Despite the success of targeted and immune therapies, many patients with advanced melanoma still die due to therapy resistance. Drug resistance is a major challenge for effective melanoma therapy. Plasticity of solid cancer plays a critical role in shaping treatment response. What determines the occurrence of phenotypically distinct tumour cell domains in solid cancers is poorly understood. Utilizing in vitro and in vivo three-dimensional models, we show that in melanoma spatial

organization of plasticity is dictated by the expression and activity of the lineage-survival oncogene microphthalmia-associated transcription factor (MITF). Mechanistically, we reveal that MITF controls extracellular matrix (ECM) composition and decreases ECM organization. This leads to reduction of Rho-ROCK-myosin signalling-driven mechanotransduction through poor focal adhesion maturation and reduced contractility of the actin cytoskeleton. The resulting altered tumour microarchitecture and structural relaxation decrease tumour solid stress and subsequently p27Kip1 expression, ultimately reducing plasticity. Consequently, selective inhibition of ROCK phenocopies the effect of MITF over-expression, demonstrating the importance of cell-ECM crosstalk in this process.

In summary, our findings place tumour cell-ECM crosstalk resulting in altered tumour microarchitecture and ROCK-driven mechanotransduction as a central driver of melanoma cell plasticity. Indeed, we show that resistance to targeted therapies is often cell cycle dependent, underlining the importance of tumour cell plasticity for successful targeted therapy. Moreover, we show that structural relaxation and decreased tumour solid stress allow deeper immune cell penetration, and thus provide potential for therapeutically targeting this phenomenon for improved immune checkpoint therapy.

P211 | Overcoming immune checkpoint inhibitor resistance to improve melanoma therapy

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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 drugs such as the proteasome inhibitor bortezomib, as demonstrated in malignant myeloma and mantle cell lymphoma, but not yet in melanoma.

We show that bortezomib 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 of tumour formation 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. Importantly, immune checkpoint inhibitor-resistance was reverted by bortezomibinduced immunogenicity.

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.

P212 | Growth and adaptation mechanisms of tumour spheroids with time-dependent oxygen availability

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Tumours are subject to external environmental variability. However, in vitro tumour spheroid experiments, used to understand cancer progression and develop cancer therapies, have been routinely performed for the past fifty years in constant external environments. Furthermore, spheroids are typically grown in ambient atmospheric oxygen (normoxia), whereas most in vivo tumours exist in hypoxic environments. Therefore, there are clear discrepancies between in vitro and in vivo conditions. We explore these discrepancies by combining tools from experimental biology, mathematical modelling, and statistical uncertainty quantification. Focusing on oxygen variability to develop our framework, we reveal key biological mechanisms governing tumour spheroid growth. Growing spheroids in time-dependent conditions, we identify and quantify novel biological adaptation mechanisms, including unexpected necrotic core removal, and transient reversal of the tumour spheroid growth phases.

P213 | Modulation of the pH in the tumor microenvironment via pH-regulating liposomes to repolarize tumor associated macrophages

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Due to high tumor cell metabolism, the pH value in the tumor microenvironment (TME) is significantly decreased compared to healthy tissue. A low pH can influence the polarization of macrophages towards the immunosuppressive M2 macrophage type, which is the predominant polarization type in tumor-associated macrophages (TAM). Additionally, a low M1/M2 ratio in the TME is associated with

poor prognosis in cancer patients, for such as malignant melanoma. M2-TAM are capable to promote tumor growth through the secretion of soluble factors and the suppression of antitumor effector cells present in the TME.

To overcome the low pH value in the treatment of cancer, we designed specially adapted pH-regulating liposomes to address the low pH in the TME and to influence TAM towards a pro-inflammatory state. By releasing an encapsulated enzyme as cargo, which cleaves excretory products like urea, the pH in the TME can be raised and in turn leads to a polarization of macrophages towards the beneficial M1 inflammatory type, thus increasing anti-tumor responses and drug uptake by drugs like doxorubicin. Meanwhile, we generated different human monocyte-derived macrophages (M1/M2) and characterized them amongst others by their surface marker expression via flow cytometry. Incubation of the macrophages by different extracellular pH values confirmed that acidic conditions promote their polarization toward the M2 type. Additionally, it could be shown, that the human malignant melanoma cell line (Ma- Mel-19) produces and excretes the desired metabolic product urea, which can be transformed by the enzyme urease. The pH-regulating liposomes, which were loaded with the transforming enzyme, were able to restore the pH in the acidified cell culture medium to its initial value within 24 h, confirming the maintenance of the enzyme's activity during encapsulation.

Based on these experiments future studies will investigate the influence of the pH modulating liposomes on the cellular composition of immune cells and their antitumor functions in the TME as well as on the efficacy of distinct drugs in more detail.

To summarize, our preliminary results show that a modulation of the pH in the TME via smart carrier systems can lead to an increase of anti-tumor responses and might improve drug uptake.

P214 | PTEN reduction in melanoma cells correlates with enhanced tumor stroma modification

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Aim: The tumor suppressor PTEN (phosphatase and tensin homolog) regulates the PI3-Kinase pathway via its lipid phosphatase function. Partial loss of the tumor suppressor PTEN is a common feature during progression of malignant melanoma. However, the impact of PTEN on melanoma cell-stroma interactions is unclear. The aim of this study was to analyze interactions between melanoma cells (with welldefined PTEN status) and tumor stroma with a focus on melanoma cell-endothelial crosstalk.

Results: We analyzed a panel of melanoma cell lines for their PTEN expression level using western blot analysis and detected either complete loss (PTEN loss), partial loss (PTEN reduced) or high expression of PTEN (PTEN high). All melanoma cell lines were examined for their ability to contract a collagen gel as a marker for matrix modification. Using melanoma cell populated 3D-collagen matrices, we detected a highly profibrotic phenotype with only PTEN reduced melanoma cell lines showing strong ability of collagen contraction and stiffening comparable to myofibroblastmediated collagen contraction. Interestingly, PTEN reduced melanoma cell lines showed no clear activation of the PI3-kinase pathway but especially of the integrin- FAK-pathway with accordingly high expression of the phosphorylated FAK (pFAK). Further molecules relevant for matrix modification showed a clear association with the PTEN status. Again, only the PTEN reduced melanoma cell lines showed high expression of collagen 1, integrin beta 1 and cadherin-11, respectively. These data suggest that the PTEN reduced melanoma cells represent a highly matrix-active phenotype.

Therefore, we were interested in possible effects upon cellular elements of the tumor stroma depending on PTEN expression in melanoma cell lines. Given that matrix stiffening has been shown to influence endothelial cell functions, we established 3D-organotypic cocultures using melanoma cell populated collagen matrices and cultivated endothelial cells (HUVECs) on top. With this model system, we are currently analyzing the influence of melanoma cell lines with well-defined PTEN status on endothelial angiogenesis and on adhesion molecule expression of endothelial cells.

Conclusion: Our data indicate that PTEN reduction in melanoma cells correlates with a phenotype showing enhanced tumor stroma activation. We suggest that especially collagen modification and stiffening by PTEN reduced melanoma cells might influence tumorigenic functions like angiogenesis. We will further characterize this distinct phenotype and its relevance in melanoma progression.

P215 | The p110 alpha isoform in the PI3K pathway as a promising target for targeted therapy for BRAF mutant malignant melanoma

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In recent years, the development of new therapeutic approaches such as immune checkpoint inhibitors or the targeted use of targeted therapy has improved the overall survival rate of melanoma patients. However, the response rates of current treatment options are limited, and the emergence of resistance mechanisms to immuno-oncology (IO) therapies such as immune checkpoint blockers has become a major challenge in the treatment of melanoma. In-house research as well as studies by colleagues have shown that the PI3K/AKT pathway is deregulated in 70% of melanomas and plays a key role in the development of resistance mechanisms to targeted and IO therapies. Therefore, PI3K may be a promising target for specific inhibitor treatment. In particular, the combination of PI3K and MEK inhibitors that simultaneously target the PI3K/AKT and MAPK pathways may be an effective therapeutic option in metastatic melanoma, as shown in previous in vitro studies by us and others. In monotherapy, the pan-PI3K inhibitor BKM120 (activity against p110 alpha, beta, gamma, and delta isoforms) is able to induce growth

inhibition and apoptosis in most of the melanoma models tested to date, whereas BYL719, an alpha-specific inhibitor, has limited anti-tumor activity as monotherapy. However, both combination treatments of PI3K and an MEK inhibitor resulted in effective growth inhibition and apoptosis in the cellular melanoma models tested, exceeding the effect of MEK inhibition as monotherapy. Initial in vivo test results (in ovo) on chick chorioallantoic membrane (CAM) showed reduced tumor burden and micrometastases by using the p110alpha-specific BYL719 in combination with trametinib. In vivo results in NSG mice injected with either BRAF or NRAS mutated cell lines also showed a synergistic effect of the combination. These data show that the combination of PI3K inhibitors with MEK inhibitors could be a new therapeutic option for melanoma patients. By using PI3Kα-selective inhibitors, potential side effects could be reduced compared to pan-PI3K inhibitors.

P216 | A specialized B cell-rich tumor microenvironment delineates cutaneous T cell lymphoma from benign inflammatory skin diseases

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Cutaneous T cell lymphoma (CTCL) is a potentially fatal clonal malignancy of T cells, presenting as skin eruptions. Lack of CTCL-specific tumour markers and similarity to non-malignant inflammatory skin diseases present major clinical challenges resulting in delayed diagnosis.

Integrating gene expression of generated and mined datasets from CTCL, atopic dermatitis, psoriasis and healthy skin of 237 patients, we detected disease-specific immune subsets and patient-specific signatures of malignant cells with high intertumour heterogeneity. However, when employing similarity mapping for CTCL donors, we identified signatures shared by all tumours, including increased glycolytic activity and stress response genes. Central memory-like malignant T cell clones were present in early CTCL stages, while effector-like clones shaped a Th2-permissive tumour microenvironment in late CTCL. Surprisingly, the CTCL microenvironment was characterized by specialized fibroblast subsets with the signature of lymphoid organ fibroblasts. Accordingly, we detected intra-tumoral B cell aggregates and tertiary lymphoid structure formation, which were not present in inflammatory skin disease. Increased B cell accumulation was associated with progressive CTCL and may present a novel histological hallmark for diagnosis and staging.

P217 | Selenoprotein O: A potential regulator of melanoma metastasis

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During metastasis, melanoma cells undergo adaptations to reduction-oxidation (redox) pathways to optimize their survival; these changes are poorly understood. Metastasizing melanoma cells migrating through the blood experience high levels of reactive oxygen species (ROS), leading to redox imbalance and stress. Consequently, metastasizing cells are dependent on cellular antioxidants including selenoproteins to survive and colonize distant organs. Selenoprotein O (SelO) is an enzyme that catalyzes a post-translational modification known as AMPylation. Even though the role of SelO in AMPylation has been studied, it remains undetermined if SelO might have functional importance in melanoma metastasis. Using a CRISPR/Cas9 system, we knocked out SelO in the Yale University Mouse Melanoma 3.3 (YUMM3.3) melanoma cell line. SelO-deficiency did not alter ROS levels, oxygen consumption, or extracellular acidification rate in vitro. However, when transplanted in an immunocompetent mouse model, SelO-deficiency had no effect on the growth of primary subcutaneous tumors but reduced the frequency of circulating tumor cells and the metastatic disease burden compared to wild-type cells. In sum, these results demonstrate a potential role for SelO in promoting metastasis, further demonstrating the relevance of antioxidant mechanism in melanoma metastasis.

P218 | Strategies for PET Imaging in Acidic Tumor Microenvironments

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Data from clinical studies have shown that changes of the pH value in the tumour microenvironment can influence metastasis, EMT and the development of senescence. The extracellular pH of solid tumors is more acidic in comparison to normal tissue as a consequence of high glycolysis and poor perfusion. It plays an important role in almost all steps of metastasis.

In this study we wanted to investigate wether it is possible to quantify changes of the pH value in the tumourmicroinveroment at different time points using a non-invasive Imaging procedure. As a tracer for pH imaging, we used FDG, which is reversibly pH dependend coupled to 4Methoxybenzylamin or other macromolecules.

The aim of this study was to establish an non-invasive imaging method to show changes of the pH value In vitro and In vivo. Our findings indicate that is it possible by an 18-F-FDG-4Methoxybenzylamin-PET based imaging modality to characterize changes of the pH value In vitro and In vivo.

Miscellaneous

P219 | Searching for new drug candidates, that aid wound healing, by screening an inhibitor library

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Non-healing, chronic wounds pose an immense health risk to patients and affect their quality of life severely. In an aging society, numbers of non-healing wounds will be increasing. As current treatment options for chronic wounds are unsatisfactory, there is a high demand for research on new treatment strategies in wound healing. Therefore, we screened 141 candidates of an inhibitor library in our refined, standardized ex vivo human skin wound healing organ culture (WHOC) model. To assess the ex vivo wound healing over the course of the culture, top-view microscopy was conducted, allowing the calculation of the top-view wound area and wound perimeter. For non-invasive wound volume determination three-dimensional, depthresolved tomograms were acquired using optical coherence tomography at the beginning and the end of the culture.

The screening revealed twelve promising candidates, which accelerated wound healing by at least 15% (relative wound area treated compared to untreated wounds after seven days of culture). Next, the effect of the promising candidates on wound healing was further validated. In this validations, three inhibitors were successful: S2891, S2149 and S1180. For S1180 the effect on the top-view wound area was significant compared to solvent control.

The three successful candidates were then tested in different concentrations to find an optimal treatment strategy for our WHOC model. Each inhibitor was tested in 0.1 µM, 1 µM and 10 µM for 24 hours or continuously in 1 µM. The effect of the different concentrations was again assessed by top-view microscopy and optical coherence tomography. Moreover, at the end of the culture the skin samples were embedded, cut and stained. Hematoxylin and eosin staining allows calculation of the microscopic area and diameter as well as length and area of the newly formed epithelial tongues. Immunofluorescence staining against cytokeratin 6 (hyperproliferation) and Pecam-1 (endothelial cells) gives a deeper insight into the mode of action of the three inhibitors.

Preliminary results show especially for 10 µM S1180 a significant decrease in topview wound area and an increase in the area and length of the epithelial tongues, indicating that this inhibitor has a positive effect on wound healing ex vivo. l

Finally, we are testing the most effective concentrations of S2891, S2149 and S1180 under pathological conditions (hypoxia, high glucose levels, insulin deficiency and oxidative stress by addition of hydrogen peroxide).

With especially S1180, but also the other two inhibitors, showing a positive effect in our WHOC model, the chances are high to find a substance that aids wound healing at least ex vivo.

P220 | Skin microbiome and pH are putative predictors for severe radiodermatitis

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Radiodermatits with varying severity is commonly observed during radiotherapy in breast cancer patients. So far, neither the underlying pathogenesis mechanisms nor biomarkers for severity prediction are fully understood. In atopic dermatitis, the disturbed skin barrier is marked by a skin microbiome dysbiosis and increased skin pH.

To investigate whether these factors are relevant in radiodermatitis and useful to predict which patients are at risk to develop a severe form of radiodermatitis, an 8- weeks long pilot study with 20 postoperative breast cancer patients was performed.

From each patient, the skin pH and skin microbiome was assessed before, during and after radiotherapy on both the affected and

non-affected bodysides on a weekly basis (360 samples) and radiodermatitis severity was determined. After five to seven weeks, four patients developed severe radiodermatitis. Interestingly, a predictor for severe radiodermatits was a combination of elevated skin pH and high levels of Corynebacteriaceae before the start of radiotherapy. Moreover, an increase in bacterial colonization compared to baseline was observed on the radiated bodyside of severe patients prior to the appearance of clinical manifestations. Throughout the radiation period, the global difference in the skin microbiome between the radiated and non-radiated bodyside increased, especially in severe patients. This hints towards radiodermatitis severity-related, rather than general radiation-induced, changes in the microbiome.

In conclusion, an impaired skin barrier marked by an increased skin pH and specific skin microbiome pattern could be predictive for radiodermatitis severity. Furthermore, the increasing bacterial colonization in patients with severe radiodermatitis potentially indicates a direct impact of the microbiome on the pathogensis of inflammation. These findings are hypothesis-generating for further studies on the impact of microbiome on radiodermatitis.

P221 | Testosterone exerts expected and unexpected responses ex vivo in hair follicles from affected and non affected scalp of androgenetic alopecia patients

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Male pattern androgenetic alopecia (mpAGA), is the most common cause of hair loss in men. It is characterized by progressive loss of terminal hair in vertex and frontotemporal scalp regions (affected), whereas occipital scalp areas remain mostly unaffected. It is generally accepted that hair follicles (HFs) from affected are highly androgen-sensitive, while HFs from occipital scalp are not. In affected scalp, testosterone (T) is converted into dihydrotestosterone (DHT), which induces premature catagen entry and the progressive miniaturization of terminal HFs. The miniaturization of HFs is suggested to be driven by a reduced derma papilla (DP) inductivity, and an imbalanced emigration and restoration of DP fibroblasts, which ultimately leads to an altered hair cycle and hair loss. However, the effect of DHT on the DP has never been investigated in the target affected organ. In this study, we treated ex vivo organ cultured, terminal (t) and intermediate (i) HFs not only from affected scalp (aff), but also from occipital scalp (occ) of mpAGA patients with T. After treatment with 30 nM T for 48 h, HFs were cryosectioned, and immunofluorescence and histochemical stainings were performed, which were thereafter assessed by (immuno-)histomorphometry. We analysed DP inductivity (alkaline phosphatase in situ activity and versican expression), hair matrix keratinocyte proliferation (Ki-67 expression), and apoptosis in the dermal cup (DC; TUNEL & cleaved caspase 3 expression), as well as cell numbers in the DP, DC and DP stalk (counterstaining of Ki-67/TUNEL). Additionally, we extracted mRNA from HFs treated with 10 nM T for 24 h and performed RNAseq analysis. We found a significant decrease of alkaline phosphatase activity and a tendential decrease of versican expression in aff iHFs, indicating a reduced DP inductivity upon T treatment. Additionally, application of T significantly increased fibroblast density in the DC of aff tHFs and tendentially decreased fibroblast density in the DP of aff iHFs, suggesting the emigration of DP fibroblast to the DC. Treatment with T significantly reduced the apoptosis in occ tHFs, while a tendential increase was seen in aff tHFs. Moreover, we investigated hair matrix keratinocyte proliferation and found a significant increase in occ iHFs in the presence of T. Lastly, we examined the transcriptomic profile of HFs under T treatment. Interestingly, we found gene expression changes after treatment with T, not only in aff HFs but also in occ HFs, incl. known androgen-regulated genes (e.g. IGF1, MMP3). However, HFs from aff and occ scalp regions had distinct transcriptomic profiles. Our data are the first to demonstrate testosterone-mediated effects on DP and DC fibroblasts in the HFs from mpAGA patients. Additionally, we demonstrate that testosterone differentially impacts on i and tHFs from affected scalp. Most intriguingly we prove that occipital HFs are not androgen-insensitive, but that testosterone rather induces an anti-apoptotic responses in DC cells and increased hair matrix keratinocyte proliferation in occipital terminal and intermediate HFs, respectively.

P222 | Activation of the olfactory receptor 2A4/7 with the odorant cyclohexyl salicylate prolongs anagen and stimulates hair follicle stem cell progeny expansion

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Hair loss and thinning, as observed in aging or androgenetic alopecia, causes severe psychological stress in affected individuals, and topically applicable non-drug agents and strategies to improve hair density are highly demanded. We have previously shown that the olfactory receptor (OR) 2AT4 agonist, Sandalore®, promotes human hair growth ex vivo and reduces telogen effluvium in in vivo. These findings prompted us to investigate whether the modulation of other ORs by non-drug agents can unfold similar properties. In this study, we focused on OR2A4/7, given its reported ability to promote proliferation in epidermal keratinocytes and differentiation in

melanocytes, as well as its selective activation by cyclohexyl salicylate (CHS), a synthetic fragrant which is widely used in, for instance, cosmeceutical products. We used in situ hybridization and immunofluorescence staining to characterize OR2A4/7 mRNA and protein expression in HFs of fresh scalp skin immediately frozen after extraction. Additionally, we organ cultured microdissected hair follicles ex vivo in the presence of the OR2A4/7 agonist CHS or vehicle control and investigated effects on hair cycle as well as hair follicle stem cells (HFSCs) and progeny, by applying (immuno-)histomorphometry. Analysis of freshly embedded human scalp skin revealed OR2A4/7 mRNA expression in the HF epithelium, especially the outer root sheath (ORS) and hair matrix (HM), while OR2A4/7 protein expression was mainly restricted to the infundibulum and bulge. However, during ex vivo HF organ culture conditions, OR2A4/7 protein expression was also detectable in the proximal ORS, hair matrix (HM) and dermal papilla (DP). These findings suggest up-regulation of intrafollicular OR2A4/7 expression under tissue stress conditions. Treatment of microdissected HFs with CHS significantly downregulated OR2A4/7 protein expression in the before mentioned compartments, delayed catagen development and tendentially increased HM keratinocyte proliferation. While CHS treatment did not impact on K15+ bulge stem cells, the percentage of CD34+ cells, i.e. their immediate progeny, was significantly increased in the suprabulbar ORS. Additionally, CD71+ transit amplifying cells (thought to derive from CD34+ cells) were also increased in the HM and suprabulbar ORS. Taken together, our data demonstrate that stimulation of OR2A4/7 with CHS promotes hair growth and expands the progeny of K15+ stem cells. Interestingly, in androgenetic alopecia the capacity of HFs to generate progeny is reduced. Therefore, our data invite the use of CHS as a novel, adjuvant strategy to inhibit hair loss and thinning.

P223 | Sphingolipid metabolism orchestrates epidermal stem cell fate - potential insights into eczema initiation

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The bioactive lipid ceramide serves as an essential building block of membranes, forming a selective barrier separating the cell from the environment and ensuring subcellular compartmentalization. Emerging work implicates ceramides also in signal transmission, but the mechanisms and relevance in controlling tissue homeostasis remain unclear. In humans, eczema is the most common chronic inflammatory skin disease affecting 15-20% of children and 1-3% of adults. Although multiple new therapeutics have been developed and are currently in phase 3 clinical studies, the underlying molecular mechanism of disease initiation is not fully understood. Our previous work shows that epidermal deletion of ceramide synthase 4 (CerS4), a key enzyme synthesizing ceramides, leads to an eczema-like phenotype in mice through mechanisms that are unclear. In the current study we aim to identify the molecular and cellular mechanisms by which alterations in ceramide metabolism drive eczema initiation. Studies of the hair follicle stem cell niche in vivo and single cell RNA sequencing of the skin of CerS4-deficient epidermis point towards abnormal hair follicle stem cell activation and differentiation in CerS4-deficient epidermis. Utilizing an ex vivo 3D hair follicle organoid culture system we observe a strong growth defect of CerS4-deficient stem cells, suggesting a stem cell intrinsic CerS4- function. Our results thus indicate that lipid metabolism plays a fundamental role in epidermal stem cell homeostasis and potentially thereby in maintaining an intact skin barrier. Understanding the function of lipids in adult epidermal stem cell maintenance and fate, may, in the long run, allow tuning stem cell behavior in vivo as therapeutic tools for skin barrier diseases.

P224 | Evaluation of the relation between calcium-ions and the ammonia-ammoniumsystem of the skin surface using a Michaelis-Menten-Model

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The investigation of the functional relationship between different molecules of the skin surface in vivo may make new insights into the complex functionality of the skin surface possible. According to their relevance for enzymes such as transglutaminases or peptide-arginine deiminases known from biochemistry, assessment of calcium ions as regulator along with molecules of the ammoniaammonium- system as products in vivo might be an approach to assess enzymatic activity in the stratum corneum from the outside. So far, we have found some relations suggesting enzymatic activity using numeric models, containing no direct molecular information. Therefore, we revisited some of the data to evaluate whether the Michaelis-Menten-Model known from enzymology might reveal some more specific data.
The data of two consecutive investigations were reviewed. In one investigation, ammonia molecules diffusing from the skin surface were assessed along with the calcium ions of the skin surface. In the second study, ammonium-ions extracted from the skin surface along with the calcium ions were included into the investigations. In both studies, determination of the parameters was performed using spectrophotometric assays. In order to evaluate a relation of the data suggesting an enzymatic influence a standard Michaelis-Menten-Model-based equation relating reaction velocity with substrate concentration as independent variable was adjusted using the MATLAB® software for a curve fitting procedure. Always, calcium-ions were applied as independent variable.
The results obtained show for the investigation considering ammonia molecules along with calcium ions a certain fit according to the Michaelis-Menten-Model with a curve showing a steep slope

in lower values and a horizontal orientation with higher values. The statistical parameters describing a possible relation, however, remained rather low (R^2 = 0.12) and do not confirm clearly the Michalis-Menten-Model. The results obtained from the second investigation revealed two subsets of values, from which one also showed a steep slope in lower concentrations and a more horizontal course with increasing concentration. When fitting this subset of the data the Michaelis-Menten-Model did show a much better fit, which could also statistically be confirmed (R^2 = 0.78), while the second subset rather showed an inverse linear relation than a Michaelis-Menten-like relation.

The data analysis based on the Michaelis-Menten-Model suggests, that at least parts of the ammonium ions and calcium ions are related to each other by enzymatic activity such as it is known from enzymes such as transglutaminases or proteinarginine deiminases in the stratum corneum. The model applied in the present study might, therefore, be of use to assess enzymatic activity more specifically in vivo or at least to filter enzyme-associated information from other information for example associated with protein binding of these ions. Further studies are required to gain more data with the same study designs as well as further studies assessing other ions or molecules, which might represent enzymatic activity in the horny layer of the skin.

None

P225 | In vitro cytocompatibility analysis of a novel porcine aortic patch for vascular reconstruction

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Background: Cardiovascular diseases and their accompanying diseases are one of the most common causes of morbidity and mortality in the modern western world. Especially for severe arteriosclerotic changes, surgical intervention is required. Although the use of autologous vessels still represents the gold standard for the restoration of an appropriate blood perfusion, not every patient has the vascular status necessary for the use of autologous bypasses. A promising alternative lies in the use of xenogeneic vascular grafts, as they are characterized by a low risk of post-implantation infection and almost unlimited availability.

The aim of the present study was the analysis of a novel xenogeneic vascular patch from decellularized porcine aorta. Therefore, the focus of this research lies in the evaluation of the efficacy of the applied purification process and its resulting influence on the microstructure and cytocompatibility of the porcine vascular patch.

Material and Methods: The newly developed porcine vascular graft from decellularized porcine aorta was analyzed in vitro for cytocompatibility according to DIN EN ISO 10993-5 with regard to cell viability and cytotoxicity. In addition, a Live-Dead staining was performed to further investigate the adhesion and proliferation properties of the material. Furthermore, a histological evaluation of the vascular patch was carried out ex vivo with particular focus on the efficacy of the applied decellularization procedure and its influence on the structural integrity of the biomaterial. A conventional bovine graft (XenoSure®), already established in vascular surgery, served as control material.

Results: In the histological ex vivo analysis, the efficacy of the novel purification procedure for the removal of xenogeneic antigen-bearing structures with minimal to none negative impact to the structural integrity of the vascular patch could be confirmed. In the in vitro analyses, a sufficient cell viability with low cytotoxicity in the sense of good cytocompatibility could be observed. The novel vascular patch achieved significantly better results in the viability assay than the already established control material. Furthermore, the Live-Dead staining confirmed the adhesion-supporting properties of the novel vascular patch.

Conclusion: In summary, the results of the present study indicate the suitability of the new
decellularization process for the tissue-conserving purification of biomaterials and a
sufficient cytocompatibility of the novel porcine vascular patch.

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