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P09.03 *CATHEPSIN S* ALTERATIONS INDUCE A TUMOR-PROMOTING IMMUNE MICROENVIRONMENT IN FOLLICULAR LYMPHOMA

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Background By targeted DNA sequencing of 305 diagnostic follicular lymphoma (FL) biopsies, we identified somatic mutations of *Cathepsin S (CTSS)* in 8% of cases (24/305), mostly clustered at Y132 (19/24) converting Y to D (16/19). Another 13% of FL had *CTSS* amplifications (37/286), associated with higher *CTSS* expression (*P=0.05*). CTSS is a cysteine protease that is highly expressed in endolysosomes of antigen presenting cells and malignant B-cells. CTSS is involved in proteolytical processing of antigenic peptides for presentation on MHC-II to be recognized by antigen specific $CD4^+$ T-cells.[1] CTSS is synthesized as an inactive zymogen, which is converted to its active form by autocatalytic cleavage of the autoinhibitory propeptide (pro-CTSS).

Materials and Methods We used CRISPR/Cas9 to introduce *CTSS* Y132D into Karpas422, a B-cell lymphoma cell line that harbors the FL hallmark translocation t(14;18). We purified pro-CTSS WT and Y132D and assayed the *in vitro* autocatalytic cleavage over time. We then tested the impact of CTSS on $CD4^+$ T-cell activation in co-culture assays, in a previously described *in vivo* model[2] which we slightly modified to reflect FL-like conditions, and in primary patient samples.

Results Single-cell derived Y132D mutant Karpas422 clones showed >3-fold higher ratios of active CTSS to pro-CTSS (N=4, *P=0.0003*). Immunoprecipitated CTSS Y132D had >3-fold higher *in vitro* substrate cleavage activity compared to CTSS wild type (WT) (N=6, *P=0.001*) which was mediated by an accelerated conversion from pro-CTSS to active CTSS (11 minutes for CTSS Y132D *vs* 17 minutes for CTSS WT; N=3, *P*=0.04). Molecular dynamics simulations showed that the Y132D mutation shortens the distances by ~2Å between the catalytic triad of active CTSS (C139, H278, N298) and a stretch of amino acids from the proform (L80, G81, D82, S94), which could facilitate intramolecular cleavage. The higher substrate cleavage activity of CTSS Y132D came along with a high capacity to stimulate antigen specific $CD4^+$ T cell responses *in vitro* and *in vivo*. Additionally, *CTSS* overexpression could phenocopy this high $CD4^+$ T cell activation. Lastly, we aimed to correlate *CTSS* aberrations with clinical outcome in patients who received standard immunochemotherapy (R-CHOP) for advanced FL (N=51 with available *CTSS* mutation and gene expression data). Compared to all other patients (N=34), patients with *CTSS* Y132 mutations or *CTSS* overexpression (N=17) had longer failure free survival (*P*=0.012).

Conclusions Here, we provide biochemical, structural, functional and clinical evidence that aberrant CTSS activity induces a supportive immune microenvironment in FL. We propose that aberrant CTSS activity can elicit a $CD4^+$ T-cell driven tumor-promoting immune response, which could be amplified within the microenvironment and substantially impact the biology and clinical course of the disease. Thus, aberrant CTSS activity is a promising biomarker and therapeutic target in FL and potentially also other tumors.

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P09.04 ONCOLYTIC H5N1 INFLUENZA STRAIN DISPLAYS SUPERIOR THERAPEUTIC PROPERTIES INDEPENDENT OF IMMUNO-STIMULATORY INTERLEUKIN-2 TRANSGENE EXPRESSION

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Background Oncolytic viruses are becoming an integral part of immunological approaches to cancer treatment. Interleukin-2 (IL-2) is known to stimulate cytotoxic T-cells, and might therefore be a reasonable cargo to enhance the therapeutic effect of such viruses. However, IL-2 is also known to promote immunosuppressive regulatory T-cells (T-reg). We investigated the impact of virally expressed IL-2 on induction of regulatory T-cells. We further investigated the effect of virally expressed IL-2 on the therapeutic efficacy of influenza A H1 and H5 subtypes.

Materials and Methods Survival of B16 melanoma xenograft bearing mice upon treatment with various oncolytic influenza viruses was examined. Effect of these viruses on PBMC gathered from 4 young healthy volunteers and murine and human melanoma cell lines was examined utilizing multiple flow cytometry protocols.

Results Viral IL-2 expression did not alter viral growth and was stable up to multiple passages in cell cultures. In human PBMC viral expression of IL-2 did not enhance differentiation