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ABSTRACTS OF THE ANNUAL CONGRESS OF THE SWISS SOCIETY OF ALLERGOLOGY AND IMMUNOLOGY

GENÈVE, SEPTEMBER 12–13, 2024

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SHORT COMMUNICATION SESSION 1.1

SC1

Circadian tumor infiltration and function of CD8+ T cells dictate immunotherapy efficacy

Q. Zeng[1], C. Wang[1], C. Scheiermann[1], Z. Melis Gul[1]

[1]University of Geneva, Geneva

The quality and quantity of tumor-infiltrating lymphocytes, particularly CD8⁺ T cells, are important parameters for control of tumor growth and response to immunotherapy. Here, we show in murine and human cancers that these parameters exhibit circadian oscillations, driven by both the endogenous circadian clock of leukocytes and rhythmic leukocyte infiltration, which depends on the circadian clock of endothelial cells in the tumor microenvironment. To harness these rhythms therapeutically, we demonstrate that efficacy of chimeric antigen receptor T cell therapy and immune checkpoint blockade can be improved by adjusting the time of treatment during the day. Furthermore, time-of-day dependent T cell signatures in murine tumor models predict overall survival in patients with melanoma and correlate with response to anti-PD1 therapy. Our data demonstrate the functional significance of circadian dynamics in the tumor microenvironment and suggest the importance of leveraging these features for improving future clinical trial design and patient care.

SC2

Regulatory T cells define affinity thresholds for CD8+ T cell tumor infiltration

M. Mohsen[1], D. Speiser[2], R. Josi[1], S. de Brot[1], M. Bachmann1

[1]University of Bern, Bern; [2]Lausanne University, Lausanne

Introduction: TCR repertoire against tumors often lack high affinity TCRs, and the available T cells with low to moderate affinity frequently fall short in effectively eliminating cancer cells. In addition, immune response is hindered by regulatory T cells (Tregs).

Methods: We developed low and high affinity vaccines using the full agonist peptide p33 and its weak agonist peptide A4Y (Y-to-A substitution at position 4). In an additional low affinity model, we targeted B16F10 melanoma with our previously described multi-target germline personalized vaccine.

Results: Our results showed limited in vivo lytic cross-reactivity against the high-affinity p33 and the low-affinity A4Y peptides. Immunization with the low-affinity vaccine failed to hinder the progression of B16F10p33 tumors. Interestingly, when A4Y vaccination was combined with Treg depletion, the low-affinity T cell induced potent local and systemic immune-response and strong or complete blockade of tumor-growth. Furthermore, we found accumulation of protective tissue-resident memory T cells. The distance between infiltrating individual T cells in the tumor were significantly reduced, indicative of increased cell clustering, and T cell migration from blood vessels was increased following Treg depletion. Importantly, we could also show enhanced anti-tumor response when combining a low-affinity germline tumor-associated antigens vaccine with Treg depletion.

Conclusion: The data presented here not only revealed a novel function of Tregs in regulating the activity of low-affinity CD8+ T cells, but also promotes the development of innovative strategies of immunotherapy.

SC3

Orthotopically transplanted organoids closely recapitulate human colonocytes in vivo

A. Hausmann[1], F. Post[2], C. Steenholdt[3], A. Mund4, O. H. Nielsen[3], M. Mann[2], K. B. Jensen[1]

[1]reNEW - NNF Center for Stem Cell Medicine, Copenhagen DK; [2]NNF Center for Proteome Research, Copenhagen DK; [3]Herlev Hospital, Copenhagen DK; [4]NNF Center for Proteome Research, Copenhagen DK

The intestinal epithelium plays a central role in human health and disease, and several chronic inflammatory disorders associate with a weakened epithelial barrier. The organoid model has been instrumental in studying primary epithelial cell behavior in homeostasis and disease. Recent advances in human organoid transplantation into mouse and human lay the base for studies of human epithelial cell behavior within tissue context, and for novel therapeutic approaches for diseases such as inflammatory bowel disease. It remained unclear how organoid transplantation into the colon affects epithelial phenotypes and protein expression, which is key to assess the suitability of this model. To address this, we employed Deep Visual Proteomics including AI-guided cell classification on images, microdissection, and high-sensitivity proteomics, on human colonic epithelial stem and differentiated cells *in vivo*, upon transplantation *in situ,* and organoids cultured *in vitro.* We find that transplanted organoids closely resemble human intestinal epithelial cells *in vivo* compared to organoids grown *in vitro*, indicating that organoid culture induces a transient shift in epithelial phenotypes, which is reversible upon reintroduction into the mucosa. Phenotypic differences between epithelial cells *in vitro* and *in situ*/*in vivo* were driven by hallmarks of high proliferation and lower functional differentiation in organoids. With this, we demonstrate that transplanted epithelial cells *in situ* represent a physiological, relevant model for studying functional aspects of mature colonocytes.

SC4

Impact of opposing signals IL4-Ra and IFN-g on neutrophils effector functions

P. A. Martinez Murillo[1], M. Diedro[1], A. Dhreher1, L. Tran[1], L. Le Lann[1], P. Y. Mantel[1]

[1]CK-CARE, Davos

Introduction: Atopic dermatitis (AD) is a chronic inflammatory skin disease with a heterogeneous clinical phenotype, affecting up to 20% of children and 3% of adults worldwide. AD is characterized by barrier dysfunction, persistent Th2 inflammation, and skin dysbiosis due to *S aureus* overgrowth. Despite being colonize by *S aureus*, skin lesions in AD patients exhibit a conspicuous absence of neutrophils, potentially attributed to the report IL-4-impairment of neutrophil functions (migration, netosis, phagocytosis). However, there is not data on the impact of co-stimulation by opposing signals on neutrophils function.

Aim: To assess functional aspects of human neutrophils adaptation after in-vitro co-stimulation with IL-4 or IL-13 and IFN-g.

Methods: We measured STAT1 and STAT6 phosphorylation by FACS, netosis by Sytox green incorporation and ROS production by ampiflu-red detection.

Results: Combining IFN-g with IL-4 or IL-13 led to simultaneous STAT-6 and STAT-1 phosphorylation. Notably, IFN-g addition to IL-4 attenuated STAT-6 phosphorylation, while its addition to IL-13 significantly increased STAT1 phosphorylation, without affecting STAT6 phosphorylation. Netosis was reduced upon

IL4-Ra signalling but was restore by the addition of IFN-g. However, IL4-Ra stimulation did not affect ROS production and the addition of IFN-g even increased it.

Conclusions: These data so far suggest a high degree of neutrophils plasticity and an immune regulatory effect of opposing signals in-vitro, which would be further evaluated in AD patients including RNA-seq and ATAC-seq profiling.

SC5

Macrophage activation syndrome induced by Toll-like receptor 9 activation requires inflammasome nucleation and caspase-1 activation

A. Huard[1], M. Harel[1], E. Rodriguez[1], C. Gabay1

[1]*University of Geneva, Geneva*

Background: Macrophage activation syndrome (MAS) is a life-threatening inflammatory condition. Elevated circulating levels of IL-18 are assumed to be pathogenic. IL-18 is initially produced as a pro-peptide, cleaved by caspase-1 (Casp1) upon inflammasome activation and naturally inhibited by IL-18 binding protein (IL-18BP) upon formation of high affinity complexes. We previously showed that severe MAS manifestations were dependent on IL-18 in IL-18BP knockout (KO), but not in WT, mice injected with CpG, a TLR9 agonist.

Aim: We aim to examine the role of inflammasome activation in CpG-induced MAS.

Methods: *Il18bp*$^{-/-}$, *Casp1*$^{-/-}$, *Gsmd*$^{-/-}$, *Il18bp*$^{-/-}$*Casp1*$^{-/-}$, *Il18bp*$^{-/-}$*Gsmd*$^{-/-}$, and WT littermates were injected i.p with CpG at days 0, 2, and 4 and followed until day 7 with daily measurement of body weight. Liver and spleen weight were assessed at day 7. Cytokines levels in organs and plasma were measured at mRNA and protein levels by RT-qPCR and ELISA.

Results: While the phenotype of *Casp1*$^{-/-}$ and *Gsmd*$^{-/-}$ did not differ from their WT littermates, we observed that the severity of MAS was markedly attenuated in *Il18bp*$^{-/-}$*Casp1*$^{-/-}$ and *Il18bp*$^{-/-}$*Gsmd*$^{-/-}$ compared to *Il18bp*$^{-/-}$ littermates, including decreased body weight loss and splenomegaly. Free IL-18 levels at day 7 were decreased in dKO mice compared to *Il18bp*$^{-/-}$ but were still detectable, suggesting the presence of IL-18 processing.

Conclusion: The severity of CpG-induced MAS in *Il18bp*$^{-/-}$ mice is dependent on the maturation of IL-18 by Casp1 and released by Gasdermin-D. However, limited IL-18 cleavage occurs in the absence of Casp1.

SC6

Cellular and Molecular Immunoprofiling of Lupus Panniculitis: Elucidating the Roles of Cytotoxic T Cells, B Cells, and Complement Activation

M. M. Ameri[1], R. Ziadlou[1], N. Li[1], E. Maverakis2, M. C. Brüggen[1]

[1]*University Hospital of Zürich, Zürich;* [2]*University of California, Davis, Davis*

Lupus panniculitis (LP) is a subtype of cutaneous lupus erythematosus that affects subcutaneous adipose tissue. The pathomechanisms underlying LP are largely unknown. In this study, we aimed to obtain deeper insights into LP through the characterization of cellular and molecular immune patterns. Using imaging mass cytometry (IMC), we analyzed the cellular infiltrates in deep skin biopsies from LP patients (n = 8) and healthy controls (n = 6). Concurrently, we performed nCounter nanostring technology to quantify the mRNA expression of immune-related markers. The IMC analysis revealed that T cells (CD3^{+}) predominated in LP More precisely, they exhibited a predominantly skin-homing (CLA^{+}), cytotoxic phenotype (CD8^{+}, granzyme B^{+}), indicating site-specific immune activation within skin tissue. B cells (CD20^{+}) were also present. They constituted a significant portion of immune cells, alongside with the presence of dendritic cells and macrophages. Differential gene expression analysis revealed an upregulation in pathways associated with adaptive and innate immune responses. This included T cell receptor signaling (upregulation of CD247, LCK), lymphocyte activation (ZAP70, CD3D), cytokine signaling (STAT1, MYD88), and MHC antigen presentation (HLA-DRA, TAP1/2). Additionally, there was an upregulation of genes associated with the Complement system (C1QA/B, C4A/B). Our results suggest that a cytotoxic T- and B-cell-predominated immune response and complement activation is involved in LP. This points towards a pathogenesis that is different from other types of panniculitis.

SHORT COMMUNICATION SESSION 1.2

SC7

Efficacy and Safety of mRNA-1345, an RSV Vaccine, in Older Adults: Results Through ≥6 Months of Follow-Up

E. Wilson[1], J. Goswami[1], P. A. Doreski[2], G. Perez Marc[3], G. Jimenez[4], F. Priddy[1], N. Lin[1], N. Le Cam[1], K. Slobod[1], S. K. Stoszek[1], C. A. Panozzo[1], A. Kapoor[1], L. Wilson[1], J. Du[1], L. Lan[1], C. Reuter[1], H. Zhou[1], J. M. Miller[1], R. Das[1], D. L. Presotto[5]

[1]Moderna, Inc., Cambridge, MA US; [2]Fundación Respirar, Vaccine Research Division, Buenos Aires AR; [3]Hospital Militar Central Cirujano Mayor Dr. Cosme Argerich, Buenos Aires AR; [4]Spotlight Research Center, LLC, Miami, FL US; [5]Basel

In the phase 3 trial primary analysis, mRNA-1345 was efficacious against RSV-associated lower respiratory tract disease (RSV-LRTD) in adults ≥60 years, with no safety concerns. We report additional analyses when >90% of participants completed ≥6-month follow-up. This ongoing phase 3, multi-country, double-blind, placebo-controlled study (NCT05127434) randomized (1:1) adults ≥60 years to receive 1 dose of mRNA-1345 (50 µg) or placebo. Primary objectives included safety and tolerability and vaccine efficacy (VE) against a first episode of RSV-LRTD with ≥2 or ≥3 symptoms between 14 days and 12 months postinjection; key secondary efficacy objectives included prevention of RSV-associated acute respiratory disease (RSV-ARD). Efficacy against RSV-LRTD with shortness of breath (SOB) was assessed as a surrogate measure of more severe disease. The analysis included 36,157 participants (mRNA-1345, n = 18,112; placebo, n = 18,045). mRNA-1345 was well-tolerated; no safety concerns were identified. At a median follow-up of 8.6 months, mRNA-1345 VE was 63.3%, 63.0%, and 53.9% against RSV-LRTD with ≥2 and ≥3 symptoms and RSV-ARD, respectively. Lower bounds of the 95% CI exceeded prespecified success criteria of 20% for all endpoints. VE was evident across RSV-A and RSV-B subtypes and was generally consistent across demographic and risk subgroups. VE was 74.6% (95% CI, 50.7-86.9) against RSV-LRTD with ≥2 symptoms, including SOB. mRNA-1345 was well-tolerated with no safety concerns, and demonstrated efficacy for the prevention of RSV disease through a median of 8.6 months among adults ≥60 years.

SC8

Tackling the HIV Reservoir with HIV-resistant Anti-PD-1 CAR-T Cells

L. Ermellino[1], N. Kadzioch[2], S. Georgakis1, R. Banga[1], A. Alcaraz-Serna[3], O. Alfageme-Abello[1], R. Porret[1], E. Lana[1], M. Perreau[1], C. Fenwick[1], C. Petrovas[1], R. Speck[2], G. Pantaleo[1], Y. Muller[1]

[1]CHUV, Lausanne; [2]USZ, Zurich; [3]CHUC, Lausanne

Aim: A successful strategy to cure HIV requires eradicating its reservoir, mainly represented by PD-1+ T follicular helper cells. Herein, we hypothesize that CAR-T cells redirected against PD-1 may represent an effective approach to target the HIV reservoir.

Methods: Second-generation CARs were cloned using the scFv of a blocking (bPD1-CAR) and a nonblocking (nbPD1-CAR) anti-PD-1 monoclonal antibody fused to an IgG4 hinge, CD28 transmembrane and 4-1BB signaling domains with a cherry reporter. Anti-CD19 CAR-T cells were used as controls. The killing activity was measured using FACS and luciferase-based assays. HIV resistance to R5 and X4 HIV strains was tested by selectively editing the CD4 receptor and/or CCR5 and CXCR4 co-receptors using CRISPR-Cas9 technologies.

Results: The PD-1 binding affinity and KD values of the two anti-PD-1 mAbs were similar. Both anti-PD-1, but not CD19-control, CAR-T cells caused delayed depletion of PD-1+ CD4+ cherry negative T cells in ex-vivo cultures. Interestingly, the bPD1-CAR but not the nbPD1-CAR mediated tonic signaling, which was efficiently prevented by editing the endogenous PD-1. Both anti-PD-1 CAR-T cells equivalently killed PD-1high transgenic cells, yet the nbPD1-CAR was significantly less efficient in killing wild-type PD-1low target cells. CD4 editing was sufficient to confer HIV-resistance against R5 and X4 HIV strains without impairing the killing activity.

Conclusion: We successfully engineered two anti-PD-1 CARs with different functional activities. In-vivo experiments in humanized HIV-infected mice are undergoing.

SC9

Complete Remission in Eosinophilic Granulomatosis with Polyangiitis (EGPA) in the MANDARA Trial of Benralizumab vs Mepolizumab

M. E. Wechsler[1], N. Agmon-Levin2, D. R. W. Jayne[3], C. Pagnoux[4], U. Specks[5], L. Börjesson Sjö[6], S. Necander[6], A. Shavit[7], C. Walton[7], P. A. Merkel[8], P. Wörner[9]

[1]National Jewish Health, Denver US; [2]Sheba Medical Center, Ramat Gan IL; [3]University of Cambridge, Cambridge GB; [4]Mount Sinai Hospital, Toronto CA; [5]Mayo Clinic, Rochester US; [6]AstraZeneca, Gothenburg SE; [7]Astra-Zeneca, Cambridge GB; [8]University of Pennsylvania, , Philadelphia US; [9]Baar

Aim: MANDARA was a Phase 3, randomised, double-blind, 52-week study (NCT04157348) of benralizumab (n = 70) vs mepolizumab (n = 70) in patients with relapsing/refractory EGPA receiving standard of care. Non-inferiority was demonstrated for remission (BVAS = 0 and oral glucocorticoid [OGC] ≤4mg/day) at both Weeks 36 and 48.

Methods: Post-hoc analyses of MANDARA assessed those achieving a more stringent definition of complete remission: BVAS = 0 and OGC dose = 0mg/day at both Weeks 36 and 48 and being relapse-free. Remission = sustained if criteria were met by Week 48 and maintained to end of the 52-week double-blind period. Investigators were encouraged to taper OGCs for patients who reached BVAS = 0 as per standard practice and clinical judgement. HR and 95% CIs were estimated using a Cox regression model with Efron method to control for ties.

Results: Adjusted rates of complete remission at both Weeks 36 and 48: benralizumab, 23.5%; mepolizumab, 11.1% (difference: 12.47 [95% CI: 0.46, 24.48]; p = 0.0418). Sustained remission was achieved by 65.7% and 64.3% of patients (HR: 1.19 [95% CI: 0.78, 1.81]; p = 0.7793) in the benralizumab and mepolizumab groups; sustained complete remission was achieved by 35.7% and 22.9% patients (HR: 1.82 [95% CI: 0.97, 3.50]; p = 0.0966), respectively.

Conclusions: Patients with EGPA achieve higher rates of complete remission with benralizumab vs mepolizumab when using a more stringent definition of the endpoint. These data highlight the possibility of achieving sustained treatment goals for patients with EGPA, including full tapering of OGCs and avoiding relapses.

SC10

Effective intralymphatic immunotherapy (ILIT) of allergic rhinoconjunctivitis with grass allergoid microcrystalline tyrosine adsorbate: a DBRCT

P. Johansen[1], L. Sosic[2], T. M. Kündig[1], H. J. Hoffmann[3], E. C. Widmer[2], S. Flory[2], M. Paolucci[2], A. Duda[2], A. Chabot[2], C. C. Lang[1], P. Schmid-Grendelmeier[1], T. Andermatt[2], S. Bösch[1], M. F. Kramer[4]

[1]University Hospital Zurich, Zurich; [2]University of Zurich, Zurich; [3]Aaurhus University, Aarhus DK; [4]Bencard, Munich DE

Aim: To test ILIT Allergen intralymphatic immunotherapy (ILIT) with a chemically modified grass allergen administered with a biodegradable depot adjuvant MicroCrystalline Tyrosine.

Methods: Sixty ARC patients were randomized to receive ILIT with placebo or grass allergen (Polvac® Rye+Grass). Three ultrasound-guided injections were given at four-week intervals into inguinal lymph nodes. The dose was 1/15th of a maintenance dose for subcutaneous immunotherapy (SCIT). The primary outcome was a daily combined symptom medication score (cSMS) during the two following grass pollen seasons. Secondary outcomes comprised quality of life questionnaires, serology, lung-function tests, SPT and adverse events (AEs).

Results: Allergen ILIT was safe, with mild AEs in 48 out of 90 injections (53.8%) and three (3.2%) moderate AEs within the 40 minutes post-treatment observation. AEs were almost all erythema, wheal, or swelling at the injection site. All reactions retracted within 1-3 days. No SAEs. A significant reduction (>20%) of cSMS in the high grass pollen season was determined in the treatment group compared to placebo. The treatment effect increased with the measured seasonal pollen load. Allergen ILIT was associated with an increase in IgG4 towards timothy grass allergens and improved lung function (FEV1).

Conclusion: ILIT with a tyrosine-based SCIT product was well tolerated. The cSMS scoring two years after ILIT showed significant symptom amelioration in the allergen-treated. ILIT allows an ultrashort, effective pre-seasonal treatment, offering unique patient convenience and motivation in the treatment regimen.

SC11

Effect of Benralizumab vs Mepolizumab on Reduction in Oral Glucocorticoid (OGC) Use in Eosinophilic Granulomatosis with Polyangiitis (EGPA)

B. Hellmich[1], M. E. Wechsler[2], P. A. Merkel3, P. Nair[4], A. Bourdin[5], D. R. W. Jayne[6], F. Roufosse[7], L. Börjesson Sjö[8], Y. Fan[9], A. Menzies-Gow[10], S. Necander[8], A. Shavit[10], A. Rahmany[11]

[1]Akademisches Lehrkrankenhaus der Universität Tübingen, Kirchheim unter Teck DE; [2]National Jewish Health, Denver US; [3]University of Pennsylvania, Philadelphia US; [4]McMaster University, Hamilton CA; [5]University of Montpellier, Montpellier FR; [6]University of Cambridge, Cambridge GB; [7]Université Libre de Bruxelles, Brussels BE; [8]AstraZeneca, Gothenburg SE; [9]AstraZeneca, Gaithersburg US; [10]AstraZeneca, Cambridge GB; [11]Baar

Aim: OGCs have been the cornerstone of EGPA treatment but are associated with adverse events and frequent relapses during tapering. Benralizumab may represent a treatment option for patients that facilitates OGC-sparing while reducing disease burden.

Methods: MANDARA was a Phase 3, randomised, double-blind, 52-week study (NCT04157348) evaluating efficacy and safety of benralizumab vs mepolizumab in adults with relapsing/refractory EGPA. Investigators were encouraged to taper OGCs for patients with BVAS = 0 as per standard practice and clinical judgement.

Results: Patients were randomised to benralizumab (n = 70) or mepolizumab (n = 70). A subset of patients achieved complete withdrawal of OGC during Weeks 48 through 52 (benralizumab, 41.4%; mepolizumab, 25.8% [difference: 15.69, 95% CI: 0.67, 30.71; p = 0.0406]). More benralizumab-treated patients than mepolizumab-treated patients had a sustained 100% reduction in OGC use (100% reduction by Week 40, maintained through to Week 52: 24.3% vs 10.0%, respectively; HR: 2.97 [95% CI: 1.26, 7.77]). Similar proportions in both treatment groups achieved sustained ≥50% reduction in OGC use (≥50% reduction by Week 40, maintained through to Week 52: 77.1% vs 70.0% in benralizumab vs mepolizumab groups, respectively; HR: 1.17 [95% CI: 0.79, 1.74]; p = 0.3852).

Conclusions: In the MANDARA study of patients with EGPA, treatment with either benralizumab or mepolizumab was associated with the ability to reduce OGC use. However, benralizumab-treated patients were more likely to fully eliminate use of OGC.

SC12

Investigating mitochondrial metabolism dysfunction in SLE NK cells and exploring therapeutic approaches with Hydroxychloroquine and Urolithin-A

N. Fluder[1], M. Humbel[1], C. Ribi[1], D. Comte1

[1]Centre Hospitalier Universitaire Vaudois, Lausanne

SLE is an inflammatory disorder characterized by the emergence of autoreactive cells. SLE patients exhibit reduced numbers of Natural Killer (NK) cells, decreased cytotoxicity, and impaired cytokine production. The molecular mechanisms underlying NK cell dysfunction in SLE remain elusive.

We compared NK cells from the peripheral blood of SLE patients and healthy controls. We found that SLE NK cells have an increased mitochondrial mass but decreased mitochondrial function, as evidenced by elevated superoxide levels and significant cristae disorganization. These alterations correlate with impaired NK cell functions.

The alterations observed are linked to an accumulation of mitochondrial DNA (mtDNA) in the cytosol of SLE NK cells, associated with a reduction in key mitochondrial clearance proteins (V-ATPase) and a higher lysosomal pH, in SLE NK cells. Inhibition of V-ATPase in healthy NK cells led to an increased mitochondrial mass by inhibiting lysosomal acidification, mirroring the phenotype of SLE NK cells. This suggests a potential link between impaired lysosomal acidification and cytosolic mtDNA accumulation. Treatment of SLE NK cells in vitro with Hydroxychloroquine (HCQ) and Urolithin A (UA) reduced mitochondrial mass and normalized lysosomal acidification, indicating a pathway for therapeutic intervention.

Our findings reveal a critical link between mitochondrial dysfunction and immunometabolic abnormalities in SLE NK cells, highlighting the potential of targeting mitochondrial and lysosomal pathways with HCQ and UA and representing a promising approach for treating SLE.

SHORT COMMUNICATION SESSION 2.1

SC13

IL-18 activates human Th2 cells in atopic dermatitis

S. Schärli[1], F. Luther[2], T. Werfel3, J. P. Thyssen[4], S. Eyerich[5], M. Gilliet[6], N. L. Bertschi[2], C. Schlapbach[2]

[1]Inselspital, Bern University Hospital, University of Bern, Bern, Switzerland, Bern; [2]Department of Dermatology, Inselspital, Bern University Hospital, Department for BioMedical Research (DBMR), University of Bern, Bern, Switzerland, Bern; [3]Department of Dermatology and Allergy, Hannover Medical School (MHH), Hannover, Germany, Hannover DE; [4]Department of Dermatology and Allergology, Gentofte Hospital, Hellerup, Denmark, Hellerup DK; [5]Center for Allergy and Environment (ZAUM), Technical University and Helmholtz Center Munich, Munich, Germany, Munich DE; [6]Department of Dermatology, CHUV University Hospital, University of Lausanne, Lausanne, Switzerland, Lausanne

Background: T helper 2 (Th2) cells are crucial contributors to the pathogenesis of atopic dermatitis (AD) by secreting high levels of interleukin (IL-)13 and IL-22. Yet, the upstream regulators that activate Th2 cells in AD skin remain unclear. IL-18 is a putative upstream regulator of Th2 cells as it is implicated in AD pathogenesis and has the capacity to activate T cells.

Objective: To decipher the role of IL-18 in Th2 responses in blood and skin of AD patients.

Results: IL-18R$^+$ Th2 cells were enriched in blood and lesional skin of AD patients. Of all the cytokines for which Th2 cells express the receptor, only IL-9 was able to induce IL-18R via a previously unknown IL-9R-TYK2-STAT1 signaling pathway. Functionally, stimulation of circulating Th2 cells with IL-18 induced secretion of IL-13 and IL-22, an effect that was enhanced by co-stimulation with IL-9. Mechanistically, IL-18 induced Th2 cytokines via activation of both NF-κB and AP-1 signaling in Th2 cells, and neutralization of IL-18 inhibited these cytokines in cultured explants of AD skin lesions. Finally, IL-18 protein levels correlated positively with disease severity in lesional AD skin.

Conclusion: Our data identify a novel IL-9-IL-18 axis that drives Th2 cell responses in AD and demonstrate a critical role of IL-9-mediated upregulation of the IL-18R via a previously unknown IL-9R-TYK2-STAT1 signaling cascade. Our findings suggest that both IL-9 and IL-18 could represent upstream targets for future treatment of AD.

SC14

Intranasal administration of a tetravalent nanovaccine inhibits growth of HPV-associated head and neck orthotopic tumors in a murine model

R. Josi[1], A. S. Casaramona[1], S. Marar1, S. de Brot[2], M. Mohsen[1]

[1]Department for BioMedical Research (DBMR), University of Bern, Bern; [2]COMPATH, Institute of Animal Pathology, University of Bern, Bern

Aim: The incidence of human papillomavirus 16 (HPV16)-associated head and neck squamous cell carcinoma (HNC) is steadily rising. Intranasal neoadjuvant vaccine against HNC is a novel and potentially highly effective approach in cancer immunotherapy to directly induce broad humoral and cellular mucosal immunity.

Methods: We utilized our previously reported Qβ-HPVag consisting of virus-like particles loaded with CPG and chemically coupled to four elongated HPV16-derived E6/E7 MHCI peptides for intranasal administration in an orthotopic murine model. Our study encompasses a range of in-vivo and in-vitro experiments as well as tissue imaging mass cytometry (ongoing) to evaluate the immune cell populations within the tumor microenvironment.

Results: Our preliminary results indicated that intranasal administration of Qβ-HPVag impeded the orthotopic tumor growth and enhanced infiltration of tumor-infiltrating lymphocytes in the tumor. Assessment of vaccinated mice lungs showed an increased CD8 T cell population, suggesting a protective potential of the intranasal vaccine. Moreover, our findings demonstrated improved tumor-free survival in the treated group after primary tumor dissection, indicating the efficacy of the neoadjuvant approach.

Conclusions: Our preliminary findings demonstrate the effectiveness of intranasal vaccination using Qβ-HPVag in an aggressive head and neck cancer orthotopic murine model. These promising outcomes pave the way for novel clinical development strategies in HNC immunotherapy, suggesting a potential transformative impact on treatment paradigms.

SC15

Metabolic regulation of epithelial RIG-I signaling in viral exacerbations of asthma

U. Radzikowska[1], I. Jardón Parages1, N. Stocker[1], G. Tan[1], P. Westermann[2], S. Johnston[3], C. Messner[2], C. A. Akdis[1], M. Sokolowska[1]

[1]Swiss Institute of Allergy and Asthma Research (SIAF), Davos; [2]Precision Proteomics Center, Swiss Institute of Allergy and Asthma Research (SIAF), University of Zürich, Davos; [3]National Heart and Lung Institute Imperial College; MRC & Asthma UK Centre in Allergic Mechanisms of Asthma; Imperial College Healthcare NHS Trust, London GB

Aim: Rhinovirus (RV) infection of airway epithelial cells from patients with asthma results in an abnormal engagement of RIG-I into RIG-I inflammasome formation, which subsequently delays RIG-I-dependent interferon responses and enhances proinflammatory signaling in asthma. The metabolic regulation of antiviral responses during those pathogenic viral infections in asthma is not well understood.

Methods: Bronchial epithelium from patients with asthma and healthy controls upon *in vitro* and *in vivo* RV infection were used to analyze the metabolic regulation of RIG-I-dependent signaling using proteomics, transcriptomics, and functional metabolism assessment (Seahorse).

Results: Bronchial epithelium of patients with asthma upon RV infection demonstrated functionally increased glycolytic ATP and decreased mitochondrial ATP production, which was accompanied by broad downregulation of mitochondrial proteins. Importantly, functional inhibition of the OXPHOS pathway led to increased RIG-I inflammasome activation. Those *in vitro* mechanistic data were confirmed *in vivo* in bronchial brushings of asthma patients and healthy controls experimentally infected with RV. In asthma, upregulated glycolysis-HIF1A pathway corresponded with increased inflammasome signaling and lack of viral clearance, whereas in healthy controls significantly downregulated OXPHOS corresponded with downregulation of type I/III IFNs and efficient viral clearance.

Conclusions: There is a strong link between aberrant metabolic reprogramming signaling in epithelium with inefficient antiviral response in asthma.

SC16

Interleukin-2 immunotherapy reveals human regulatory T cell subsets with distinct functional and tissue-homing characteristics

M. E. Raeber[1], D. Caspar2, Y. Zurbuchen[2], N. Guo[3], J. Schmid[2], J. Michler[4], A. C. Martin[2], U. C. Steiner[2], A. E. Moor[4], F. Koning[3], O. Boyman[1]

[1]University of Zurich, University Hospital Zurich, Zurich; [2]University Hospital Zurich, Zurich; [3]Leiden University Medical Center, Leiden NL; [4]ETH Zurich, Basel

Due to its potential to stimulate immunomodulatory CD4+ regulatory T (Treg) cells, low-dose interleukin-2 (IL-2) immunotherapy has gained considerable interest for the treatment of autoimmune diseases. Motivated by these preliminary results, we conducted an investigator-initiated phase 2 clinical trial of low-dose IL-2 immunotherapy in systemic lupus erythematosus (SLE) patients (ClinicalTrials.gov ID: NCT0331233). IL-2 treatment not only increased the number of circulating Treg cells but also improved disease activity, as measured by validated clinical scores, thereby meeting its primary and secondary endpoints. To further investigate immunological changes, we conducted an in-depth analysis of circulating and cutaneous immune cells in IL-2-treated SLE patients using imaging mass cytometry, high-parameter flow cytometry, single-cell RNA sequencing with cellular indexing, ATAC sequencing, and targeted serum proteomics, creating a comprehensive atlas of in vivo human immune responses to IL-2. This analysis identified distinct IL-2-driven Treg cell activation programs, including gut-homing CD38+ Treg cells, skin-homing HLA-DR+ Treg cells, and highly proliferative, inflammation-homing CD38+ HLA-DR+ Treg cells. Moreover, skin-homing Treg cells were observed in the skin of SLE patients, interacting closely with endothelial cells, suggestive of a gatekeeper function. These data identify distinct and functionally characteristic Treg cell subsets in human blood and skin, including the Treg cell subsets most responsive to IL-2 immunotherapy, thus providing unprecedented insight into Treg cell biology during IL-2 treatment.

SC17

AhR agonism by tapinarof regulates TH2 and TH17 cell function in human skin

F. Luther[1], N. L. Bertschi2, O. Steck[2], S. Schärli[2], L. Taylor[3], S. Radonjic-Hoesli[4], N. Yawalkar[4], D. Simon[4], C. Schlapbach[4]

[1]Inselspital, Bern; [2]Inselspital, Institute of Dermatology, Bern; [3]Interfaculty Bioinformatics Unit and Swiss Institute of Bioinformatics, University of Berne, Bern; [4]Inselspital Institute of Dermatology, Bern

The aryl hydrocarbon receptor (AhR) is a transcription factor for skin homeostasis and barrier function. Tapinarof, a topical AhR agonist, has shown impressive clinical efficacy in psoriasis (PSO) and atopic dermatitis (AD), inducing long-lasting remissions. However, tapinarof's anti-inflammatory mechanism remains unclear. We aimed to investigate tapinarof's effects on T cells in healthy skin, AD, PSO and allergic contact dermatitis.

Using a short-term human skin explant model, we cultured skin biopsies from PSO, AD and ACD with tapinarof for 24 hours. We observed elevated cytokine levels in disease-driving populations of T_{RM} (IL-13$^+$CD4$^+$ T_{RM} in AD and IL-17a$^+$CD8$^+$ T_{RM} in PSO), validating our model. Tapinarof significantly reduced IL-13 and IL-17a in the respective diseases and populations. In ACD, tapinarof decreased IL-13 levels in T_{RM} and CD4$^+$ T cells without affecting IFN-g.

Transcriptomic analysis on tapinarof-treated T cells showed reduced metabolic enzymes, T cell activation and a reduction of *IL13* and *IL17A*. Single-cell RNA-seq on tapinarof-treated T cells from AD and PSO biopsies showed similar metabolic impairments. Preliminary mechanistic studies revealed reduced glycolysis and oxidative phosphorylation in resting and activated T cells after tapinarof treatment.

In conclusion, our ex vivo model demonstrated tapinarof's impact on skin T cells, with significant reduction in disease-relevant cytokines in AD, PSO, and ACD. Lastly, tapinarof directly affected T cells and impairs glycolysis and oxidative phosphorylation, revealing a previously unknown mechanism of action.

SHORT COMMUNICATION SESSION 2.2

SC18

Lymphatic-derived oxysterols promote immunity and response to immunotherapy in melanoma

M. Sun[1], L. Garnier[1], C. Wang[1], J. Angelillo[1], J. Montorfani[1], M. Roumain[2], D. Brighouse[1], N. Fournier[3], T. Petrova[4], C. Jandus[1], D. Speiser[5], M. Kopf[6], C. Pot[7], C. Scheiermann[1], G. Muccioli[8], A. Garg[9], S. Hugues[1], M. Roumain[2], N. Fournier[3], A. Garg[9], M. Roumain[2], N. Fournier[3], A. Garg9, M. Roumain[2], N. Fournier[3], A. Garg[9], M. Roumain[2], N. Fournier[3], A. Garg[9], M. Roumain[2], N. Fournier[3], A. Garg[9], M. Roumain[2], N. Fournier[3], A. Garg[9], M. Roumain[2], N. Fournier[3], A. Garg[9], M. Roumain[2], N. Fournier[3], A. Garg[9]

[1]CMU, University of Geneva, Geneva; [2]Université catholique de Louvain, Brussels BE; [3]Swiss Institute of Bioinformatics, Lausanne; [4]Université de Lausanne, Lausanne; [5]University of Lausanne, Lausanne; [6]ETH, Zurich; [7]Lausanne University Hospital and University of Lausanne, Lausanne; [8]Université catholique de Louvain, Bruxelles BE; [9]KU Leuven, Leuven BE

In melanoma, lymphangiogenesis correlates with metastasis and poor prognosis and promotes immunosuppression. However, it also potentiates immunotherapy by supporting immune cell trafficking. We show in a lymphangiogenic murine melanoma that LECs upregulate the enzyme Ch25h, which catalyzes the formation of 25-hydroxycholesterol (25-HC) from cholesterol and plays important roles in lipid metabolism, gene regulation, and immune activation. We identify a new role for LECs as the main source of extracellular 25-HC in tumors inhibiting PPAR-γ in intra-tumoral macrophages and monocytes, preventing their immunosuppressive function and instead promoting their conversion into proinflammatory myeloid cells that support effector T cell functions. In human melanoma, LECs also upregulate Ch25h, and its expression correlates with the lymphatic vessel signature, infiltration of pro-inflammatory macrophages, better patient survival, and better response to immunotherapy. We identify here in mechanistic detail a novel LEC function that supports anti-tumor immunity, which can be therapeutically exploited in combination with immunotherapy.

SC19

Interplay of Interferon alpha receptor signaling in IL-33 release in Fibroblastic reticular cells: Is cell death the way out for IL-33?

J. A. Villegas1, L. Scarpellino[1], P. Broz[1], D. Pinschewer[2], S. Luther[1]

[1]Universite de Lausanne, Epalinges; [2]University of Basel, Basel

Secondary lymphoid organs are critical sites for mounting T cell responses. We have previously shown that T zone fibroblasts (FRC) constitutively express the nuclear cytokine (alarmin) IL-33, which is critical to amplify the CD8+ T-cell response to LCM-virus (LCMV). Currently, the way FRCs release this nuclear cytokine is not understood. Most studies suggest that IL-33 release happens after activation of pyroptosis and necroptosis with few studies suggesting IL-33 release by live cells.

Analysis of publicly available scRNA-Seq data indicate that at day 3 after LCMV infection IFNAR signaling in FRCs activates cell death pathways as well as IL-33 expression. Thus, we tested the virus-specific CD8+ T-cell expansion and function in mice deficient in IFNAR selectively in FRCs using a low dose LCMV-WE infection model. Indeed, the T cell response was significantly decreased. To investigate whether the IL-33 release occurs via necroptosis or pyroptosis we infected mouse strains deficient in some of these pathways, but T cell expansion remained unaffected. Histological investigation of the FRC network confirmed an intact network in which T cell expansion was occurring, at times when also the cleaved hyperactive form of IL-33 became detectable. In summary, our data suggest that FRC sense LCMV infection via their IFNab-receptors, leading to IL-33 release from living FRCs, thereby ensuring functional tissue organization necessary for development of an efficient antiviral CD8 T cell response

SC20

Unlabeling the Influence of type 2 cytokines (IL-4 and IL-13) and IL-22 on epithelial barrier dysfunction and skin inflammation in Ex-Vivo human skin

Y. Mitamura[1], P. D'avino[1], M. Li[1], P. Gessner[1], J. Kim[1], E. Barletta[1], P. Westermann[1], N. Gaudenzio[2], C. Messner[1], C. Akdis[1], Y. Mitamura[1]

[1]Swiss Institute of Allergy and Asthma Research, Davos; [2]Toulouse Institute for Infectious and Inflammatory Diseases (Infinity), INSERM UMR1291, CNRS UMR5051, University Toulouse III, Toulouse FR

Aim: Atopic dermatitis (AD) is a chronic skin disease characterized by type 2 inflammation resulting in skin eczema, itching, and barrier dysfunction. Previously, our group demonstrated that electrical impedance spectroscopy (EIS) is a useful tool for detecting skin barrier integrity in mouse models and human AD patients. *Ex-vivo* human skin (NativeSkin®) is a model that exhibits normal skin barrier function and contains almost all cell types.

Objective: We aimed to investigate the distinct effects of representative type 2 cytokines, IL-4 and IL-13, on the skin barrier and resident cells in normal human skin. We compared the role of type 2 cytokines with another key cytokine, IL-22.

Methods: The *ex-vivo* skins were stimulated with 100 ng/mL of IL-4, IL-13, IL-4+IL-13 (100 ng/mL each), IL-22, and PBS for 24 hours. The epithelial barrier integrity was assessed on *ex-vivo* skins by EIS, and RNA sequencing and untargeted proteomics analysis by mass spectrometry were performed with skin lysate.

Results: IL-4, IL-13, and IL-22 treatments impair the skin barrier integrity in ex-vivo human skin. Th2 cytokines generally lead to the upregulation of eosinophil chemotaxis and activation of the ERK1/ERK2 signaling. Remarkably, all stimulation downregulated the pathways involved in the skin barrier and keratinization. Interestingly, IL-22 induced the upregulation of innate immune response. Proteomics analysis demonstrated that IL4, IL13 and IL-22 downregulate barrier molecules, such as CLDN1.

Conclusions: We showed the detailed effects of crucial cytokines on the pathogenesis of AD.

SC21

Adaptive MR1T cells recognise self-metabolites

A. Chancellor[1], Q. Yang1, D. Constantin[1], G. Berloffa[1], V. Nosi[1], R. Columbo[1], R. Jakob[1], J. p. Loureiro[1], D. Joss[1], M. Pfeffer[2], V. Schaefer[1], A. Vacchini[1], L. Brunelli[3], A. Zippelius[1], E. Davoli[3], D. Haussinger[1], T. Maier[1], L. Mori[1], G. de Libero[1]

[1]University of Basel, Basel; [2]University of Basel, Basel; [3]Istituto di Ricerche Farmacologiche Mario Negri, Milan

MR1 presents altered metabolites to T cells. The capacity of the immune system to surveil cellular metabolic integrity opens a new paradigm in immunology. Thus, a detailed understanding of the MR1-restricted T cell repertoire is required. We identified a cell endogenous carbonyl-adduct of adenine, 8-(9H-purin-6-yl)-2-oxa-8-azabicyclo[3.3.1]nona-3,6-diene-4,6-dicarbaldehyde (M_3Ade) that is presented by MR1 to T cells. Structural

analysis showed that it binds in the A' pocket of MR1 via a Schiff base. We generated MR1-M_3Ade tetramers to identify and isolate reactive MR1T cells, which were polyfunctional and broadly reactive to different healthy and tumour cell types. Single cell-sequencing and high-dimensional flow cytometry revealed that MR1T cells display features of adaptive T cells, following a functional differentiation pathway from naïve to recently activated and then to memory or effector cells. Some MR1T cells are expanded *in vivo,* indicating their Ag experience. These data suggest that MR1T cells are involved in surveilling metabolic alterations in healthy and cancer cells. Their physiological functions and contribution to diseases are yet to be understood.

SC22

Accessory lymph nodes support local control of tumor growth

C. Papadopoulou[1], E. Bugar[1], S. S. Ring[1], M. Lütge[1], C. Engetschwiler[1], S. Caviezel-Firner[1], A. Besse[2], U. Islander[3], S. H. Yun[4], S. J. Jang[4], J. Bae[5], G. Y. Koh[5], B. Ludewig[1], L. Onder[1]

[1]Institute of Immunobiology, Kantonsspital St. Gallen, St. Gallen; [2]Experimental Oncology, Kantonsspital St. Gallen, St. Gallen; [3]Krefting Research Centre, Department of Internal Medicine and Clinical Nutrition, University of Gothenburg, Gothenburg SE; [4]Wellman Center for Photomedicine, Massachusetts General Hospital, Harvard Medical School, Cambridge US; [5]Center for Vascular Research, Institute for Basic Science (IBS), Daejeon KR

Lymph nodes (LNs) are strategically positioned throughout the mammalian body and provide local base camps for immune cells. The establishment of complex microenvironments for cellular interactions in LNs is of utmost importance for the defense against invading pathogens and the control of tumor growth. Here, we have addressed the hypotheses that the activation of dormant LN anlagen and the induction of accessory LNs could be exploited to foster the initiation and maintenance of anti-tumor immunity. Specifically, we dissect the developmental pathways and cellular interactions that underpin accessory LN formation and functional impact of accessory LNs on immune reactivity. We found that accessory LNs contributed efficiently to the generation of anti-viral immune responses and the control of local tumor growth. The presence of serially connected LNs in the vicinity of tumors modified the microenvironment of the tumor, including heightened tumor fibroblast activation and T cell activity. In sum, our findings provide novel avenues towards improvement of immune reactivity and options to interfere with immune-regulatory circuits during malignant diseases.

SC23

Anti-allergen antibodies can act synergistically with peanut allergen immunotherapy

N. Wuillemin[1], P. Gasser[1], C. Arena[1], J. Konrad[1], R. Repke[1], S. Götschi[1], D. Bieli[1], T. Sonati[1], A. Barberis[1], N. Pengo[1]

[1]Mabylon AG, Schlieren

Aim: Peanut allergy is one of the most common food allergies and has a high unmet medical need, given the risk of sudden and severe allergic reactions due to accidental peanut exposure. Allergen immunotherapy (AIT) is demanding, has a slow onset of protection, and has significant safety risks. We hypothesize that a combination of anti-allergen antibodies together with AIT will enhance the safety and efficacy of AIT.

Methods: We have developed MY006, a cocktail of anti-peanut antibodies by exploiting our human antibody discovery pipeline for cloning peanut allergen-specific human antibodies from allergic patients. First, we investigated the impact of MY006 on antigen presentation and suppression of Th2 cell activation *in vitro*. Second, we tested if MY006 modulates peanut-specific IgG and IgE levels during peanut AIT and suppresses mast cells activation in a mouse model of peanut allergy.

Results: MY006 suppressed the binding of IgE-facilitated allergen binding to the low-affinity IgE receptor CD23 on primary human B cells. In patient-derived Ara h 2-stimulated mononuclear blood cells, MY006 suppressed TH2 inflammatory cytokine release and increased the anti-inflammatory cytokine IL10 as well as the frequency of T reg cells. Combining MY006 with AIT *in vivo* increased peanut allergen-specific IgG levels, decreased peanut allergen-specific IgE levels, and suppressed mast cell activation.

Conclusions: Besides protecting from allergic reactions, MY006 in combination with AIT will induce tolerance thus offering a disease-modifying therapy with a simpler, safer, and more effective approach than AIT alone.

SHORT COMMUNICATION SESSION 2.3

SC24

Enhanced Diagnostic Accuracy for Peanut Allergy using the Hoxb8 Mast Cell Activation Test

A. Eggel[1], N. Bachmeier-Zbären[1], A. Celik[2], R. van Brummelen[1], N. Roos[1], M. Steinmann[1], J. A. Hoang[2], X. Yin[3], C. M. Ditlof[3], L. Duan[3], J. E. Upton[3], T. Kaufmann[1], T. Eiwegger[3]

[1]University of Bern, Bern; [2]Hospital for Sick Children, Toronto CA; [3]The Hospital for Sick Children, Toronto CA

Aim: Accurate food allergy diagnosis is essential for effective disease management. Here, we present data from a prospective clinical performance study using a novel Hoxb8 mast cell (Hoxb8 MCs) assay on a pre-validated peanut allergy cohort.

Methods: Serum samples from peanut-allergic patients and controls were used to sensitize Hoxb8 MCs for subsequent dose-titration experiments with peanut allergen. Degranulated Hoxb8 MCs were quantified by flow cytometry. Results from this Hoxb8 mast cell activation test (Hoxb8 MAT) were compared to standard diagnostic assays, including skin prick test (SPT), allergen-specific IgE (sIgE) measurements, and basophil activation test (BAT). Further, we evaluated BAT non-responder samples.

Results: Dose-dependent Hoxb8 MC activation was observed for peanut allergic samples, with optimal cut-off <5.2% and maximal activation >89%. ROC curve analyses highlighted highest diagnostic accuracy at allergen concentrations ≥100 ng/ml, with an AUC of 0.97, and diagnostic sensitivity and specificity of 93% and 96%, respectively, outperforming SPT and sIgE. Remarkably, Hoxb8 MAT results significantly correlated with disease severity and accurately classified BAT non-responder samples, highlighting its potential for peanut allergy diagnosis.

Conclusions: Overall, the Hoxb8 MAT demonstrated high diagnostic precision for peanut allergy and is currently evaluated for multiple other allergies. Advantages over existing tests include enhanced patient safety, logistical simplicity, retrospective analysis capability, and accurate identification of BAT non-responders.

SC25

Diagnostic performance of the ALEX2 multiplex test for Hymenoptera venom allergy

M. Emmenegger1, I. A. F. M. Heijnen[1], C. M. Berkemeier[1], A. M. Willimann[1], F. Gerhard[1]

[1]Division of Medical Immunology, Laboratory Medicine, University Hospital Basel, Basel

Aim: Multiplex specific immunoglobulin E (sIgE) testing has become increasingly popular, but screening-based methodologies face critique on different levels. A major risk concerns the incidental detection of sIgE, e.g., to Hymenoptera venom, in the absence of clinical relevance. We sought to evaluate the performance of the ALEX^2 multiplex assay for bee and wasp venom allergy and to compare it with ImmunoCAP (IC).

Methods: We enrolled 75 patients with clinically diagnosed bee and/or wasp venom allergy and, additionally, 25 controls without allergy to Hymenoptera venom. We utilised IC and ALEX^2 to measure sIgE to whole extracts and components. We computed Spearman ρ and calculated the diagnostic performance on dichotomised data.

Results: IC sIgE correlated well with ALEX^2, with ρ between 0.76 and 0.88. However, sIgE ranges were distorted with systematically higher IC (Api m extract, Api m 1, Ves v extract, Ves v 1, Ves v 5) or ALEX^2 values (Api m 10), suggesting that absolute values (expressed in kUA/l) are not comparable. When assessing combined test performances, sensitivity for bee and wasp allergy was higher with IC than ALEX^2 (0.86 and 0.91 vs. 0.74 and 0.81, respectively), while specificity was higher with ALEX^2 (0.91 and 0.86 vs. 0.85 and 0.81, respectively), resulting in a higher likelihood ratio for ALEX^2.

Conclusions: ALEX^2 displayed a good diagnostic performance and, importantly, did not produce additional false-positive results as compared to IC. Moreover, our data caution that ALEX^2 values, albeit bearing the same unit, are not to be interpreted as interchangeable.

POSTER

P1

Dysregulated innate immune cell activity in children with periodic fever, aphthous stomatitis, pharyngitis, adenitis

G. Blanchard-Rohner[1], A. Dernstedt[2], P. Girard[2], E. Sallansonnet[2], E. Arsac[2], P. Fontannaz[2], W. Adouan[2], S. Lemeille[2], A. Didierlaurent[2]

[1]University Hospital of Geneva, Genève; [2]University of Geneva, Geneve

Aim: Periodic fever, aphthous stomatitis, pharyngitis, adenitis (PFAPA) is an autoinflammatory syndrome primarily affecting children between 2-5 years. While immune dysregulation is considered central, the innate immune cell phenotype in PFAPA remains poorly understood. We assessed the functionality of monocytes, dendritic cells (DCs), and natural killer cells in PFAPA children compared to controls.

Methods: Blood samples were obtained from PFAPA children during and between flares (n = 13) and age-matched healthy controls (n = 10). Flow cytometry assessed innate immune cells at baseline and after stimulation with lipopolysaccharide (LPS), Gardiquimod, or interferon (IFN)-γ. We also assessed cytokines and transcriptomics.

Results: During PFAPA flares, there was decreased T cells, B cells, and innate cells frequencies. PFAPA children showed increased TNFα production in DCs post-LPS and Gardiquimod stimulation compared to controls, also seen in flare-free periods. CD14+CD16+ monocytes showed elevated TNFα and IL12/23 production post-LPS stimulation and at baseline between flares. Serum analysis revealed increased IL-1RA, IL-15, IL-6, and IL-10 during flares, returning to baseline between flares, albeit with lower GM-CSF and IL-10 levels compared to controls. Blood transcriptomics suggested upregulated innate antiviral response pathways during flares, reverting to levels similar to controls between flares.

Conclusion: This study highlights dysregulated responsiveness to innate stimuli in PFAPA children's DC and monocyte subsets. Further investigation into these cells is necessary.

P2

Maintenance of spike-specific CD8 T cells after SARS-CoV-2 mRNA vaccination in anti-CD20 treated patients

N. Heikkila[1], N. Madelon[2], P. Fontannaz2, W. Adouan[2], G. Breville[2], K. Lauper[2], R. Goldstein[2], A. Grifoni[3], A. Sette[4], C. A. Siegrist[2], A. Finkch[2], P. Lalive[2], R. Genolet[5], A. Didierlaurent[2], C. Eberhardt[2]

[1]University of Geneva, Genève; [2]University of Geneva, Geneve; [3]La Jolla Institute of Immunology, San Diego; [4]La Jolla Institute of Immunology, Geneve; [5]University of Lausanne and Lausanne University Hospital, Lausanne

Individuals on anti-CD20 therapy are susceptible to severe COVID-19, but vaccination protects them from hospitalizations and death. While the vaccine induces weaker antibody responses in anti-CD20 treated individuals than in immunocompetent controls, it elicits robust T-cell responses. We studied spike-specific CD8 T cells 4-6 months after the 2nd and the 3rd dose of SARS-CoV-2 mRNA vaccination in 23 uninfected anti-CD20 treated patients (18/23 on ocrelizumab for multiple sclerosis, 5/23 on rituximab for rheumatoid diseases) and 10 uninfected controls. Using activation-induced marker assay, we observed a higher frequency of spike-specific CD8 T cells in anti-CD20 treated patients than in controls after both doses. After two doses, the distribution of effector/memory subsets in spike-specific CD8 T cells was skewed in patients compared to controls with an increased frequency of terminal effector memory cells (CD45RA+CCR7-) at the expense of effector memory cells (CD45RA-CCR7-). After three doses the differences in memory subset distributions were restored. T-cell receptor (TCR) analysis of spike-specific CD8 T cells showed similar diversity and clonality in patients and controls. In contrast, the TCR repertoires after two versus three doses contained fewer overlapping clonotypes in patients than in controls. Altogether, our data suggest altered maintenance mechanisms of antigen-specific CD8 T-cell population in anti-CD20-treated individuals compared to controls and help optimizing the vaccine recommendations for immunocompromised individuals.

P3

Analytical Performance of a Novel, Fully Automated Multiplexed Microarray Immunoassay Prototype for the Detection of 15 antibodies Associated with CTD

G. Gomez[1], D. Bijlsma[1], E. Lukasik1, M. Hausmann[1], C. Fischer[1], P. Ghillani-Dalbin[2], M. Miyara[3], E. Moreau[1]

[1]AliveDx, Eysins; [2]Sorbonne Université, Assistance Publique Hôpitaux de Paris (AP-HP), Hôpital Pitié-Salpêtrière, Département d'Immunologie, Paris FR; [3]Sorbonne Université, Inserm, Centre d'Immunologie et des Maladies Infectieuses (CIMI-Paris), Assistance Publique Hôpitaux de Paris (AP-HP), Hôpital Pitié-Salpêtrière, Paris FR

Aim: To assess the analytical performance of a novel, single-use, multiplexed microarray immunoassay prototype (MosaiQ AiPlex CTDplus; AliveDx, Eysins, Switzerland), used with the fully automated MosaiQ® system, for the simultaneous detection of fifteen autoantibodies associated with CTD, compared with selected CE-marked devices.

Methods: Serum samples characterized as reactive to ≥1 autoantibody (n = between 23 and 101 per analyte) or non-reactive (n = 100) by CE-marked devices were included. For CCP, only reactive samples were included. Each individual non-reactive sample was tested with the investigational device once; reactive samples were tested in duplicates. Positive percentage agreement (PPA) and negative percentage agreement (NPA) for individual analytes were calculated.

Results: Compared with relevant CE-marked devices, the investigational prototype showed, respectively, the following positive (PPA) and negative (NPA) percent agreement per analyte: CCP 86%/NA, chromatin 75%/95%, CENP-B 98%/97%, DFS70 94%/100%, dsDNA 95%/97%, Jo-1 93%/96%, Ribosomal P 98%/96%, RNAP3 76%/95%, Scl-70 81%/95%, Sm 88%/95%, Sm/RNP 98%/98%, SSA-60 99%/100%, SSB 93%/97%, TRIM21 99%/100%, U1RNP 97%/98%.

Conclusions: The investigational prototype demonstrated substantial agreement with the compared CE-marked devices. Further studies will allow for expanded performance assessment of the investigational device. This fully-automated multiplexed platform has the potential to contribute to optimizing CTD evaluation by simplifying complex testing pathways and analyzing large number of samples per day.

P4

Performance of a novel, fully automated immunoassay microarray prototype for the serological detection of specific IgE directed against Bet v 1

G. Gomez[1], Y. Ataman-Önal[1], B. Steele2, R. Pasion-Galvan[1], J. Santiago[3], M. Hausmann[1], C. Fischer[1]

[1]AliveDx, Eysins; [2]AliveDx, Edinburgh GB; [3]AliveDx, Chicago US

Aim: To assess the performance characteristics of a novel, single-use, microarray immunoassay prototype (MosaiQ Allergy Bet v 1) (Bet-v-1-MA), used with the fully automated MosaiQ® system, for the detection of Bet v 1 specific IgE (sIgE), compared with ImmunoCAP™ Specific IgE (Phadia AB). Assay's reproducibility and repeatability were also assessed.

Methods: 163 serum samples, characterized by the comparator method as reactive (n = 63) or as non-reactive (n = 100) were tested with Bet-v-1-MA. Magazine lot reproducibility was assessed over 5 days/3 lots/2 instruments; instrument reproducibility was evaluated over 5 days/1 lot/3 instruments; repeatability was assessed on 1 instrument and 1 magazine lot, 2 runs/day/5 days.

Results: After the protocol exclusion of 1 reactive sample, Bet-v-1-MA identified as reactive 60 out of 62 characterized reactive samples by the comparator and all 100 non-reactive samples; for a positive, negative and overall agreement of 96.8% (95%CI: 88.8%, 99.6%), 100% (95%CI: 96.4%, 100%) and 98.8% (95%CI: 95.6%, 99.9%), respectively. Agreement of Bet-v-1-MA with expected results in the evaluations of reproducibility (by lot and by instrument) and repeatability (869, 435 and 291 data points, respectively) were all 100%.

Conclusion: Bet-v-1-MA showed high concordance with the compared device for detecting Bet v 1 sIgE. Bet-v-1-MA demonstrated a high degree of precision in the reproducibility and repeatability evaluations. This device/platform has the potential to multiplex; further ongoing steps include the addition of other allergens to the microarray.

P5

A Swiss Model of Collaborative Care for Patients with Immunodeficiency by Integrating Specialized Private Practice and University Center Expertise

O. V. Hausmann[1], M. Kühn[1], F. Poletti[2], J. Köppen[2], M. Recher[2]

[1]Löwenpraxis, Luzern; [2]University Center for Immunology, University Hospital Basel and Immunodeficiency Laboratory, Department of Biomedicine, University of Basel, Basel

Background: Effective management of primary and secondary immunodeficiency disorders requires experienced centers which are limited in number and funding. Synergizing specialized private practices and university centers enhances patient care, expedites access to specialized care and comprehensive laboratory analysis, and facilitates participation in prospective patient cohorts including genetic investigations.

Methods: From an initial cohort of 18 patients (median age 52 yrs, 8 females), we present two cases illustrating the collaborative care model. Case 1 showcases a syndromal immunodeficiency with musculoskeletal involvement, emphasizing the role of private practitioners in diagnosis and management. Case 2 depicts late-onset antibody deficiency with pulmonary manifestations, highlighting university resources for advanced diagnostics and genetic analysis.

Results: Collaboration reduces diagnostic delay (median 4.5 yrs), tailors treatment plans, and provides access to cutting-edge diagnostics and research. Integrating clinical immunologists in private practices and university centers optimizes patient care, increases local awareness, fosters ongoing research and monitoring for immunodeficiency disorders.

Conclusion: Integrating specialized clinical immunologists in private practices and university centers optimizes patient care for immunodeficiency disorders. This seamless collaboration not only addresses immediate clinical needs but also ensures inclusion in prospective cohorts for ongoing research and long-term monitoring, ultimately improving outcomes for patients.

P6

Exploring cross-reactive T cell immunity in Guillain-Barré syndrome

C. Capelle[1], L. Súkeníková[1], D. Latorre[1], P. Piffaretti[1], S. Edalat[1], B. Schreiner[2], P. Ripellino[3]

[1]Institute of Microbiology, ETH Zurich, Zurich; [2]Department of Neurology, University Hospital Zurich, Zurich, Switzerland, Zurich; [3]Department of Neurology, Neurocenter of Southern Switzerland EOC, Lugano

Guillain-Barré syndrome (GBS) is an autoimmune disorder of the peripheral nervous system (PNS) usually associated with preceding infections, which are believed to serve as a trigger for its development. We have recently revealed the presence of autoreactive memory $CD4^+$ T cells targeting PNS-myelin proteins in GBS patients. Intriguingly, such cells showed a certain degree of cross-reactivity between distinct PNS-myelin antigens and, in patients associated with primary cytomegalovirus (CMV) infection, with CMV antigens (Súkeníková L et al, Nature, 2024). However, the cellular and molecular bases of such cross-reactivity remain elusive. Here, we use in vitro T cell screenings, TCR sequencing and computational tools to investigate the degree of cross-reactivity of autoreactive T cell clones in GBS patients. By screening over 200 autoreactive $CD4^+$ T cell clones, encompassing over 50 different TCR Vb clonotypes, isolated from five patients, three of which had a known preceding infection (1 CMV and 2 of unknown origin), we describe that the vast majority of clonotypes displayed some degree of cross-reactivity between distinct PNS-myelin and/or CMV antigens. We are currently employing computational tools and in vitro functional screenings to predict and identify potential epitopes targeted by the cross-reactive T cell immunity in GBS patients. Overall, these results suggest that autoreactive and broadly cross-reactive T cells are a common feature of GBS patients and provide evidence that virus infections could trigger the disease through T cell cross-reactivity.

P7

Understanding the connections between cellular metabolism, MHCI regulation, and the immune response

S. Nakka[1], G. Guarda[2], A. Zenobi[2], H. Khameneh[2], A. Rinaldi[3], B. Fischer[4]

[1]Institute for research in biomedicine, Bellinzona, Bellinzona; [2]Institute for research in biomedicine, Bellinzona; [3]Institute for research in oncology, Bellinzona; [4]Karolinska Institutet, Stockholm SE

MHCI molecules play a central role in adaptive immunity. They sample peptides generated within the cell and signal if the cell is transformed or infected to effector cells of the immune system. Studies are lacking on how cellular metabolism might further impact antigen presentation.

We thus investigated the effect of altered metabolism on MHCI levels using mouse bone marrow-derived dendritic cells (BMDCs). Mouse BMDCs grown in galactose-containing medium, or in the presence of inhibitors of the glycolysis pathway exhibited higher MHCI surface expression as compared to the levels of BMDCs cultured in glucose-containing medium. As the total bolus of MHCI molecules was not increased, we assessed

the kinetics of MHCI surface export. BMDCs cultured in the presence of galactose and inhibitors of glycolysis recruited MHCI faster to the surface. Notably, RNA-sequencing results highlighted a significantly higher expression of genes related to the anterograde transport from the endoplasmic ER to the Golgi apparatus and downregulation of genes related to ER-Golgi retrograde transport in BMDCs grown in galactose, in line with the altered trafficking of these molecules between the Golgi and cell surface. This increased surface expression of MHCI is correlated with the enhanced activation potential of CD8+ T cells in vitro.

Collectively, these results show that the metabolic state of DCs regulates the MHCI through post-translational mechanisms and influences the activation of T cells. These findings might be highly relevant to diseases such as metabolic disorders, cancer, and infections

P8

T-safe: a tolerogenic niche for T-reg priming to prevent graft-versus-host disease after allogeneic hematopoietic cell transplantation.

N. Kallal[1], S. Hugues[1], F. Bonini[2], F. Simonetta[1], F. Fico[2], C. Bernardi[1]

[1]UNIGE, GENEVE; [2]UNIGE, Geneve

Allogeneic hematopoietic stem cell transplantation (allo-HSCT) is a proven therapy for hematologic diseases, but it remains a challenge due to graft-versus-host disease (GvHD). Despite advancements in pharmacological and graft engineering techniques, current treatment of GvHD rely predominantly on broad-spectrum immunosuppression. This approach heightens the risk of other serious complications such as cancer relapse and systemic infections. The T-Safe project aims to develop an injectable and bioactive biomaterial scaffold to induce tolerogenic regulatory T-cells (T-regs) capable of long-term reduction or prevention of GvHD. The underlying research hypothesis is that confined uptake of apoptotic cell debris by professional phagocytes (DCs and/or macrophages) in a tolerogenic environment will lead to durable T-regs induction. Our preliminary data show that after implantation in mice, the biomaterial scaffold successfully promotes vascularization and attracts endogenous cells. *In vivo* loading of T-Safe with apoptotic cells shows that local APCs can phagocytize these cells, a crucial step for antigen presentation and subsequent T-reg generation. Lastly, injecting T-regs into the scaffold has shown promising results, with data indicating that these cells can migrate from the biomaterial scaffold to secondary lymphoid organs (SLOs), main site of T-reg-mediated GvHD suppression of GvHD. Altogether, our data indicate that T-Safe acts as a tolerogenic niche for T-reg generation, with adequate vascularization for immune cell entry and T-reg exit to draining lymph nodes.

P9

Human-plasma derived-IgG and recombinant IgG Fc fragments modulate the effector functions of NK cells

D. Reis Galvão[1], M. Freitas Monteiro[1], R. Spirig[2], G. Puga Yung[1], J. D. Seebach[1]

[1]Division of Immunology and Allergy, Department of Medicine, Faculty of Medicine, University Hospitals Geneva, Geneva; [2]CSL Behring AG, Bern

The clinical indications for Intravenous immunoglobulins G (IVIG) are steadily increasing. However, there is a limited supply of human plasma to produce it. Alternatives include engineered recombinant molecules, e.g. hexamer (HEX) composed of Fc from IgG1 fused to IgM µ-tailpiece. One of the mechanisms of action of IVIG is blocking Fc-gamma receptors (FcγRs) thereby inhibiting antibody-dependent cell-mediated cytotoxicity (ADCC) mediated by natural killer (NK) cells.

The goal of this study was to measure the effect of IVIG and HEX-variants on NK cell viability and function. NK cell viability was tested by vital cell dye exclusion and Annexin V apoptosis assay (flow cytometry). The cytokine release by NK cells was measured after culture with IVIG or HEX-variants. NK cytotoxicity (ADCC and direct cytotoxicity) were assessed by non-radioactive release assays after NK culture in the presence of IVIG or HEXs at variable times. Targets consisted in Daudi plus anti-CD20 or K562 cells for ADCC and direct cytotoxicity, respectively.

HEX was the most potent inhibitor of ADCC (90% inhibition), after both 1h and 16h of culture with human NK cells, followed by IVIG. Remarkably, direct cytotoxicity was also inhibited by the tested molecules, with a higher inhibition mediated by IVIG (55% inhibition). Both IVIG and HEX induced limited cell death (<20%). All molecules but not Fc monomer triggered the release of TNFα and IFNγ by NK cells.

In conclusion, HEX inhibit NK cell effector functions and may have the potential to substitute IVIG for the treatment of autoantibody-mediated diseases.

P10

IgG-Epitope Mapping of Birch-Pollen Bet v 1 Allergen in Allergic and Treated Patients for the Design of Hypoallergenic Immunotherapy Allergens

M. Paolucci[1], L. Šošić[2], A. Streuli[3], D. Melillo[2], G. Sousa-Augusto[4], C. C. Lang[5], A. Duda[6], T. Jiang[7], R. Campana[7], M. Bachmann[4], P. Schmid-Grendelmeier[5], K. Eyer[3], T. M. Kündig[5], P. Johansen[5]

[1]University of Zurich, Zurich; [2]Department of Dermatology, University of Zurich, Zurich; [3]Laboratory for Functional Immune Repertoire Analysis, Institute of Pharmaceutical Sciences, ETH, Zurich; [4]Department of Immunology RIA, University Hospital Bern, Bern; [5]Department of Dermatology, University Hospital Zurich and University of Zurich, Zurich; [6]Department of Dermatology, University Hospital Zurich, Zurich; [7]Department of Pathophysiology and Allergy Research, Center for Pathophysiology, Infectiology and Immunology, Medical University of Vienna, Vienna AT

Allergen immunotherapy (AIT) is the sole curative treatment for allergies, promoting the production of allergen-specific neutralizing IgG antibodies and tolerance. However, AIT is often linked with allergic adverse events. This project aims to pinpoint IgG-binding epitopes on the major birch pollen allergen Bet v 1 to generate hypoallergenic peptides, enhancing AIT's safety and efficacy.

Blood samples from 5 healthy and 30 birch-pollen allergic patients (20 with subcutaneous or sublingual immunotherapy) were collected. Rhinitis Quality of Life Questionnaires (RQLQ) quantified patient symptoms. Sera were assessed for allergen-specific IgE, IgG, and IgG4, and for basophil degranulation inhibition. IgG- and IgE-secreting B cells were analyzed via single-cell DropMap microfluidics. Linear and conformational IgG epitopes on Bet v 1 were determined using overlapping peptides and CLIPS technology. Candidate peptides were synthesized using Fmoc technology, and allergenicity was evaluated in a basophil degranulation assay.

All allergic patients had Bet v 1-specific IgE and higher RQLQ scores. AIT patients showed elevated IgG and IgG4 levels and stronger Bet v 1-specific basophil degranulation inhibition. Four non-overlapping Bet v 1-specific IgG-binding sites were identified. The peptides, designed from these regions, did not induce degranulation in sensitized basophils.

This study utilizes a patient-derived approach to identify clinically relevant IgG-binding Bet v 1 epitopes for the design of hypoallergenic peptides. This method could pave the way to a safer and more effective AIT.

P11

Sphingomyelin complexed cholesterol controls Lymphocyte function-associated antigen-1 mediated effector T cell function.

K. A. Patommel[1], A. A. Martí I Líndez[1], J. Jaenicke[1], S. Verma[1], J. Edwards-Hicks[2], C. Hess[3], L. Laraia[4], S. Wilson[1], M. Donde[1], J. Lötscher[5], L. M. Booty[6]

[1]University of Cambridge, Cambridge GB; [2]University of Edinburgh and University of Cambridge, Edinburgh GB; [3]University of Basel and University of Cambridge, Basel; [4]Technical University of Denmark, Kongens Lyngby DK; [5]Universitätsspital Basel, Basel; [6]GSK, Stevenage

Introduction: Lymphocyte function-associated antigen 1 (LFA-1) is a T cell integrin regulating adhesion, immune synapse formation and effector function. LFA-1 is increasingly selected as target for cancer immunotherapy. The *affinity* and *avidity* of LFA-1 are regulated by interactions with lipid rafts stabilised by sphingomyelin complexed cholesterol.

Hypothesis: We hypothesise that perturbation of plasma membrane (PM) cholesterol alters integrin mediated T cell adhesion and function.

Aim: Establish PM cholesterol perturbation models to study the biophysical mechanisms by which PM cholesterol controls LFA-1 mediated T cell function.

Methods: PM cholesterol distribution and content were perturbed in Jurkat and primary effector T cells using autogramin-2, a sterol-derived synthetic compound, and methyl-b-cyclodextrin complexed cholesterol (MbCD-chol) respectively. The effect on T cell adhesion, and effector function was assessed.

Results: Increasing total PM cholesterol content with MbCD-chol altered neither LFA-1 mediated adhesion nor anti-tumour cytotoxic killing. Yet, perturbing PM cholesterol distribution with autogramin-2 significantly reduced adhesion and LFA-1 mediated immune cell function. This phenotype was recapitulated through liberation of sphingomyelin complexed cholesterol.

Conclusion: These results suggest that stabilisation by sphingomyelin-complexed cholesterol is necessary for LFA-1 dependent effector T cell function. The implications of these findings for cancer-immunotherapy are subject of further study.

P12

Innate immune cell dysregulation in children with periodic fever, aphthous stomatitis, pharyngitis, adenitis (PFAPA)

A. Dernstedt[1], P. Girard[1], E. Sallansonnet[1], E. Arsac[1], P. Fontannaz[1], S. Lemeille[1], A. Didierlaurent[1], G. Blanchard-Rohner[1]

[1]University of Geneva, Geneva

Aim: Periodic fever, aphthous stomatitis, pharyngitis, adenitis (PFAPA) is a cyclic autoinflammatory syndrome that typically manifests in young children. Our aim is to assess monocyte, dendritic cell (DC), and NK cell functionality in children with PFAPA.

Methods: Whole blood from children with PFAPA during and between flares (n = 13), and age-matched healthy controls (n = 10) was used for flow cytometric characterisation of innate immune cells at baseline and after six hours stimulation with LPS, a TLR7-agonist, or IFN-γ. We quantified production of IL-12/23, TNFα, IFNα, IFNγ, and IP10 upon stimuli. We also transcriptomic analysis on whole blood cells and measured serum cytokine levels by multiplex.

Results: We found decreased frequencies of most innate cells during PFAPA flares. Cell stimulations showed increased TNFα production in DCs and CD14+CD16+ monocytes after stimulation with LPS in PFAPA subjects compared to controls. TNFα and IL12/23 production was also increased between flares without stimulation. In serum, IL-1RA, IL-15, IL-6, and IL-10 levels were increased during flares, while levels between flares were similar to controls, except IL-10 and GM-CSF which were lower between flares than in controls. Blood transcriptomics showed upregulated pathways associated with innate antiviral responses during flares, while controls and between flares were similar.

Conclusion: Our initial analyses show that DC and monocytes in PFAPA may respond differently to innate stimuli. Further functional and genetic analyses of these cells could reveal pathways involved in this dysregulation.

P13

High-affinity omalizumab variants with optimized disruptive efficacy accelerate allergic effector cell desensitization

A. Eggel[1], L. F. Pennington[2], D. Brigger[1], R. van Brummelen[1], P. Guntern[1], T. S. Jardetzky[3]

[1]University of Bern, Bern; [2]Stanford University, Stanford US; [3]Stanford University, Bern

Background: Omalizumab, a therapeutic monoclonal anti-IgE antibody, is successfully used in several allergic conditions. Its moderate affinity for IgE and weak disruptive efficacy, however, necessitate frequent dosing over prolonged periods. Here we characterized two new high-affinity omalizumab variants with improved disruptive efficacy, which could potentially optimize treatment outcome.

Methods: We compared binding characteristics and treatment efficacy of the new anti-IgE antibodies C03-H1L2 and C03-H2L2 with omalizumab. Surface plasmon resonance was used to measure binding kinetics and stoichiometry was assessed by size exclusion chromatography. In vitro assays were employed to evaluate receptor inhibition profiles, disruptive efficacy and anaphylactogenicity. In vivo efficacy was explored in a passive systemic anaphylaxis mouse model.

Results: Both omalizumab variants demonstrated increased affinity to human IgE. While immune complex formation and receptor inhibition profiles remained similar to omalizumab, the variants showed significantly improved disruptive efficacy. Both C03 antibodies removed IgE with faster kinetics from its high-affinity receptor FcεRI than omalizumab. In the systemic anaphylaxis mouse model, the C03 antibodies reached complete desensitization within 36 hours of single dose application.

Conclusions: These findings demonstrate that successfully established therapeutic anti-IgE biologicals, like omalizumab, can be optimized. Such antibody variants could yield novel and potentially more efficient therapeutic options for the treatment of allergies.

P14

Characterization of anti-IgE molecules to inhibit IgE: receptor interactions and suppress IgE production in B-cells.

P. Guntern1, L. Pennington[2], S. Nyffeler[1], T. Jardetzky[2], A. Eggel[1]

[1]University of Bern, Bern; [2]Stanford University School of Medicine, Stanford US

Aim: Binding of IgE to FcεRI and CD23, contributes to different immunological processes involved in the development of allergic disease. Allergen-induced crosslinking of IgE-FcεRI complexes on allergic effector cells leads to cellular degranulation. Recently, we have described a fast-acting anti-IgE molecule,

termed KIH_E07_79, that rapidly disrupts IgE-FcεRI complexes, inhibits pre-activated human basophils *ex vivo* and stops pre-initiated systemic anaphylaxis in mice *in vivo*. Here, we further compared the capacity of KIH_E07_79 to neutralize IgE, inhibit receptor binding and suppress IgE production in primary human B-cells *in vitro* to classical anti-IgE antibodies.

Methods: IgE-receptor interactions were assessed by ELISA, SPR measurements and flow cytometry. Human B-cells were isolated from whole blood and stimulated with IL-4 and an anti-CD40 antibody. IgE production was assessed by ELISA, qPCR and flow cytometry.

Results: KIH_E07_79 efficiently inhibited IgE binding to both IgE receptors on primary human basophils and B-cells, respectively. Interestingly, KIH_E07_79 suppressed IgE production and reduced the number of IgE$^+$ cells in primary human B-cells *in vitro*.

Conclusions: The disruptive anti-IgE molecule KIH_E07_79 efficiently inhibits IgE-binding to FcεRI and CD23. In addition to active desensitization of allergic effector cells, KIH_E07_79 inhibits IgE production and reduces IgE$^+$ cells in primary human B-cells. These additional modes-of-action could potentially be of clinical relevance and might increase the efficacy of such a multi-level IgE targeting approach.

P15

Blunting of CD8+ T cell response to COVID-19 mRNA vaccine in JAK inhibitor-treated patients is associated with reduced interferon signaling.

N. Madelon[1], R. Morse[1], N. Heikkila[1], G. Ruiz Buendia[2], P. Girard[1], W. Adouan[1], P. Fontannaz[1], R. Goldstein[3], K. Lauper[4], A. Finckh[4], C. Eberhardt[3], C. Berger[5], A. Didierlaurent[3]

[1]Geneva Vaccine Center, Faculty of Medicine, University of Geneva, Geneva; [2]Swiss Institute of Bioinformatics, Geneva Vaccine Center, Faculty of Medicine, University of Geneva, Lausanne; [3]Geneva Vaccine Center, Faculty of Medicine, University of Geneva, Geneva University Hospitals, Geneva; [4]Department of Medicine, Division of Rheumatology, Geneva University Hospitals and University of Geneva, Geneva; [5]Department of Biomedicine, Translational Immunology, University of Basel, Basel

JAK inhibitors (JAKi) target inflammatory cytokine signaling, potentially reducing efficacy of vaccines and the induction of persistent cellular immunity. We performed a comprehensive analysis of the innate and adaptive immune responses in 32 healthy participants and 22 rheumatic disease (RD) patients treated with JAKi who received a third dose of mRNA COVID-19 vaccine.

Levels of SARS-CoV-2 spike-specific antibodies were significantly lower in JAKi-treated patients as compared to controls before and 1 month after the 3rd dose, although the overall response remained high in both groups 6 months later. In contrast, spike-specific CD8+ T cell responses in JAKi-treated patients were either no detectable or lower than controls at all time points, correlating with lower serum levels of IP10, IL1Ra, MCP1 measured 1 day after vaccination. Gene-set enrichment analysis of gene expression in whole blood at the same time point revealed the absence of JAK-STAT and interferon-related pathways in patients. Potential defects in innate cell function were assessed at time of vaccination by stimulating PBMC with different TLRs or cytokines *in vitro.* Interferon-gamma stimulation of NK cells from JAKi-treated RD patients was impaired, suggesting that these cells play a critical role to CD8+ T cell response induced by mRNA vaccine *in vivo*.

In conclusion, CD8+ T cell response to mRNA vaccine is impaired in JAKi-treated patients. Our in-depth immune profiling identified key innate pathways associated with immune response to mRNA vaccines.

P16

Elevated serum levels of IL-18 discriminate Still's Disease from other autoinflammatory conditions: results from a unique European cohort

C. Girard-Guyonvarc'h[1], E. Rodriguez[2], A. Caruso[2], C. Gabay[1]

[1]HUG - UNIGE, Genève; [2]UNIGE, Genève

Aim: Autoinflammatory diseases (AIDs) pose diagnostic challenges due to their rarity and severity. IL-1β and IL-18 (a strong IFN-γ inducer) are pro-inflammatory cytokines of the IL-1 family that are secreted upon inflammasome/caspase pathway activation and therapeutic targets in AIDs. We aimed to measure the serum levels of IL-1β, IL-18, their inhibitors, IL-6 and IFN-γ in a large European cohort of AID patients (ImmunAID consortium).

Methods: We prospectively collected serum from 274 patients with monogenic or genetically undiagnosed AIDs, alongside 49 healthy controls. Commercial ELISAs measured IL-18, IL-18BP, IL-1Ra, IFN-γ, and IL-6, while a homemade ELISA quantified free IL-18 levels. IL-1β levels were assessed using an electrochemiluminescence assay. Correlations were established between cytokine levels and reported clinical and laboratory data. ROC curves were drawn to assess the diagnostic value of various parameters.

Results: Total IL-18 and free IL-18 were higher in Still's disease than in other AIDs. IFN-γ was the most elevated in Still's disease and IFN-γ levels correlated with total and free IL-18 ($r = 0.37$ and 0.30 respectively, $p<0.0001$). In contrast, IL-1β, IL-1Ra, IL-18BP, and IL-6 levels did not differ between AIDs patients. Total and free IL-18 correlated strongly with ferritin ($r = 0.44$ and 0.52 respectively, $p<0.0001$) and served as promising predictors of Still's disease (AUC: 0.92 and 0.85, respectively).

Conclusion: Our findings highlight the diagnostic potential of IL-18 in Still's disease and support targeting the IL-18-IFN-γ axis in its management.

P17

Effect of radiotherapy on the immune microenvironment of muscle-invasive bladder cancer

G. Zanette[1], E. Desponds[1], M. Leblond[1], V. Grégory[1]

[1]University of Lausanne, Ludwig Institute for Cancer Research, Lausanne

Bladder cancer was ranked as the 4th most frequent cancer in males and 14th in females in America and Europe in 2022. Among all the patients, 20% is diagnosed with muscle-invasive bladder cancer (MIBC), characterized by disruption of the urothelium and invasion of the muscle layer by tumor cells. The gold standard treatment is cisplatin-based chemotherapy followed by radical cystectomy, but it confers patients a survival of only 15 months. The use of immunotherapy, including antibodies targeting the PD-1/PD-L1 axis, has been approved but the response rate is ranging from 15% to 24%, which highlights the urgent need to develop new therapeutic options. Radiotherapy (RT) is used in BC for bladder-sparing protocol, which include very few patients who will undergo high dose RT combined with chemotherapy. However, low-dose RT has been investigated for its capacity to stimulate the TME and could be used in combination with immunotherapy.

Our project aims to detail the effects of different doses of RT on the TME of MIBC. We used a mouse model of MIBC that recapitulates features of the human disease, presents a highly suppressive TME and resistance to anti-PD-1 treatment. MIBC-bearing mice were irradiated with different doses of X-rays and the immune compartment was characterized by flow cytometry 7 days post-RT. Preliminary data showed an increase of anti-

tumor immune cells, especially CD8 T cells, 7 days post-RT with low dose of X-rays compared to untreated mice. The beneficial effect of RT can lead to the development of novel therapeutic solution for MIBC patients, especially combined with immunotherapy treatments.

P18

Fine tuning CD19-CAR sensitivity by modulating the CD28 transmembrane and intracellular domains

O. Alfageme-Abelló[1], E. Lana[2], R. Porret[1], L. Ermellino[1], A. Alcaraz-Serna[1], S. Georgakis[3], C. Petrovas[3], L. Perez[1], R. Cecchin[1], Y. Muller[1], P. Nunes-Hasler[4]

[1]Service of Immunology and Allergy, Department of Medicine, CHUV, Lausanne; [2]CHUV, Lausanne; [3]Institute of Pathology, Department of Medicine and Pathology, CHUV, Lausanne; [4]Department of Pathology and Immunology, Faculty of Medicine, University of Geneva, Geneva

CD19-CAR T cells are emerging as promising therapies yet exposed to on- and off-target toxicities possibly resulting from the formation of CAR-CD28 heterodimer. Modulations and functional implications of CAR-CD28 interactions have not been thoroughly studied.

We engineered 2nd generation CD19-CARs with either a CD28ζ or 4-1BBζ intracellular-domain (ICD) with selected mutations in the CD28 transmembrane-domain (TMD) targeting: (A) the CD28 evolutionary conserved YxxxxT motif (M1b = T171L), (B) the CD28-core polar amino acids (CYSxxxT->LLLxxxL = M4), (C) the GxxxA dimerization motif (to YxxxL = M5). Control wild-type CD28 TMD-CAR (WT) was used. CARs were linked to an EGFRt reporter and inserted into the TRAC locus of primary human T cells to control genomic integration.

Except for the M5 construct, all four mutants significantly reduced CAR-CD28 formation independently of the ICD assessed by the division index to anti-CD28 mAb stimulation and confirmed using a split fluorescent protein system. Mutating the threonine (M1b) strongly reduced cell-surface CAR expression and sensitivity against CD19 -low but not -high targets. CAR expression and sensitivity was progressively restored in the M4 CAR construct, although it remained inferior to WT-CAR and was ICD dependent.

Our data suggest that the TMD conformation dictates CAR expression level and its interaction with endogenous CD28. CAR T cell sensitivity can be modulated using selected TMD-ICD pairs, opening the perspectives to engineer safer CAR-T cell products for non-oncological applications.

P19

High-resolution KIR genotyping and its implication in allogeneic hematopoietic stem cell transplantation

J. Villard[1], A. Schäfer[1], S. Buhler[1], T. Farias[2], K. Kichula[2], Z. Calderin Sollet[1], M. P. Hervé[1], S. Ferrari-Lacraz[1], Y. Chalandon[1], S. Masouridi[1], D. Schneidawind[3], V. Mesquita[3], T. Gungor[4], O. Kursteiner[5], G. Nair[5], J. Halter[6], H. Baldomero[6], J. Passweg[6], P. Norman[2]

[1]Geneva University Hospital, Geneva; [2]University of Colorado School of Medecine, Colorado US; [3]Zurich University Hospital, Zurich; [4]Zurich Kinderspital, Zurich; [5]Swiss Blood Stem Cells, Liebefeld; [6]Basel University Hospital, Basel

The success of allogeneic hematopoietic stem cell transplantation (alloHSCT) partly relies on the beneficial graft-versus-leukemia effect, mediated by donor-derived alloreactive NK cells through their killer-cell Immunoglobulin-like receptor (KIR). Conflicting results have been reported with a scarcity of data interrogating the effect of KIR allelic polymorphism. With the aim to fill this gap, donor KIR genes derived from a national cohort of 1292 donor/recipient alloHSCT pairs were genotyped at a high-resolution and combined with the recipient HLA genotype to test their association with various transplant outcomes. In univariate analysis, we observed a lower progression-free survival (PFS) (p = 0.01) and higher transplant-related mortality (TRM) (p = 0.012) in donor/recipient pairs bearing KIR2DS4**00101*$_D$ - HLA-C2/A11$_R$ interactions, which was confirmed by multivariate analysis (PFS: hazard ratio [HR], 1.38, p = 0.0025; TRM: HR = 1.53, p = 0.018). PFS was also significantly influenced by the number of KIR2DL3$_D$ – HLA-C1$_R$ interactions (HR = 1.08, p = 0.02). In addition, these recipients showed a higher risk of developing chronic graft-versus-host disease with KIR2DS4**00101*$_D$ - HLA-C2/A11$_R$ interactions (HR = 1.31, p = 0.021) or by the strength of KIR2DL2/L3$_D$ - HLA-C1$_R$ interactions (HR = 1.21, p = 0.009). Recipients lacking a KIR2DS2$_D$ – HLA-C16$_R$ interaction had a lower rate of relapse (p = 0.0023), although the significance was lost in multivariate analysis. Our study indicates the potential detrimental effect of KIR activating interactions, especially KIR2DS4, potentially due to its sustained expression in an overactivated environment.

P20

Mechanistic evaluation of JAK inhibitor-mediated impairment of vaccine response in mouse models.

P. Torres Rodríguez[1], Y. Donati[2], G. Ruiz Buendia[2], P. Fontannaz[2], N. Madelon[2], A. M. Didierlaurent[2]

[1]University of Geneva, Geneva; [2]University of Geneva, Genève

Janus Kinase inhibitors (JAKi) are a novel class of disease-modifying antirheumatic drugs that selectively target JAK kinases to prevent/reduce the inflammation associated with the pathology. However, because JAKs are directly involved in the signalling of inflammatory cytokines needed for vaccine mode of action, their use have been associated with lower vaccine response. Our group recently showed that the cellular response to COVID-19 mRNA vaccine is impaired in patients treated with JAKi (see Madelon et al.)

Here, we investigated the impact of systemic JAKi therapy on the local tissues targeted by the vaccines (muscle and lymph node (LN)) in mouse models. We assessed the immune response to mRNA-based and adjuvanted vaccines because, they efficiently mobilise innate immunity to promote robust and long-lasting vaccine response, and this may be impacted by JAKi. We hypothesised that some tissue-resident cells, particularly those in the LN, are less affected due to limited drug accessibility to the tissue or variations in drug sensitivity at the cellular level. Our preliminary observations indicate that JAKi exert their effect mainly in blood and muscle, while the LN is relatively preserved from the inhibition of JAKI-dependent signalling. Additional research will identify which immune cells or pathways are most affected by the JAKi in these organs and and how this translates into a weakened immune response to vaccinations. . Our data will complement observation in in vaccinated RD patients treated with JAKi and shed light on how these drugs impairs immunity in different organs.

P22

HLA-E protects genetically engineered porcine endothelial cells from the lysis of natural killer cells in 2D and 3D microfluidic systems.

T. Tran[1], V. Galdina[1], O. Urquidi Gandarillas[1], R. Rieben[2], E. Wolf[3], E. Kemter[3], G. Puga Yung[1], J. Seebach[1], T. Adachi[1]

[1]University of Geneva, Geneva; [2]University of Bern, Bern; [3]Ludwig-Maximilian-University of Munich, Munich DE

Aim: Natural Killer (NK) cells are crucial in transplant rejection. Introducing inhibitory ligands for NK cells on pig cells could offer an effective means to suppress NK cell cytotoxicity. Cells expressing HLA-E can interact with human NK cells, inhibiting their destructive activity. Previous studies showed HLA-E transgenic pigs reduced NK cell lysis of porcine endothelial cells (PAECs). Our study examines HLA-E's protective effects in PAECs using 2D and 3D microfluidic systems.

Methods: Human NK cells were activated with IL-2 and co-cultured with WT and HLA-E/CD46 transgenic PAECs. Porcine TNF was used for endothelial activation to simulate the clinical conditions of xenografts. Live cell tracking was conducted in 2D and 3D microfluidic systems.

Results: HLA-E/CD46 transgenes reduced NK cell lysis of PAECs. Analysis of our 2D live cell tracking data revealed that NK cells killed PAECs mainly via apoptosis. NK cells exhibited greater attachment to WT PAECs, characterized by more constrained movements in single-cell trajectory analysis, whereas interactions with TG PAECs resulted in longer trajectories. In the 3D microchannel system, more attached NK cells and higher number of dead PEACs were observed in under flow conditions in WT PAEC.

Conclusion: This study demonstrated that introducing HLA-E molecules into genetically modified porcine endothelial cells resulted in effective inhibition of human xenoreactive NK cells. These findings could inform future genetic modifications in pigs aimed at mitigating rejection mediated by human NK cells.

P23

Antigen recycling in mast cells for the regulation of the immune response

J. Miguez Vazquez[1], P. Engeroff[1], M. Vogel[1], M. Bachmann[1]

[1]Universität Bern, Bern

Aims: Mast cells (MCs) are long-lived tissue resident cells capable of storing a wide range of biologically active mediators in their granules. Recent studies have uncovered the antigen storage capabilities of MCs, which, coupled with their extended lifespan, suggest a potential immunological antigen storage function similar to that observed in lymphocytes.

Methods: Antigen storage capabilities of mouse and human MCs were investigated through flow cytometric analysis. *In vitro* cultured MCs were tested for the uptake and storage of Fel d 1, Fel d 1 coupled to Qb Virus-like particles (VLPs; Qb-Fel d 1), and immune complexes of Fel d 1/Qb-Fe l d 1 with IgG as well as IgE. Additionally, we investigate antigen-transfer from MCs to other immune cells, such as Dendritic Cells (DCs), and lymphocytes. Antigen localization will furthermore be investigated through imaging flow cytometry.

Results: Our data confirms the antigen storage capabilities of MCs up to 21 days. Storage duration was significantly enhanced in presence of IgE antibodies. Moreover, the role of MCs as antigen storage cells is currently being investigated *in vivo* to discern their impact on shaping the immune response. Preliminary results show *in vitro* MC antigen storage. Furthermore, *in vitro* antigen transfer from antigen-bearing MCs to DCs was observed.

Conclusion: The role of MCs as long-term antigen reservoirs, along with their ability to transfer antigens to DCs, implies a novel contribution to immune mechanisms that has yet to be fully understood, potentially impacting various diseases, including autoimmune conditions.

P24

Is NLRC5, a transcriptional regulator of MHCI genes, controlled by the same conformational changes of inflammasome-forming NLRs?

J. Guerra[1], L. Simonelli[1], R. Thakur[1], A. Zenobi[1], P. Cejka[1], D. Morone[1], L. Varani[1], G. Guarda[1]

[1]Insitute of Research in Biomedicine, Bellinzona

NOD-like receptors (NLRs) are best known for their role as innate immune sensors. Upon cellular perturbations, NLRs like NLR CARD-containing (NLRC) 4 and NLR pyrin-containing (NLRP) 3, switch from an inactive/close conformation to an open one, allowing the formation of a multimeric platform known as inflammasome. NLRC5, despite belonging to the NLR family, exerts a completely different function; it transcriptionally regulates Major Histocompatibility Complex class I (MHCI) genes, the core genes of the adaptive immune system. This NLR shuttles to the nucleus where it occupies the promoter of MHCI genes by interacting with epigenetic and transcription factors to regulate transcription. Despite its emerging role in anticancer immunity, the molecular mechanisms underlying NLRC5 function are still unknown. NLRC5 shows the same domains organization of other NLRs, which are known to undergo conformational changes once activated. It is therefore temping to hypothesize that similar structural configurations to those identified in inflammasome-forming NLRs regulate NLRC5 activity.

In this work, by using both biochemical and molecular biology approaches we aimed to identify key residues, in NLRC5, which will allow us to clarify NLRC5's structure and function. Understanding the conformational changes of NLRC5 will shed light on its mode of action and will open new avenues for the development of modulators of NLRC5 activity and, thus, of MHCI levels, which is highly relevant to cancer immunotherapy.

P25

Transglutaminase 2 governs phospholipid metabolism and ferroptosis in type 2 immunity

B. Spitzlberger[1], J. Esser-Von Bieren[2], F. Henkel[3], E. Ben Salah[2], R. Zhigang[4], S. Bohnacker[2], S. Schindela[1], F. Hartung[1], A. Köberle[4], C. Ohnmacht[1], P. Murray[3]

[1]TU Munich and Helmholtz Munich, Munich DE; [2]UNIL Lausanne, Lausanne; [3]MPI Munich, Munich DE; [4]Michael Popp Institute, Innsbruck AT

Ferroptosis is a form of regulated cell death induced by iron-dependent lipid peroxidation. Alternatively activated macrophages (AAM) play key roles in type 2 immunity against helminth parasites, despite being highly susceptible to lipid peroxidation and ferroptotic cell death. Here, we identify transglutaminase-2 (TG2), a conserved marker of human and murine AAM, as a central driver of ferroptosis susceptibility. When infected with a parasitic nematode, wildtype mice show a profound increase of lipid peroxidation and ferroptotic cell death in the small intestine, while mice lacking hematopoietic TG2 are protected from ferroptosis. While products of arachidonic acid oxidation (eicosanoids and 4-hydroxynenal (4-HNE)) as well as membrane lysophospholipids are reduced in the absence of hematopoietic TG2, oxidized PE species accumulate, indicative

of a TG2-dependent pro-ferroptotic lipid remodeling. In line, AAM from TG2 deficient mice show an increased PUFA/ MUFA ratio in membrane phospholipids as well as exaggerated accumulation of oxidized PE species and reduced cell death upon ferroptosis induction. Thus, TG2 drives ferroptosis susceptibility in type 2 immunity by remodeling the phospholipid metabolism of a key immune cell type.

P26

Why do NK-92 cells lack the surface expression of CD16A?

M. Andreani[1], M. Freitas Monteiro[1], A. Réal[1], G. Puga Yung[1], J. Seebach[1]

[1]University of Geneva, Geneva

Natural Killer (NK) cells patrol for virally infected and malignant cells. Clinical trials using chimeric antigen receptors (CAR)-NK therapy are showing promising results, especially in hematological tumors. This therapy is based on the NK-92 cell line expressing both CAR and the high-affinity Fc receptor 3A variant (CD16A, *FCGR3A* gene). The human non-Hodgkin lymphoma-derived NK-92 cell line lacks the surface expression of CD16A presumably due to a non-functional promoter; according to literature, *FCGR3A* mRNA is not expressed. Incidentally, by RT-PCR, we found the expression mRNA covering the extracellular domains of CD16 in NK-92 cells, yet at lower levels compared to freshly isolated human NK cells. Furthermore, we confirmed the absence of CD16A using several monoclonal antibodies by flow cytometry. This study aims to understand why CD16A surface expression is absent in NK-92 cells. We hypothesize that *FCGR3A* expression may be regulated at the post-transcriptional level, namely by micro-RNA (miRNA). By using miRNA-inhibiting oligonucleotides, we targeted two miRNAs overexpressed in CD16A-negative human primary NK cells. Preliminary results indicate that miRNA inhibition partially rescues CD16A expression in NK-92 cells. Additional factors potentially affecting CD16A surface expression in NK-92 cells, such as the lack of the transmembrane domain, are being addressed. This study could elucidate the mechanisms involved in the expression of CD16A and suggest the NK developmental stage from which NK-92 cells originated.

P27

Role of SHP-2 and PD-1 in lymphoid and myeloid antitumor responses.

I. Buzzago[1], P. Ventura[1], H. Javanmard Khameneh[1], A. Zenobi[1], F. Silini[1], L. Brossay[2], G. Guarda[1]

[1]Institute for Research in Biomedicine (IRB), Bellinzona; [2]Brown University, Providence US

When immune cells infiltrate the tumor, their functions can be altered. For example, T cells can reach a state of “exhaustion”, characterized by expression of inhibitory receptors, such as programmed cell death protein 1 (PD-1). SH-2 domain-containing phosphatase (SHP)-2 is reported to be an important component of the inhibitory effects of PD-1 by interacting with it. Paradoxically, SHP-2 is best known as a positive regulator downstream of growth factor receptors and inhibitors targeting this phosphatase are currently under clinical evaluation to dampen cancer progression.

To date, the biological meaning of the interplay between SHP-2 and PD-1 and their downstream signalling remains an open question. Whereas their interaction is thought to be essential for T cell exhaustion, *in vivo* data from our lab indicate that Shp-2 is dispensable for PD-1 signaling in T cells and that its homologous phosphatase SHP-1 does not play a redundant function. This brought us to:

- generate mice lacking PD-1 in the T cells, which better control tumor and will enable us to further study the underlying molecular mechanism.
- evaluate the role of the PD-1/SHP-2 axis in other immune cells mediating anticancer responses; single deletion of these proteins in myeloid cells delays tumor growth, supporting their function in establishing tumor suppression.

We are currently investigating how PD-1 and Shp-2 diverge or converge into a tumor-supportive role of lymphoid and myeloid cells, hoping that our results will highlight the potential benefits and risks for future anti-cancer therapies.

P28

Xenotransplantation: Innovative approaches for functional assessment of human anti-pig cytotoxic T cells in vitro

V. Galdina[1], T. Tran[1], R. Rieben[2], E. Wolf[3], E. Kemter[3], G. Puga Yung[1], J. D. Seebach[1]

[1]University of Geneva, Geneva; [2]University of Bern, Bern; [3]Ludwig-Maximilian-University of Munich, Munich DE

The use of pig organs is closer to become an alternative to the shortage of human organs, while the modulation of the acquired immunity is being explored. Our aim was to develop tools to assess the xenospecific responses of $CD8^+$ T cells (CTL) to monolayers of porcine aortic endothelial cells (PAEC) from wild type (WT) and transgenic (TG) animals.

Human CTL were characterized and purified from PBMC that were stimulated with irradiated PAEC for 3 weeks. CTL’s specificity was tested by conventional cytotoxicity assay using primary $PAEC_{WT/TG}$ as targets. In addition, cytotoxicity was analysed by live-cell imaging under static (2D) and microfluidic (3D) co-cultures at an effector to target (E:T) ratio of 1:1. Read outs were the numbers of CTL’s adhered and lysed PAEC (apoptotic bodies and necrotic $Draq7^+$ cells).

After stimulation with PAEC, CTL phenotype underwent a transition from naïve to memory. In conventional assay, CTL were highly specific for $PAEC_{WT}$, and their cytotoxicity was dependent on the number of effector cells. Yet, the effect was evident only at high E:T ratios (≥ 5:1). In 2D live-cell imaging, CTL lysed $PAEC_{WT}$ mostly via apoptotic pathways (11.26 ± 0.55%) compared to necrosis (6.02 ± 1.73%). Human PD-L1 in $PAEC_{TG}$ protected them from apoptosis (0.13 ± 0.12%) and necrosis (1.17 ± 0.73%). In 3D perfusions, CTL adhered less to $PAEC_{TG}$, but the number of lysed $Draq7^+$ $PAEC_{WT}$ was similar.

Human CTL and PAEC interactions were assessed using a more physiological, sensitive, and reliable 2D and 3D live-cell imaging method under static and flow conditions than conventional assays.

P29

Probing Monoclonal Antibodies for Targeted Alzheimer’s Vaccine Design

A. C. Vogt[1], M. Vogel[2], M. Jendrach[3], A. Förster[3], A. Haake[3], C. Wong[3], A. Zeltins[4], F. Heppner[3], M. Bachmann[2]

[1]University Hospital Bern, Bern; [2]Department for BioMedical Research, Bern; [3]Department of Neuropathology, Berlin DE; [4]Latvian Biomedical Research & Study Centre, Riga LV

Alzheimer’s disease (AD) is the most common form of dementia and may contribute to 60–70% of cases. Accumulation of extracellular plaques containing amyloid-b peptide characterizes one feature of the neuropathology of AD.

The pathogenesis of the disease appears 10–20 years before AD's clinical manifestation. Thus, the goal of innovative treatments should be to postpone or stop the disease before it reaches the preclinical stage.

Recently, 2 mAbs have been approved for the treatment of AD, namely Aducanumab and Lecanemab. In the current project, we design vaccines to induce polyclonal antibodies of the same specificity as the approved mAbs. We developed a vaccine based on virus-like particles (VLP) derived from the cucumber mosaic virus ($CuMV_{TT}$) fused with different epitopes of $Abeta_{1-42}$. Our experiments have now shown that genetic fusion of different epitopes of Ab ($Abeta_{3-6}$, $Abeta_{1-6}$ and $Abeta_{1-7}$) to the surface of $CuMV_{TT}$ resulted in three vaccine candidates which are highly immunogenic and induced IgG antibodies against the full-length $Abeta_{1-42}$. Moreover, IgG generated by the $CuMV_{TT}$-$Abeta_{3-6}$ vaccine, showed the same recognition profile as approved mAb Aducanumab, preferably binding oligomers. Additionally, the generated antibodies specifically bound Abeta plaques in human brains as well as in mouse brain tissue. Treatment with the $CuMV_{TT}$-$Abeta_{3-6}$ vaccine, in transgenic mice prone to the disease, resulted in a decrease in the amount of Abeta plaques in the brains of these mice. In addition, we are now testing $CuMV_{TT}$-$Abeta_{1-16}$ for the induction of Lecanemab type antibodies.

P30

TLR7-9 adaptors TASL and TASL2 mediate IRF5-dependent antiviral responses and autoimmunity.

M. Rebsamen[1], A. Drobek[2], L. Bernaleau[2], M. Delacrétaz[3], S. Calderon Copete[4], J. Korzeniowski[2], M. Monguio Tortajada[2], M. Longepierre[2], J. Marquis[4]

[1]University of Lausanne - Dpt of Immunobiology, Epalinges; [2]University of Lausanne, Epalinges; [3]University of Lausanne, Epalinges; [4]University of Lausanne, Lausanne

Endosomal nucleic acid sensing by TLR7-9 plays a central role in antimicrobial immunity and in several autoimmune conditions such as systemic lupus erythematosus (SLE). We recently identified a novel innate adaptor, TASL, which controls TLR7/9-induced responses by assembling in an IRF5-activating signaling complex with the endosomal solute carrier SLC15A4 (Heinz et al, Nature 2020). We further demonstrated that this complex is “druggable” (Boeszoermenyi et al, Nat. Commun. 2023). Notably, all the components of this signaling axis are associated to SLE in GWAS. Here, we address the pathophysiological role of TASL and its murine paralogue TASL2 *in vivo*.

Newly generated *Tasl*, *Tasl2* and *Tasl* double knockout mice were characterized with *Slc15a4*-deficient mice by monitoring TLR7/9 responses *ex vivo* and *in vivo*. The impact of *Slc15a4* and *Tasl* deficiency was further investigated in chronic LCMV infection and in the pristane-induced SLE model.

Here we show that TLR7/9-induced IRF5 activation and cytokine production are impaired in $Slc15a4^{KO}$ and $Tasl^{DKO}$ primary immune cells. *In vivo*, $Slc15a4^{KO}$ and $Tasl^{DKO}$ show a profound defect in type I IFN and cytokine production upon stimulation with TLR7/9 agonists. Accordingly, $Slc15a4^{KO}$ and $Tasl^{DKO}$ mice displayed impaired antiviral responses to LCMV infection, while being strongly protected from disease in the pristane-induced SLE model.

This study demonstrates the critical role of SLC15A4 and TASL for TLR7/9-driven inflammatory responses *in vivo*, further supporting the therapeutic potential of targeting this complex in SLE and related diseases.

P31

Role of type 1 IFN signaling in trained type 2 immunity

E. Ben Salah[1], F. Hartung[2], S. Bohnacker[3], B. Spitzlberger[4], F. Alessandrini[4], M. Kulagin[3], J. Esser-Von-Bieren[3], F. Hartung[2], F. Hartung[2], F. Hartung[2], F. Hartung[2], F. Hartung[2], F. Hartung[2], F. Hartung[2], F. Hartung[2]

[1]Université de Lausanne (UNIL), Lausanne; [2]Center of Allergy and Environment (ZAUM), Technical University of Munich and Helmholtz Center Munich, 80802, Munich, Germany., Munich DE; [3]Department of Immunobiology, University of Lausanne, Epalinges, Switzerland, Lausanne; [4]Center of Allergy and Environment (ZAUM), Technical University of Munich and Helmholtz Center Munich, Munich, Germany, Munich DE

Trained immunity of innate immune cells confers protection against pathogens, however, the contribution of innate immune memory to type 2 immune responses such as allergy or helminth infection is only beginning to emerge. We previously showed that intranasal house dust mite (HDM) exposure alters bone marrow (BM) progenitor cells implicating central trained immunity in a murine model of allergic airway inflammation (AAI).

To assess the reprogramming of innate immune cells in BM progenitors as well as locally in the lung in two settings of type 2 immunity.

We studied the transcriptional reprogramming of bone marrow-derived macrophages (BMDM) and alveolar macrophages (AMs) in *in vivo* models of AAI and helminth (*Heligmosomoides polygyrus bakeri* (Hpb)) infection in wildtype and transgenic ($Ifnar^{fl/fl}$ x $Vav1^{Cre}$) mice using RNA seq and qPCR.

RNA seq analysis of BMDM from HDM-sensitized WT mice revealed a strong induction of type 1 IFN signaling. Global Ifnar KO mice showed no reduction of AAI whereas the deficiency in type 1 IFN signaling in the hematopoetic compartent resulted in reduced inflammation. $Ifnar^{fl/fl}$ x $Vav1^{Cre}$ mice displayed lower BAL cell counts and reduced airway eosinophils compared to WT during AAI. RNA seq data of AMs from Ifnar KO mice indicated that type 1 IFN might contribute to airway remodeling. We further observed an upregulation of several markers of type 2 immunity in BMDMs from $Ifnar^{fl/fl}$ x $Vav1^{Cre}$ mice infected with Hpb.

Type 1 IFN signaling drives type 2 inflammation and regulates the reprogramming of innate immune cells during AAI and helminth infection.

P32

How nociceptors shape the tumor microenvironment in melanoma and breast cancer

L. Garnier[1], M. Madec[1], L. Basso[2], S. Hugues[1]

[1]University of Geneva, Geneva; [2]Infinity, Toulouse FR

Tumor immunity arises from complex interactions between tumor cells, stromal cells and immune cells of the tumor microenvironment (TME). Tumor cells escape immunosurveillance by inhibiting directly on immune effector cells or acting on stromal cells, such as cancer-associated fibroblast, lymphatic and blood endothelial cell, playing key role in anti-tumor responses. Nociceptors are emerging in the literature as an integral part of the TME. Their main role is to convey noxious stimuli from the periphery to the central nervous system leading to pain sensation. When activated, these peripheral sensory neurons can secrete neuropeptides from their peripheral axons, known to act on stromal and immune cells in several contexts. Nociceptors, often present in solid tumor, can impact tumor growth by modifying anti-tumor immunity in different mouse model of cancer. However, the influence of nociceptors on tumor stroma is poorly described. By using a genetically-ablated nociceptor mouse model, we showed that sensory neurons promote tumor

growth and alter TME (immune cell and stromal cell composition and phenotype) in orthotopic lymphangiogenic melanoma and breast cancer. The better characterization of TME modulation by nociceptors in different cancers may unveil new potential targets to enhance cancer treatments and response to immunotherapy.

P33

TCR, WHERE ARE YOU? AN INTRIGUING CASE OF A PATIENT WITH AN ABERRANT T LYMPHOCYTE POPULATION OF CD3+/CD4-/CD8-/TCRαβ-/TCRγδ- AND IGG2 DEFICIENCY

C. Ballerini[1]

[1]University of Milan, Milano IT

Aim: Double negative (DN) T cells are a subpopulation of T lymphocytes involved in immune response and regulation lacking the surface expression of the CD4 and CD8 co-receptors. They normally express the T cell receptor (TCR)-γδ or, in small proportion, -αβ.

Method: We report the case of a 4-year-old boy with an aberrant T lymphocyte population of CD3+/DN/TCRαβ-/TCRγδ- and IgG2 deficiency.

Results: The patient came to our attention at the age of 4 months, when he was hospitalized for an incomplete Kawasaki disease and a post-natal CMV infection treated with Valganciclovir. A complete immunological work-up showed normal serum immunoglobulin levels but an IgG2 deficiency. Lymphocyte subpopulations presented an increased percentage of DN T cells (10% of lymphocytes), with an aberrant TCRαβ-/TCRγδ- population, equal to about 30% of DN cells and 4% of total CD3+. Further investigation on this subset showed a proper expression of the other T cell lineage markers (CD2,CD5,CD7), the absence of CD56, and a polyclonal rearrangement of TCR gene. A deeper characterization excluded an expansion of the recent thymic emigrant lymphocytes or of activated T cell fraction.

Conclusions: We are dealing with a case of a patient with IgG2 deficiency and a CD3+/DN/TCRαβ-/γδ- population, stable in percentage, and where the normal expression of T cell markers together with the absence of a clonal TCR rearrangement seems to exclude an immuno- or onco-hematological pathology. This population of unknown origin and significance is still under investigation and further analysis are scheduled.

P34

Does c-MYC modulate metabolism and effector functions in TLR activated B cells?

M. Pezzoli[1], R. Steiner[1], G. Bantug[1], C. Hess[1]

[1]University Hospital Basel, Basel

To exert their innate-like function B cells depend on pattern recognition receptors, such as Toll-like receptors (TLRs). Upon TLR stimulation and activation of downstream signaling pathways B cells undergo metabolic changes which prompt their effector maturation. c-MYC is a known driver of metabolic reprogramming in lymphocytes. Here we investigate the role of c-MYC in TLR stimulated B cells, particularly in regards to its impact on metabolism, cell activation, and cytokine production with the help of transcriptomics, metabolomics, and biochemical studies.

To elucidate how TLR engagement prompts B cells stimulation, we performed transcriptomics and metabolomics analysis of primary B cells following treatment with a TLR-ligand. Integration of transcriptomics and metabolomics analysis highlighted glucose metabolism as the most enriched pathway, with targets of the transcription factor MYC being the most prominently altered gene sets. Using mitochondrial stress assays, we observed that the increase in both mitochondrial respiration and glycolysis observed after TLR stimulation of B cells, is limited when cells are additionally treated with the c-MYC inhibitor 10058-F4 for 48 hours. Similarly, inhibition of c-MYC lead to a decrease in the expression of the activation marker CD71 and in cytokines TNF and IL-6 compared to controls.

These findings position c-MYC as a significant contributor of B cell metabolism and effector function, especially with regards to cell activation and cytokine production, and provide new perspectives on the regulation of innate-like functions of B cells.

P35

Harnessing lymphatic endothelial cells to improve anti-tumor immunity

M. Madec[1], L. Garnier[2], S. Hugues[2], J. Angelillo[2]

[1]UniGe, Genève; [2]UniGE, Genève

Lymphatic endothelial cells (LECs) form lymphatic vessels (LVs) that expand upon inflammation, a process called lymphangiogenesis. They play an important role in cancer as they can drain immune cells to the tumor and have been shown to be beneficial for immunotherapy. However, LECs can also promote metastasis and inhibit anti-tumor immune response. This project aims at harnessing LECs to improve anti-tumor activity in lymphangiogenic tumors. The laboratory engineered several mouse tumor cell lines to overexpress the lymphangiogenic factor VEGF-C (VC) to increase the lymphangiogenesis associated to the tumor growth. Preliminary data have shown that some molecules that might be implicated in the ability of LECs to regulate metastasis or immune cells are upregulated in LECs from highly immune infiltrated tumors such as the colorectal model MC38ovaVC, while they are downregulated in less immune infiltrated tumor models such as the B16ovaVC melanoma model. CSF-1 and IL33 are 2 of these molecules that are being studied. CSF-1, colony stimulating factor 1, is a hematopoietic growth factor. Proliferation, survival, and differentiation of macrophages depend on this cytokine. IL33 is a cytokine expressed by endothelial cells, epithelial cells, and fibroblastic reticular cells (FRCs). Known as an alarmin, its role can be very interesting in the modulation of the immune response. Harnessing the LEC functions by modifying the expression of these molecules could improve anti-tumor activity and/or decrease metastasis.

P36

SHAECS: The Swiss Hereditary Angioedema Cohort Study

I. Morales[1], W. A. Wuillemin[2], S. Zeerleder[3], B. Lucas[4], K. Hartmann[5], M. Fontana[6], P. Jandus[7], L. Arlettaz[8], L. Jörg[1], C. Ribi[9], B. Ballmer-Weber[10], A. Neff[10], C. Weber-Chrysochoou[11], H. Hengartner[12], U. C. Steiner[13]

[1]Division of Allergology and Clinical Immunology, Department of Pneumology and Allergology, Inselspital, Bern University Hospital, University of Bern, Bern, Switzerland, Bern; [2]Division of Hematology and Hematology Laboratory, Cantonal Hospital of Lucerne and University of Bern, Switzerland, Lucerne; [3]Division of Hematology and Hematology Laboratory, Cantonal Hospital of Lucerne and University of Bern, Lucerne; [4]Medignition Inc., Research Consultants, Zürich, Switzerland, Zürich; [5]Division of Allergy, Department of Dermatology, University Hospital Basel and University of Basel, Switzerland., Basel; [6]Allergology and Clinical Immunology Service, Ospedale Beata Vergine, Mendrisio, Ticino, Switzerland., Mendrisio; [7]Service d'Immunologie et d'Allergologie, Hôpitaux Universitaires de Genève, Geneva, Switzerland., Geneva; [8]Allergology and Clinical Immunology, Hôpital du Valais, Sion, Switzerland, Sion; [9]Service Immunologie et Allergie, CHUV, Lausanne, Switzerland, Lausanne; [10]Clinic for Dermatology and Allergology, Cantonal Hospital of St. Gallen, St.Gallen, Switzerland, St.Gallen; [11]Department of Dermatology and Allergology of University Hospital Zurich, Zurich; [12]Pediatric Hematology-Oncology Unit, Children's Hospital of St. Gallen, St. Gallen, Switzerland;

[13]Department of Rheumatology and Immunology, Department of Clinical Chemistry, Inselspital, Bern University Hospital, University of Bern, Switzerland, Bern

Background: In recent years new medications have improved the treatment options for patients affected by HAE. The Swiss HAE Cohort study started in March 2023 and we aim to assess the clinical characteristics and quality of life of patients with HAE living in Switzerland.

Methods/Design: This prospective, longitudinal, multi-center cohort study includes ten centers in Switzerland. Patients affected by hereditary angioedema Type 1, Type 2, HAE with normal C1-Inhibitor (nC1-INH HAE) and acquired angioedemas are included. Clinical data including attack-rate, medication use, comorbidities and quality of life with the Angioedema Control Test (AECT) and Angioedeme Quality of Life test (AeQoL) are collected at inclusion and at annual follow-ups.

Results: Mean age of the 62 patients (42 women and 20 men) is 45.9 [SD 20.39] years. Fourty-eight patients are under prophylactic therapy and 14 have therapy on demand. The AECT score for women is 13.5, for men 13.3. The AeQoL score for women is 16.5 and 11.5 for men. The overall attack rate in the last 6 months before inclusion is 1.5 [SD 1.9], with 1.8 for women and 1.0 for men. The most common comorbidities are arterial hypertension, cancer, dyslipidemia, cardiopathy, and depression/anxiety.

Discussion: HAE is well controlled in women and men. Quality of life in women is slightly decreased compared with men, this may reflect the fact that women are more affected by HAE. In the international context Swiss HAE patients showed the best results for AE-QoL, which could be associated with the high availability of the newest treatments.

P37

Towards deciphering how inflammatory response to biomaterials modulates bone formation

S. Ardicli[1], S. Wawrocki[2], H. Babayev[1], E. I. Bektas[3], O. Ardicli[1], D. Yazici[1], Y. Pat[1], B. Rückert[1], A. Heider[1], Z. Zhou[4], M. D'este[3], E. Della Bella[3], T. Serra[3], C. Ligorio[5], A. Mata[5], M. Akdis[1], M. Stoddart[3], C. Akdis[1]

[1]Swiss Institute of Allergy and Asthma Research (SIAF), Davos Wolfgang; [2]Swiss Institute of Allergy and Asthma Research, Davos Wolfgang; [3]AO Research Institute Davos, Davos; [4]Guangdong Provincial Key Laboratory of Orthopedics and Traumatology, The First Affiliated Hospital of Sun Yat-sen University, Guangzhou CN; [5]Department of Chemical and Environmental Engineering, University of Nottingham, Nottingham GB

Aim: The importance of immunomodulation in bone healing is widely recognized, and the field of osteoimmunology is rapidly growing in significance. Large bone defects often need additional material to fill voids and support bone formation. In this study, we investigate the immunomodulatory properties of novel bone-forming materials using high-throughput techniques.

Methods: The response of PBMCs to hydrogels based on tyramine-modified hyaluronic acid gel (THA), agarose, and fibrin sealant, as well as beta-tricalcium phosphate and hyaluronic acid particles, was examined in monolayer and transwell cultures. Our experiments included measuring cell proliferation using thymidine assay, cell viability with propidium iodide in flow cytometry, and targeted proteomics using Proximity Extension Assay.

Results: The data showed a slight increase in cell proliferation when exposed to THA gels, agarose, or fibrin sealant. In the targeted proteomics data, fibrin sealant and bone particle combinations led to upregulated proteins associated with bone remodeling and low inflammatory response. Conditions related to agarose and bone particles did not induce significant alterations in our biomarker panels. We identified the matrisome, plasmacytoma, apoptosis, and immune response-related pathways for the fibrin sealant as well as its combination with filtrated bone particles.

Conclusions: PBMC response to fibrin sealant and its combination with filtered bone particles point out reduced bone resorption and potentially increased bone formation.

P38

A helminth enzyme subverts macrophage-mediated immunity by epigenetic targeting of prostaglandin synthesis

S. Bohnacker[1], F. D. Henkel[2], F. Hartung[3], A. Geerlof[4], S. Riemer[3], U. F. Prodjinotho[5], E. Ben Salah[6], A. Santos Dias Mourão[4], S. Bohn[7], T. Teder[8], D. Thomas[9], R. Gurke[9], C. Boeckel[10], M. Ud-Dean[10], A. C. König[11], A. Quaranta[12], F. Alessandrini[3], A. Lechner[3], B. Spitzlberger[3], A. M. Kabat[13], E. Pearce[13], J. Z. Haeggström[8], S. M. Hauck[11], C. E. Wheelock[12], P. J. Jakobsson[14], M. Sattler[15], D. Voehringer[16], M. J. Feige[17], C. Prazeres Da Costa[18], J. Esser-Von Bieren[6]

[1]University of Lausanne, Epalinges; [2]Center of Allergy and environment (ZAUM), Technical University of Munich and Helmholtz Munich, Munich DE; [3]Center of Allergy and Environment (ZAUM), Technical University of Munich and Helmholtz Munich, Munich DE; [4]Protein Expression and Purification Facility (PEPF), Institute of Structural Biology, Helmholtz Munich, Neuherberg DE; [5]4Institute for Microbiology, Immunology and Hygiene, Technical University of Munich, Munich DE; [6]Department of Immunobiology, Université de Lausanne, Epalinges; [7]Department of CryoEM Technology, Max-Planck-Institute of Biochemistry, Martinsried DE; [8]Department of Medical Biochemistry and Biophysics, Division of Chemistry II, Karolinska Institutet, Stockholm SE; [9]Goethe University Frankfurt, Institute of Clinical Pharmacology, Faculty of Medicine, Frankfurt DE; [10]Institute of Computational Biology, Helmholtz Munich, Neuherberg DE; [11]Metabolomics and Proteomics Core, Helmholtz Munich, Neuherberg DE; [12]Division of Physiological Chemistry 2, Department of Medical Biochemistry and Biophysics, Karolinska Institute, Stockholm SE; [13]Max Planck Institute for Immunobiology and Epigenetics, Freiburg DE; [14]Department of Medicine, Division of Rheumatology, Karolinska Institutet & Karolinska University Hospital at Solna, Stockholm SE; [15]Cryo-Electron Microscopy Platform and Institute of Structural Biology, Helmholtz Munich, Neuherberg DE; [16]Infektionsbiologische Abteilung, Universitätsklinikum Erlangen, Friedrich-Alexander-Universität, Erlangen-Nürnberg DE; [17]Center for Functional Protein Assemblies (CPA), Department of Bioscience, TUM School of Natural Sciences, Technical University of Munich, Garching DE; [18]Institute for Microbiology, Immunology and Hygiene, Technical University of Munich, Munich DE

Mechanisms and molecules associated with the evasion of the immune response by parasitic helminths can be exploited for the treatment of type 2 immune response disorders. Key mediators in type 2 inflammatory diseases are bioactive metabolites of arachidonic acid. Here, we identified the immune-modulatory glutamate dehydrogenase (GDH) in the larval extract of the helminth *Heligmosomoides polygyrus bakeri* (*Hpb*). We particularly assessed whether helminthic GDH (heGDH) regulates allergy or anti-helminth immunity by modulating immune cell metabolism.

Effects of heGDH on the metabolism of monocyte derived macrophages (MDM) were quantified by LC-MS/MS (eicosanoids, TCA metabolites) and seahorse analysis. Furthermore, heGDH treated MDM were subjected to RNA- and ChIP sequencing to assess effects on gene expression profiles and epigenetic changes.

In macrophages, heGDH induced the production of prostanoids and 2-hydroxyglutarate, which contributed to the suppression of pro-inflammatory cysteinyl leukotrienes. Moreover, heGDH treated MDM showed an induction of regulatory genes, which depended on histone acetylation via p300. Treatment of mice with heGDH attenuated house dust mite- induced allergic airway inflammation in mice, while the treatment during *Hpb* infection resulted in chronicity and PGE_2 production in host macrophages.

Our findings thus suggest that heGDH mediates immune evasion by inducing the p300-prostaglandin axis. Thus, anti-inflammatory modulation of macrophages by heGDH may be translated into new immunomodulatory strategies for the treatment of inflammatory diseases.

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No Grass Pollen Allergy in sub-Saharan Africa? Analysis of Sensitization Patterns to Pollen in Swiss & sub-Saharan African Atopic Dermatitis Patients

D. Fehr[1], M. Rentschler[2], F. Sendrasoa[3], N. Li[1], A. White[1], C. Clemens[1], C. Lang[1], G. Masenga[4], N. Mosha[4], G. Semango[4], T. Rasamoelina[5], A. H. Soankasina[5], F. Rapelanoro Rabenja[3], D. Mavura[4], J. E. Masenga[4], P. Schmid-Grendelmeier[1], M. C. Brüggen[1]

[1]University Hospital Zurich, Zurich; [2]University Hospital Zurich, Zurich; [3]Centre Hospitalier Universitaire Joseph Raseta Befelatanana, Antananarivo MG; [4]Regional Dermatology Training Center, Kilimanjaro Christian Medical Center, Moshi TZ; [5]Centre d'Infectiologie Charles Mérieux, Antananarivo MG

Background/Aim: Sensitizations to pollen or house dust mites can directly impact atopic dermatitis (AD). Some patients report an AD exacerbation upon exposure. This study aims to gain more insights into the sensitization patterns to pollen allergens of AD patients in Europe compared to sub-Saharan Africa (SSA).

Methods: We included 20 AD patients and 10 healthy controls (HC) from each center in Switzerland (CH), Tanzania (TZ) and Madagascar (MD) in this case-control study. We analyzed the sera with the ALEX^2 Allergy Explorer, measuring total IgE and 300 specific IgE antibodies.

Results: The prevalence of ARC and allergic asthma in AD patients was similar in all countries (RCA: 60% in TZ, 70% in CH, 75% in MD; asthma: 25% in TZ, 30% in CH, 20% in MD). Total IgE levels were significantly lower in the CH than in both SSA HC groups (TZ vs. CH: p = 0.03, MD vs. CH: p = 0.04). We found major differences in sensitization patterns to inhalative allergens, especially to grass pollen allergens. Swiss AD patients were sensitized to various grass pollen such as bahia grass (Pas n), bermuda grass (Cyn d, Cyn d 1), common reed (Phr c), perennial ryegrass (Lol p 1), rye (Sec c_pollen), and timothy grass (Phl p 1, Phl p 2). However, no AD patient or HC subject from the SSA cohort was sensitized to the tested grass pollen.

Conclusions: The absence of grass pollen sensitizations in SSA is most likely due to a lack of commercially available sIgEs tailored to the African environment. More research is needed to identify local types and counts of pollen to develop suitable diagnostics and therapies.

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Investigation of the role of IL-33 for intestinal tumorigenesis

A. Herbst[1], V. Vu[2], M. H. Wasmer[2], S. J. Eugster[2], P. Krebs[2], A. Chatziioannou[3], E. Pilalis[4]

[1]University of Berne, Bern; [2]Institute of Tissue Medicine and Pathology, Bern; [3]Center of Systems Biology, Biomedical Research Foundation of the Academy of Athens, Athens GR; [4]e-NIOS, Athens GR

Aim: Colorectal cancer (CRC) is one of the leading causes of cancer-related deaths, with chronic inflammation in the intestine being a prominent catalyst. Interleukin-33 (IL-33) signaling plays a controversial role in intestinal inflammation and CRC, and its exact mode of action in the tumor environment is unclear. Here, we want to investigate the dual role (nuclear/ST2-independent vs soluble) of IL-33 in CRC.

Methods: CRC was induced by treating WT, *St2*$^{-/-}$, *Il33*$^{-/-}$ and *St2*$^{-/-}$*;Il33*$^{-/-}$ mice with azoxymethane (AOM) and dextran sodium sulfate (DSS). Bulk RNA sequencing has been performed on tumor vs. adjacent tumor-free intestinal tissue of these mice. MC38 cell lines transduced with different IL-33 isoforms were injected s.c. in WT, *St2*$^{-/-}$, *Il33*$^{-/-}$, *St2*$^{-/-}$*;Il33*$^{-/-}$ mice to observe tumor growth.

Results: *St2*$^{-/-}$ mice treated with AOM/DSS were protected from intestinal tumors, whereas *Il33*$^{-/-}$ and *St2*$^{-/-}$*;Il33*$^{-/-}$ mice developed colorectal tumors with similar numbers and load to WT controls. RNA sequencing revealed a distinct patternfor *St2*$^{-/-}$ mice. In MC38 tumors, nuclear IL-33 showed a pro-tumorigenic effect, while soluble IL-33 acted anti-tumorigenic. Similar to the AOM/DSS model, *St2*$^{-/-}$ mice injected with MC38 cells showed reduced tumor growth compared to *Il33*$^{-/-}$ and WT mice.

Conclusion: IL-33 has a dual role in the AOM/DSS CRC model: pro-tumorigenic when soluble (ST2-dependent) and anti-tumorigenic in the nucleus (ST2-independent). In a model of s.c. injected MC38 cell lines, nuclear IL-33 has a pro-tumorigenic effect in tumors and an anti-tumorigenic effect in non-tumor tissue.

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Control of dendritic cell dynamics in the skin by the sympathetic nervous system

V. Oliva[1], R. Pick[1], C. Scheiermann[1]

[1]Université de Genève, Genève

The sympathetic nervous system (SNS) has been shown to strongly impact immune responses. SNS fibers innervate all tissues and release the catecholamine noradrenaline locally, which binds cell surface alpha- or beta-adrenergic receptors. The skin is densely innervated by SNS fibers and represents one of the first lines of defense of an organism against pathogens. It harbors numerous immune cells that act as sentinels, reacting to pathogens or malignant cell proliferation by mounting a robust inflammatory response. Leukocyte migration through the lymphatic vessels network is crucial to mount an efficient adaptive immune response. The aim of this project is to dissect how dendritic cell (DC) trafficking in the skin is governed by the SNS.

To study the impact of the noradrenergic signaling on DC trafficking, both pharmacological inhibition of the B2 adrenergic receptor (B2AR), as well as a B2AR full KO (B2KO) mouse model were used, as this is the main adrenergic receptor expressed on leukocytes. Different *ex vivo* and *in vivo* cell trafficking methods were employed to study DC draining into lymphatic vessels.

Our data point to a role of noradrenergic signaling in modulating DCs migration, as the pharmacological inhibition or the lack of B2AR, induces a higher DC migration capacity towards lymphatic vessels and draining lymph nodes, compared to controls.

This study will gain fundamental insights into how the immune system is tuned by the SNS, which should lead to the pharmacological optimization of immune responses to infections, vaccination regimes as well as anti-tumor therapies.

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Association of cytokines with Myasthenia Gravis in Algerian patients

R. Raache[1], N. M. Bouchtout[2], E. Attal[3], C. Touil-Boukoffa[4], N. Attal[3]

[1]University of Sciences and Technology (USTHB), University of Alger 1 Benyoucef Benkkada, Alger DZ; [2]University of Sciences and Technology (USTHB), Alger DZ; [3]Immunology Department, Pasteur Institute of Algeria, Alger DZ; [4]University of Sciences and Technology (USTHB),, Alger DZ

Aim: Myasthenia gravis (MG) is an autoimmune disease of multifactorial etiology, results from the deleterious effects of autoantibodies directed against components of the postsynaptic membrane of the neuromuscular junction, in which genetic factors and cytokines seem to play an important role. The aim of this study was to investigate potential associations of cytokines (SNPs) and MG in Algerian patients.

Materiel: We performed a case–control study that included 27 patients and 74 healthy subjects. Genomic DNA was extracted using the salting out method. DNA quality was assessed on agarose gel, and its concentration was adjusted using a Nanodrop spectrophotometer. Cytokines SNPs genotyping was performed by the polymerase chain reaction sequence–specific primers (PCR-SSP) method and the amplification bands were visualized using a gel imager.

Results: Our results showed that the TNF-α −308G/A (P < 0.005) and TGF-β1 +869T/T (P < 0.05) genotypes were more frequent among patients with MG compared with healthy individuals, whereas TNF-α −308G/G (P < 0.0001), TGF-β1 +869T/C (P < 0.05), and IFN-γ +874A/A (P < 0.05) were less frequent. Our results showed that IL-10 and IL-6 SNPs did not show any significant difference in distribution between patients and healthy individuals.

Conclusion: We report the existence of associations between the polymorphisms of cytokines (TNF-a, TGF-b1, and IFN-g) and myasthenia gravis in Algerian patients.More comprehensive studies involving a larger number of patients and cytokines levels measurements will be required to further validate our observations

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Association of TNF-α -308A/G SNP with kidney allograft rejection in Algerian population

R. Raache[1], A. Chebine[2], H. Amroun[3], C. Touil-Boukoffa[2], N. Attal[4], A. Benziane[5]

[1]University of Sciences and Technology (USTHB), University of Alger 1 Benyoucef Benkkada, Alger DZ; [2]University of Sciences and Technology (USTHB),, Alger DZ; [3]Central Laboratory of Biology, University Hospital Center Nafissa Hamoud, Algiers, Algeria, Alger DZ; [4]Immunology Department, Pasteur Institute of Algeria, Alger DZ; [5]Department of Nephrology and Transplantation, Mohamed Amine Debbaghine Hospital, Alger DZ

Aim: Rejection events (REs) are commonly caused by acute and chronic injuries that alter graft function, leading to kidney allograft rejection (KAR) and graft failure. REs are triggered following allorecognition of the alloantigen (donor human leukocyte antigens [HLA]) by T cells. Our study aimed to investigated the association between the TNFA-308A/G SNP and KAR in Algerian patients who underwent kidney transplantation and to evaluate the possible associations of SNP with PG-a-HLA-Ab.

Methods: Genotyping of the *TNFA* -308A/G SNP was performed for 116 patients selected randomly using a case- control strategy from transplantation centers in Algeria and 197 healthy individuals (HI). All patients received transplants from living donors. Genomic DNA (gDNA) was extracted using the salting out method. The *TNFA* -308A/G SNP genotyping was performed using real-time polymerase chain reaction (PCR) with a TaqMan 5′ nuclease assay.

Results: The frequencies of *TNFA* -308A allele and AA genotype were higher in the PWR than in the HI groups (p = 0.001, OR = 2.26, p = 0.0004, OR = 5.53, CI- 1.89-16.6 respectively)., particularly among PWR patients with de novo anti-HLA antibodies. However, the frequency of *TNFA* -308G allele was lower in the PWR than in the PWoR (p = 0.001, OR = 0.3, and the HI group (p = 0.001, OR = 0.44)

Conclusion: Our results suggest an association of the T*NFA* -308A allele who have PG-a-HLA-Ab might have a higher risk. Thus, therapeutic strategies can be adapted to minimize KAR risk in patients who have a genetic proclivity for increased pro-inflammatory TNF-α activity.

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Matrix metalloproteinase and oxidative stress in systemic lupus erythematosus

S. Louahchi[1], N. Khaldoun2, A. Galleze[3], R. Raache[3], F. Hanni[2], C. Touil-Boukoffa[3]

[1]Laboratory of Cellular and Molecular Biology, Cytokine and NO Synthase Team, Faculty of science biology, University of sciences and technology Houari Boumedienne, Algiers DZ; [2]Rheumatology Department, BoukhroufaAbdelkader Hospital, Ben Aknoun,, Algiers DZ; [3]Faculty of science biology, University of sciences and technology Houari Boumedienne, Algiers DZ

Aim: Systemic lupus erythematosus (SLE) is an autoimmune disease characterized by chronic systemic inflammation and the production of autoantibodies against nucleus. The Matrix metalloproteinases (MMP) and oxidative stress have been demonstrated to be essential mediators of SLE pathogenesis. The aim of this study was to analyze the activity of MMP-2 and MMP-9 in SLE patients and to investigate the effects of oxidative stress by analyzing the production of nitric oxide (NO) and malondialdehyde (MDA).

Methods: Activity levels of MMP-2 and MMP-9 were measured by zymography in plasma from 30 SLE patients and 50 healthy subjects. Plasma MDA and NO concentrations were determined using the thiobarbituric acid reactive substances technique and the Griess method.

Results: Our results showed a significant increase in MMP-9, NO, and MDA serum levels in lupus patients compared to controls (p = 0.01; p $<10^{-3}$). However, no significant difference was found in MMP2 levels between the two groups (p >0.05). Furthermore, our results showed a remarkable increase in the plasma expression level of MMP-9 in patients with joint, skin, and kidney involvement. Furthermore, SLE patients with anti-RNP antibodies had higher serum concentrations of MMP-2 than anti-RNP-negative patients. The high MMP-9 levels correlated with the disease activity indice SLEDAI. No correlation was found between our patients' NO, MDA, and MMP production.

Conclusion: This study suggests that oxidative stress markers (NO, MDA) and MMP-9 could be useful inflammatory biomarkers for the progression and exacerbation of SLE.

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Characterization of adjuvants for optimal time-of-day effects of vaccines

Z. M. Gul[1], C. Scheiermann[1]

[1]University of Geneva, Geneva

Vaccines are powerful tools against various immune-regulated diseases, including cancer. The magnitude of vaccine-induced immune responses is known to vary with the time of day (TOD)

of vaccination in both mice and humans. However, the mechanism behind these TOD-dependent responses remains to be elucidated. In this study, we investigated how different immunological adjuvants affect TOD-dependent vaccination responses and whether we can manipulate them to increase vaccine efficacy. To identify adjuvants that elicit strong time-dependent responses, we screened for receptors with time-dependent gene expression and stimulated them with their ligands at different times of the day. Preliminary results show that the adjuvants LPS and Poly I:C induce a stronger immune response on primary mouse bone marrow-derived dendritic cells (mBMDCs) when administered at a specific time of day, measured by qPCR analysis. Together, we aim to uncover TOD-dependent immune responses to adjuvants and assess a comprehensive network of genes and proteins that drive this response. When completed, this work will provide a mechanistic understanding of TOD-dependent vaccine responses that could be used to improve vaccination strategies in human trials.

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Acute and chronic myocardial inflammatory disease - the explorative prospective ImmpathCarditis study

E. S. Payne[1], A. Joachimbauer[2], N. A. Cadosch[3], C. Gil Cruz[3], C. Perez-Shibayama[3], K. Frischmann3, F. Tanner[1], F. Ruschitzka[2], B. Ludewig[1], D. Schmidt[1]

[1]Department of Cardiology, University Hospital Zurich, Zurich; [2]Department of Cardiology, University hospital Zurich, Zurich; [3]Institute of Immunobiology, St. Gallen

Aim: Myocarditis is an inflammatory heart disease which can lead to fibrotic remodeling of the myocardium culminating in heart failure. Myocarditis is caused mainly by the activation of $CD4^+$ T cells against MHC class II-binding peptides derived from the contractile protein myosin heavy chain 6 (MYH6). The aim of the ImmpathCarditis study is to elucidate the pathogenesis of myocardial inflammation using longitudinal high-dimensional analysis from prospective patient cohorts.

Methods: The ImmpathCarditis study is a prospective exploratory study, which currently recruits acute myocarditis patients and patients with chronic inflammatory cardiomyopathy. A high-dimensional integrative data analysis of clinical, serological and immunological parameters including HLA sequencing and flow cytometry of peripheral blood mononuclear cells will be hierarchically structured to perform multiparametric phenotyping. To extend the longitudinal proteomic profiling of inflammatory markers in sera, we will use established ELISA methods to determine the reactivity of IgG serum antibodies against MYH6 proteins and analyze cytokines, inflammatory chemokines, growth factors such as bone morphogenic proteins.

Results: Patient recruitment and clinical longitudinal data acquisition are in progress. Serum antibody profiling of patient samples will commence soon.

Conclusions: The main hypothesis underlying this research project is that phenotypical and functional changes of serologic immune parameters, as well as heart-specific T cells critically determine the disease course during myocardial inflammatory disease.

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T-Cell Receptor Precision Editing of Regulatory T-Cells for Celiac Disease

R. Porret[1], A. Alcaraz Serna[1], B. Peter[1], M. Hafezi[2], J. Bernier-Latmani[2], F. Du Pré[3], O. Alfageme-Abello[1], L. Ermellino[1], E. Lana[1], R. Cecchin[1], D. Golshayan[1], D. Velin[1], J. Eyquem[4], Q. Tang[4], T. V. Petrova[2], G. Coukos[1], M. Irving[2], C. Pot[1], G. Pantaleo[1], L. Sollid[3], Y. D. Muller[1]

[1]CHUV, Lausanne; [2]UNIL, Lausanne; [3]University of Oslo and Oslo University Hospital, Oslo NO; [4]University of California San Francisco, San Francisco US

Aim: In celiac disease, HLA-DQ2.5 facilitates the presentation of deamidated gluten-derived peptides to antigen-specific CD4+ T-cells, triggering immune activation and enteropathy. The adoptive transfer of engineered (e) gluten-specific regulatory T-cells (Tregs) may suppress the effector function of pathogenic T cells.

Methods: Five TCRs recognizing the immunodominant DQ2.5-glia-a1a and glia-a2 epitopes were tested in a TCR-deficient NFAT-luciferase cell line. We next replaced the endogenous TCR of human primary T-cells and Tregs through homology-directed repair targeting the TCR alpha and beta constant loci using AAV and CRISPR-Cas9. The same strategy was applied to murine T-cells and Tregs, and evaluated in HLA-DQ2.5+ C57BL/6 transgenic mice exposed to gliadin via oral gavage.

Results: Jurkat cells transfected with the mRNA of any of the five TCRs exhibited peptide-specific NFAT activity. Human primary eCD4+ T cells displayed a similar mean functional avidity (EC50) for either specific peptide but not for glia-a1a/a2 overlapping epitopes. Human eTregs demonstrated superior suppressive activity compared to polyclonal Tregs in an EC50-dependent manner. In vivo, eCD4+ T-cells migrated and proliferated in the small intestine and draining lymph nodes. This proliferation was suppressed only in the presence of gluten-specific eTregs.

Conclusion: Redirecting Tregs to a single immunodominant gliadin-derived peptide could be sufficient for selective trafficking into the gut and draining lymph nodes. These cells hold therapeutic potential for restoring gluten tolerance in celiac patients.

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Cutting-Edge: Unleashing the Potential of γδ T Cells with Novel Nanoparticles for Cancer Immunotherapy Applications

A. Solé Casaramona[1], M. O. Mohsen[1], R. Josi[1], A. Ogrina[2], I. Balke[2], S. Marar[1], S. de Brot[1], E. Sevick[3]

[1]Universität Bern, Bern; [2]Latvian Biomedical Research and Study Centre, Riga LV; [3]The University of Texas Health Science Center at Houston, Houston, US

Aim: Recent research demonstrates specific variants of gamma delta (γδ) T cells possess innate properties, previously deemed only adaptative. Our understanding of the innate-like behavior of γδ T cells upon encountering virus-like nanoparticles (VLPs) remains limited. Exploring the interaction between γδ T cells and VLPs presents an intriguing avenue for research, offering insights into how VLPs can serve as an effective platform to enhance their expansion and activation.

Methods: We created novel plant-derived VLPs, engineered to incorporate TLR ligands to effectively stimulating the innate immune system. The study encompasses in vivo and in vitro assays, FC analysis and RNA sequencing.

Results: Our findings demonstrate robust uptake of our novel VLPs by γδ T cells, leading to significant expansion in draining lymph nodes upon subcutaneous injection. Interestingly, in

mice lacking TLR7 or C3, γδ T cell expansion was reduced, suggesting their involvement in the immune response triggered by our VLPs. Subsequent analysis of γδ T cell Vγ1 and Vγ4 subtypes post-VLP administration revealed substantial expansion and distinct activation profiles. Ongoing RNA sequencing will unveil activation pathways, shedding light on the molecular mechanisms underlying their response to our innovative VLPs.

Conclusions: Our novel data reveals the innate-like response of γδ T cells to our VLPs, loaded with different innate stimuli. This highlights the rapid and crucial role of γδ T cells in early immune responses, offering insights for potential immunotherapeutic strategies against cancer.

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Investigation of the detailed mechanisms of skin barrier dysfunction and inflammation caused by surfactants

Y. Mitamura[1], M. Li[1], P. D'avino[1], H. Babayev[2], D. Yazici[3], P. Gessner[3], P. Westermann[1], N. Gaudenzio[4], C. Messner[1], S. Simmons[5], A. Almada[5], C. Akdis[1], Y. Mitamura[1]

[1]Swiss Institute of Allergy and Asthma Research, Davos; [2]Swiss Institution of Allergic and Asthma Research, Davos; [3]Swiss Institution of Allergy and Asthma Research, Davos; [4]Toulouse Institute for Infectious and Inflammatory Diseases (Infinity), INSERM UMR1291, CNRS UMR5051, University Toulouse III, Toulouse FR; [5]SEED Health, Los Angeles US

Aim: The impairment of the epithelial barrier function is associated with various allergic and inflammatory skin disorders. The electrical impedance spectroscopy (EIS, Nevisense®) is a non-invasive tool to detect skin barrier integrity. We recently developed a method to evaluate skin barrier integrity in ex-vivo human skin (NativeSkin®) that exhibits normal skin barrier with almost all cell types. We investigated the mechanisms of epithelial barrier dysfunction and inflammation in human skin caused by surfactants which is an active ingredients of house cleaning products.

Methods: NativeSkins were treated with different types of surfactants, sodium dodecyl sulfate (SDS), cocoyl methyl glucamide (CMG), and cocamidopropyl betaine (CAPB) at several dilutions. Skin barrier function was assessed by EIS. RNA sequencing and targeted multiplex proteomics analyses in skin lysate samples were performed. Treatment effects of an antioxidant, N-acetylcysteine (NAC), were investigated in the air-liquid interface cultured normal human epidermal keratinocytes.

Results: The epicutaneous application of SDS/CMG/CAPB demonstrated the downregulations of EIS in a dose-dependent manner. Even CMG and CAPB showed a disruptive effect on the skin barrier integrity. SDS activated the pathways related to Oxidative stress and Endoplasmic reticulum stress. NAC treatment mitigated the increased permeability caused by surfactants.

Conclusions: We demonstrated that exposure to surfactants can impair the skin barrier. Oxidative stress may play a key role in the skin barrier damage induced by surfactants.

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Decoding polygenetic complexity in primary immunodeficiency

A. Martí[1], C. Hess[1], K. Patommel[1], O. Omarjee[1], B. Wingham[2], D. Lau[3], T. Elliott[3], J. Lötscher[4], T. Kuijpers[5], J. Edwards-Hicks[1]

[1]University of Cambridge, Cambridge GB; [2]Lumicks, Amsterdam NL; [3]University of Oxford, Oxford GB; [4]University of Basel, Basel; [5]Department of Pediatrics, Academic Medical Center, Amsterdam NL

Monogenic primary immunodeficiencies (PIDs) exist, yet more often pathogenesis is polygenic in nature. Unravelling how polygenic defects interrelate and contribute to clinical phenotypes is challenging, and key to fully understanding pathogenesis, nonetheless. Here we explore a pathway-centric interrogation to uncover the contribution of distinct, but functionally interlinked, genes to PID pathogenesis and clinical heterogeneity.

Testing this hypothesis in a cohort of >900 PID patients led us to identify a family characterized by Mendelian inheritance of a gain-of-function mutation in CXCR4 and a rare, uncharacterized mutation in LFA-1. Specifically, pathway interrogation flagged LFA-1 inside-out signalling as a downstream event of CXCR4 activation.

The identified CXCR4 mutation (L321Pfs*1) causes a syndrome variably characterized by a combination of warts, hypogammaglobulinemia, infections and myelokathexis (WHIM syndrome). Cell-surface expression of the identified LFA-1 variant (αL434P) was abolished in T-cell lines, highlighting its functional significance. Within the affected family, disease manifestation is heterogeneous and, intriguingly, segregates with co-inheritance of wild type vs. mutated LFA-1 – the latter being associated with reduced severity. Pathway interrogation thus identified genetic variations in two functionally-linked genes, associating with distinct clinical phenotypes.

Pathway-centric interrogation of genomic data may emerge as a novel approach to disentangling the polygenic complexity of PID, with the potential to define unexpected and novel therapies.

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Intranodal injection of Immune Activator demonstrates antitumor efficacy in an adjuvant approach

M. Mohsen1, R. Josi[1], A. Sole[1], A. Ogrina[2], I. Balke[3], S. de Brot[1], M. Mohsen[1]

[1]University of Bern, Bern; [2]Latvia Biomedical Research and Study Centre, Riga LV; [3]Latvian Biomedical and Research Center, Riga LV

The tumor-draining lymph nodes (tdLN) are the initial site of metastases and are the prime site for generating robust antitumor responses. In this study, we explored the efficacy of a universal immune activator (ImmAct) targeted to the tdLN. This approach can be viewed as an attempt to turn a cold unresponsive tdLN, into a hot, responsive site. The adjuvant antitumor efficacy of our novel intranodal injection was evaluated in an aggressive metastatic mammary carcinoma murine model. The cancer cells were inoculated subcutaneously in the lower quadrant of the mouse to provoke the tdLN (inguinal lymph node). The study encompasses a range of methodologies, including *in vivo* and *in vitro* assays and high-dimensional flow cytometry analysis. Our findings demonstrated that intranodal administration of ImmAct following the dissection of the primary tumor led to improved tumor-free survival and minimized weight loss. ImmAct led to both local and systemic alterations in the cellular and humoral immunity. Additionally, after ImmAct treatment, non-responders showed a higher rate of exhausted $CD8^+$ T cells compared to responders. Indeed, our innovative approach surpassed the gold standard surgery of sentinel lymph node excision. Overall, intranodal administration of ImmAct yielded a robust antitumor immune response, offering protection against micrometastases and relapse.

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Metal allergies – an investigation of sensitization and allergy patterns in patients treated at the University Hospital of Zürich 2011-2022

T. El Saadany[1], B. Illi[2], P. Furrer[3], P. Schenk[4], S. Fucentese[3], N. Li[1], P. Schmid-Grendelmeier[1], C. Lang[1], K. Bärenfaller[5], D. Zhakparov[5], M. C. Brüggen[1]

[1]University Hospital of Zürich, Zürich; [2]Stadtspital Zürich Triemli, Zürich; [3]Balgrist University Hospital, Zürich; [4]Uster Hospital, Uster; [5]Swiss Institute of Allergy and Asthma Research, Davos

Aim: 10-20% of all prosthesis patients suffer from implant-related complications within 1-2 years after surgery. Our study aimed to explore the characteristics of metal allergies and potential associated risk factors in prosthesis patients.

Methods: We conducted a retrospective monocentric study at the University Hospital of Zürich. We identified patients who underwent patch testing for metal/additive allergies between 2011 and 2021 and had a joint or dental prosthesis.

Results: A total of 225 patients with a mean age of 72 were included, with 34.2% being male and 65.8% female. Pain was the most common symptom (84.8%), followed by eczematous skin lesions and joint instability (both around 20%), and redness/swelling (13-16%). In 67.1% of the patients undergoing patch testing, an allergy was diagnosed, with nickel being the most common allergen (31.5%), followed by vanadium chloride (13.7%) and gentamycin (an antibiotic that is often added to bone cement). 60.3% of the metal/additive-allergic patients had a previously known contact allergy to metals (25.7%) or other substances (34.6%). Nickel was the most common allergen (19.5%), followed by silver (3.1%).

Conclusion: A previous contact allergy may be an indicator of a prosthesis-related metal/additive allergy. In contrast, the clinical presentation or consideration of atopic/non-atopic comorbidities do not seem to provide helpful clues for the diagnosis. Our findings suggest that metal/additive allergies might be an underestimated cause of postoperative complaints.

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