ABSTRACT BOOK

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ÖGDV
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Session 1, 1

A novel xenografting mouse model to study human cutaneous γδ T cells in health and disease

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Gamma delta (γδ) T cells play important roles in the surveillance of cellular stress, tumors, and infection, that help maintain tissue integrity and modulate adaptive responses to these stimuli. γδ T cells can recognize malignant cells via surface molecules and exert killing activity upon activation. γδ T cells respond to a variety of solid and hematological tumors, and the presence of tumor infiltrating γδ T cells was the most significant favorable prognostic immune population among 39 human cancer types. In clinical trials, transfer of ex vivo expanded γδ T cells lead to temporary tumor regression and increased survival of leukemia patients. Hence, γδ T cells are promising candidates for anti-tumor immune therapeutic approaches. It is technically challenging to isolate sufficient γδ T cells from human skin, and thus most functional studies on cutaneous γδ T cell biology were one in mouse skin, which differ significantly from their human counterparts. To fill this gap and to elucidate human γδ T cell biology, we used novel methodologies to expand functional human cutaneous γδ T cells ex vivo. These expanded cells were then adoptively transferred into immunodeficient NSG mice that had received xenografted engineered human skin or squamous cell carcinoma (SCC), which histologically resembled tumors. In this model, γδ T cells engrafted in the spleen, healthy engineered skin and the tumor tissue, and we studied γδ T cell migration, maintenance, and phenotypic adaptation in these tissues in vivo. γδ T cells that were isolated from the tumor mass displayed an activated phenotype and function. This model enables functional and mechanistic studies of cutaneous γδ T cells in the tumor microenvironment. Additionally, the in vivo tumor model can be utilized to study the therapeutic potential of γδ T cells in cutaneous carcinomas, paving the way to novel anti-tumor treatments.

Session 1, 2

Cancer-associated fibroblasts shape myeloid cell response to chemotherapy- induced immunogenic signals in primary complexed tumor organoid culture

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Introduction: Patient-derived colorectal cancer organoids (PDO) have significantly advanced our understanding of cancer treatment responses. However, the absence of cells from the tumor microenvironment in current ex vivo models limits their ability to capture the complex interplay between different cell types. This study addresses the critical need for more comprehensive models by incorporating cancer-associated fibroblasts (CAFs) and primary monocytes into a primary co-culture assay.

Results: Our complex primary co-culture system, comprising PDO, CAFs, and primary monocytes as surrogate tumor-associated macrophages (TAMs), was established to investigate the interplay between tumor cells and the surrounding microenvironment. Single-cell RNA sequencing (scRNA-seq) and functional assays revealed that the presence of CAFs in the co-culture with monocytes induced this phenotypic switch characterized by the expression of major immunosuppressive markers comparable to TAMs in colorectal cancer (CRC). Notably, treatment with the chemotherapeutic agents oxaliplatin and 5-fluorouracil (5-FU) induced a polarization of macrophages towards a pro-inflammatory phenotype, mirroring the immunogenic effects observed with oncolytic virus treatment.

Conclusions: Our findings demonstrate that primary CAF-containing cultures successfully model TAM-like phenotypes ex vivo, allowing for the assessment of their functional and phenotypic changes in response to different therapies. The ability to replicate the complex interactions between cancer cells, CAFs, and immune cells in a controlled in vitro environment provides a valuable platform for investigating the dynamic nature of the tumor microenvironment.

Session 1, 3

The role of tissue-resident memory T cells in anticancer skin defenses of organ transplant recipients

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Background: Life-long immunosuppressive therapy is required for Organ transplant receivers (OTR) to prevent graft rejection. This puts them at an increased risk for keratinocyte cancers (KC), namely squamous cell carcinoma (SCC) and basal cell carcinoma (BCC)). Despite immunosuppressive therapy, not all OTR develop KC in the post-transplant period (PTP). Additionally, the common trends to have more BCC than SCC in the general population is reversed in OTR This indicates that the immune defenses against human papillomavirus (HPV) may be weakened in some patients, pointing to skin specific immune players in the protection against KC in OTR. Tissue resident memory T cells (TRM), especially CXCR3+ TRMs, are known contributors to anti-viral and anti-cancer defenses at skin level. This project aims at understanding their possible role in KC cancer prevention in OTR.

Methods: We selected a matched cohort of OTR patients, matched for age, gender, post-transplant period and immunosuppressive therapy. The first OTR cohort (OTR_A) was defined by having had more than 5 histologically confirmed KC in the PTP. The matched cohort (OTR_B) had no KC in the PTP. We obtained skin biopsies from the two OTR groups, OTR_A (n=10), OTR_B (n=10) and Healthy controls (n=10). After embedding in OCT and snap freezing, we performed cryosectioning, and immunofluorescence, with a staining for CD3, CD8, CD4, CD69 and CXCR3 to identify TRMs.

Results and Conclusion: Preliminary results show that the number of CD4+ TRMs remains the same in all patients group, in spite of immunosuppression. However, OTR patients that are protected from SCC show increased number of CXCR3 + CD8+ TRMs, which have been linked to anti-HPV response in humans. These results speak for the presence of a long-term protection against SCC conferred by CD8+ TRMs in some patients. Further studies will evaluate if the CXCR3+ TRMs are also HPV specific.

Session 2, 1

The hair canal serves as an EGFR-regulated antimicrobial gatekeeper

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Hair follicles are crucial to maintain mammalian skin barriers and protect against physical stressors of the environment. They represent a unique niche for commensal skin bacteria but unchecked proliferation of microbiota within hair follicles initiates folliculitis and fosters cutaneous dissemination of infections. This clinical implication is exceptionally displayed in cancer patients receiving anti-EGFR therapy that evokes papulopustular eruptions with concomitant bacterial Staphylococcus aureus superinfections.

In this study, we identified the specific cell population facilitating the pivotal microbial gatekeeping function of the hair follicle. We exploited single-cell datasets, deciphered epidermal growth factor receptor (EGFR)-dependent transcriptional signatures, and conducted targeted knock-out experiments in genetically engineered mice to pinpoint the significance of the EGR2/K79-positive hair canal as essential antimicrobial bastion. EGFR orchestrates the expression of antimicrobial peptides (AMPs) such as beta-defensin1/6 and SPRR1a/4 within the hair canal via the ERK signaling pathway. Notably, the presence of EGFR in fully differentiated sebocytes is expendable for the homeostatic defense mechanism of the hair follicle.

Our investigation further revealed that the identified AMP profile translates to the human skin, as AMP homologues are also concentrated in K79-expressing cells, and their overexpression is evident in EGFR-ERK-dominant psoriatic skin conditions. These findings provide crucial mechanistic insights into the microbial defense strategy employed by the hair follicle, with direct therapeutic implications for addressing folliculitis associated with EGFR-inhibitor-based anti-cancer therapy.

Session 2, 2

The impact of ER stress on gamma delta T cells in the tumor microenvironment

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γδ T cells are unconventional T lymphocytes with a role in the immune surveillance of cellular stress. γδ T cells respond to a wide range of tumors and tumor infiltrating γδ are the most significant favorable prognostic immune population among 39 cancer types. However, a suppressive tumor microenvironment (TME) can impair γδ function, and the mechanisms are not fully understood. Thus, we aim to elucidate cellular communication between tumor cells and tissue resident γδ T cells to provide a basis for the development of effective anti-tumor therapies. The TME is often characterized by increased endoplasmic reticulum (ER) stress, a cellular response to an accumulation of un-/misfolded proteins in the ER lumen that can be promoted by several environmental insults. To restore cell homeostasis, the ER activates pathways collectively known as unfolded protein response (UPR), and an enhanced UPR correlates with poor clinical outcomes in cancer patients. Crucially, the UPR can support tumor growth by regulating immune cell function. We hypothesize that ER stress is a mechanism used by tumor cells to dysregulate the activation and function of tumor-infiltrating cutaneous γδ T cells. To this end we induced ER stress pharmacologically in primary human cutaneous squamous cell carcinoma cell lines using thapsigargin and treated cutaneous γδ T cells with the conditioned medium of these cells. This induced the upregulation of activation and cytotoxic markers and concomitantly reduced the expression of NK cell receptors (NKRs) and the production of pro-inflammatory cytokines by γδ T cells. These findings support the hypothesis that cutaneous γδ T cells respond to ER stress within the TME and this modulates their activation and function. Our findings will provide important new insights into the immune surveillance and anti-tumor function of cutaneous γδ T cells under ER stress and help optimize their future clinical applications for cancer therapies.

Session 2, 3

The role of a specific S100a8/a9 positive macrophage subset in murine skin aging

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One putative key player of skin aging are macrophages, as they contribute to clearing of dead cells, thereby restoring tissue homeostasis. This study focuses on a specific macrophage subtype expressing S100a8/a9, previously implicated in pro-fibrotic processes, such as idiopathic subglottic stenosis and pulmonary fibrosis in humans.

To investigate skin aging on a transcriptional and cellular level, we conducted single-cell RNA-sequencing (scRNA seq) analysis of the back skin of 8-week, 26-week and 52-week-old male C57/BL6 mice. 3 animals were pooled per condition. Further we have used a published human scRNA seq dataset of young (n=3, <30 years) and aged (n=4, >60 years) human females for comparative analysis to our mouse data.

We identified a distinct subcluster of macrophages exclusively present in aged murine skin, termed age-associated macrophages, expressing S100a8 and S100a9 as prominent marker genes. These cells exhibited a pro-inflammatory phenotype, characterized by upregulated genes associated with responses to lipopolysaccharides and inflammation. Interestingly these cells did not cluster with the classical pro-inflammatory M1 macrophages. In addition, fibroblasts in aged murine skin showed an upregulation of various collagen genes such as Col1a1 and Col3a1. This collagen over-production was recently attributed to S100A8/A9 expressing macrophages in fibrotic tissue. In contrast to our murine data, aged human skin exhibited an increased total macrophage count, but no distinct cluster of age-associated macrophages was identified. Human macrophages, present in aged skin, displayed a pro inflammatory phenotype, more similar to classical M1 macrophages. Of note, S100A8/A9 expression was not detected in this data set.

Together, an alteration in macrophage numbers in the skin was observed in both mouse and human, yet these cells seem to differ on the molecular level. The pro-inflammatory nature of these cells in both species suggests a potential role in inflammaging. Their exact contribution to skin aging is currently investigated in ongoing experiments.

Session 3, 1

Investigating the role of the extracellular matrix to induce “pioneer melanoma cells”

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Due to its significant metastatic potential, melanoma accounts for the highest mortality rates among skin cancers. The migratory behavior of cancer cells has been shown to depend on the mechanical properties of the extracellular matrix (ECM), sensed by cellular components, such as actin-rich membrane protrusions called filopodia. Nevertheless, the mechanisms driving cancer dissemination and the interplay between cancer cell motility and the mechanical properties of the ECM remain elusive. To further understand the correlation between the mechanical characteristics of the ECM and its effect on melanoma cell migration, we compared a highly metastatic melanoma cell line with its poorly metastatic parental cell line with regard to cell morphology and migratory behavior. We used immunofluorescence staining to investigate the cell morphology on substrates of varying stiffness, and migration assays to explore collective and single cell migration. Further, spheroids incorporating both cell lines were embedded in 3D matrices and served as in vitro disease models. When seeded on a glass surface, highly metastatic cells developed a more mesenchymal-like cell morphology compared to the overall rounder shape of the parental cancer cells. Remarkably, when cultured on soft substrate (1.5 kPa), only highly metastatic cells responded with an increased round morphology, while their parental counterparts remained unchanged, indicating high mechanosensitivity of metastatic cells. Further, highly metastatic cells displayed more rapid collective cell migration, yet the migration pace of individual cells without cell contact remained similar between the two cell lines. Additionally, melanoma spheroids, comprising both cell lines, offered valuable insights into the dynamic behavior of these cells in 3D. Our findings underscore significant differences in morphology and migration between poorly and highly metastatic cancer cells, potentially contributing to tumor dissemination. Further exploration of cell behavior in 3D collagen matrices promises to deepen our understanding of cell migration and the formation of cancer metastasis.

Session 3, 2

Borrelia burgdorferi spirochetes contribute to vascular dysfunction and neurogenic inflammation in Erythema migrans.

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Background: The predominant manifestation of Lyme disease, which is caused by Borrelia burgdorferi (B. burgdorferi) spirochetes and transmitted by ticks, is the Erythema migrans (EM). The EM typically appears as a characteristic target-shaped rash and can progress to persistent skin inflammation and localized neurologic manifestations if left untreated.

Methods: This research intends to elucidate the dissemination and translocation of B. burgdorferi in EM. Additionally, it aims to observe borrelia interactions with local dermal vessels and sensory nerve fibers. For this purpose, the glycoprotein von Willebrand factor (vWF) serves as a marker of potential endothelial dysfunction, and the neuropeptide Calcitonin Gene-Related Peptide (CGRP) is used as an indicator for neurogenic inflammation and systemic neuropeptide-immune cell interplay in EM.

Results: Our findings indicate a higher concentration of B. burgdorferi within the central region of the lesion compared to the border. The majority of B. burgdorferi is proximal to endothelial vessel cells, which exhibit decreased vWF levels, suggesting compromised vascular function. Dermal nerve fiber surrounding Schwann cells, which co-localize with B. burgdorferi show elevated CGRP expression compared to those without bacterium proximity. Single-cell RNA sequencing analysis reveals increased expression of the CGRP receptor CALCRL in lesional skin compared to healthy skin, particularly within lymphocytes, dendritic cells, and fibroblasts.

Conclusions: These findings demonstrate dermal translocation of B. burgdorferi from the primary tick bite site towards the border of EM lesions and systemic dissemination via vessels, accompanied by endothelial dysfunction. Additionally, the CGRP release by Schwann cells co-localized with B. burgdorferi and the heightened CALCRL expression in immune cells suggest neurogenic modulation of the local inflammatory response to B. burgdorferi infection.

Session 3, 3

Melatonin significantly regulates arylhydrocarbon receptor and downstream signaling molecules with consecutive impact on UVR-induced inflammation, skin aging and carcinogenesis

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UV-radiation (UVR) is a known skin stressor leading to inflammation, photoaging and skin cancer. Melatonin is one of the most potent UV protective agents in the skin. The UV-mediated damaging role of the arylhydrocarbon receptor (AhR) has previously been described. However, the influence of melatonin on this UVR-activated transcription factor and related events is not known yet.
We investigated human ex vivo full skin irradiated with different UVA/B doses (0, 100, 300 mJ/cm2) at 0, 24, 48h post UV exposure and compared skin pre-incubated with melatonin with non-pre-incubated skin. Skin cryosections were analysed by immunofluorescence staining for AhR and downstream signaling molecules such as the tumor suppressor p27 and phosphorylated H2AX, a DNA double strand break marker. Furthermore, gene expression levels of AhR and p27 as well as cyclooxygenase-2 (COX-2), p38α and the metalloproteinases MMP2 and TIMP1 were analysed by real-time PCR.

UVR increased AhR positivity compared to non-UV-exposed skin with a peak at 24h post UVR which was counteracted by melatonin at all time points.
The tumor suppressor p27 was slightly decreased by UVR, while melatonin increased it after 300mJ/cm2 at 24h post UVR.
UVR enhanced pH2AX positivity, whereas melatonin prominently decreased pH2AX positivity directly after irradiation (0h post-UVR) and 24h and 48h post UVR.

Gene expression showed similar results. Melatonin lead to a downregulation of AhR by 21.2% ($p<0.01$) at 24h post 300mJ UVR. At the same time point and UV dose melatonin down-regulated p27 by 24.8% ($p<0.01$) and COX-2 by 42.9% ($p<0.001$). Further, melatonin reduced MMP2 gene expression by 8,2% ($p<0.05$) and p38α gene by 6,6% ($p<0.05$). TIMP1 expression was reduced by 10,2% by melatonin (n.s.).

In conclusion, melatonin prevents the UV-dependent activation of AhR, thus significantly reducing AhR-mediated inflammation, cellular aging and carcinogenesis on the protein and gene level in UV-irradiated human skin.

Session 4, 1

IFNAR-JAK-STAT Signaling Induces Melanoma Cell-Intrinsic PD-1 and its Blockade Disrupts Immune Checkpoint Inhibitor Efficacy

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Introduction: Melanoma is among the most common cancers worldwide, and its incidence is increasing. With the advent of PD-1 immune checkpoint inhibitor (ICI) therapies, treatment for advanced melanoma has been revolutionized. The Schatton laboratory was the first to show that PD-1 is not only expressed by T- cells, but also by melanoma cells. While regulation of T cell-PD-1 has been studied in detail, the factors governing PD-1 expression by cancer cells, including melanoma, have not been defined.

Methods: Melanoma cell lines were treated with type I interferons (IFN). Inhibition assays were performed with FDA-approved anifrolumab (IFNAR1), upadacitinib (JAK1), deucravacitinib (TYK2), ruxolitinib (JAK1/2) or STAT1/2 knockdown. Gene expression analyses were done by RT-qPCR and protein expression was assessed by flow cytometry. ATAC-seq and ChIP-seq were used to characterize at transcriptional level. Immunocompetent and -compromised mouse models were performed to investigate translational aspects.

Results: Here, we show PD-1 induction on melanoma by stimulation with type I IFNs through the IFNAR-JAK-STAT signaling pathway combined with increased chromatin accessibility of a PDCD1 enhancer region. Inhibition or knockdown of IFNAR1, JAK1/2, TYK2 or STAT1/2 significantly suppresses type I IFN-induced PD-1 gene- and protein expression on melanoma cells and ICI efficacy is disrupted in murine melanoma models, indicating an important role of the IFNAR-JAK-STAT pathway in regulation of PD-1 in melanoma.

Conclusion: Our findings reveal a molecular pathway responsible for tumor cell-intrinsic PD-1 regulation, IFNAR-JAK-STAT, and raise caution about combining PD-1 ICI therapy with JAK, STAT or IFNAR inhibitors in current common clinical use for other indications.

Session 4, 2

Targeting the hyperactive STAT3/5 Pathway in Cutaneous T-Cell Lymphoma: superior efficacy of multi-kinase inhibitor IQDMA over conventional PUVA therapy

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Introduction: Cutaneous T-cell lymphoma (CTCL), particularly its tumor stage mycosis fungoides (MF) subtype, presents a considerable therapeutic challenge, with current treatment modalities showing limited efficacy. This study aims to address the acute unmet need for novel targeted therapies by focusing on the inhibition of the STAT3/5 pathway, which is found to be hyperactive in CTCL.

Methods: Utilizing a murine model with intradermally grafted malignant T-cell lymphoma cells, we compared the efficacy of IQDMA, a multi-kinase inhibitor, with the conventional, topical psoralen (PUVA) phototherapeutic regimen.

Results: Our data show that IQDMA reduced tumor volume ($p=0.0001$) and was significantly more effective than PUVA ($p=0.0074$). Immunohistological analysis revealed that IQDMA treatment resulted in decreased CD3+ tumor cell infiltration ($p=0.03$) and induced apoptosis, evidenced by elevated cleaved-caspase-3 levels. Furthermore, IQDMA treatment led to a significant decrease in KI67+ cells ($p=0.03$), indicating a reduced rate of tumor cell proliferation. A remarkable reduction was observed in both total STAT5 ($p=0.05$) and STAT3 ($p=0.01$) levels of the infiltrated tumor cells. A positive correlation was identified between total STAT5 levels and CD3+ tumor cell infiltration, confirming the role of the STAT3/5 pathway in the disease's pathogenesis. Intriguingly, while the vehicle-treated group showed a positive correlation between phospho-STAT5 and total STAT5 levels, this correlation turned negative in the IQDMA-treated group. As IQDMA targets PAK-kinase, a nuclear transporter for phospho-STAT5, we observed a subcellular localization shifts in pY-STAT5 from the nucleus to the cytoplasm, corroborating our initial hypothesis.

Conclusions: This study bridges a critical gap in CTCL therapy, particularly for the tumor-stage MF subtype, by demonstrating the superior efficacy of IQDMA over conventional PUVA therapy in reducing tumor volume, inducing apoptosis, and attenuating the hyperactive STAT3/5 pathway. These key findings establish the properties of IQDMA as a potent targeted therapy for CTCL and offer compelling evidence for its clinical evaluation.

Session 4, 3

Targeted impacts of JAK-inhibitors during a multifaceted skin inflammation

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Janus kinase (JAK) inhibitors have been approved for the treatment of a wide range of inflammatory skin diseases including atopic dermatitis, vitiligo and alopecia areata. While efficacious, we currently lack a detailed mechanistic understanding of how they work due to their broad spectrum of potential targets in different cell types.

In a mouse model of EGFR-inhibitor-induced rash and subsequent scarring hair loss, we could recently show that the hair follicle-intrinsic JAK-STAT1 pathway is responsible for loss of immune privilege and destruction of hair follicle stem cells. Further, we could show an involvement of both microbial dysbiosis and cytotoxic lymphocytes in aggravating this stem cell loss via the IFN gamma-JAK-STAT1 pathway. Importantly, broad therapeutic JAK1/2 inhibition of all cell types in the epidermis with topical ruxolitinib treatment reduced inflammation, rescued the hair follicle stem cells and induced novel hair growth. Furthermore, JAK1/2 inhibition with oral baricitinib hindered the progression of scarring alopecia in a folliculitis decalvans patient, demonstrating the relevance of JAK1/2-driven inflammatory pathways in human alopecia.

We now performed single cell RNA-sequencing of wildtype and mutant mice during progressive hair loss that were treated with topical ruxolitinib or vehicle for two weeks. Here, we could show that JAK1/2 inhibition preferentially suppresses Type 1 and Type 2 immune responses and cell types, resulting in a shift towards a more Type 17 dominated immune landscape in the epidermis. Our study indicates that JAK1/2 signaling orchestrates a defined arm of the immune system in the context of a multifaceted inflammatory insult.

Session 4, 4

A novel human 3D organotypic skin model to study differentiation of human cutaneous T cells.

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Human skin is populated by tissue resident T lymphocytes (TRM), which are not only crucial for immune surveillance and tissue homeostasis but also responsible for various skin pathologies. However, their functional and phenotypic adaptations upon tissue entry as well as their mechanisms of maintenance are poorly understood. There are currently no suitable in vitro model systems to address these questions and in vivo studies of human cutaneous T cells are limited by technical hurdles or for ethical reasons. Here, we have established a novel reductionist 3D organotypic skin culture system, in which immortalized human skin fibroblasts and keratinocytes form fully differentiated skin and blood derived immune cells can be incorporated in these cultures. We found that skin structural cells supported the survival of antigen-presenting cells, γδ T cells or CD4+ T cells without the requirement of any additional stimulation. Further, skin structural cells also maintained the ability of CD4+ T cells to produce effector cytokines in long term cultures (> 21 days). Interestingly, upon co-culture with skin structural cells, CD4+ T memory cells (but not naïve T cells) assumed a cutaneous TRM phenotype. Specifically, effector memory T (TEM) cells that were sorted from human blood upregulated their expression of CD69, CXCR6 and PD1 more effectively than central memory (TCM) or naïve T cells, suggesting the TEM are the circulating memory T cell precursors that display the greatest aptitude to differentiate TRM in the skin. Further, we also aim to understand the basic mechanisms involved in tissue residency, which could be targeted systemically in therapeutic settings. In summary, we have generated a simple yet powerful model with a broad range of applications that can be utilized to decipher disease mechanisms in various skin pathologies.

Session 5, 1

Phenotyping of immune and non-immune cells in cutaneous sarcoidosis compared to homeostatic conditions and after mTOR inhibition

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Cutaneous sarcoidosis is an inflammatory disease characterized by the formation of granulomas consisting of organized cellular structures of immune and structural cells. Granuloma-associated cells show an up-regulation of the mTOR pathway, which is instrumental for granuloma formation as evidenced by a recent clinical study with the mTOR inhibitor sirolimus in cutaneous sarcoidosis patients.

Punch biopsies were gathered from lesional and non-affected regions before and after a 4-months treatment period with systemic sirolimus. We assessed the spatial distribution of T cells, macrophages, fibroblasts (n=10) and activated fibroblasts (FAP+) (n=3) by immunofluorescence staining. In addition, we performed FACS analysis of single-cell suspensions from skin to quantify immune and structural cells and evaluate their expression of activation markers (HLA-DR, CD86, CCR7).

Relative and absolute numbers of macrophages and T cells are increased in granulomatous tissue identified with immunofluorescence and flow cytometry ($p<0.001$). Lesional macrophages ($p<0.0001$) and T cells ($p<0.001$) show a higher expression of the chemokine receptor CCR7 and other activation markers including CD86 compared to non-lesional macrophages ($p<0.0001$). Fibroblasts exhibit an increased mean intensity of CD90 ($p<0.05$) and HLA-DR expression ($p<0.001$) in lesional compared to non-lesional tissue.
After treatment, the characteristic granulomatous structures are decreased (n=3) or completely resolved (n=4) in clinical responders to mTOR inhibition. Importantly, even if granuloma-like structures are still visible, the typical spatial organisation of cells is lost. The arrangement of fibroblasts surrounding granulomas is disrupted, leading to a dispersion of immune cells in the dermis. Fibroblast activity, evaluated by intensity of FAP+ and HLA-DR expression in non-immune cells, decreased ($p<0.05$).

We identified a higher proportion of activated cells in lesional sarcoidosis tissue. Especially fibroblasts, play an important role in providing structural integrity to granulomas. mTOR inhibition results in a loss of fibroblast activation and disruption of granuloma organisation, suggesting fibroblasts as new targets for granuloma-specific therapeutic interventions.

Session 5, 2

Diverse virus-specific tissue-resident T cells are detected in the oral mucosa of healthy volunteers

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Introduction: In healthy individuals, SARS-COV-2 infection and current COVID-19 vaccines elicit a broad immune response. A reliable, long-lasting protection is particularly based on the generation of memory T cell populations within the affected tissues. Consequently, the assessment of specific T cell subpopulations in the upper respiratory tract mucosa is of utmost importance for the evaluation of adaptive cellular immune responses.

Methods: We performed flow cytometry analysis and single cell RNA sequencing via the 10X-Genomics protocol on blood and oral mucosa samples from healthy, SARS-CoV2 recovered individuals 1 month after infection. Three donors were vaccinated against Yellow-Fever-Virus >3 years prior to sampling. Cell types were annotated based on widely accepted marker genes and Python package CellTypist. Differential gene expression and TCR receptor analysis were performed using the Python toolkits SCANPY and SCIRPY.

Results: We found that the majority of SARS-CoV2-specific T cells in blood samples were central memory T helper cells (TCM) and, in oral mucosa samples, cytotoxic tissue-resident memory T cells (TRM). While SARS-CoV2-specific mucosa T cells had a balanced ratio of Type-1 helper cells and cytotoxic TRM cells, Epstein-Barr virus- and Yellow-Fever-virus-specific cells consisted predominantly of cytotoxic TRM cells. The most diverse phenotypic repertoire including regulatory T cells was found among Influenza- and Cytomegalovirus-specific cells. The differential gene expression between SARS-CoV2-specific T cells and other virus-specific T cells enabled the distinction between gene programs involved in early and late tissue residency, as well as circulating T cells.

Conclusion: Our data provide valuable insights into the distribution of T cell subpopulations and their respective TCR-specificity in healthy oral mucosa. This ongoing project may contribute to further understanding of T cell responses at effector sites following viral infection and vaccination.

Session 5, 3

Single-cell RNA sequencing characterization of primary cutaneous B cell lymphoma reveals distinct entities

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Introduction: B-cell lymphomas (BCL) are a heterogeneous group of cancers affecting millions of people worldwide and arise from developing B lymphocytes. In contrast to systemic lymphomas, which by definition affect extracutaneous tissues, the majority of primary cutaneous lymphomas show clinically visible lesion formation only in the skin and mostly exhibit an indolent course. However, the cellular and molecular basis underlying the striking differences in clinical course and prognosis remains unclear.

Methods: We performed single-cell RNA sequencing (scRNA-seq) analyses combined with B cell receptor sequencing (BCRseq) on lesional punch biopsies from patients with primary cutaneous marginal zone lymphoproliferative disorder (MZLPD), follicle center lymphoma (FCL), and diffuse large B-cell lymphoma leg type (DLBCL-LT). The raw data were processed using CellRanger. Expression data were processed using R and Seurat. Clonality was defined using BCRseq. We compared these data to biopsies from gastric MALT lymphoma and publicly available datasets containing data from samples of systemic FCL and extracutaneous DLBCL.

Results: Our analysis showed that clonally expanded B cells in primary cutaneous MZLPD, FCL, and DLBCL-LT exhibited distinct molecular markers from their systemic counterparts. In MZLPD, clonally expanded B cells followed canonical differentiation trajectories, which were not observed in any other entity. MZLPD showed only minimal clonal expansion in contrast to gastric MALT. Similarly, clonally expanded FCL B cells were strictly limited to phenotypically normal germinal center B cells without any signs of plasticity. This is in stark contrast to our findings in gastric MALT and systemic FCL. Finally, DLBCL-LT exhibited highly aberrant B cell phenotypes, rendering this entity most similar to its non-cutaneous counterpart.

Conclusion: Our findings demonstrate distinct differences between cutaneous BCL types and their systemic counterparts in terms of B cell phenotypes and clonal abundance. These data form the basis for clear diagnostic criteria to differentiate the subtypes of primary cutaneous BCL.

Session 6, 1

A patient-derived xenograft model to validate ex vivo and in vivo gene editing in epidermolysis bullosa

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Introduction: CRISPR/Cas9-induced gene repair represents a promising tool for editing mutations in any subtype of epidermolysis bullosa (EB). EB, a genetic skin disorder characterized by blister formation, is caused by mutations in genes encoding dermal-epidermal junction proteins, which are responsible for flexibility and stability of the skin. An ongoing challenge in EB research is the small patient cohort and the limited access to patient biopsies, which are indispensable to develop new therapeutic regimens. Therefore, we aimed to establish a patient-derived xenograft model to study ex vivo and in vivo gene editing treatments.

Methods: Engineered human skin equivalents are generated on the back of NSG mice using a grafting chamber injected with a suspension of previously isolated human fibroblasts and keratinocytes. Immunofluorescence stainings were set up to distinguish not only between secreted human and murine proteins, but also between human and murine tissue in general.

Results: We successfully established a transplantation technique based on grafting a human keratinocyte and fibroblast suspension onto the back of immune-deficient NSG mice. Fibroblasts and keratinocytes were able to self-organize and differentiate to full-thickness human skin within a few weeks. We could set up a staining protocol using human and murine-specific antibodies, allowing the exact discrimination of human and murine tissue. We were able to generate recessive dystrophic EB (RDEB) and junctional EB (JEB) skins as well as skins from a healthy donor. Characterization of ex vivo-gene-edited primary JEB keratinocytes revealed auspicious protein restoration efficiencies and the accurate deposition of the restored protein in vivo.

Conclusions: We established a highly efficient, robust and reproducible humanized skin mouse model, which can be used for all subtypes of EB. This opens the way for functionality tests of ex vivo-treated skins in vivo and topical in vivo treatments.

Session 6, 2

Efficient COL7A1 repair via prime editing in recessive dystrophic epidermolysis bullosa

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Introduction: Prime editing (PE) is a relatively new, CRISPR/Cas9-based gene editing technique. In contrast to most classical gene editing techniques, PE allows for traceless correction of disease-causing mutations. This is achieved via a combination of a Cas9 nickase (Cas9n)-reverse transcriptase fusion (RT) protein and a sgRNA extended by a reverse transcriptase template (RTT).
Epidermolysis bullosa (EB) is a monogenetic skin disease characterized by extreme skin fragility. In this study, we aim for the correction of a prevalent A>G (425A>G) mutation in exon 3 of COL7A1 (C7) leading to recessive dystrophic EB (RDEB). Patients affected by this mutation show almost complete loss of type VII collagen (C7).

Methods: RDEB patient keratinocytes were nucleofected with mRNA encoding an improved Cas9n-RT fusion protein and mutation-specific prime editing sgRNA (pegRNA) and nicking sgRNA. Following treatment, patient cells were analysed via immunofluorescence (IF) staining, digital droplet PCR (ddPCR), Western blot and next-generation sequencing (NGS) analysis.

Results: IF stainings revealed the re-expression of C7 in ~90 % of patient keratinocytes. ddPCR and Western blot analyses showed increased C7 mRNA and protein levels comparable to that of healthy keratinocyte donors. Furthermore, NGS revealed no detectable off-targets as well as accurate on-target editing.

Conclusion: Our data show that PE can achieve high mutation correction efficiencies, resulting in a promising C7 restoration at DNA, RNA and protein level. In addition, our NGS data revealed precise and uniform repair pattern. In theory, it is suitable for most EB-causing mutations and can achieve high correction efficiencies upon tuning of the molecules involved. Therefore, we consider it as a highly relevant future gene editing tool for the correction of monogenetic skin diseases such as EB.

Session 6, 3

Primary cutaneous follicle center lymphoma spans yet unrecognised subtypes including polyclonal reactions

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Introduction: Primary cutaneous B-cell lymphomas are a subgroup of extranodal non-Hodgkin lymphomas divided into three subtypes: Primary cutaneous follicle centre lymphoma (PCFCL), primary cutaneous marginal zone lymphoma / lymphoproliferative disease (MZLPD), and diffuse large B cell lymphoma-leg type (DLBCL-LT). Due to the indolent nature of MZLPD, it is commonly considered lymphoproliferative disease. However the similarly indolent PCFCL is still considered a lymphoma requiring a complete staging and continuous follow-up visits to exclude systemic disease.

Methods: We obtained lesional punch biopsies from 8 patients histologically diagnosed as PCFCL and used single cell RNA sequencing (scRNA-seq) combined with B cell receptor sequencing to characterize the phenotypes of the lesional B cells. Furthermore, we collected systemic FCL scRNA-seq datasets from public databases.

Results: Surprisingly, although several thousand B cells were characterized in each sample, only four samples from PCFCL exhibited a clearly expanded clone. Furthermore, the clone was exclusively of germinal center B cells in these samples. This observation is unexpected, given our current understanding of B cell physiology, which anticipates the subsequent differentiation of B cells into memory or plasma cells. In contrast, clonally expanded B cells in systemic FL samples showed a wide range of phenotypes.

Conclusions: We observed a previously unrecognized heterogeneity of PCFCL based on the clonal expansion. Moreover, the direct comparison with systemic FL suggests that PCFCL is a distinct entity. This highlights that PCFCL is a yet insufficiently understood disease and may encompass distinct yet unrecognized subtypes.

POSTERS

P1

Selumetinib, identified by transcriptome-guided drug repurposing, with anti tumor potency against highly aggressive RDEB-SCCs

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Background: Cutaneous squamous cell carcinomas (SCCs) arising in patients with recessive dystrophic epidermolysis bullosa (RDEB) are highly aggressive and often lead to premature death. Addressing the urgent need for effective treatments, we conducted a transcriptome-guided computational drug screen, identifying Selumetinib, a MEK inhibitor, as a potential drug candidate. To substantiate the rationale for repurposing Selumetinib for the treatment of RDEB-SCC, we assessed its anti-tumor effects in vitro and in vivo.

Methods: The impact of Selumetinib on tumor cell viability and cytotoxicity was evaluated in cell-based assays. The treatment's effect on the cellular phenotype and tumor-specific pathways was analysed using Western Blot. Functional migration assays were conducted to assess the drug's impact on cell motility. Furthermore, in a xenograft model, we investigated the efficacy of Selumetinib in targeting tumor growth and assessed target engagement using sq-RT-PCR and Western Blot.

Results: In vitro, Selumetinib exerted a dose-dependent cytostatic effect on RDEB-SCC cells. Treatment with Selumetinib led to a significant reduction in ERK phosphorylation, a downstream target of MEK, in RDEB-SCC cells. Additionally, Selumetinib induced a phenotypic shift from rather mesenchymal towards epithelial characteristics, exemplified by upregulation of E-cadherin. Functionally, Selumetinib treatment attenuated cell motility, suggesting its potential in impeding tumor progression. In vivo experiments showed efficient inhibition of tumor growth without apparent toxicity. Analysis of xenograft tumors revealed reduced MAPK signaling, evidenced by decreased ERK phosphorylation and downregulation of downstream transcript biomarkers AURKA/B and DUSP6.

Conclusions: Selumetinib, identified by computational drug screening, demonstrated promising efficacy against RDEB-SCCs in vivo, suggesting its potential for clinical use. Our findings also support the transferability of generalized in silico data to specific conditions, like RDEB-SCCs. Integrating such computational methods in drug discovery holds promise to expedite the identification and clinical translation of effective treatments for rare diseases.

P2

Allele specific silencing of a missense Keratin 9 gene mutation restores intermediate filament integrity

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Epidermolytic palmoplantar keratoderma (EPPK) is a rare, autosomal dominantly inherited skin disorder, caused by heterozygous point mutations in the keratin 9 gene (KRT9), e.g. p.N161S (c.482A>G). We suggest that the integration of mutant keratin 9 into the intermediate filament (IF) network results to massive thickening of the palmoplantar epidermis.
In this work, we report a previously unrecognized mono-allelic mutation of KRT9 p.E283X (c.847G>T), generating a STOP codon without symptoms phenotypically comparable to EPPK. Therefore, monoallelic expression of wild type KRT9 should be a feasible gene-editing goal for the severe form.
We therefore tested whether a STOP codon can be introduced allele specifically into primary keratinocytes derived from patients with EPPK (p.N161S). A ribonucleoprotein-based double-nickase approach was chosen and after harvesting clones with an intact wild type allele and a frameshift-induced STOP codon on the mutated strand, we used immunofluorescence staining to demonstrate improved keratin 9 integrity, comparable to p.E283X or wild type cells. Upon heat stress 90% of p.N161S keratinocytes exhibited abnormal keratin aggregates, whereas in p.E283X keratinocytes and in gene edited p.N161S keratinocytes the frequency of keratin aggregates was low. Because gene editing poses the risk of unintended insertions/deletions or translocations we employed comprehensive CAST-Seq and NGS analyses, which did not reveal any off-target mediated translocations or mismatches. A digital modelling of IF interactions between keratin 9 and keratin 1 shows that the smaller size of serine in the mutant keratin 9 inhibits the formation of hydrogen bonds, which could be a possible explanation for the fragility of the mutant keratin 9 IF network.
Our results obtained in primary patient-derived cells transfected by carrier-free electroporation of double-nickase allele-specific ribonucleoptroteins demonstrate restoration of keratin 9 IF integrity with an excellent safety profile and major therapeutic potential.

P3

Integrative omics analysis to elucidate potential antitumor mechanisms of a ketogenic diet in melanoma

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Dietary interventions, in particular the use of a low-carbohydrate, high-fat ketogenic diet, are highly attractive approaches to target the metabolic vulnerabilities of tumor cells. Using metabolic profiling, we have recently shown that human melanoma cells engrafted into mice present distinct metabolomes partially independent of genetic driver mutations, such as BRAF and NRAS. Moreover, we demonstrated that treatment of these genetically and metabolically heterogeneous melanoma xenografts with ketogenic diet effectively reduces tumor growth. Targeted metabolomics of plasma and tumor samples revealed distinct alterations in amino acid and lipid metabolism induced by the ketogenic diet. In order to study the effect of the ketogenic diet on gene expression in melanoma and to integrate different omics data sets, we analyzed the transcriptome of xenografts. RNA sequencing revealed that ketogenic diet induced changes in gene expression in melanoma, however, distinctly throughout different melanoma xenograft models, potentially indicating that most metabolic regulations induced by ketogenic diet occur on the post-translational level. Therefore, we investigated the expression of key proteins of ketone body metabolism (OXCT1), fatty acid metabolism (ACC, CPT1, FASN), branched chain amino acid metabolism (BCKDE1A), glycolysis (HK2, LDH), and the PI3K/Akt pathway (FAK, Akt, AMPK) in the xenografts by Western blot analysis. We found that BRAF-mutant, NRAS-mutant, as well as BRAF/NRAS wild-type melanoma xenografts lack the expression of the rate limiting ketolytic enzyme OXCT1, suggesting that melanoma cells cannot utilize ketone bodies as energy substrate during ketogenic diet intervention. Moreover, ketogenic diet reduced the expression of HK2, BCKDE1A and FASN, possibly contributing to its anti-proliferative effects. Further correlation analysis of gene/protein expression levels, pathways and metabolite concentrations will help us to understand the crosstalk between cancer-related transcriptional as well as posttranscriptional regulators, metabolism and the tumor microenvironment and, potentially, to identify possible mechanisms underlying the anti-proliferative activity of the ketogenic diet in melanoma.

P4

Deciphering the crosstalk between cancer-associated fibroblasts and T cells in melanoma

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Cancer-associated fibroblasts (CAFs) are crucial elements within the tumor microenvironment (TME), showing significant diversity based on the tissue of origin. Recent studies categorize CAFs into inflammatory CAFs and myofibroblastic CAFs, characterized by distinct phenotypes and secretomes. In non-melanoma and melanoma skin cancer, further subgroups emerge: immunomodulatory CAFs (iCAFs), matrix-CAFs (mCAFs), and RGS5+ CAFs. iCAFs, expressing proinflammatory factors, are highly abundant in aggressive skin tumors and serve as the major source of chemokines in the TME, indicating that they play a role in immune cell recruitment and activation. We could show that the secretome of melanoma cells is capable of transforming healthy dermal fibroblasts into cytokine-producing iCAFs in vitro in an IL1β-dependent manner. Furthermore, we could inhibit IL-1R1 signaling in iCAFs using the IL-1R1 antagonist. We further investigated the potential of iCAFs to trigger an antitumor response examining their capacity to stimulate CD4+ and CD8+ T cell activation and proliferation. Employing a T cell activation assay involving co-culture of iCAFs and melanoma cells with naïve CD4+ and CD8+ T cells, and subsequent analysis of proliferation and T cell activation markers, we observed increased CD4+ and CD8+ T cell activation and proliferation induced by iCAFs within the first 24 hours. However, this effect diminished for CD8+ T cells after 96 hours. Examining the gene expression profile of iCAFs, with a focus on genes related to T cell activation, our results suggested that CD4+ T cell activation was likely dependent on direct cell-to-cell contact with iCAFs. Additionally, we showed that melanoma cells inhibited the proliferation of activated CD4+ and CD8+ T cells, while iCAFs sustained high levels of T cell proliferation. Altogether, these findings highlight the immunomodulatory role of fibroblasts, and provide valuable insights into the crosstalk between CAFs and T cells, which can contribute to advancing melanoma immunotherapy strategies by targeting iCAFs.

P5

MMP8 Contributes to Pulmonary Glycocalyx Derangement in Burn Injury

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Burn injuries cause significant trauma, with lung damage and respiratory failure being severe complications post-injury. Our study aimed to elucidate how burn trauma causes pulmonary glycocalyx derangement, thereby potentially exacerbating lung injury and triggering acute respiratory distress syndrome (ARDS).

Key glycocalyx constituents, including Syndecan-1 (SDC1), Syndecan-4 (SDC4), and Hyaluronan (HA), were quantified in the sera of 28 burn patients and 8 controls via ELISA. Our findings revealed that SDC1 levels were significantly elevated in burn patients from day 1, peaking on day 14, indicating a prolonged glycocalyx disruption following burn trauma. These elevated SDC1 levels showed a notable correlation with the risk of inhalation injury. Further, we identified potential mechanisms involved in glycocalyx shedding post-burn by analyzing scRNA-seq data from a murine burn-tenotomy model and human DNA microarray data from 244 burn patients and 35 healthy controls. Gene expression analysis revealed a significant increase in neutrophil activation-related genes post-burn, with matrix metalloproteinase-8 (MMP8) showing the highest fold change. This finding was corroborated by scRNA-seq analysis from murine data, revealing Mmp8's systemic presence and conservation across species in response to burn trauma. Target validation in our study cohort was achieved through Proteome Profiler Antibody Array and ELISA, which revealed an increase in MMP8 protein levels post-burn. Additionally, in-vitro treatments of 3D lung models and small airway epithelial cells with recombinant human MMP8 validated its role in SDC1 shedding, substantiating the proposed mechanism in pulmonary glycocalyx derangement.

Together, our data suggest that burn injury can lead to pulmonary glycocalyx derangement through MMP8-mediated SDC1 cleavage. This mechanism could significantly contribute to post-burn complications such as respiratory failure and ARDS.

P6

HDAC1-regulated effects of Tfh cells on B cell differentiation and function

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The skin forms the first line of defense against invading pathogens and environmental threats. Thus, processes controlled by immune cells such as CD4+ T cells have to be tightly regulated to allow for fine-tuned immune responses while maintaining immune homeostasis. Histone deacetylases (HDACs) are enzymes removing acetylation marks on lysine residues from histones and non-histone proteins. HDACs regulate CD4+ T cell development, differentiation and function. However, the role of HDAC1 in follicular T helper (Tfh) cells and the impact on Tfh-guided B cell differentiation and antibody production in skin autoimmunity has not yet been studied.

To analyze the role of HDAC1 in Tfh cells, we utilized a mouse model of CD4+ T cell-mediated skin autoimmunity (K5/TGO), in which expression of ovalbumin (OVA) in the epidermis was induced upon tetracycline administration. Adoptive transfer of naïve HDAC1-WT or HDAC1-deficient (HDAC1-cKO) CD4+ OTII T cells into K5/TGO recipient mice induced skin inflammation, associated with differentiation of effector CD4+ T cells (including Tfh). Interestingly, disease was more severe in HDAC1-cKO recipients compared to HDAC1-WT recipients.

In addition to Tfh cells, which had differentiated in skin draining lymph nodes of recipient mice as early as 6 days post-transfer, we revealed B cell differentiation into plasma cells upon adoptive transfer and also OVA-specific IgG in the serum. Direct immunofluorescent staining of skin sections indicated the presence of dermal IgG in inflamed skin of K5/TGO mice. HDAC1-deficiency in CD4+ T cells led to elevated levels of cutaneous IgG2c 40 days post-transfer compared to HDAC1-WT recipients and IgG2c levels in the skin correlated with disease severity. Therefore, Tfh differentiation and formation of antigen-specific antibodies might be key determinants of skin inflammation. We thus aim to better understand the contribution of B cells in CD4+ T cell-mediated skin autoimmunity and elucidate the role of HDAC1 and Tfh cells herein.

P7

HDAC1 as a regulator of CD4+ T cell expansion in skin autoimmunity

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The skin constitutes the first line of defense against pathogens and toxins derived from the environment. It mounts host-protective responses while maintaining immune homeostasis. These functions are fulfilled by immune cells, including CD4+ T cells. Histone deacetylases (HDACs) regulate the acetylation status of histones and non-histone proteins by removing acetylation marks on lysine residues. Hence, HDACs control several biological processes, such as differentiation and function of CD4+ T cells. However, the exact role of HDAC1 in CD4+ T cells, specifically in skin autoimmune diseases, is not well understood.
To assess the role of HDAC1 in the regulation of cutaneous CD4+ T cells, we utilized a well-established mouse model of experimental skin autoimmune disease (K5/TGO) in which ovalbumin (Ova) is expressed by keratinocytes in a tetracycline-dependent manner. We adoptively transferred HDAC1-WT or HDAC1-deficient (HDAC1-cKO) naïve Ova-specific CD4+ OTII T cells in K5/TGO/TCRα-/- recipient mice. Transferred OTII cells responded to the neo-self-antigen Ova in the skin and elicited inflammation. Recipients of HDAC1-cKO T cells displayed increased skin inflammation. This was in line with a decreased fraction of peripherally induced Foxp3+ Treg in HDAC1-cKO OTII recovered from K5/TGO recipients and with increased numbers of HDAC1-cKO T cells in the skin-draining lymph nodes (sdLNs).
Surprisingly, in a competitive setting of HDAC1-WT and HDAC1-cKO T cells, HDAC1-cKO T cells showed a disadvantage for expansion both in sdLNs and skin as well as reduced activation and skin-homing, which might indicate that HDAC1 impacts the potential of OTII to interact with antigen-presenting cells. To test this, we will study early events of T cell activation and proliferation and chemokine receptor regulation of HDAC1-cKO versus WT CD4+ T cells. With the help of already obtained scRNA-sequencing data, we hope to further understand transcriptional changes and determine HDAC1-dependent regulators of T cell expansion and TCR signaling in skin autoimmunity.

P8

Squamous Cell Carcinoma in patients with Recessive Dystrophic Epidermolysis Bullosa is associated with dysregulated T-cell function

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The skin of patients with Epidermolysis bullosa (EB) is highly fragile due to inherited mutations in genes that encode proteins with roles in keratinocyte integrity and cellular adhesion. Minimal trauma causes mucocutaneous blister formation in these patients. In patients with the severe form recessive dystrophic EB (RDEB) these wounds are commonly infected with opportunistic pathogens and often become chronic. The chronicity of the wound microenvironment seems to provide an ideal environment for the formation of highly aggressive cutaneous squamous cell carcinomas (SCC), which are one of the most feared complications in RDEB patients. We hypothesize that chronic inflammation results in T-cell dysfunction which promotes RDEB SCC tumors. Indeed, the levels of multiple cytokines (e.g.,IL-17A, IL-21, IL-22, TNF-α, GM-CSF) were significantly increased in the plasma and skin blister fluids of RDEB patients compared to healthy donors, suggesting a mixed inflammatory response. However, among skin tropic CD103+CLA+CD4+ circulating tissue-resident memory T cells only the fraction of IL-17A producing cells was significantly increased in RDEB patients with SCC compared to RDEB patients without SCC or healthy donors. scRNA sequencing further supported the notion that cutaneous IL-17 producing T cells are increased in RDEB SCC tissue compared to RDEB skin, and IL-17A-induced signature genes were enriched within a specific fibroblast cluster in RDEB SCC tumors. Interestingly, using novel 3D organotypic skin/tumor cultures we revealed that the elevated IL-17 production within the SCC tissue seems to be induced by the tumor microenvironment, supporting the notion that IL-17A is induced within the tumor tissue and further impacts the tumor microenvironment. We thus hypothesize that the altered cutaneous cytokine milieu in RDEB patients promotes the aggressive nature and growth of RDEB SCC. To test this hypothesis and elucidate cellular communication between structural cells and immune cells we are using 3D cultures and RDEB SCC xenograft mouse models.

P9

Dissecting the role of cancer-associated fibroblasts and extracellular matrix dynamics in skin cancer

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Cancer-associated fibroblasts (CAFs) exert significant influence on cancer initiation, progression, and response to therapy. The tumor microenvironment (TME) harbors multiple CAF subsets with tumor-promoting or tumor-suppressing properties. Understanding CAF heterogeneity within the TME is crucial for defining their complex roles in skin cancers. We identified two CAF subtypes which modulate tumor immune surveillance. While immunomodulatory-CAFs (iCAFs) significantly contribute to immune cell recruitment and activation, matrix-CAFs (mCAFs) encapsulate tumor nests and modulate extracellular matrix (ECM) dynamics, possibly influencing immune cell infiltration.

Employing in vitro experiments with melanoma cell lines, alongside in situ staining, we investigated the functional role of mCAFs in melanoma and non-melanoma skin cancer (basal and squamous cell carcinoma). Utilizing conditioned media from melanoma cells, we assessed the capacity of tumor cells to transform primary healthy dermal fibroblasts into CAFs. Furthermore, we examined the influence of ECM composition on CAF subset differentiation and immune cell invasion.

We observed differential expression of ECM proteins by CAFs across different skin cancer types. Intriguingly, a dense mCAF-derived ECM-network correlated with T-cell exclusion from tumor nests. Treatment of healthy fibroblasts with melanoma cell-conditioned media induced an iCAF- but not a mCAF-like phenotype, while Collagen I coatings resulted in a slight decrease of iCAF-marker expression. Invasion assays revealed increased invasiveness of peripheral blood mononuclear cells (PBMCs) into matrices composed of Collagen I mixed with Collagen IV, Laminin or Fibronectin compared to Collagen I alone, indicating that ECM composition affects immune cell marginalization.

Our findings highlight the capacity of cancer cell-derived factors to induce iCAF-like phenotypes in fibroblasts and suggest a role for ECM composition in modulating immune cell behavior within the TME. Investigating the role of mCAFs and their ECM components will enhance our understanding of immune surveillance mechanisms and could lead to novel approaches to improve responses to immunotherapy.

P10

CRISPR/Cas9-mediated COL17A1 reframing in a patient-derived xenograft model

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CRISPR/Cas9-based gene editing represents an auspicious tool for the correction of disease-causing mutations in epidermolysis bullosa, a monogenetic skin disorder characterized by blistering upon minor mechanical trauma. Junctional EB (JEB) is associated with mutations in genes encoding laminin-332, integrin-α6β4, or type XVII collagen (C17). Our aim is to restore functional C17 expression in patient-derived JEB keratinocytes employing a CRISPR/Cas9-based gene reframing strategy to override the effect of a heterozygous frameshift mutation (c.3569insG) in exon 50 of COL17A1.

We designed a mutation-specific CRISPR/Cas9-based approach. Cas9 was introduced into JEB keratinocytes as ribonucleoprotein (RNP) via electroporation or as mRNA via lipid nanoparticles (LNPs). To assess gene editing outcomes, NGS analysis at genomic and transcriptional level was performed. C17 restoration was analysed via immunofluorescence microscopy, flow cytometry and Western blot. Furthermore, the correct in vivo deposition of restored C17 was assessed in a patient-derived xenograft model.

Upon Cas9 treatment of JEB keratinocytes we obtained C17 restoration efficiencies of ~30% (RNP) and ~10% (Cas9 mRNA). NGS analysis of the COL17A1 target site revealed the presence of nucleotide deletions with a G deletion as the most frequent repair outcome. Interestingly, Cas9-induced deletions led to exon 50 skipping at RNA level possibly caused by the loss of splicing elements at the targeting site. Restored C17 was detected via immunofluorescence staining, flow cytometry and Western blot analysis. Furthermore, transplantation of ex vivo-corrected cells on immunodeficient mice showed the accurate deposition of C17 within the basement membrane zone with an increasing fraction of C17-expressing basal keratinocytes over time, indicating a proliferative advantage of gene-reframed, C17+ keratinocytes over C17-deficient JEB cells.

CRISPR/Cas9-mediated gene correction of a JEB-causing mutation led to restoration of C17 in patient-derived keratinocyte monolayers in vitro and correct C17 deposition in a xenograft model in vivo, providing a promising gene therapeutic strategy for patients with JEB.

P11

Development of an immunocompetent 3D-bioprinted skin-on-a-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.
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, prostaglandin E2 and IL-6. When CD14+ monocytes were embedded into hydrogels and differentiated by GM-CSF and IL-4, they upregulated HLA-DR and downregulated expression of CD14.
These preliminary results suggest that in hydrogel embedded moDC or monocytes 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.

P12

Genome-scale investigation of stop codon readthrough inducing drugs in model systems of severe junctional and dystrophic epidermolysis bullosa

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Background: Epidermolysis bullosa comprises a heterogenous group of rare genodermatoses with skin fragility. Despite ongoing efforts, curative treatment options are just beginning to emerge, while current disease mangement mainly focuses on symptoms relieve. Drug induced stop codon readthrough (SCR) is a promising approach that offers the opportunity for local and systemic application. Prediction and mitigation of adverse side effects during drug treatment, however, remains a major challenge.

Methods: We established a keratinocyte-based dual luciferase reporter (DLR) assay system to test putative SCR-inducing compounds in the context of the LAMB3 hotspot mutation c.1903C>T (p.R635X) at high capacity. For subsequent validation, we performed ribosome profiling (Ribo-Seq), a modern NGS technology, that allows genome-scale detection of drug-induced SCR events and perturbations to the translatome at nucleotide resolution.

Results: We used the DLR system to test a broad spectrum of SCR-inducing compounds, including aminoglycoside antibiotics, non-aminoglycoside antibiotics, and non-aminoglycoside compounds. While there was no indication of induced SCR upon treatment with ataluren or amlexanox, we observed a marked induction of SCR upon treatment with the nucleoside analog clitocine. Clitocine treatment resulted in a 30 fold SCR induction, compared to a 10 fold induction achieved with the bona fide SCR-inducer G418. In a HaCaT model system of junctional EB as well as in immortalized recessive dystrophic EB patient cells we performed Ribo-seq analysis for global detection of gentamicin-induced SCR. Surprisingly, gentamicin induced significant SCR at normal termination codons (NTCs), but at the same time failed to induce detectable levels of SCR at premature termination codons (PTCs).

Conclusion: We established a system for high throughput screening of SCR-inducing compounds in keratinocytes and identified the nucleoside analog clitocine as a potent SCR-inducer in the context of EB. Gentamycin-mediated induction of global SCR at NTCs may explain the frequently observed side effects that result from its clinical use.

P13

Revealing long-lasting epigenetic alterations: the impact of chronic TGF-β exposure on fibroblasts from patients with recessive dystrophic epidermolysis bullosa

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Introduction: Epidermolysis bullosa, a monogenic skin condition marked by severe skin fragility, displays diverse phenotypic variations even among individuals sharing identical genetic defects. Previous studies highlighted the potential correlation between transforming growth factor beta 1 (TGFB1) signaling and disease severity in recessive dystrophic epidermolysis bullosa (RDEB), as observed in twins having the same mutation but diverse severity. In this study, we aimed to replicate conditions resembling severely affected skin by continuously stimulating RDEB fibroblasts with TGFB1 and analyze the resulting long-term changes to identify potential modifiers influencing disease expressivity in RDEB.

Methods: RDEB fibroblasts were treated with TGFB1 for four weeks and maintained in culture for additional four weeks post-treatment; at both timepoints DNA, RNA, and, protein samples were collected. DNA was bisulfite converted and loaded onto Illumina's Infinium EPIC bead chip array for DNA methylation analyses. RNA samples underwent external RNA sequencing. Differential methylation (differentially methylated CpGs [DMCs]) and gene expression (DEGs) were assessed and integrative analyses showed inverse correlations between DMCs and DEGs. Further validation studies of targets were performed, including sqRT-PCR, Western Blot, and immunofluorescence.

Results: Persistent TGFB1 exposure significantly changed the methylation pattern and transcriptome. Filtering genes based on correlated methylome and RNA-seq data identified 221 genes with significant upregulation and hypomethylation, while 165 genes showed downregulation and hypermethylation. We further focused on epigenetic changes that persisted even after TGFB1 withdrawal and found 15 genes that remained deregulated. EnrichR analysis revealed pathways and ontologies like focal adhesion, extracellular matrix interaction, syndactyly, or abnormal skin morphology. Notably, the targets GREM1 and PMEPA1 were further analyzed in correlation with a more severe prognosis.

Conclusion: TGFB1 treatment of RDEB fibroblasts induced alterations in the methylome and transcriptome. The study identified persistent epigenetic changes even after cytokine withdrawal, providing insights into potential therapeutic targets for managing severe cases of RDEB.

P14

Disrupted keratinocyte differentiation and increased chemokine and antimicrobial peptide expression in palmoplantar keratoderma due to keratin 9 mutation

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

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

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

P15

Psoriasis Phototherapy Treatment Adherence: Exploring the Role of Disease Severity and Treatment Alternatives

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Background: Phototherapy is a highly effective treatment for psoriasis. However, the factors influencing phototherapy treatment adherence remain to be determined.

Methods: We conducted an observational, retrospective cohort study using data from the Phototherapy Registry Graz and the Austrian Psoriasis Registry (PsoRA). Phototherapeutic adherence, patient characteristics, and disease severity were analysed for 735 psoriasis patients (05/1997 – 12/2023), irrespective of type or number of UVB and/or PUVA phototherapy cycles. Adherence rates were determined from the initiation of phototherapy to the end of follow-up or discontinuation of treatment due to lack of efficacy and/or prescription of systemic agents such as oral small molecules and/or biologics.

Results: The overall 3-year phototherapy survival rate was ~63% (95% confidence interval, 61–52%). Palmoplantar pustulosis (hazard ratio [HR] 1.80, $p < 0.001$), arthritis (HR 1.49, $p < 0.05$) and palmar and/or plantar plaque psoriasis (HR 1.34, $p < 0.05$) were significantly associated with reduced phototherapy adherence. On the other hand, gender ($p = 0.56$), nail involvement ($p = 0.44$), scalp involvement ($p = 0.14$), inverse involvement ($p = 0.39$), disease duration ($p = 0.33$) and patient age ($p = 0.1$) did not exhibit statistically significant impact on treatment adherence. The introduction of highly effective systemic treatment alternatives such as biologics was associated over time with a significantly lower phototherapy adherence (HR > 1.8, $p < 0.001$).

Conclusion: Phototherapy demonstrates comparable adherence rates to biologics, but its continuous utilization is influenced by the availability of systemic treatment options. The disease characteristics, particularly palmoplantar pustulosis, arthritis and palmar and/or plantar plaque psoriasis, should be considered when selecting phototherapy for psoriasis treatment.

P16

The p-rpS6-zone delineates wounding responses and the healing process

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Introduction: The spatial boundaries of tissue response to wounding are unknown and difficult to visualise. We have identified a novel wound marker: the ribosomal protein S6 (rpS6) is phosphorylated in response to skin injury forming a zone of activation enveloping the initial insult.

Method: The zone encapsulates markers of the healing process, including proliferation, angiogenesis and senescence, which was confirmed with immunohistochemistry. This wound response is global: using a model of skin injury in pig, mouse and human ex vivo skin we were able to confirm that the wound response is conserved in mammals, and shows up in response to various injuries (burns, excision wound, needle prick). A transgenic mouse model unable to phosphorylate rpS6 shows an initial acceleration of wound closure, but results in disrupted healing, confirming an important role for p-rpS6 in healing.

Results: This p-rpS6-zone forms immediately after wounding and is present until healing is complete. Along with its role in healing, the zone is an ideal wound marker as it correlates with healing progress over weeks and accurately reports on the status of dermal vasculature.

Discussion: The p-rpS6-zone is a promising diagnostic tool which divides an otherwise homogeneous tissue into regions with different properties, clearly separating tissue undergoing a wound response from unaffected tissue.

P17

BRAF inhibitors differentially affect vascular endothelial cell-cell junctions

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Melanoma often bears BRAF mutations, which contribute to uncontrolled proliferation and survival of tumor cells. Targeted therapies inhibiting mutant BRAF or its downstream effectors are an effective treatment to suppress this pathway in patients. However, treatment success is impaired by resistance and adverse events, such as rash, pyrexia, diarrhea and edema. Endothelial cells are likely to take part in these events, as they line the innermost layer of blood vessels and come in contact with high plasma levels of the inhibitors. However, data that describe the effect of BRAF inhibitors on endothelial cells are missing. Thus, we aimed to characterize off-target effects of different clinically approved BRAF inhibitors (BRAFi) on the vascular endothelium. We found that cell-cell junctions of human primary dermal microvascular endothelial cells were visibly disrupted by Vemurafenib and the next-generation dimerization inhibitor PLX8394 in a dose-dependent manner. This is consistent with our findings, that endothelial permeability for fluorescent tracers was compromised upon treatment with the same BRAFi. We assessed markers of endothelial cell-cell junctions in archived skin biopsies from melanoma patients before and during BRAFi therapy. Three patients who received Dabrafenib/Trametinib showed similar levels of junction markers before and during treatment. Vemurafenib monotherapy was associated with a decreased signal from cell-cell junctions in blood and lymphatic vessels of one patient. Together, these findings provide insights on the differential effects of BRAFi on endothelial junction architecture and barrier integrity in cell culture and in patient samples. This knowledge could inform future studies on the development and application of targeted therapies for cancer patients.

P18

Single-cell responses to mTOR inhibition in patients with granulomatous disease

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Introduction: Granulomatous diseases are distinguished by the presence of granulomas, organized clusters of immune and structural cells, which participate in an aberrant immune response. The mTORC1 (mechanistic target of rapamycin complex 1) pathway, which regulates processes like metabolism and autophagy, is implicated in granuloma formation and maintenance.
Recently, we conducted a clinical trial using sirolimus, an mTOR inhibitor, to successfully treat cutaneous sarcoidosis, a granulomatous disease. Skin and blood samples from different timepoints of the trial were assayed by single-cell RNA sequencing and spatial transcriptomics to uncover transcriptional changes in the tissue upon mTOR inhibition and underlying patterns which determine treatment response.

Methods: Scanpy and scvi-tools software packages were used to process the sequencing data and scCODA and Milo were used for compositional analysis. Differential expression analysis was performed using limma after clustering of the dataset.

Results: We find all expected immune and structural cell subsets of the skin in our transcriptomics data as well as distinct disease-specific cell populations with significant changes in abundance throughout treatment. Various cytokines and chemokines as well as transcriptional regulators were found to be differentially expressed by macrophages and T cells between treatment conditions. Cell populations enriched after treatment showed a notable decrease in several metabolic pathways and an increase in longevity-reated genes. Comparison of responders and non-responders revealed differences in pathways related to metabolism and immunity, such as reduced transendothelial migration of leukocytes and antigen presentation in responders.

Conclusions: The cell composition and expression profile of granulomatous tissue appear to change markedly after mTOR inhibitor treatment. As expected, mTOR inhibition results in changes to metabolic pathways, but also immune function is strongly affected. Additionally, by disentangling differences between responders and non-responders we aim to predict response of future sarcoidosis patients.

P19

Establishment of an in vivo RDEB-SCC humanized xenograft mouse model to study the effect of IL-17A on tumor progression

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Epidermolysis bullosa (EB) is a group of rare genetic skin disorders with mutations in genes responsible for mucocutaneous structural integrity. Even minor mechanical insults result in blistering of skin and mucous membranes. Most patients with recessive dystrophic EB (RDEB), a severe form of EB develop Squamous Cell Carcinomas (SCC), which are highly aggressive in nature. SCC in EB patients typically arise in inflamed chronic skin wounds. We thus hypothesize that cutaneous inflammation is promoting SCC tumors. In line with this notion, we found that the levels of IL-17A were significantly increased in RDEB patients compared to healthy controls both systemically (in plasma) and locally (in skin blister fluids). Additionally, the fraction of IL-17A producing skin tropic CD103+CLA+CD4+ circulating tissue-resident memory T cells (cTRM) was significantly increased in RDEB patients with SCC compared to EB patients without SCC or healthy controls. The observed elevated amount of Th17 skin-resident T cells in SCC patients was further supported by an increased expression of IL-17A mRNA by T cells in SCC tissue compared to RDEB patient skin (in scRNA sequencing). To further elucidate the role of IL-17A in regulating the growth of RDEB SCC, we developed a human RDEB-SCC xenograft mouse model. Specifically, we injected primary RDEB-patient derived SCC cells (along with patient-matched cancer associated fibroblasts) into the skin of immunodeficient NSG mice, which lead to the formation of solid cutaneous SCC tumors that share histological features with SCC of RDEB patients. This in vivo RDEB-SCC mouse model can be exploited to study the effect of IL-17A on tumor growth. We further plan to utilize the model to study the efficacy of IL-17A neutralization as a novel approach to treat the highly aggressive RDEB-SCC in patients.

P20

Gamma delta T cell and macrophage interplay within the ER stress tumor microenvironment

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γδ T cells are T lymphocytes which combine an adaptive potential, by recognizing MHC related molecules through their TCR, with a rapid innate response upon recognition of self- and non-self antigens. γδ T cells exert anti-tumor immunity against a wide range of solid and hematological tumors. However, γδ T cells can also have a pro-tumorigenic function, depending on the tumor microenvironment, but the mechanisms behind these contradictory functions are not fully understood. It is well established that endoplasmic reticulum stress (ER stress), an accumulation of un-/misfolded proteins in the ER lumen, promotes tumor growth and alters the function of several immune cell types. Treatment of γδ T cells with conditioned media of ER stressed cutaneous squamous cell carcinoma (SCC) cells induced the upregulation of genes that are involved in monocyte recruitment and macrophage differentiation. Moreover, ER stressed conditioned γδ T cells released increased amounts of IL-4 and IL-10, which can induce M2 macrophage polarization. Interestingly, one of the major components of tumor infiltrating leukocytes are tumor associated macrophages (TAM), which promote tumor growth and angiogenesis by their strong M2-like signature. Additionally, the tumor microenvironment is associated with ER stress. We thus hypothesize that ER stress within the tumor microenvironment diverts the function of γδ T cells, which in turn affects the differentiation of macrophages towards a pro-tumorigenic function. So far, no studies have been performed on the interplay between γδ T cells and macrophages in the tumor microenvironment. Here, we aim to investigate the role of tumor-derived ER stress conditioned γδ T cells in macrophage differentiation and polarization into M1 or M2 phenotype. This study will give us new insights on the role of γδ T cells in regulating the macrophage anti-tumor response in the context of an ER stress microenvironment, which can be targeted for future cancer immune therapies.

P21

Investigating cellular crosstalk in non-infectious granulomas of the skin

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Granulomas are aggregates composed of immune and structural cells that cause chronic inflammation and tissue fibrosis, eventually leading to organ failure. Most often granulomatous inflammation is studied in the context of infection. However, non-infectious granulomatous inflammation - as found in sarcoidosis - is a cause of worldwide morbidity and mortality in both adults and pediatric patients.
While granulomas form as a dysregulated response of activated immune cells, such as macrophages, we lack a mechanistic insight into disease regulating targets, which is hindered by the inability to faithfully emulate non-infectious granulomas in vitro and in vivo.
Using a combination of unbiased system-wide single-cell transcriptomic analyses of skin biopsies from sarcoidosis patients, we recently found that macrophages and fibroblasts jointly contribute to a granuloma-specific microenvironment.
In order to understand the local and spatially coordinated immunoregulatory relationships between structural- and immune cells, we now conduct spatial transcriptomics with subcellular resolution and established an in vitro human granuloma model. The model is based upon spheroids of defined primary sarcoidosis cell subsets, namely primary macrophages and fibroblasts. This enables us to recapitulate key aspects of non-infectious granulomas encompassing the complex genetic and spatial composition of tissue granulomas.
Importantly, due to the incorporation of fibroblasts, the model accounts for the contribution of stromal cells in granulomas. Since fibroblasts are known to recruit and differentiate macrophages in granulomatous diseases, we are thus able to differentiate between the cell autonomous versus the non-cell autonomous effect on dysregulated macrophage activation in non-infectious granulomas.

P22

Antisense oligonucleotide-mediated splicing modulation in recessive dystrophic epidermolysis bullosa

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In this study we investigated the potential of short 2'-O-(2-Methoxyethyl) oligoribo-nucleotides (2'-MOE ASOs) to modulate splicing pattern within cells derived from patients afflicted with recessive dystrophic epidermolysis bullosa (RDEB). These patients have a common splicing mutation (c.425A>G) in COL7A1, encoding type VII collagen (C7), which is crucial for the formation of anchoring fibrils that tether the epidermis to the underlying dermis. Mutations in COL7A1 lead to dysfunctional or absent C7, resulting in severe blistering and wound formation upon minor mechanical trauma. The severe nature of RDEB underscores the urgent need for innovative therapeutic approaches. The c.425A>G variant negatively affects splicing at the COL7A1 exon 3/intron 3 junction, ultimately leading to the production of non-functional C7 transcripts. Despite this disruption, we observed residual levels of accurate splicing, indicating a potential avenue for therapeutic intervention.
Therefore, we transfected RDEB keratinocytes with a wide range of 2'-MOE ASOs binding in a region ranging from exon 3 to the intron 3/exon 4 junction of the COL7A1 gene. ASOs capable of enhancing the amount of correctly-spliced COL7A1 transcripts were identified by sqPCR, RT-PCR and ddPCR and were further validated at cellular level via immunofluorescence staining. In addition, the most promising ASO was applied on RDEB-derived skin equivalents, resulting in an augmentation of full-length C7 expression and its accurate deposition along the basement membrane zone (BMZ). This promising outcome underscores the therapeutic potential of splicing modulation via ASOs. A minor increase of C7 levels within the patient's skin is probably sufficient to partially revert the disease phenotype in RDEB.

P23

Single-cell transcriptomics unravel cellular heterogeneity in epidermolysis bullosa

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Introduction: Recessive dystrophic epidermolysis bullosa (RDEB) is a rare genetic skin disease characterized by repeated cycles of wounding, fibrosis, inflammation and infection, that promotes the development of life-threatening skin cancer in early adulthood. To map the complex and manifold processes that drive chronically-damaged skin to malignant transformation, we sought to build an integrated transcriptomic single cell atlas using patient samples representing various phenotypic stages of the disease.

Methods: We collected 33 samples from 8 different RDEB patients and 3 healthy volunteers that included skin, fibrotic/wounded tissue, wound bandage cells, tumor tissue and blood. In total, the transcriptomic profiles of 171,481 single cells were used to build an integrated map of various cell populations in RDEB.

Results: Our integrated dataset encompasses 11 major cell clusters including both structural and immune cells. RDEB skin cell composition appears to be dominated by immune cells, with fewer keratinocytes compared to heathy donor skin. These data are consistent with recent data from the hypomorphic Col7a1 RDEB mouse model. Further unsupervised subclustering of fibroblasts and neutrophils based on their differential gene expression signatures allowed us to discern distinct subpopulations e.g. myofibroblasts vs inflammatory fibroblasts subsets. Moreover, we could observe a shift in the abundance of an activated neutrophil subset and inflammatory cancer-associated fibroblast cluster according to disease state, particularly in fibrotic and SCC tissue. Notably, the neutrophil cell population exhibits gene signatures associated with S. aureus infection, TNF-alpha signalling and NET formation, mechanisms known to shape the tissue microenvironment into a pro-tumorigenic state.

Conclusions: Our data confirm and contribute to previous findings, validating the EB Cell Atlas as a unique and meaningful tool to interrogate molecular and cellular drivers of disease progression that may be amenable for future drug development.

P24

Transglutaminase 2 deficient sebocytes display disturbed autophagy and lipid secretion.

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The main cell type situated in sebaceous glands (SG) are sebocytes, which produce sebum in a terminal differentiation process called holocrine secretion. The lipids contained in the sebum contribute to physical and chemical homeostasis of hair and skin. An imbalance in SG lipids resulting from disturbed sebocyte differentiation is however implicated as actively contributing to skin diseases such as acne and pemphigus foliaceus.
A protein with a potential role in sebocyte differentiation and cell integrity is transglutaminase 2 (TGM2). Lack of TGM2 was recently indicated for affecting differentiation, autophagy and thereby lipid composition in sebocytes. This study aims to investigate if TGM2 deletion affects the composition of SG lipids and if oxidative stress introduced in the form of oxidized phospholipids impacts autophagy in sebocytes.
HPLC-MS/MS epi-lipidomics showed increased phospholipid hydroperoxide levels in extracts of TGM2 knockout cells as compared to wildtype cells and increased aldehydophosphate lipid levels in cell lysates as well as supernatants of TGM2 knockout cells. SZ95 cells were subjected to oxidative stress, and the TGM2 knockout cells showed a decreased LC3II/I ratio as well as an accumulation of high molecular weight P62, indicative of impaired autophagy induction and -flux.
Our initial data suggests elevated redox stress and an impairment of autophagic flux in SZ95 TGM2 knockout cells resulting in increased levels of potentially proinflammatory lipid mediators being secreted from TGM2 defective sebocytes.

P25

Unraveling the role of CD4+ tissue-resident memory T cells in cervical dysplasia

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Human papilloma virus (HPV) is the most common sexually transmitted pathogen with approximately 90% of sexually active individuals being infected during their lifetime. Although the infection is efficiently resolved by the immune system in most cases and vaccination protects reliably, persistent HPV infection can cause a variety of mucosal (pre-)cancerous lesions. We could show that the number of CXCR3+CD4+ tissue-resident memory T cells (TRM) in barrier tissues is important for the development of HPV-associated anal high-grade squamous intraepithelial lesion (HSIL). As previous studies focused mainly on CD8+ T cells in HPV immune response, we aim to investigate the CD4+ T cell compartment in cervical and anal HSIL.
We recruited HIV negative women with HSIL and women undergoing hysterectomy for benign reasons to obtain cervical biopsies and PBMC. TRM were profiled by flow cytometry and immunofluorescence staining. Samples from HPV lesions show reduced numbers of TRM compared to healthy cervix samples. This effect was especially pronounced in the CD4+ TRM compartment. While CD103+ TRM were present in similar levels in HSIL and control samples, we observed a selective reduction of CD69+ TRM. Additionally, we detected lower numbers of CXCR3-expressing CD4+ TRM. As it is still unclear if the CD69-expressing TRM subset is depleted or if only the marker CD69 is downregulated, we plan to establish an in vitro model of infected cervical epithelial cells and T cells.
CD4+ TRM in the cervical mucosa seem to be essential during the immune response against HPV. We aim to determine if patients with low numbers of CD69+ TRM are at higher risk for dysplasia or if loss of CD69+ TRM occurs because of dysplasia. We will assess TRM in HSIL patients before and after receiving local imiquimod treatment to shed light on the role of CD69+ TRM in HPV clearance in responders and non-responders.

P26

A pilot study to investigate the influence of long-term metformin treatment on wound healing and cutaneous squamous cell carcinoma development

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Introduction: Patients with recessive dystrophic epidermolysis bullosa (RDEB), a severe hereditary skin fragility disorder, experience repeated wounding and inflammation, which promotes the development of aggressive cutaneous squamous cell carcinoma (cSCC) in wounded areas. Since this life-threatening cancer leads to an increased risk of death of 76 % by the age of 45, strategies for prevention or delay of cSCC development that do not interfere with wound healing are needed.

We previously investigated the anti-neoplastic effect of the anti-diabetic drug metformin in in vitro and in vivo models of fast-growing cSCC. Our data showed that acute metformin treatment appears to have an anti-tumorigenic effect on aggressive cSCC. However, according to recent literature, long-term treatment with metformin may reduce tumor development more effectively than an acute treatment.

Methods: The effect of metformin treatment (5 mg/ml administered topically or in the drinking water for the entirety of the study) on wound healing was investigated in two rounds of wounding using a medical skin biopsy punch. Wounds were photographed and analysed using ImageJ. Following wound closure, mice were challenged with the syngeneic SCC-VII tumor line in previously-wounded vs. never-wounded skin. Tumor development and general health of mice were monitored regularly.

Results and conclusions: Metformin treatment appeared to slow individual wound closure rates in the first week after wounding. Nevertheless, >85 % of all wounds in each cohort were completely healed within 14 days. We observed no difference in tumor onset across the different settings, but observed a tendency for more rapid growth in tumors developing in previously-wounded skin. Comparing tumor growth in never-wounded skin, we observed a tendency towards increased survival in oral metformin treated mice compared to placebo. The molecular events underlying these observations need to be investigated in order to determine the potential clinical impact to patients.

P27

Deciphering the Role of Immunogenic Cell Death in Extracorporeal Photopheresis

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Extracorporeal photopheresis (ECP) is a photoimmunotherapy providing both immunity against dysfunctional T cells and suppression of immune reactions. Although it has been successfully used in the clinic for over three decades, the mechanism of action is not entirely clear. We found indications that immunogenic cell death (ICD) might play a role in the mechanism of action of ECP in cutaneous T cell lymphoma (CTCL) patients, however, ICD in other ECP-treated diseases, such as solid organ transplantation, graft versus host disease (GvHD), and systemic sclerosis (SSc), is yet to be explored.

The induction of ICD-associated danger signals was investigated in solid organ transplantation, GvHD, as well as SSc patient samples that received ECP treatment. Samples were incubated for up to 72 hours without additional immunostimulation. ICD-related danger signals (calreticulin, HMGB1, IFNα2, etc.) were investigated using multiplex FACS analysis in different cell populations and qPCR.

In our cohort of ECP-treated patients we found significantly increased expression of calreticulin in B and natural killer (NK) cells post compared to pre ECP. Remarkably, in T cell subsets only solid organ transplantation patients showed relevant changes in ICD signal. Interestingly, bulk qPCR displayed an increase in various ICD signals post compared to pre ECP for all indications except SSc.

Proving the induction of ICD in various ECP-treated diseases grants a new perspective for elucidating the bimodal mechanism of action of ECP. This might pave the way towards a better understanding of the pathogenesis of the so far not well understood diseases.

P28

The impact of the tumor micromilieu on gamma delta T cell function in aggressive squamous cell carcinoma in Epidermolysis bullosa

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Epidermolysis bullosa (EB) is an inherited skin disorder, characterized by mucocutaneous fragility and blister formation upon minimal trauma. Patients who suffer from the severe form, recessive dystrophic EB (RDEB), often develop highly aggressive cutaneous squamous cell carcinomas (SCC). SCC in RDEB patients has a higher morbidity and mortality compared to SCC in patients without RDEB, although they share similar driver mutations. The patho-mechanisms are still largely unknown and currently there is no effective therapy available. Therefore, we analyzed whether the aggressive nature of SCC in RDEB patients is associated with a dysfunction in tumor immune surveillance. Gamma Delta (γδ) T cells display anti-tumor functions and the presence of tumor infiltrating γδ T cells was the most significant favorable prognostic immune population among 39 human cancer types. We discovered that the fraction of IFN-γ producing cells was reduced among circulating and tumor infiltrating γδ T cells isolated from RDEB patients with SCC. Based on these results, we hypothesize that γδ T cells are dysfunctional, and that this dysfunction is mechanistically linked to the formation of SCC. Whereas γδ T cell function has been studied in various skin cancer entities, their role in regulating the growth of the uniquely aggressive SCC in RDEB patients has not been investigated. To fill this knowledge gap, we will mechanistically dissect how the SCC tumor microenvironment modulates the function of γδ T cells in RDEB patients. We will utilize our innovative and unique in vitro 3D organotypic skin and in vivo tumor xenografting models to elucidate the cellular communication between γδ T cells and tumor cells. This study will provide comprehensive and mechanistic insights on the anti-tumor function of γδ T cells in RDEB patients with SCC. Furthermore, our results will contribute to the development of effective immuno-therapies against the highly aggressive SCC in RDEB patients.

P29

Exploring the impact of metabolic inhibitors in melanoma in vitro

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Although immunotherapy and targeted therapy have revolutionized the treatment of advanced melanoma, there is still need for improved treatment options. A possible approach could be combination of established treatment alongside metabolic inhibitors to target tumor metabolism. Therefore, we aimed to elucidate the effect of 8 promising metabolic inhibitors impeding mitochondrial oxidative phosphorylation (OXPHOS), glycolysis, glutaminolysis or lactate transport in human BRAF-mutant A375 and WM47, NRAS-mutant WM3000, and BRAF/NRAS wild-type WM3311 melanoma cells. Human dermal fibroblasts and human embryonic kidney 293 cells were serving as non-cancerous controls. Cells were treated for 72 hours with various concentrations of IACS-010759 (complex I inhibitor), ONC212 (complex I and II inhibitor), atovaquone (complex III inhibitor), DX3-213B (complex I inhibitor), 2-deoxyglucose (2-DG; hexokinase inhibitor), dichloroacetate (DCA; pyruvate dehydrogenase kinase inhibitor), CB839 (glutaminase inhibitor) and diclofenac (monocarboxylate transporter (MCT) inhibitor) and cell proliferation was quantified using the CyQUANTTM assay. The effect of CB839 and DX3-213B on respiration was assessed using the Seahorse XFe96 analyzer. IACS-010759, ONC212 and DX3-213B as well as CB839 suppressed melanoma cell proliferation at nanomolar concentrations, although sensitivity varied between melanoma cell lines. Nevertheless, proliferation of human dermal fibroblasts was not affected by these compounds. In contrast, the administration of 2-DG and DCA as well as diclofenac also inhibited proliferation of control cells, suggesting general toxicity. Combining the two most promising inhibitors CB-839 and DX3-213B displayed additive effects on growth inhibition. Moreover, CB-839 and DX3-213B reduced respiration in BRAF-mutant A375 melanoma cells. Our data indicate that response to metabolic inhibitors in melanoma cell lines differed independently of their mutational genotype, most likely attributable to heterogeneity of adjunct metabolic phenotypes. Combination of metabolic inhibitors impeding multiple metabolic pathways at once might tackle this issue. Taken together, combating tumor metabolism may require patient-specific approaches, due to the observed heterogenic responses.

P30

Visual explainability of 250 skin diseases viewed through the eyes of an AI-based, self-supervised Vision Transformer – a clinical perspective

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Background: Conventional deep learning approaches mostly focus on a small range of skin diseases. Recently, self-supervised Vision Transformers (ViTs) have emerged, capturing complex visual patterns in hundreds of classes without any need for tedious image annotation.
Objectives. This study aimed to form the basis for an inexpensive and explainable pipeline targeted at the vastness of clinical skin diagnoses: By comparing a self-supervised and supervised ViT's inner-visualizations on 250 skin diseases.

Methods: Using a public dataset containing images of 250 different skin diseases, three small ViTs were: pre-trained self-supervised for 300 epochs (=ViT-SS), and fine-tuned supervised from ImageNet-weights for 300 epochs (=ViT-300) and for 78 epochs due to heavier regularization (=ViT-78). These models then each visualized areas of high interest in 250 input images (=self-attention-maps, one for each diagnosis). All 750 self-attention-maps were analyzed manually in a blinded manner using a proposed "DermAttention" score. Finally, models were compared. Diagnostic metrics and t-SNE visualizations of various training protocols were logged for additional evaluation.

Results: Visual analysis revealed that ViT-SS delivered superior self-attention-maps. It scored a significantly higher accuracy of attending to disease-defining lesions [88%; CI 95%: 0.840-0.920] compared to ViT-300 [78.4%; CI 95%: 0.733-0.835; $p<0.05$ – McNemar-test = 12.023; $p<0.001$] and ViT-78 [51.2%; CI 95%: 0.450-0.574; $p<0.05$ – McNemar-test = 76.676; $p<0.001$]. It also exceeded in all three subcategories of "DermAttention". These discriminative abilities were further confirmed by superior clustering patterns after supervised fine-tuning (=ViT-fine-tuned-300). ViT-300 consistently yielded better diagnostic metrics than ViT-fine-tuned-300.

Conclusions: Self-supervised pre-training did not translate to better diagnostic performance when compared to conventional supervision. However, it led to more accurate visual representations of varying skin disease images. These findings may pave the way for large-scale, explainable computer-aided skin diagnostic in an unfiltered clinical setting. Further research is needed to leverage those superior visual embeddings for improved clinical performance.

P31

Easy access to curated public datasets via ReactomeGSA

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Introduction: ReactomeGSA, is a widely used multi-omics pathway analysis platform within the Reactome knowledgebase. The integration of pathway analyses greatly simplifies comparing experiments across various 'omics technologies. Here we present an update of ReactomeGSA that enables researchers to quickly integrate public data of different ‘omics types into their analysis.

Methods: In order to simplify public data reuse from within ReactomeGSA, we developed several new features for this update. First, we integrated the grein_loader python application that now enables ReactomeGSA to directly fetch GEO datasets from the GREIN resource. Secondly, we developed a novel search function that enables users to quickly search for relevant datasets in both GREIN as well as EBI’s ExpressionAtlas from within ReactomeGSA. Finally, we completely redeveloped the ReactomeGSA web interface to improve its ease of use.

Results: The major update introduces an easy, user-friendly approach for accessing and integrating public datasets. Data is made consistently retrievable with the search function and is easily queried, extending available datasets in ReactomeGSA to over 25 000. Furthermore, the search function allows users to efficiently locate and integrate relevant public datasets into their analysis, which allows access to data without prior bioinformatics knowledge or advanced algorithm techniques.

Conclusions: This update simplifies the reuse and integration of public datasets in quantitative, comparative pathway analyses. The incorporation of the grein_loader plugin and its extensive data, coupled with the search function, allows browsing a vast amount of curated data. Extending ReactomeGSA with these new features allows users without extensive bioinformatics knowledge to perform complex pathway analysis and integrate public data within a few steps.

P32

Differential distribution of dendritic cells and T cells in the skin tumor microenvironment.

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Introduction: Immunophenotyping of tumor infiltrating immune cells has become increasingly pivotal for skin cancer diagnosis and treatment. However, the establishment of immunofluorescence (IF) staining procedures of formalin-fixed paraffin-embedded (FFPE) tissue samples can be challenging due to tissue condition and antibody availability and affinity.

Methods: To elucidate the tumor immune microenvironment, we aimed to determine the abundance and spatial distribution of dendritic cells (CD1a+) and T cells (CD3+) within different skin cancer types, actinic keratosis, squamous cell carcinoma, basal cell carcinoma, and melanoma, using immunofluorescence. In each FFPE tumor sample, we investigated four distinct areas: intratumoral, tumor margin, intraepidermal, and intradermal. From each area, 5-8 images were selected, and CD1a+ cells and CD3+ cells were counted using the ImageJ software.

Results: Our findings confirmed the presence of both CD1a+ and CD3+ cells in all examined skin cancer types and areas. Intriguingly, the spatial distribution of CD1a+ dendritic cells and CD3+ T cells varied. A predominance of CD1a+ dendritic cells was observed within the tumor and the epidermis, while CD3+ T cells were predominantly localized near the tumor margin.

Conclusion: Our study highlights distinct infiltration patterns of dendritic and T cell within skin cancers. As a subsequent step, we aim to establish a multiplex staining procedure for simultaneous detection of various immune cell types. Additionally, we intend to compare this data with results from flow cytometry analysis, enriching our understanding of the immune landscape in skin cancers.

P33

Diagnostic performance of neural networks in dermoscopic assessment of melanocytic lesions: context is critical

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Introduction & Objectives: Artificial intelligence holds immense promise as a diagnostic tool for clinicians, particularly in the identification of melanomas among high-risk patients and those with multiple suspicious lesions. Here, we examine the utility of convolutional neural network models for the dermoscopic diagnosis of melanoma.

Materials & Methods: We compare a model trained on a dataset that included images from European and American sources (CNN-1) to one that had also been pre-trained on an Australian dataset and was otherwise identical (SMARTI). Dermoscopic images were collected from prospectively recruited cohort of 210 lesions (from 191 patients) suspected to be melanomas from an Australian skin cancer clinic. Each lesion was diagnosed independently by five pathologists to establish ground truth. These were compared to diagnoses given by the two AI models.

Results: The CNN-1 model yielded an area under the receiver-operator curve of 0.682 while SMARTI's yielded 0.725. CNN-1 had a specificity of 0.35 (95% confidence interval (CI) 0.27-0.45) and sensitivity of 0.91 (CI 0.84-0.96). Whereas SMARTI demonstrated a specificity of 0.26 (CI 0.19-0.35) at a sensitivity of 0.95 (CI 0.88-0.98). We observed a higher inter-rater agreement for lesions correctly classified by SMARTI (Fleiss' Kappa 0.788) relative to lesions misclassified by SMARTI (Fleiss' Kappa 0.406). So, lesions misclassified by the AI model were also divisive for pathologists.

Conclusion: These results demonstrate the impact of population relevant training data on the performance of a dermoscopic CNN. Our study examined a cohort of lesions biopsied for suspicion of melanoma which often pose a greater challenge for even the experienced dermoscopist. We find that lesions that provoke discordant diagnoses between the 2 AI models were those that provoked disagreement between the pathologists. This highlights the importance of incorporating multiple independent diagnosticians to establish ground truth for training datasets.

Conflict of Interest: HPS is a shareholder of MoleMap Pty Ltd.

P34

ECIS as a real-time assessment tool of endothelial barrier function

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Introduction: Therapies targeting mutant BRAF and downstream MAPK signaling components are effective options against advanced BRAF-V600E positive melanoma. Vemurafenib, as one clinically approved treatment option, has many off-targets and detrimental effects on endothelial cells and the tumor microenvironment. We have found in a previous study that it induced barrier dysfunction in dermal microvascular endothelial cells and have identified off-targets and affected pathways. Endothelial barrier function is important for homeostasis and the regulation of the exchange between bloodstream and the surrounding tissue. Impaired vascular functions are a characteristic of many cancer therapies and dysfunctional vessels can lead to swelling and signs of inflammation in the tumor microenvironment.

Aim: Our aim is to verify off-targets and to characterize their functional consequences for endothelial barrier function.

Method: Therefore, inhibitors of relevant pathways were screened for their ability to alter endothelial barrier function and junction integrity. Here, Electric Cell-Substrate Impedance Sensing (ECIS) was employed as a real-time screening method that uses electrical impedance of endothelial cell monolayers on a gold electrode as a measure of ion flux through the cellular barrier.

Results: We were able to confirm a long-lasting barrier disruption for 24 hours upon treatment with Vemurafenib. However, the barrier signal returned to baseline after washout of the compound. In contrast, Thrombin resulted in a short-lived barrier breakdown of less than one hour. Importantly, intervention in relevant pathways with established compounds that control signaling did not result in an improvement or loss of the endothelial barrier.

Conclusion: Compounds used as targeted therapy for melanoma can have a considerable effect on the vascular endothelium. Identifying novel compounds that rescue the Vemurafenib-induced barrier dysfunction may support the development of therapies against mutant BRAF melanoma.

P35

Untargeted plasma and serum metabolomics of dupilumab-associated conjunctivitis in atopic dermatitis patients

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Background:
Atopic dermatitis (AD) is an inflammatory skin disease characterized by recurrent, localized, pruritic eczema with an estimated prevalence of 10% in adults, with 50% suffering moderate to severe manifestations. Dupilumab, an IL-4/IL-13 inhibitor, is approved for treating moderate to severe AD. Dupilumab-associated ocular surface disease (DAOSD) emerges in up to 60% of dupilumab-treated patients, constituting a major AD-specific adverse event. DAOSD pathogenesis has not been fully understood yet.

Methods: In this prospective single-center cohort study, 20 AD patients underwent dupilumab therapy, with six developing DAOSD. Serum and plasma samples were collected at baseline, 4 and 16 weeks post-treatment initiation, or during the conjunctivitis episode. Additionally, 10 age- and sex-matched healthy controls were sampled solely at baseline. High resolution mass spectrometry was employed for metabolomics and lipidomics analysis of all blood samples.

Results: Targeted metabolomics and lipidomics identified 138 metabolites and lipids, while untargeted analysis revealed 7931 features. Multivariate analysis unveiled significant metabolic and lipidic disparities between untreated AD patients and healthy controls, as well as between AD patients who later developed DAOSD. The metabolic and lipidic profiles were significantly influenced by ongoing treatment, with distinct temporal progressions observed between AD and DAOSD patients. Untargeted pathway enrichment analysis highlighted significant differences in amino acid metabolism between AD and DAOSD patients.

Conclusion: Metabolomics and lipidomics analysis represent valuable tools for advancing our comprehension of DAOSD pathogenesis. Furthermore, these findings suggest the feasibility of identifying biomarkers predictive of DAOSD occurrence.

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Antibiotic subcutaneous tissue penetration in septic versus healthy subjects

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Introduction: Little data exists regarding antibiotic exposure to subcutaneous tissue. This study aims to determine whether meropenem and linezolid standard dosing achieve the concentrations associated with maximal activity in septic patients versus healthy volunteers.

Methods: Patients with sepsis received either 2000 mg of meropenem every 8 hours (n=10) or 600 mg of linezolid every 12 hours (n=9). 2 healthy volunteers per antibiotic were included as a control group. Antibiotic concentrations were determined in blood and subcutis. Free plasma concentrations were calculated using the plasma protein binding (PPB) determined with ultrafiltration. Free tissue concentrations were obtained using microdialysis.
The non-compartmental analysis determined PK parameters and penetration ratios were calculated as AUC0-8_tissue/AUC0-8_free_plasma. The EUCAST clinical breakpoints were used for PK/PD analysis.

Results: Linezolid showed a higher subcutaneous penetration in septic patients (89 ± 33%) than in healthy (68 ± 33%). Meropenem penetration was comparable in septic (120 ± 60%) and healthy (121 ± 21%). Still, AUC variability was very high in septic, especially in subcutis (53.43% for linezolid and 64.43% for meropenem). Regarding PK/PD analysis in septic patients, meropenem T>MIC2 mg/L was 89.81 ± 12.87 % in plasma-free and 84.75 ± 11.35 % in subcutis. Linezolid AUC0-24h> MIC4 mg/L was 59.87 ± 35.19 in free plasma 61.15 ± 59.73 in subcutis.

Conclusion: Both meropenem and linezolid demonstrate high tissue penetration and correlation with plasma levels. We could observe a significantly higher variability in septic patients than in healthy volunteers, indicating that some patients might need dosing adjustments.

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Integration of Spatial Transcriptomics into Multimodal Imaging of Skin Aging

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In the last decade, spatial transcriptomics emerged as a new analysis technique, allowing a full transcriptome analysis while retaining the spatial information of the tissue. Still, several challenges like limitations in detection rate, resolution, coverage, and ambient RNA contamination, with this method remain.
Our group attempted to use spatial transcriptomics for the comparative analysis of young and aged skin, and while we are able to extract basic information on the expression patterns of several genes, a more detailed analysis was unfeasible due to resolution of the transcriptomics. This project aims to both address these resolution challenges as well as simultaneously implement spatial transcriptomics into a multimodal imaging workflow covering in addition immunofluorescence microscopy and mass spectrometric imaging of the same tissue.
Duplicate skin paraffin sections from young and aged donors were analysed using the Visium platform from 10X Genomics. Utilizing a combination of MATLAB R2023b, Python, and StrataQuest Tissuecytometry software we aim to generate an analysis pipeline, incorporating data preprocessing, dimensionality reduction, clustering, and variable gene analysis. This pipeline will then be further used to analyse approximated cell-level spatial transcriptomics.
The data for the approximated cell-level transcriptomics will be generated by combining a cell map created in StrataQuest as well as a full-coverage heatmap over the whole tissue, using the individual gene expression measurement form the spatial transcriptomics.
Using the approximated cell-levels, we hope to improve the resolution of the data. Additionally, this approach will enable the integration with multimodal imaging, facilitating correlation between local gene expression and diverse imaging modalities.

P38

Teledermatology in Styria: A Practical Triage System Bridging the Gap in Rural Healthcare

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Background: Access to dermatological care in rural areas can pose significant challenges. The Teledermatology in Styria project aimed to address this issue by establishing a digital connection between general practitioners (GPs) and dermatologists.

Methods: Fifteen GPs in Styria received digitally recommendations from two experienced dermatologists since January 2020 thanks to the funding from the Styrian Medical Association, the Health Fund, the ÖGK and the University Clinic for Dermatology. Patient satisfaction and the potential for GPs to learn from dermatologists were evaluated using questionnaires and a retrospective analysis of 2,552 completed cases (first 3 years).

Results: Over 80% of cases were resolved without the need for further dermatologist consultations, with 19% requiring no treatment, 63% being managed with teldermatological advice by the GP and only 1.2% receiving urgent clinical care. Only 32% of diagnoses were consistent between GPs and dermatologists. Diagnosis corrections were less common for cases requiring no treatment and more frequent for those amenable to GP management. Intensive GP users (>60 cases) did not exhibit a significant improvement in diagnostic accuracy. Patient satisfaction (548 questionnaires) was excellent, with 96% rating the rapid diagnosis and treatment provision as highly positive.

Conclusion: Teledermatology in Styria demonstrated the effectiveness of digital triage for dermatological, reducing waiting times, improving patient satisfaction and catching urgent clinical cases. While diagnostic accuracy between GPs and dermatologists was not consistently high, the system facilitated appropriate patient management. Consistent and coherent communication between physicians and across specialties remains crucial for optimizing telemedicine projects and enhancing patient care.

Conflict of Interest: Rainer Hofmann-Wellenhof is co-owner of the teledermatology company e-derm-consult, Graz, Austria. The following authors declare no conflict of interest: Elena Hofmann-Wellenhof, Edith Arzberger, Natalie Bordag.

P39

Enhancing data integration from GEO into ReactomeGSA

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Background: ReactomeGSA, is a widely used multi-omics pathway analysis platform within the Reactome knowledgebase. It supports the direct analysis and comparison of proteomics, transcriptomics, and microarray datasets. Even though microarray is no longer widely used, there is a large number of large, clinical datasets that are still relevant as a comparison for novel results. Here we present a novel feature facilitating the seamless integration of Gene Expression Omnibus (GEO) microarray datasets into ReactomeGSA. Thereby, users are able to directly load these datasets into their ReactomeGSA analysis.

Methods: The feature was implemented in a Python environment, which queries data from GEO using the GEOparser library. The library allows fetching the desired data from GEO using the unique GEO-ID and further temporarily storing the data locally until a cleanup is triggered.
The approach aligns with existing public data source fetchers within the platform, which already give access to a vast amount of public data.

Results: The update introduces an additional feature which allows an user-friendly approach for accessing and integrating data, which is consistently retrievable from the well-established GEO data source. By using the GEOparser library the samples within a data source are automatically downloaded and returned in a consistent format. Thereby, both the expression data as well as the matching metadata are directly available. By integrating this tool within the workflow an additional amount of 218,143 data series with 7,006,270 samples can be retrieved and included within the analysis.

Conclusion: This new feature greatly simplifies the reuse of public microarray data. Instead of time-consumingly downloading and converting the data, users can now directly fetch these datasets through ReactomeGSA. We believe that this vast amount of additionally available datasets will greatly simplify the reuse of public datasets in current research projects.

P40

Comorbidities of ichthyosis – a retrospective analysis of an Austrian ichthyosis cohort

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Ichthyoses are monogenetic skin diseases characterized by dry, thickened and scaly skin associated or not with inflammation. Comorbidities in these patients are not well documented. Thus, the aim of this study was to identify comorbidities in patients with ichthyosis. 92 adult patients with a primary diagnosis of ichthyosis - ichthyosis vulgaris, X-linked ichthyosis, autosomal recessive congenital ichthyosis, keratinopathic ichthyoses and syndromic ichthyoses like Netherton syndrome, keratitis ichthyosis deafness syndrome or ichthyosis prematurity syndrome - were included in this retrospective study. Information on medical history, blood parameters and genetic mutations was assembled using KIS powerchart software and data statistically analyzed using IBM SPSS statistics. Results showed that the majority of patients suffer from at least one comorbidity. Data revealed an association between ichthyosis and elevated serum IgE as well as atopic diseases such as atopic dermatitis, allergic rhinoconjunctivitis and allergic asthma, although to a lower extend. Other nonatopic comorbidities included mainly cardiovascular, metabolic and infectious diseases and cancer. Whereas patients with ichthyosis vulgaris and syndromic ichthyosis mainly develop comorbidities, most of patients with autosomal recessive ichthyosis are devoid of other diseases. Thus, this study uncovered a tendency of ichthyotic patients, regardless of the genetic mutations, to develop atopic symptoms, putatively owing to severe epidermal barrier impairment in these patients.

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The Transformative Power of PRP Therapy in Aesthetic Dermatology

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Introduction: In the evolving landscape of aesthetic dermatology, Platelet-rich plasma (PRP) therapy marks a revolutionary stride toward natural, regenerative beauty enhancements. Leveraging the body's intrinsic healing mechanisms, PRP therapy offers a promising solution for skin rejuvenation, anti-aging, and hair restoration. This autologous procedure utilizes concentrated platelets from the patient's blood, rich in growth factors, to stimulate tissue regeneration and collagen production, paving the way for a myriad of dermatological improvements.

Methods: The procedure begins with the collection of a small blood sample from the patient, which is then subjected to centrifugation to separate and concentrate the platelets in the plasma. This Platelet-rich plasma is meticulously injected into targeted areas of concern, whether for skin revitalization or hair regrowth. The growth factors released by the platelets initiate a series of regenerative processes, including the stimulation of collagen synthesis, enhancement of tissue repair, and activation of hair follicle growth.

Results: Clinical applications of PRP therapy have demonstrated significant improvements in skin texture, elasticity, and hydration, effectively reducing the appearance of wrinkles, fine lines, and scars. In the realm of hair restoration, patients have reported increased hair density, thickness, and stimulation of dormant hair follicles, particularly in cases of androgenetic alopecia. Furthermore, the combination of PRP with other aesthetic treatments has shown to enhance healing, reduce recovery time, and improve overall treatment efficacy, highlighting the versatile nature of PRP in augmenting aesthetic procedures.

Conclusions: PRP therapy stands as a testament to the advances in regenerative medicine within the field of aesthetic dermatology. Its ability to promote natural healing and rejuvenation through a minimally invasive procedure underscores the potential of utilizing the body's own biological mechanisms for aesthetic enhancement. The safety, efficacy of PRP therapy make it a valuable and increasingly popular option for patients seeking anti-aging solutions and hair restoration treatments.

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Vitiligo Prevalence Study: a Case Study of Investigation Samples over 42 Thousand

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Introduction: Based on clinical cases, dermatologists in Shaanxi Province, China assumed the publications before 2007, especially the ones based on population surveys, showed that the presumed vitiligo prevalence of 1–2% was overestimated. Therefore, this study analyzed the vitiligo prevalence in Shaanxi Province via a specifically designed population survey.

Methods: Approximately 0.1% of the 36.05 million people living in Shaanxi Province in 2002 were selected through stratified four-stage cluster sampling. They lived in 180 demographic census units and supported a door-to-door survey. Vitiligo and suspected vitiligo patients were marked in the basic questionnaire. They were encouraged to complete a well-prepared questionnaire and send it back to the investigation center. The questionnaires assigned to the investigators contained questions about vitiligo characteristics, such as the area affected, number of areas, and whether or not the affected areas were covered by scurf. At the end, professional dermatologists verified these results.

Results: There were 42,833 people in 180 demographic census units. The sex, residence, and educational level of these individuals were representative of the population of Shaanxi Province. The investigation team reported 43 vitiligo patients and 14 suspected vitiligo patients. During the verification period, 3 patients and all suspected vitiligo patients were excluded. In total, there were 40 patients (17 women and 23 men).

Conclusions: The prevalence of vitiligo in Shaanxi Province is 0.093% (95% confidence interval, 0.067–0.127%). No significant difference was found between males and females or between urban and rural residents.

P43

Decoding Linear Morphea: Clinical Insights and Treatment Paradigms from a Retrospective Patient Cohort Analysis at a Tertiary Care Center

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Aims: Morphea (Localized Scleroderma) is characterized by indurated plaques, with linear morphea often leading to severe disfigurement. We sought to delineate the clinical profile and treatment response in linear morphea cases.

Methods: This study employed a retrospective chart review of patients diagnosed with linear scleroderma and morphea at the Medical University of Vienna between 2007 and 2024. Patient data were meticulously extracted from electronic health records, ensuring a comprehensive analysis of clinical presentations, treatment modalities, and outcomes.

Results: Over the specified period our institution attended to a diverse cohort comprising over 200 cases of localized scleroderma and 33 instances of linear scleroderma. Notably within the linear scleroderma subgroup, females predominated with 28 cases, compared to 5 males. The age at diagnosis varied widely, spanning from 3 to 68yrs (median 23yrs). The most prevalent manifestation was facial linear scleroderma (coup-de-sabre), affecting 12 patients (36.4%). This was followed by lower extremity involvement in 9 patients (27.3%) and facial hemi atrophy (Parry-Romberg Syndrome) in 8 patients (24.2%). Occurrences on the trunk and upper extremities were comparatively uncommon, each observed in 2 patients. Treatment regimens were diverse, with phototherapy and methotrexate both being utilized for 12 patients (42.9%), and photopheresis for 2 patients (7.1%). Local treatments were also employed in select cases. In total, 16 patients necessitated a change in therapeutic approach. Overall, at the final follow-up, 24 patients were in remission, while only four patients exhibited active disease. Cosmetic interventions, predominantly autologous fat transfer, were undertaken in nine patients.

Conclusion: The need for medication changes and post-treatment cosmetic procedures in a substantial number of cases underscores current limitations. Enhancing diagnostic strategies and therapeutic options guided by critically necessary molecular understanding of the disease is crucial to mitigate scarring and atrophy.

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