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

[1,2]A Kratzert*, [1,2]E Morrish, [1,2]SJ Keppler, [1,2]J Ruland. *[1]TranslaTUM, Center for Translational Cancer Research, Technical University of Munich, Munich, Germany; [2]Institute of Clinical Chemistry and Pathobiochemistry, School of Medicine, Technical University of Munich, Munich, Germany*

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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

[1,2,3]S Oganesian*, [1]M Funk, [1,2,3]A Hadzic, [1,2,3]M Kirmaier, [4]G Piontek, [2]J Watter, [1]M Heide, [2]S Krebs, [2]S Mages, [2]H Blum, [5]C Posch, [1]O Weigert, [2]J Klughammer, [4]M Rudelius, [1,3]M Bergwelt-Baildon, [1,2,3]S Theurich. *[1]LMU MED3, Munich, Germany; [2]LMU Gene Center, Munich, Germany; [3]German Cancer Consortium (DKTK/DKFZ), Munich/Heidelberg, Germany; [4]LMU Pathology Institute, Munich, Germany; [5]Siegmund Freud University, Department of Dermatology, Vienna, Austria*

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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