PDO,patient matched CAF and monocytic cells and subjected to single cell RNA seq,flow cytometry and cytokine analysis.
Results We discovered that CAF co-culture resulted in apartial differentiation of monocytes into macrophages and a phenotypic switch,characterized by the expression of major immunosuppressive markers comparableto TAMs in CRC. Macrophages did not appear to gain TAM-like features from CAFco-culture. Oxaliplatin and 5-FU, the standard of care chemotherapy for CRC,induced polarization of TAM-like cells to a pro-inflammatory phenotypecomparable to the immunogenic effects of treatment with an oncolytic virus.
Conclusions Thus, primary CAF-containing triple culturessuccessfully model TAM-like phenotypes *exvivo* and allow the assessment of their functional and phenotypic changes inresponse to treatments following a precision medicine approach.

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05. Immunometabolism

P05.01 SENSITIZING IMMUNOTHERAPY REFRACTORY PROSTATE CANCER WITH OPTIMIZED KETOGENIC DIET REGIMEN AND EPIGENETIC REPROGRAMMING

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Background Resistant to immune checkpoint blockade (ICB) therapy, especially in a few solid tumor types including advanced prostate cancer, represents a formidable clinical challenge. The ketogenic diet can enhance ICB therapy in some cancer models (prostate cancer not included yet). However, the adverse effect associated with continuous KD was also observed, demanding better mechanistic understanding and optimized regimens of using KD as an immunotherapy sensitizer.
Materials and Methods We developed a series of ICB-resistant murine prostate cancer cell lines from an ICB-sensitive prostate cancer cell line previously developed from the *PB-Cre*$^{+}$ *Pten*$^{L/L}$ *p53*$^{L/L}$ *Smad4*$^{L/L}$ mouse model of metastatic prostate cancer. C57BL/6 mice inoculated with dual ICB-resistant subline PPS-ICBR were treated with epigenetic modulators or specialized diets in the presence or absence of anti-PD1 and anti-CTLA4 antibodies. The effect of the therapies were evaluated at both phenotypical levels and single cell profiling (including both CyTOF and scRNA-seq). Emerging mechanisms were tested with loss of function and gain of function experiments.
Results The major histocompatibility complex (MHC) class I protein levels were downregulated in the resistant sublines compared with the parental cell line. Using the most ICB-resistant subline model, we demonstrated that synergistic efficacy was achieved by combining anti-PD1 and anti-CTLA4 antibodies with histone deacetylase (HDAC) inhibitor vorinostat, a cyclic ketogenic diet (CKD), or supplementation of ketone body β-hydroxybutyrate (BHB, endogenous HDAC inhibitory metabolite) via 1,3-butanediol-admixed food. CKD and BHB supplementation delayed prostate cancer tumors as monotherapy, and both BHB and adaptive immunity are required for the anti-tumor activity of CKD. Single-cell transcriptomic and proteomic profiling revealed that the HDAC inhibitor and ketogenesis-enhanced ICB therapy was effectuated through both cancer-cell-intrinsic (upregulated MHC class I molecules) and extrinsic mechanisms ($CD8^{+}$ T cell chemoattraction, M1/M2 macrophage rebalancing, monocyte differentiation toward antigen presenting cells, and diminished neutrophils).
Conclusions Epigenetic modulation with pharmacological HDAC inhibitor vorinostat or endogenous HDAC inhibitor BHB (via CKD or BD) sensitized ICB-refractory prostate cancer to PD1/CTLA4 dual ICB therapy. The two combination therapies share underlying mechanisms such as cancer cell MHC-I upregulation and concerted innate and adaptive immune landscape remodeling. Among the strategies, 1,3-butanediol-supplemented-diet may represent the most attractive option to enhance ICB therapy due to its high chance of patient compliance, low cost, and minimal toxicity.

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P05.02 INVESTIGATING THE KINETICS OF CD3 BINDING AND TRAFFICKING IN REAL-TIME

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Background T-Cell Receptor complex (TCR) is an immune structure aimed at antigen recognition and initiation of immune response. It is bound to three protein dimers, known as Cluster of Differentiation 3 (CD3), whose role is related to signal transduction. Since the immune response is precisely tuned, mobilization of the TCR complex is highly regulated, as the complex undergoes several rounds of positioning on the cell membrane, endocytosis, and recycling. This study was thus aimed at understanding the kinetics of recycling and internalization of the CD3ϵ subunit, by targeting it with the antibody SPV-T3a. The final goal would be the development of a mathematical model of trafficking kinetics, which would not only help understanding the metabolic effect of endocytosis in inflammation processes, but also be a game changer in drug development.
Materials and Methods Lymphoblastic cell line Jurkat overexpressing CD3ϵ, and K562 (negative control) were used for the experiments. Both were grown in RPMI1640 media with 10% HiFBS and 1% PeSt. The antibody SPV-T3a was labeled with either ATTO488 or the pH dependent dye pHrodo Green. Receptor trafficking was studied in real-time assays using LigandTracer Green and evaluated with TraceDrawer software.
Results Binding of ATTO488-SPV-T3a to CD3 receptors showed a peculiar profile, which reflected an ongoing endocytosis pattern. The plateau phase of the curve at equilibrium was replaced by a section of linear growth in which the slope was constant and independent of antibody concentration. This slope is believed to reflect the kinetics of CD3 recycling on the cell surface. Dissociation was also ligand-independent, with a biphasic profile where a first initial detachment of the complex was followed by a stable signal, given by the fluorophores accumulated in the cells. Labeling SPV-T3a with

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pHrodo enabled a more precise analysis of the internalization pattern, which showed a linear intake. This together with the fluorescent signal growth in the dissociation phase verified how the internalized SPV-T3a gets degraded through time. A fast initial endocytosis was observed for higher antibody concentrations, that then slowed until a plateau phase before restarting linearly. When using lower concentrations, internalization happened in linear fashion. To investigate the endocytic pathway, cells were treated with various chemicals, in which a 1:1 binding curve was only obtained when treating the cells with 0.5 M sucrose.

Conclusions The study showed that the recycling patterns of CD3 are mostly unmodified by the targeting process. The linear internalization kinetics indicates a fast receptor recycling and antibody degradation. Sucrose experiments showed that the clathrin dependent pathway is primal for CD3 internalization, but not exclusive, as more pathway-specific chemicals did not modify the shape of the curve.

P. Zucchetti: A. Employment (full or part-time); Significant; Ridgeview Instruments AB. **S. Bondza:** A. Employment (full or part-time); Significant; Ridgeview Instruments AB. **S. Lundsten Salomonsson:** A. Employment (full or part-time); Significant; Ridgeview Instruments AB.

06. B cells in IO

P06.01 GAIN-OF-FUNCTION CARD11 SIGNALLING IN B CELLS CAUSES PATHOLOGICAL LYMPHOPROLIFERATIVE DISEASE IN MICE WHICH IS STRICTLY DEPENDENT ON MALT1 ACTIVITY

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Background The B cell receptor is activated together with co-receptors to regulate crucial signalling and metabolic pathways that mediate B lymphocyte growth, survival and expansion. Central to these pathways is the CBM complex, composed of the molecules CARD11, BCL10 and MALT1. Importantly, alongside serving as a scaffold protein, MALT1 is a paracaspase which regulates gene expression through its activity on RNases. Gain-of-function CARD11 mutations, for example CARD11^{L225LI}, drive aberrant CBM complex activation and are recurrently detected in human B cell lymphomas, including diffuse large B cell lymphoma.

Materials and Methods Here we have generated the novel mouse model CARD11$^{L225LI;mb1\text{-}CreERT2}$ which enables controlled GOF CARD11 expression only in the B cell lineage using Tamoxifen mediated Cre activity. We determined the role of MALT1 activity in these mice by generating mouse models that specifically lack MALT1 function. We performed transcriptomic analyses of splenic B cells of the different genetic models.

Results Acute B cell intrinsic expression of GOF CARD11 in CARD11$^{L225LI;mb1\text{-}CreERT2}$ mice resulted in a pathological expansion of transgenic B cells leading to B cell lymphoproliferative disease, increased immunoglobulins and high levels of inflammatory cytokines. These CARD11^{L225LI} expressing B cells also had increased survival and proliferation. Next, we determined the role of MALT1 paracaspase action on this pathology and observed that the absence of MALT1 activity rescued B cell lymphoproliferative disease upon CARD11^{L225LI} expression. To functionally understand the role of MALT1 in GOF CARD11 signalling, we completed RNAseq analysis and found upregulation of gene sets that correspond to plasma cells and lymphoid tumours, as well as NF-κB and Myc activation upon CARD11^{L225LI} expression, independent of MALT1 function. Therefore, we next completed differential expression analysis of individual genes and observed that genes known to be critically involved in B cell survival and proliferation were upregulated in cells with MALT1 activity, but not without MALT1 activity. Protein analysis validated these findings, demonstrating increased BCL-xL, Cyclin D2 and Cyclin E expression following acute CBM complex activation in a MALT1 dependent manner.

Conclusions Through our novel genetic system, we show that GOF CARD11^{L225LI} signalling induces a pathological B cell lymphoproliferative disease which is strictly dependent on MALT1 activity. We also show that MALT1 selectively affects the expression of a series of proliferation and survival factors that are known to mediate physiological and pathological B cell expansion. Lastly, these data highlight the role of paracaspase-mediated regulation of immune signalling events in B cells.

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P06.02 SPATIO-FUNCTIONAL CHARACTERIZATION OF TUMOR-INFILTRATING B CELLS IN THE TUMOR MICROENVIRONMENT OF CUTANEOUS T CELL LYMPHOMA

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Background The majority cutaneous T cell lymphoma (CTCL) run an indolent clinical course. However, advanced stages (>=IIB) or certain CTCL subtypes such as folliculotropic Mycosis Fungoides (FMF) and Sézary Syndrome (SS) are associated with significantly worse prognosis and treatment options are limited. In previous studies, our group has described increased numbers of CTCL tissue infiltrating B cells as an adverse prognostic factor. However, the underlying mechanisms are still poorly understood.This project therefore aims to characterize the role of B cells within the CTCL Tumor Microenvironment with a special focus on spatial distribution and immunometabolic and cellular interaction patterns.

Materials and Methods An independent cohort of clinically annotated, Formalin fixed and Paraffin embedded (FFPE) clinical CTCL probes were analyzed by multiplex Immunohistochemistry (IHC) to characterize the entire tumor and immune cell infiltrate and to determine spatial distribution and interaction patterns. Gene expression within these probes was quantified from consecutive tissue sections by Nanostring© and spatial transcriptomic analyses via the 10x Genomics© and