NSI Annual Meeting 2023 Abstract book

Title #1: Understanding the role of T-cell receptor repertoire in T1D status

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Although Islet autoantibodies (AAb) are clinically used in the diagnosis of disease, AAb only serve as a marker of autoantigen presentation and not involved in disease pathogenesis. However, T cells are hypothesized to be directly pathogenic and responsible for the destruction of β-cells, highlighting the importance of developing a T-cell biomarker for T1D.We have sequenced 2286 TCRβ repertoires containing 1112, 720, 68 and 386 repertoires for T1D, first degree relatives, second degree relatives and healthy controls, respectively. The standard repertoire analysis (e.g. diversity profile, Morisita-Horn similarity index etc.) were not able to classify the T1D repertoires from the healthy ones. Therefore, we implemented a Deep learning model entitled “DeepRC” to classify the T1D and healthy repertoires and identify the biomarker associated with T1D. The DeepRC method showed classification accuracy of ~80% on the test dataset. We also tested conventional ML approaches, which had lower prediction performance compared to DeepRC. We also looked into the association between HLA alleles and CDR3β phenotypes and observed that CDR3 risk score was able to classify different T1D status.

Title #2: Infection with P. gingivalis as an etiological factor in Alzheimer’s disease

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Alzheimer’s disease, the most prevalent form of dementia, represents an unmet challenge in the aging population. Infectious agents have been identified in the Alzheimer’s disease patients’ brains and several bacterial species were postulated to be an etiologic factor, but robust evidence of causation has not yet been established. Among them P. gingivalis, a keystone pathogen in the development of chronic periodontitis, has been identified as significant risk factor in the development of Alzheimer’s disease.

Our study aims to delineate the effect of P. gingivalis and its virulence factors on microglia cells. We observed strong up-regulation of the proinflammatory factors, such as NOS2, COX2, IL1b, TNFa in microglia upon exposure to P. gingivalis that strongly correlated with the presence of arginine or lysine specific cysteine proteinases (known as gingipains), having been recognized as a major virulence factor. Using mass cytometry, we evaluated the gingipains' impact on the phenotype of microglia by profiling 36 antigens potentially engaged both in the pathogenesis of bacterial infection and Alzheimer's disease. We have identified 15 different populations (clusters) and among them the ones with the high expression of CD68, PD-L1, CD40, CD44, CD18 and others. To list one, a cluster highly expressing CD68 and PD-L1 exhibited four times higher frequency of cells in P. gingivalis wild type condition than when microglia were exposed to P. gingivalis without gingipains (48-h exposure). In conclusion, microglia cells undergo gingipain-dependent phenotypic shifts upon exposure to P. gingivalis supporting the role of that periodontopathogen in Alzheimer's disease development and progression.

Title #3: Biopsy proteome scoring to determine mucosal remodeling in celiac disease

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Celiac disease (CeD) is diagnosed and monitored by histological evaluation of duodenal biopsies, but histological measurements require good biopsy orientation and suffer from poor inter-observer reproducibility. Tissue proteome analysis of formalin-fixed paraffin-embedded (FFPE) biopsy sections capture disease processes in the CeD intestine. We aimed to transform complex proteome data into a numerical score for use as an observer-independent measure of gluten-induced mucosal damage in CeD. To enable use in clinical settings we developed a simple protocol for MS-based proteome analysis of single, glass-mounted FFPE biopsy sections. Proteome data were converted to numerical scores using two different computational approaches, taking advantage of previously published CeD proteome datasets. The score approaches were validated using an independent patient cohort of CeD patients comparing biopsies collected at diagnosis to biopsies collected after treatment with gluten-free diet (GFD). Proteome scoring discriminated between biopsy samples taken from CeD patients before and after treatment on GFD. The two proteome score approaches correlated well with each other, and also with histology-derived villus height to crypt depth (Vh:Cd) ratio. Change in proteome score upon treatment with GFD correlated with change in Vh:Cd ratio. Both unstained and H&E stained sections can be used for proteome scoring, and proteome score can determine disease state of poorly oriented biopsies not suitable for image-based histology. The proteome score represents a much-needed observer-independent molecular histology measure to supplement image-based histology in CeD. The pipeline is simple and can readily be implemented by non-expert laboratories and scaled for clinical trial settings.

Title #4: Targeting carbohydrates in adenocarcinomas: STn-CAR T cells

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Chimeric Antigen Receptor (CAR) T cells provided critical improvements in therapeutic outcome in blood malignancies. CAR T cells also harness high potential for the treatment of solid tumors, but some challenges remain to be addressed. Several proteins have emerged as targets for CAR T cell therapy against solid carcinomas with promising results in ongoing clinical trials. Nonetheless off-tumor toxicities, antigen-loss and availability of a suitable target remain critical considerations. Post-translational modifications of surface proteins are highly regulated and essential for the protein structure and function. Among these secondary modifications, alterations in glycan biosynthesis lead to the expression of aberrant carbohydrate residues. One such residue is the Sialyl-Tn (STn) truncated O-glycan. STn truncated O-glycans are frequently and selectively expressed in adenocarcinomas (80% of human epithelial-derived cancers). We have developed three monoclonal antibodies (mAb) specific for STn through hybridoma technology and assessed their specificity. They displayed high specificity towards STn antigen, STn+ tumor cell lines, primary tumor cells of several carcinomas and no reactivity towards a wide range of healthy tissues. We identified the sequences of the mAb and designed single chain variable fragments (scFv) then cloned into a second generation CAR backbone. These STn-CARs were expressed in a Jurkat reporter cell line and in primary T cells. STn-CARs demonstrated potent and specific functionality in in vitro assays. Lastly, STn-CAR T cells showed remarkable control of tumor growth in murine xenograft models.Our results show that STn-CAR T cells are promising candidates for treatment development in a large number of solid tumors.

Title #5: single-cell RNA sequencing atlas of colorectal cancer

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Colorectal cancer (CRC) stands as a major global health challenge, necessitating a deeper understanding of its cellular heterogeneity and immune microenvironment. In this project, we generate a comprehensive single-cell RNA sequencing (scRNA-seq) atlas comprising data sourced from eight publications on colorectal cancer. Our integrated dataset encompasses 905,842 cells obtained from 186

patients, capturing both tumor cells and cells from the adjacent normal colon. Utilizing state-of-the-art bioinformatics techniques, particularly leveraging Python and scVI integration, we seek to delineate the complex landscape of tumor-infiltrating immune cells. Specifically, we focus on T cells and macrophages, discerning high and low abundance groups within our patient cohort. Through this stratification, we aim to unravel potential prognostic implications based on recent findings by members of our group (Majid et al. unpublished). Preliminary analyses reveal a high degree of immune cell heterogeneity within the tumor microenvironment, emphasizing the intricate interplay between tumor cells and the immune system in CRC. We anticipate that our study will shed light on novel avenues for therapeutic intervention and offer valuable insights into the prognostic value of immune cell composition in CRC. This work represents a significant step towards a more complex understanding of colorectal cancer, exemplifying the power of integrative scRNA-seq approaches in deciphering intricate biological systems.

Title #6: Challenges of phenotype reproducibility in animal models: investigating the phenotype of TSAd knockout mice

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Credibility in research is an integral part of scientific advancement, where reliable data lies at the heart of it. In recent years, there have been growing concerns in the scientific community regarding the challenges of reproducing reported findings. In this study, we address the issues of data reproducibility concerning the T cell specific adaptor protein (TSAd), by revisiting a knockout mouse model, which has been made to elucidate the protein's functional role. TSAd is encoded by the *Sh2d2a* gene and is expressed in activated T and B cells, natural killer cells and in subtypes of endothelial cells. It has been reported that TSAd KO mice display autoimmune-like phenotypes and impaired viral clearance, while being protected in tumour challenge models. Nevertheless, the role of TSAd in T cells remains elusive as the phenotypes have often been mild and inconsistent when compared to more recent observations. We explore these discrepancies and hypothesise that the cleanliness of housing facilities or mouse strains, might contribute to the immunological status. Here, we show preliminary data on wild-type versus TSAd KO mice housed in specific-pathogen free (SPF) or in "dirty" conditions (feralised) and compare their immune phenotypes. Taken together, these observations may suggest why discrepancies between reported findings exist, which might partially explain the general phenomena of data irreproducibility in experimentation using animal models.

Title #7: Improved cancer immunotherapy in solid tumor through CXCR4 modulation

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Despite the emergence of cancer-immunotherapy, numerous challenges are encountered, particularly in the treatment of solid tumors, which account for over 90% of cancers, and of which 35 to 40% have a poor prognosis. The C-X-C motif chemokine receptor 4 (CXCR4) has become an attractive target due to its expression in most solid tumors and its involvement in migration. Indeed, the binding of its ligand, CXC chemokine ligand 12 (CXCL12) present in the tumor microenvironment, has been described as a driver of tumor growth and metastasis. Our collaborators have previously developed a specific inhibitor, R54, able to affect tumor cell migration. Since CXCR4 is also expressed on immune cells, we assessed the effect of R54 on immune effector cells (T and NK cells). Our results confirm that R54 is highly specific against CXCR4 in immune cells and blocked their migration. From a therapeutic point of view, the use of R54 could have a negative effect on the natural immune system or in combination with immunotherapy. To overcome this effect, a migration enhancer was expressed in immune cells, circumventing R54- inhibited migration in a CXCL12 gradient. We have extended our analysis to chimeric antigen receptor (CAR) expressing T cells in a metastatic model of osteosarcoma and aim at testing therapeutic combination. This work could pave the way to a combination therapy where the metastatic spreading will be limited without affecting CAR-based immunotherapy.

Title #8: CRISPR/Cas9 T cell pipeline to introduce custom edits in patient T cells

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Emerging CRISPR/Cas9 gene therapy trials demonstrate the therapeutic potential of correcting mutations in defected patient cells. However, as these trials are largely focused on targeting common hematologic diseases, such a sickle cell disease, little is known about how to optimize CRISPR for other monogenic mutations. To address this challenge, we developed a CRISPR/Cas9-based T cell editing pipeline that streamlines CRISPR tool discovery for highly efficient and safe correction of mutations in ADA2, AIRE and

RMP loci in patient T cells.Our data demonstrate a rapid and selective expansion of CD3+ T cells from patient and healthy donor peripheral blood mononuclear cells (PBMC) after 8-day cytokine stimulation. CRISPR guide-RNAs (gRNA) for ADA2, AIRE and RMRP were screened directly in patient T cells, and off-target safety of best performing gRNAs was assessed using genome-wide, unbiased identification of double-strand breaks enabled by sequencing (GUIDE-seq), where no gRNA off-targets were identified for any of the loci. Finally, we tested multiple strategies to increase precise homology-directed repair (HDR) outcomes and developed an HDR-optimized T cell pipeline that can achieve as high as 80% mutation correction levels in patient T cells.In conclusion, our work demonstrates that the CRISPR/Cas9 T cell pipeline is robust and suited for creating small custom edits, such as mutation correction, in patient T cells. Furthermore, it has the potential to be converted into a GMP-compatible workflow and scaled up to fit clinical gene therapy needs.

Title #9: Crystalline Silica Particle Induced Immune Effects on Human Whole Blood using High Dimensional Immune Cell Profiling

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Environmental exposure to crystalline silica (CS) has been linked to adverse immune effects and is a possible risk factor for lung and systemic autoimmune diseases. However, the understanding of the cellular mechanisms linking CS toxicity to autoimmune diseases remains elusive. This study is part of the EXIMIOUS project, aiming to characterize and quantify multiple, combined environmental exposures during a person's lifetime with high-dimensional immunophenotyping and to develop immune fingerprints for occupational and autoimmune disease cohorts. Specifically, it characterizes the early invitro effects of CS exposure on the phenotype and function of innate and adaptive immune cells in human whole blood. Using an indirect exposure model, whole blood was exposed to conditioned media from CS treated THP-1 macrophages followed by analysis of immune cell responses by CyTOF. The antibody panel consisting of 42 markers has been optimised for identifying innate and adaptive immune-cell subsets as well as their functional states. The indirect exposure model was also treated with a stimulation cocktail mix consisting of TLR stimulants and PMA/Ionomycin to study the impact of CS on immune stimulated cells. Multiplex analysis of blood plasma after indirect exposure revealed induction of specific patterns of secreted cytokines and chemokines. IL-1β, IL-18, IL-4, IL-23, TNFα, CCL2, CXCL8, CXCL10 were induced with indirect exposure of CS. Preliminary single cell CyTOF analysis suggest changes in phenotype and cytokine expression in specific immune cell subpopulations. These findings

invitro may point to mechanisms and biomarkers and will support causality, if similar profiles are identified in EXIMIOUS cohorts.

Title #10: Longitudinal analysis of anti-tumor T-cell responses in long-term survivor glioblastoma patients and immune correlates of response to immunotherapy

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Glioblastoma (GBM) is the most aggressive primary brain tumour in adults with a median survival of 12–15 months. None of the immunotherapies have shown clinical efficacy. We previously performed a clinical trial investigating dendritic cell (DC)-based vaccine targeting cancer stem cells (CSC) and universal tumour antigens (telomerase and survivin) in GBM. Prolonged overall survival was shown for vaccinated patients, with three extreme long term survivors. To link survival with immune response, we investigated the T-cell landscape at different locations in one long term survivor. We detected vaccine-specific T-cell proliferation in peripheral blood post-vaccination and survivin-specific CD4+ T cell clones were isolated. T cell receptor (TCR) sequencing (TCR-seq) revealed overlap between the TCR repertoire in blood and leukapheresis product pre-and post-vaccination. Tumour infiltrating lymphocytes (TILs) demonstrated high clonality and overlap with post-vaccination samples. Interestingly, TILs from a biopsy taken upon tumour progression showed increased TCR diversity and phenotypic heterogeneity indicating reduced tumour specificity. Additionally, we isolated tissue-resident memory T cells, which reacted against HLA-matched CSC from GBM patients. Work is ongoing to identify the target antigens and TCRs of these TILs. In summary, tumour-specific T cells were induced by vaccination and could be detected both in the periphery and in the tumour. A randomized study comparing DC vaccine and standard treatment is now ongoing. Additional samples are currently investigated to confirm immune correlates of response to immunotherapy. This will provide insight into how a successful anti-tumour response is constituted in GBM and guide the design of more efficient immunotherapy approaches.

Title #11: An APC-targeted influenza H1-mix DNA vaccine induces cross-protective immunity in mice

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Influenza continues to be a threat to global health, causing several cases of respiratory deaths and severe illness worldwide. Since the influenza virus mutates constantly, influenza vaccines must be updated annually and usually have an efficacy of only 20-60%. Predicting the next season's circulating strains is a challenge. Novel approaches to generate vaccines with a broader range of protection are as such of high priority. One approach is to use DNA vaccines, which are rapid and cheap to construct and produce. In a novel DNA vaccine, we have combined multiple HAs from H1N1 virus strains. Four H1 variants were selected from viruses known to have circulated in humans. These antigens were expressed as proteins, fused to a heterodimerization motif, and a single chain variable fragment (scFv) that targets the vaccine to antigen-presenting cells (APCs) via MHC class II. In this H1-mix vaccine, only dimers with two different H1s is formed. In mice, the H1-mix vaccine induced high amounts of antibodies against the HA-stem and antibodies that cross-reacted to several HAs not included in the vaccine. The cross-reactive antibodies were mainly of IgG2a subtype and activated antibody-dependent cellular cytotoxicity. High amounts of H1-specific IFN-γ producing cells were present in the spleen of H1-mix vaccinated mice. Finally, mice were completely protected when challenged with heterologous H1N1 virus, with a significant reduction of viral load in the lungs. In summary, this H1-mix vaccine can be used to skew immune responses towards conserved HA epitopes and broaden protection against influenza.

Title #12: Tumor microenvironment hosts game-changers for the development of metastatic melanoma

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Melanoma is the cancer form with the steepest increase in incidence and mortality in Norway. Patients with lymph node metastasis (stage III disease) get neoadjuvant and adjuvant treatment with immune checkpoint inhibitors in addition to surgery. This reduces the risk of recurrence for half of the patients, but the treatment is associated with severe side effects. It is also evident that a significant proportion of the patients would have been cured by surgery alone. Prognostic biomarkers, identifying patient without risk of recurrence, and predictive biomarkers, enabling treatment stratification, will reduce the chance for over-treatment of patients without need, and spare patients without benefit from unnecessary side effects. We have characterized patient tumor biopsies at both transcriptome and single cell level. A

unique 24 gene signature was identified, capturing tumor-immune interactions, able to distinguish tumors at risk of recurrence from tumors with exceptionally good prognosis.

Title #13: Transient Delivery of an Osteosarcoma-specific CAR for clinical use

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Osteosarcoma (OS) is an aggressive tumour, prevalent in children and young adults, representing 5-10% of paediatric and adolescent cancer patients. Combination therapies have proved beneficial to patient survival, however OS metastases - including to the lung – have a dismal prognosis. We have recently published a new Chimeric Antigen Receptor (CAR), which targets an isoform of alkaline phosphatase (ALPL-1) that is highly and specifically expressed in primary and metastatic OS. While retroviral CAR therapies provide stable and persistent cellular therapies, the path from the bench to the clinic is long, and entails additional testing prior to approved CAR therapies in the clinic. Transient CAR expression can allow earlier testing of CAR efficacy in patients, while bypassing certain of the risks and delays associated with retroviral transduction. In this study, we assessed delivery of CARs via electroporation of CAR mRNA to primary T cells. We examined these transient CAR T cells in vitro, and in vivo, and demonstrated that they retain the efficacy and efficiency of retroviral delivery. These results indicate that transient CAR T cells can provide a functional alternative to stably-transduced CAR T cells. This may expedite clinical testing of these CAR therapies, and may also provide therapeutic options where retroviral-based therapies are unfeasible.

Title #14: Immune responses to repeated SARS-CoV-2 vaccination and breakthrough infections in patients on TNF inhibitor treatment

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Patients on TNF inhibitor (TNFi) therapies have attenuated vaccine responses and are prone to severe infections. Identification of protective SARS-CoV-2 antibody and T cell responses after repeated vaccination and breakthrough infections (BTI) is important for future vaccination recommendations in this vulnerable patient group. Adults (n=144) with Crohn's Disease (CD), ulcerative colitis (UC) or arthritis on TNFi treatments gave blood samples before vaccination and 2-4 weeks after vaccination for doses 2-5 and 1-6 months after BTI from the third vaccine dose onwards. Antibody levels and CD4 and CD8 T cell responses to spike and non-spike SARS-CoV-2 peptides were analysed by flow cytometry to assess vaccine and/or infection responses and determine magnitude and quality of immune responses. After three vaccine doses, cellular responses in CD/UC patients were comparable to healthy controls while humoral responses were attenuated. Spike-specific CD4 T cell frequencies plateaued after two doses, whereas CD8 T cell responses continued to improve up to four doses. BTI generated strong cellular responses against nucleocapsid and membrane peptides. Cellular responses waned slightly in the six months after a fourth vaccine dose or an infection but rebounded to previous levels after a fifth dose. CD4 and CD8 T cell responses showed different dynamics after vaccination and eventually plateaued after four vaccine doses. Although arthritis patients had lower responses to vaccination than CD/UC patients, a subsequent infection removed the differences in response between these groups. These data indicate that a combination of vaccination and infection is sufficient to generate and maintain cellular responses over time.

Title #15: Improving precision editing outcomes in human CD34+ hematopoietic stem and progenitor cells in clinically relevant ADA2 locus

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Primary Immune Deficiencies (PIDs) are represented by various genotypes and phenotypes complicating diagnostic and therapeutic intervention. Combined prevalence creates great opportunities for employing a promising novel gene therapy strategies based on CRISPR/Cas9. DADA2 (Deficiency in ADA2) is one of the childhoods PID. One of the bottlenecks of precise gene correction is insufficient HDR levels in relevant primary healthy hematopoietic stem and progenitor cells (HSPCs). Here, we employed classical CRISPR/Cas9 approach using ssODN as our repair template format and CD34+ HSPCs. We introduced innocent mutations in ADA2 gene where most common Finnish founder mutation, R169Q, is localized. Previously, we observed HDR edits dropped after initial base line levels after 12 weeks in vivo. We hypothesized increasing HDR baseline levels would preserve edits in vitro and in vivo later as well. To tackle presented problem, firstly we titrated Cas9, sgRNA and ssODN to pick the best editing conditions. Secondly, we screened several chemically modified ssODNs to improve HDR further. Lastly, we applied and titrate DNA repair pathway inhibitor in HSPCs to boost HDR. Combining these optimized steps, we were able to improve overall HDR up to 2-3x fold time (up to 80% HDR) in healthy donor HSPCs. Strikingly, we observed significant changes in Colony Forming Unit (CFU) assay output between inhibitor-treated and inhibitor-free treated HSPCs and validate liquid CFU as an alternative method to golden standard semisolid CFU. To conclude, more thorough assessment of the toxicity caused by the inhibitor must be performed.

Title #16: Single-cell transcriptomic profiling of liver T cells reveals enriched gamma-delta and regulatory T cells in primary sclerosing cholangitis

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Primary sclerosing cholangitis (PSC) is an immune-mediated bile duct disease lacking curative treatment that often leads to end-stage liver disease. Pathogenesis remains unclear but repeated demonstrations of robust HLA associations prototypic of antigen-driven diseases alongside strong lymphocyte infiltration suggests a key role for T cells. To determine if PSC patients have unique T cell subsets, liver T cells were isolated by negative bead selection (StemCell Technologies) from PSC (n=12) and non-PSC controls (primary biliary cholangitis, PBC: n=9, alcoholic liver disease, ALD: n=3), and analyzed using 5' single-cell RNA sequencing (10X Genomics). Relative to all hepatic T cells, PSC livers had a larger median proportion of gamma-delta (γδ) T cells (*TRDC*+TCR-αβ) than PBC (PSC: 12.5%, PBC: 6.1%; P=0.049, Mann Whitney) and similar frequencies to ALD (8.5%, P=0.49). By contrast, proportions of *FOXP3*+ regulatory T cells (Tregs) in PSC livers were similar to PBC (PSC: 3.2%, PBC: 2.1%, P=0.54) but higher than ALD (0.4%, P<0.01). Liver γδ T cells from all phenotypes analyzed together expressed higher levels of the chemokines *CCL3* (8.6-fold, P<0.001, Benjamini-Hochberg) and *CCL4* (3.9-fold, P<0.001), and the cytotoxic markers *GZMK* and *PRF1* (2.5-fold, P<0.001) compared to non-γδ T cells. Tregs from all livers were also transcriptionally comparable and marked by high immunosuppressive *IL10* (16-fold, P<0.001) and *CTLA4* expression (24-fold, P<0.001) relative to non-Tregs when samples were analyzed together. Overall, our findings suggest that γδ T cells and Tregs are involved in PSC pathogenesis and warrant future studies addressing the clonality and antigen specificity of these enriched cell types

Title #17: The Tankyrase Inhibitor OM-153 Demonstrates Antitumor Efficacy and a Therapeutic Window in Mouse Models

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The catalytic enzymes tankyrase 1 and 2 (TNKS1/2) alter protein turnover by poly-ADP-ribosylating target proteins, which earmark them for degradation by the ubiquitin–proteasomal system. Prominent targets of the catalytic activity of TNKS1/2 include AXIN proteins, resulting in TNKS1/2 being attractive biotargets for addressing of oncogenic WNT/β-catenin signaling. Although several potent small molecules have been developed to inhibit TNKS1/2, there are currently no TNKS1/2 inhibitors available in clinical practice. The development of tankyrase inhibitors has mainly been disadvantaged by concerns over biotarget-dependent intestinal toxicity and a deficient therapeutic window. Here we show that the novel, potent, and selective 1,2,4-triazole–based TNKS1/2 inhibitor OM-153 reduces WNT/β-catenin signaling and tumor progression in COLO 320DM colon carcinoma xenografts upon oral administration of 0.33–10 mg/kg twice daily. In addition, OM-153 potentiates anti–programmed cell death protein 1 (anti–PD-1) immune checkpoint inhibition and antitumor effect in a B16-F10 mouse melanoma model. A 28-day repeated dose mouse toxicity study documents body weight loss, intestinal damage, and tubular damage in the kidney after oral–twice daily administration of 100 mg/kg. In contrast, mice treated oral–twice daily with 10 mg/kg show an intact intestinal architecture and no atypical histopathologic changes in other organs. In addition, clinical biochemistry and hematologic analyses do not identify changes indicating substantial toxicity. The results demonstrate OM-153–mediated antitumor effects and a therapeutic window in a colon carcinoma mouse model ranging from 0.33 to at least 10 mg/kg, and provide a framework for using OM-153 for further preclinical evaluations.

Title #18: Investigating the Health of Tissue Surrounding the Colorectal Cancer

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The classification of tissue adjacent to colorectal cancer as 'normal' has long been a subject of debate in the field of oncology. We aim to shed new light on this question by harnessing the power of single-cell RNA sequencing (scRNA-seq) and advanced data integration techniques. Our study focuses on the comprehensive analysis of integrated scRNA-seq datasets from normal and healthy colon tissues to discern whether the so-called 'normal' tissue is truly healthy at the single-cell level. In addition to computational analyses, we are leveraging multiplex immunostaining, a robust technique that allows for the simultaneous detection of multiple protein markers. We are also employing super-resolution imaging techniques to provide enhanced spatial information and precise localization of specific molecules. This holistic approach will not only provide comprehensive insights into the nature of normal tissue adjacent to colorectal cancer but also enhance our understanding of the complexities involved. By addressing this fundamental question, our research carries significant implications for the field of oncology, potentially guiding future treatment strategies and diagnostic approaches.

Title #19: Dude, where's my CAR ?! – the untold story about troubles with tinkering under the hood of your CAR

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The discovery that chimeric antigen receptors (CARs) allow targeting of cancer cells by T-cells, thus providing potential cure of the disease, has been a major scientific breakthrough in the last decade. CAR consists of an extracellular antibody-based part and an intracellular part based on the T-cell receptor (TCR) ζ-chain combined with intracellular domains of costimulatory receptors CD28 or/and 4-1BB. Therefore, CAR-transfected T-cells can kill specific cells based on their surface markers, which are recognized by the given antibody. However, since the initial discovery, it has been appreciated that CAR T-cells can fulfil their role only to a certain degree. Recently, it was pointed out that flaws in the downstream signalling led to a blunted CAR T-cell response. As the intracellular part of the CAR cannot fully recapitulate the TCR signalling, it is vital to understand how to improve it. Moreover, it should be

equally interesting to manipulate the signalling in order to tailor it to the therapeutical needs. However, creating a library of defined modules requires a systematic and functional testing of various intracellular domains. Here, we tested the known Lck adaptor proteins sequences i.e., Lck-recruiting motifs of LAT, LIME1, SKAP1, and SH2D2A in the 2nd generation CARs against CD19. Lck-binding motif of CD3ε was used as a positive control. However, we quickly discovered that unstructured regions of proteins hide a secret, a molecular signature, which is vital to understand before employing them in CARs.

Title #20: Genomic and proteomic antibody repertoire analysis at single-cell and single-molecule resolution

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The diversity of the antibody repertoire is crucial to broad pathogen recognition. Therefore, investigating the relationship between the genomic (BCR) and phenotypic (serum antibody) diversity of antibodies is crucial for understanding human adaptive immunity. The capability of accurately predicting and describing the entire antibody repertoire has the potential to dramatically alter vaccine development and disease diagnostics. However, despite advances in high-throughput BCR sequencing, a joint characterization of antibody complexity at the genomic single-cell and proteomic single-molecule level remains elusive due to the fact that mass spectrometry (MS) analysis of the serum antibody repertoires has remained underdeveloped. Specifically, it remains unclear to what extent the blood B-cell receptor repertoire differs from the serum antibody repertoire or, even more fundamentally, what the number of unique antibody clonotypes in the blood is. To address these questions, we isolated blood-borne B cells from a healthy donor and sequenced the BCR repertoire at bulk and single-cell level. Simultaneously, serum antibodies of all major isotypes were isolated, digested, and sequenced with tandem MS, ensuring that the antibody repertoire is covered comprehensively. Systems immunology analysis showed high concordance between bulk- and single-cell sequencing. MS was able to identify CDRH/L3 peptides linked to specific clonotypes in the sequencing libraries, demonstrating how to reconstruct antibody clonotypes from proteomics data at single-molecule resolution. In addition, performance benchmarking of antibody MS revealed that relative CDR3-peptide concentration differences can be measured with high sensitivity. In conclusion, we developed a platform that connects bulk sequencing, single-cell sequencing, and mass spectrometry enabling the quantification of the serum antibody repertoire at unprecedented precision thereby removing a blank spot in our understanding of antibody adaptive immunity.

Title #21: Modeling human gut disease using patient derived organoids

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The gastrointestinal tract is an organ that takes in food, digests it, and absorbs food-derived nutrients. However, the gut also protects against insults after exposure to numerous pathogens. Intestinal epithelial cells generate multiple barriers to protect the intestinal mucosa from invading pathogenic microorganisms. Because of the cellular and molecular complexity of the intestine, elucidating the intrinsic mechanisms underlying the regulation of epithelial homeostasis is difficult. Thus, the current three-dimensional gut organoid culture has revolutionized fundamental and biomedical science research. Organoids are mini-guts and show high regeneration potential from normal and diseased primary tissues and mimic the physiological features of their tissue of origin. I have developed healthy duodenal organoids from five patients undergoing Whipple surgery at Rikshospitalet. I observed that proliferation and differentiation could be controlled by high (50 % Wnt3a and 20 % R-Spondin 1) and low (5 % Wnt3a and 5 % R-Spondin 1) concentrations of growth factors. We observed an MUC2+ Goblet cell number increase by decreasing the concentration of Wnt3a & R-Spondin 1 and in the absence of Nicotinamide and SB202190- a p38 MAPK inhibitor. We tried to map the transcriptional landscape of the human duodenal region using organoids as a model. We have used seven cytokines to study immune-epithelial interactions. We found that IFN-γ induces significant upregulation of genes responsible for Celiac disease in human patients. Therefore, in this direction, we performed single-cell RNA-sequencing in biopsies derived from Celiac disease patients and in IFN-γ treated duodenal organoids. In the future, our single cell sequencing datasets could reveal more complex relationships between genes and track the distinct cell lineages in disease development.

Title #22: β2 or not β2: Connecting the Lck-adaptor TSAd to integrin β2 via a combined wet lab & bioinformatic approach

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The influx of high-quality, high-depth single-cell RNA-sequencing (scRNA-seq) datasets of PBMCs following the onset of the COVID-19 pandemic has opened up the possibility of repurposing these datasets to produce new data outside the initial purview of these studies, where earlier studies lacked the depth or breadth to produce statistically significant results. TSAd, expressed by the gene SH2D2A, is a protein primarily expressed in NK and T cells. TSAd is known to interact with the important immune kinase Lck and thus is thought to contribute to signalling in NK and T cells, but the details of this

contribution remain largely unknown. We here demonstrate the possibility to repurpose pre-existing scRNA-seq datasets to produce novel data for genes whose function remain largely obscured by first connecting gene expression of TSAd to integrin β2 (ITGB2), producing a workflow that can be re-applied to produce similar output for any other gene of interest. We then verified this connection at the protein level in the wet lab by visualising the co-localisation of TSAd, Lck, and ITGB2 at homotypic T-T junctions in primary human T cells, suggesting that TSAd may play a role related to that of ITGB2 in these synapses. Pulldown of TSAd from primary T cells, and identification of pulled-down proteins via MassSpec, further confirmed the larger macromolecular interaction of TSAd and ITGB2. What does TSAd bind in this synapse—β2, or not β2: that is now the question.

Title #23: B cell and antibody responses in the context of repeated influenza vaccinations

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The purpose of vaccination is to stimulate formation of pathogen-specific memory responses able to efficiently respond to a future exposure. Specific antigen recognition is mediated by highly diverse receptors on T and B cells. Following receptor recognition of antigen, an immunological cascade may be activated to result in development of long-lasting immunity. In the context of Influenza, antibodies against the surface glycoprotein hemagglutinin (HA) are the most commonly used correlate of protection. HA is highly mutagenic, necessitating annual vaccine updates. Here, we will address current knowledge gaps on the impact of repeated vaccinations against influenza. Healthcare workers are recommended for annual influenza vaccination. To quantify the impact of influenza vaccination during the 2022/23 season in this group, blood samples were collected before and one month after vaccination. As expected, the vaccination induced an increase in the hemagglutination inhibition (HI) titers against the H1N1 vaccine strain, even in individuals who already had protective HI titers before vaccination. Regarding HA specific IgG levels against H1N1 HA variants from previous seasons, the titers were already high prior to vaccination and we did not observe a difference in the samples collected before and after vaccination, nor for their neutralizing capacity. At present, we are evaluating antibody responses against more influenza subtypes and strains. Further, we are isolating HA-specific B-cells to evaluate germinal center formation as evidenced from B cell receptor sequences present before and after vaccination. The aim is to shed light on germinal center activation in response to repeated antigenic exposures, as such providing information that is key for development of future vaccination strategies against variable viruses such as influenza.

Title #24: High dimensional mass cytometry reveals correlates of vaccine heterogeneity in the elderly

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The recent COVID-19 pandemic has caused a significant burden on global healthcare systems, with unprecedented high levels of morbidity and mortality. With the large-scale deployment of multiple rounds of vaccines, much of the severe clinical manifestations were mitigated in affected individuals, and transmission was reduced to more manageable levels. However, heterogeneity in vaccine response remains an area of concern, particularly in vulnerable populations that include immunocompromised individuals and the elderly. In the present study, we performed a 40-parameter deep profiling of immune cell phenotype and function to identify cellular correlates of vaccine responsiveness in selected low and high-vaccine responders from a cohort of Norwegian older adults. We aimed to identify both inherent differences in immune cell frequencies and function of various subpopulations before vaccination, as well as qualitative and quantitative differences after two doses of vaccination. Unsupervised clustering followed by statistical analysis revealed differences in immune cell frequencies and function at baseline as well as after vaccination, which associated with vaccine response. Our results are expected to provide a valuable resource for targeted decisions of follow-up vaccinations regimes.

Title #25: CellFit: T cells fit to fight cancer

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The use of T lymphocytes in adoptive cell therapy (ACT) shows great promise for hard-to-treat cancers. Nevertheless, one of the largest challenges faced in cell-based cancer therapy is to provide an efficient and scalable production. Whilst the main focus of development has been on finding tumour targets and improving the genetic modifications of the immune cells, less effort has been made to efficiently produce the optimal subset of immune cells, which ideally is more homogenous and stem-cell like, for treatment. To realise broader clinical applications for efficacious ACT and enhance the current therapeutic approach, the immunotherapy field has a great need to further improve therapeutic T-cell manufacturing methods. In this project, we aim to define optimal growth conditions for improved culture of adapted therapeutic T cells required for solid tumour treatment. The T-cell culture methods will be translated from manual to robotic set-ups where all pipetting, cell culturing and assay read-outs will be performed in 96-well plate formats with robots coupled to incubators, spectrophotometer and high-content confocal microscope. Moreover, we will test a panel of phenotypic markers using state-of-the-art technologies such as mass cytometry (CyTOF) and cellular metabolism (Seahorse analysis) to be able to compare different culture conditions and characterise the heterogeneity of the T-cell population. The data acquired by these high-throughput systems will be analysed with a customized pipeline designed for this project and including various modern computational cytometry techniques. We will present the pipeline of the project to establish an optimal T cell product and a high throughput screening platform for cellular therapies.

Title #26: Retroelement decay by the exonuclease XRN1 is a viral mimicry dependency in cancer

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Viral mimicry describes the immune response induced by endogenous stimuli such as dsRNA formed by endogenous retroelements. Activation of viral mimicry has the potential to kill cancer cells and augment anti-tumor immune response. Cancer cells frequently present a dysregulated epigenome, leading to increased expression of retroelements. We previously found that ADAR1p150 is required to tolerate high retroelement-derived dsRNA levels by editing dsRNA. Here, we systematically identified novel mechanisms of viral mimicry adaptation associated with cancer cell dependencies. We correlated the gene knockout sensitivity from the DepMap dataset and interferon stimulated gene expression in the Cancer Cell Line Encyclopedia dataset of 1005 human cell lines and identified pathways such as RNA modification and nucleic acid metabolism. Among the top hits was the RNA decay protein XRN1 as an essential gene for the survival of a subset of cancer cell lines. XRN1-sensitive cancer cell lines have a high level of cytosolic dsRNA and high ISG expression. Furthermore, sensitivity to XRN1 knockout was mediated by MAVS and PKR activation, indicating that the cells die due to XRN1-dependent induction of viral mimicry. XRN1-resistant cell lines had low basal dsRNA levels but became synthetically dependent on XRN1 upon treatment with viral mimicry inducing drugs such as 5-AZA-CdR or palbociclib. Finally, XRN1-dependency is partly independent of ADAR1 activity. These results confirm the potential for our ISG correlation analysis to discover novel regulators of viral mimicry and show that XRN1 activation is an adaptive mechanism to control high dsRNA stress induced by dysregulated retroelements in cancer cells.

Title #27: A novel non-invasive FcRn-targeted mucosal vaccine strategy

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While the established route for vaccines is intramuscular, it may be preferable to deliver vaccines intranasally to secure mucosal protection at the site of infection. This will limit the spread of the virus, ease administration and likely improve vaccine acceptance. Here, we present a subunit vaccine strategy based on engineered human albumin fused to immunogens, which upon intranasal delivery targets FcRn followed by induction of robust mucosal and systemic antibody responses beyond that gained by intramuscular delivery. This needle-free vaccination principle protects against challenge with SARS-CoV-2 and influenza A, and as such should be an attractive strategy for design of subunit vaccines targeting respiratory diseases.

Title #28: Investigating the importance of CD4+ T cell activation after DNA vaccination for protection against multiple myeloma in MOPC315.BM.luc mouse model.

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Multiple myeloma (MM) is a hematologic cancer caused by uncontrolled proliferation of malignant plasma cells characterized by their secretion of monoclonal immunoglobulins (M-proteins). The M?proteins have tumor-specific idiotypes and are thereby a potential target for immunotherapy. Prophylactic DNA vaccination studies directed at these tumor-specific epitopes have shown promising results in the MOPC315 tumor model. Although preliminary results indicate that vaccine-induced protection against MOPC315.BM.luc tumor challenge was dependent on activation of CD8+ T cells, coactivation of CD4+ T cells was required to obtain a stronger effect. The DNA vaccine used combine the tumor antigen with a targeting unit that binds to antigen presenting cells (APC) via a heterodimerization unit. This vaccine will be translated into a dimeric vaccine protein with two arms. These arms can be similar or different, allowing us to compare vaccines with only CD8 epitopes or with both CD4 and CD8 epitopes in the same vaccine molecule. In addition, more epitopes of either CD8 or CD4 can be included. We will use antigenic units as so-called “string of beads” containing several consecutive epitopes, either as whole single chain variable fragments (scFv) or as peptide epitopes. The various DNA vaccines are under construction. In this project we investigate whether increasing the number of CD4+ T cell activating epitopes in the vaccine construct can increase tumor specific immune response. We have tested an optimalization of the peptide epitopes, to increase translation into vaccine protein. In addition, we have vaccinated mice with DNA constructs containing different numbers of CD8+ T cell and CD4+ T cell epitopes prior to MOPC315.BM.luc tumor challenge. Tumor growth was followed by non-invasive imaging and M-protein blood levels. Preliminary results confirm that CD4+ T cells are needed for efficient protection, however, more experiments are needed to see if additional CD4 epitopes can enhance protection

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Title #29: Tissue-specific NK cell responses during acute GvHD

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Acute graft-vs-host disease (GvHD) is a life-threatening inflammatory disease where T cells from a donor allo-hematopoietic cell transplant attack recipient tissues. Donor NK cells rapidly reconstitutes from the graft, and their role in this disease is not fully understood. We addressed in this study NK cell infiltration of tissues and their activity in a rat model of allogeneic full MHC-mismatch transplantation model with

delayed allogeneic donor T cell infusion 14 days post transplantation of T-cell depleted bone marrow. MHC-matched transplantations were done as comparisons. Full donor chimerism of NK cells was established in all tested tissues (blood, spleen, liver, lung, skin, small intestine, colon, mesenteric lymph nodes) prior to donor T cell injection. There was an increased NK-cell infiltration of lung and intestinal tissues, and a reduction in blood and spleen. Lung NK cells were more activated in animals with GvHD. Using 10X sequencing of intestinal tissues, we found an increase in an allogeneic subset of NK cell in rats suffering from GvHD, which expressed markers for cellular activation and exhaustion. Together these results indicate that NK cells can have an inflammatory role in GvHD and can lead to the perpetuation of the disease.

Title #30: Single-cell characterization of blood and expanded regulatory T cells in autoimmune polyendocrine syndrome type 1

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APS-1 is a rare monogenic disorder, caused by mutations in the AIRE gene. AIRE induces tolerance through negative selection and is thought to be involved in the generation of regulatory T cells (Tregs). Tregs are important mediators of peripheral tolerance, as they are able to suppress the function of other immune cells. Proper functioning Tregs are crucial to avoid autoimmune disease. We aimed at using single-cell transcriptomics and proteomics to characterize Tregs sorted from blood and in vitro expanded Tregs in patients with APS-1 compared to controls. CD4+CD25+CD127- Tregs were sorted from 4 APS-1 patients and 4 controls prior to single-cell sequencing. In vitro expanded Tregs from 8 APS-1 patients and 8 controls were characterized by flow cytometry and CyTOF using a panel of Treg markers. Further, these in vitro expanded Tregs were TCR and single-cell sequenced using a targeted Human Immunology Panel. Single-cell sequencing revealed two downregulated (CD52 and LTB) and one upregulated (TXNIP) as consistently differentially expressed genes when comparing naïve and expanded Treg datasets. There were no large differences in the TCR-repertoire of expanded Tregs between the cohorts, but some patients showed a more restricted use of specific clonotypes. Flow and mass cytometry revealed a lower CD4+CD25+FOXP3+CD127- population in APS-1 compared to healthy controls, in addition to a lower expression of Helios and CD31 and a higher expression of CD57 and CD161. Taken together our findings

do not support inherent Treg dysfunctionality in APS-1 patients, as their Tregs were able to expand with retained functions of Tregs.

Title #31: High seroprevalence in all age groups with pattern of Omicron infection in children and hybrid immunity in adults: A nationwide cross-sectional study of humoral immunity to SARS-CoV-2 in Norway, autumn 2022

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Background: In Norway, 91% of individuals ≥16 years had received ≥1 dose of COVID-19 vaccine by mid-July 2022, while less than 2% of children <12 years were vaccinated. Confirmed COVID-19 was reported for 27% of the population. Infections were probably widely underreported, thus we conducted a nationwide cross-sectional study to explore the humoral immunity to SARS-CoV-2 in Norway in August 2022.
Methods: 1,914 anonymized residual sera and 243 NorFlu-cohort sera with reported infection/ vaccination status were analyzed for antibodies against spike and the receptor-binding domain (RBD) of the ancestral Wuhan strain (RBD_W), Omicron BA.2 RBD (RBD_BA2) and nucleocapsid (N). Samples were also tested for antibodies inhibiting RBD-ACE2 interaction. Neutralization assays were performed on subsets of residual sera.
Results: The seroprevalence estimate in children increased from 83.3% (Wuhan-antibodies) to 97.7% when including samples with anti-RBD_BA2 antibodies. The national seroprevalence estimate was 99.1% (95% CrI 97.0%-100.0%). 38.1% of all residual sera were positive for anti-N antibodies. Sera neutralizing both Wuhan and BA.2 strains showed higher levels of anti-N antibodies than sera only neutralizing Wuhan, suggesting hybrid immunity, and also neutralized newer Omicron variants. Reactivity profiles against Wuhan and BA.2 were used to infer infection and vaccination history in anonymized sera and validated using cohort sera.
Conclusions: Most of the Norwegian population had SARS-CoV-2-specific antibodies. Almost all children had been infected. Assays based on Wuhan-antibodies might underestimate seroprevalence in individuals with primary Omicron infection. Sera displaying hybrid immunity could neutralize Omicron variants emerging after sample collection suggesting that infections induce cross-reacting antibodies.

Title #32: Generation of an immunoglobulin knock-in mouse to study gluten-specific T cell – B cell collaboration in celiac disease.

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Celiac disease (CeD) is an autoimmune enteropathy driven by dietary intake of gluten. A hallmark of the disease is the development of highly specific antibodies to modified (deamidated) gluten peptides (DGP) as well as autoantibodies to the enzyme mediating the modification, transglutaminase 2. In CeD, these autoantibodies have a strong association to the HLA allotypes HLA-DQ2.5, HLA-DQ2.2 and HLA-DQ8, suggesting that T cell – B cell collaboration is a key part of disease pathogenesis. We have generated an immunoglobulin knock-in (Ig KI) mouse expressing a prototypic, celiac-patient derived anti-DGP antibody, recognizing the immunodominant epitope of DGP (QPEQPFP) and crossed these mice to HLA-DQ2.5 KI mice. We show that most B cells express the transgenic B-cell receptor and that BCR-mediated uptake of DGP results in efficient proliferation of DGP-specific CD4+ T cells as well as DGP-specific B cells in vitro. B cells from anti-DGP Ig KI mice were adoptively transferred, with gluten-specific CD4+ T cells, into HLA-DQ2.5 KI recipient mice that were immunized orally with DGP mixed with cholera toxin (CT) as adjuvant. While the oral immunization with DGP and CT induced a robust antibody response to DGP in serum, no response was triggered in the gut. However, when DGP was chemically coupled to CT, priming of the DGP-specific responses occurred in the Peyer's patches and resulted in high levels of DGP-specific IgA plasma cells in the small intestinal lamina propria, secretory anti-DGP IgA in intestinal lavage fluid, as well as anti-DGP serum antibody response. These findings suggest that priming of the anti-DGP (and anti-TG2) plasma cell responses as seen in the gut lesion of CeD patients occurs in Peyer's patches. The strategy of immunizing with coupled peptide antigen-adjuvant also has implications for mucosal vaccine design in which protective immunity can be induced locally at sites of infection.

Title #33: Novel combinatorial CARs efficiently and selectively target malignant cells

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Chimeric antigen receptor (CAR) T cells have revolutionized cancer immunotherapy, showing a great potential against relapsed and refractory leukemia. Traditional CD19 CAR T cells effectively eliminate B cells in patients, however, they do not discriminate normal and leukemic B cells, leading to B cell aplasia that increases the risk of infection. On the other hand, a solution to avoid side effects reminiscent of

on-target/off-tumor toxicity, combinatorial CAR design can be exploited. These combinatorial CARs are composed of a subunit that consists in a single chain variable fragment (scFv) of an antigen specifically expressed on malignant cells followed by CD3ζ signaling domain; and a second subunit with a scFv of a high efficiency CAR followed by 4-1BB co-stimulatory domain. Thus, we designed a combinatorial CAR composed by IGK scFv – CD3ζ (Kz) and CD19 scFv – 4-1BB (19BB) (Kz-19BB CAR) for the treatment of B-cell lymphoma and/or leukemia, and TAG72 scFv – CD3ζ (TAG72) and K101 scFv – 4-1BB (undisclosed target; K101) (TAG72-K101 CAR) for the treatment of ovarian cancer. These designs provide the restriction of specificity toward IGK+ or TAG72+ targets only, while maintaining the level of CD19 and K101 CAR efficacy, respectively. Both combinatorial CAR T cells showed high efficacy and selectivity against malignant cells in vitro. Furthermore, their in vivo functionality will be tested using immunodeficient mice transplanted with malignant cells and treated with the two combinatorial CAR T cells, with the aim of reducing tumor burden. Taken together, our results confirm the efficiency and selectivity of combinatorial CAR T cells in vitro, which appear to be a promising safe therapy for the treatment of both, hematological and solid tumors.

Title #34: Positive selection of B cell convertants that express the MOPC315 idiotope: the role of Id-specific Th cells in the germinal center

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Idiotope-driven T-B cell collaboration may contribute to the development of autoimmune diseases and even B cell lymphomas. Idiotopes (Id) are parts of the variable (V) regions of B cells receptors (BCRs). Id-peptides are displayed as pId:MHCII complexes on the B cell surface for $CD4^+$ T cell recognition. Using a unique mouse model, we have demonstrated that upon BCR ligation, naive B cells can upregulate pId:MHCII complexes and interact with Id-specific $CD4^+$ T helper cells, leading to Id-driven T-B cell collaboration. The YST to FRN amino acid triplet change as found in the 2 light chain of MOPC315 enables Id-driven T-B cell collaboration. The aim here is to investigate whether Id-specific T cells with a defined specificity for FRN can positively select FRN-converted B cells in thegerminal center reaction. The result should be expansion of Id^+ (FRN^+) B cells that differentiate to Id^+ memory B cells and to Id^+ plasma cells that secrete Id^+ antibodies. This process will be studied in a novel mouse model, which encodes FSN in the BCR V region. We aim to induce the conversion to FRN by antigen priming with BCR ligands. This will be combined with the induction of Id-specific $CD4^+$ T cells by vaccination with APCs-targeted vaccibody containing the Id, as well as through the transfer of Id-specific T $CD4^+$naïve cells from transgenic mice. The conversion will be followed by Id-specific ELISA, FACS and BCR V-region sequencing.

Preliminary results suggest that conversion may happen at low frequencies in Island mice after antigen priming. T cell help may enhance the selection of convertants. In conclusion, we have detected instances of conversion in the BCR V-region in Island mice. More experiments are in the process to see if this is a spontaneous phenomenon or if it can be induced by Id-specific T cells and BCR ligation.

Title #35: Morphological profiling of polarized macrophages

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In image-based morphological profiling, stained cells are imaged using multi-channel fluorescence microscopy, and image analysis is used to extract thousands of morphological features from the imaged cells. This yields highly resolved and reproducible single-cell profiles that can be used to analyze and classify cell types and cell perturbations with applications in basic research and drug discovery. Here, we explore image-based morphological profiling of in vitro polarized macrophages using the Cell Painting assay, showing that pro-inflammatory and anti-inflammatory phenotypes can be distinguished in an unbiased manner using image-based morphology alone. Since the method can be run with very high throughput at relatively low cost, we believe it can be used to complement traditional methods such as ELISA or quantitative RT-PCR for the analysis of macrophage phenotypes, and possibly other immune cells.

Title #36: High dimensional immune profiling of disease severity in patients hospitalized with influenza

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Influenza epidemics represent a huge health burden in terms of hospitalization, morbidity and deaths and there is a need for improved understanding of the immune responses leading to severe disease. The aim was to identify an immune profile associated with severe influenza. Blood was sampled from adult hospitalized influenza patients during acute infection and after 5-11 months, and from healthy controls. High dimensional immune profiling of PBMCs by mass cytometry with unsupervised clustering (FlowSOM) and negative binomial regression were applied to reveal cell populations with different frequencies between moderately and severely ill patients. Patients with severe disease had lower

frequencies of CD4+ memory Tfh cells, MAIT cells and memory B cells, but higher frequencies of plasma cells, activated monocytes and NK cells, compared to moderately ill patients during acute infection. Analysis of PMA/ionomycin-stimulated PBMCs revealed higher frequencies of cytokine-producing NK cells, as well as NKT cells and lower frequencies of central memory CD4+ and cytokine-producing CD8+ cells in severe compared to moderate disease. At convalescence, as well as compared to healthy controls at infection, NK and monocyte frequencies were still higher, and the memory B cell frequency was still lower in the severely ill patient group, while the cell subpopulation frequencies in the moderately ill group resembled the controls. High dimensional single cell protein analyses identified a severity-associated immune profile for influenza disease characterized by altered frequencies of innate and adaptive immune cell populations reflecting an inflammatory-like condition, that partly still were present at convalescence.

Title #37: Tracing the intrathecal B-cell response in Multiple Sclerosis from germline IGH loci to immunoglobulin transcripts

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Mechanisms driving the B cell response against specific antigens are not very well known. We have previously found that B cells in the cerebrospinal fluid (CSF) of multiple sclerosis (MS) patients have a restricted and shared usage of IGHG1 alleles and IGHV genes. Here, we utilize Oxford Nanopore Technology (ONT) to sequence the complete germline IGH locus and combine this with single-cell RNA-seq data of B cells from CSF. This will allow us to create a personalized and phased assembly of the IGH germline repertoire and link this to the ongoing B-cell response in the CSF of the same patients. High molecular weight gDNA is isolated from monocytes of MS patients and sequenced with ONT PromethION platform. This generates data of ultra-long reads (N50 > 50 Kb) which enables the bioinformatic separation of reads from sister chromosomes. We have successfully established a bioinformatic pipeline and used this to assemble haplotype-resolved and annotated IGH loci. Using reads with N50= 70 Kb, mean coverage of 30x and a personalized reference, we were able to create two contiguous assemblies >2 Mb. De novo assembly of the two haplotypes will enable us to not only assess the usage of IGH genes in B cells, but also investigate intergenic regions and promoters of our patients' IGH loci. The IGH locus has only been fully resolved in a handful of people previously due to its complex sequence and structure. We have shown that our method has comparable accuracy to- and better contiguity than established methods.

Title #38: Development of a Humanized Chimeric Antigen Receptor and Bispecific T cell Engager for Treatment of Osteosarcoma

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Osteosarcoma is an aggressive malignancy that primarily affects children and young adults. Despite its rarity in the general population, it comprises approximately 5–10% of cancer diagnoses in pediatric and adolescent patients, thus emphasizing the need for a safe and effective treatment. Currently, the condition is difficult to treat via immunotherapy as a result of intra-tumor heterogeneity and widespread expression of potential target proteins. However, the development of a humanized bispecific antibody engager (BiTE) and chimeric antigen receptor (CAR) with specificity for isoform 1 of alkaline phosphatase (ALPL-1), a highly expressed surface protein in OS cell lines, has the potential to act as a treatment for those afflicted by OS. To develop these OSBiTEs and OSCARs, the sequence of two murine monoclonal antibodies, TP-1 and TP-3, were utilized. These antibodies were isolated after inoculating mice with a human OS cell line, and subsequently humanized. Current results from preclinical models indicate that these humanized OSBiTEs and OSCARs can be efficiently produced and are capable of eliciting an in vitro immunological response without compromising the binding of the original murine scFv. Therefore, humanized OSBiTEs and OSCARs have the potential to revolutionize OS treatment if these findings can be successfully translated into a clinical treatment.

Title #39: Broadly reactive vaccines against seasonal infectious diseases

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Seasonal influenza and coronavirus variants will continue to circulate in the future. We need prophylactic vaccines to protect our population, but the currently used vaccines are designed to protect against only very limited viral variation. Both influenza and coronaviruses are prone to antigenic drift, so we have developed a new vaccine that will broadly protect against both past and future virus variants. The key is to include different antigens that can raise both relevant antibodies and T-cell responses, and combine these in a single seasonal vaccine that can protect broadly against influenza and coronavirus.

Title #40: IMMUNOmodel: Coming together to model immunotherapy response and toxicity in cancer

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The IMMUNO-model COST Action aims to foster research and innovation in the field of preclinical immunooncology models with the ultimate goal of advancing in the treatment of cancer patients by improving their outcomes and quality of life. Cancer immunotherapy has revolutionized treatment of cancer patients, as illustrated by the spectacular results obtained in previously incurable malignancies, such as metastatic melanoma and B-cell leukemia. However, the widespread use of these therapies has been hindered by their limited effectiveness in solid tumors and associated toxicities. A better understanding of the complex interactions between tumour cells and the immune system is critical to address these problems, and to develop more effective and safer immunotherapies. However, one of the most important obstacles in immuno-oncology research is the scarcity of preclinical models that recapitulate the human paradigm and contribute to accurately trial new immunotherapeutics concepts, characterize biomarkers of therapeutic response and toxicity, and generate reliable data on drug synergies.IMMUNO-model will bring together European researchers from diverse sectors (academia, clinical, industry) with the common goal of establishing a Network that endorses immuno-oncology research by specifically promoting the sharing, standardization and application of immunotherapy preclinical models. This Action will allow the implementation of a broad, creative and collaborative hub through the organization of community-building activities, the creation of synergies among European and non-European scientists, and the training of future researchers in the field. As of today, 28 European countries are dedicated to the development of this COST-action.

Title #41: Influence of genetic background in vaccine response

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The purpose of vaccination is to stimulate the formation of pathogen-specific memory responses through highly diversified receptors on T and B cells. However, the formation of an effective immune response following vaccination differs within a population. The major histocompatibility complex (MHC) molecules have genetically endowed differences with respect to which peptides they can bind and present. Here, we are looking at how different MHC class II molecules can influence B cell and antibody

responses in context of the genetic backgrounds of different mouse strains. More specifically, BALB/C, C57BL/6, and C57BL/6-DQ2.5 transgenic mice were immunized intramuscularly with Pandemrix. B cell responses and antibody formation in sera were evaluated using flow cytometry, ELISpot, ELISA, and microneutralization assay. Results showed a significantly elevated IgG response against hemagglutinin from A/California/07/2009 (H1N1) (vaccine matching influenza strain) in BALB/C and C57BL/6 mice as compared to C57BL/6-DQ2.5 mice after one vaccine dose, but no significant differences after a second dose. These data corresponded well with the presence of germinal center B cells after vaccination in the different mouse strains. Further work aims to evaluate B and T cell responses more in depth using ELISpot and Flow cytometry, and also assess the antibody repertoires formed for epitope usage.
