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

¹JA Hildebrand*, ¹D Bararia, ¹S Stolz, ¹S Häbe, ²F Osorio-Barrios, ³MD Bartoschek, ¹E Gaitzsch, ¹V Jurinovic, ⁴K Rautter, ⁵C Ludwig, ³S Bultmann, ³H Leonhardt, ⁶S Eustermann, ⁶K Hopfner, ⁷W Hiddemann, ⁷M Bergwelt, ⁸M Schmidt-Suppran, ⁹MB Sárosi, ¹⁰M Rudelius, ¹¹V Passerini, ¹¹J Mautner, ¹O Weigert. ¹Experimental leukemia and lymphoma research, LMU, university clinic, Munich, Germany; ²Hematology and Oncology, TUM, Klinikum rechts der Isar, Munich, Germany; ³Department of Biology II, LMU, Center for Integrated Protein Science Munich (CIPSM), Munich, Germany; ⁴Immunanalytics: Research Group Tissue Control of Immunocytes and Core Facility, Helmholtz Zentrum, Munich, Germany; ⁵Bavarian Center for Biomolecular Mass Spectrometry (BayBioMS), TUM, Munich, Germany; ⁶Gene Center, LMU, Munich, Germany; ⁷Department of Internal Medicine III, LMU, Munich, Germany; ⁸Hematology and Oncology, Klinikum rechts der Isar, TUM, Munich, Germany; ⁹Institute of Inorganic Chemistry, Faculty of Chemistry and Mineralogy, Leipzig, Germany; ¹⁰Institute of Pathology, LMU, Munich, Germany; ¹¹Helmholtz Zentrum, Munich, Germany

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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 proteolytic 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* model² 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

¹J Kabiljo*, ¹I Kuznetsova, ¹J Homola, ¹S Proding, ^{1,2}J Laengle, ¹M Sachet, ³A Egorov, ^{1,2}M Bergmann. ¹Division of General Surgery, Department of Surgery, Comprehensive Cancer Center Vienna, Medical University of Vienna, Waehringer Guertel 18-20, A-1090 Vienna, Austria, Vienna, Austria; ²Ludwig Boltzmann Institute Applied Diagnostics, Medical University of Vienna, Waehringer Guertel 18-20, 1090 Vienna, Austria, Vienna, Austria; ³Research Institute of Influenza, Russian Academy of Medical Sciences, Prof. Popova Str. 15/17 196376 St. Petersburg, Russia., St. Petersburg, Russian Federation

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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

of T-cells into a regulatory phenotype. In a murine B16 xenograft model IL-2 expression significantly enhanced therapeutic effects of an H1 oncolytic influenza virus. Expressed within the background of H5 hemagglutinin, IL-2 did not lead to a significant enhancement of therapeutic efficacy. Interestingly, the empty influenza H5 subtype was significantly more potent in treating B16 xenograft tumors than the H1 subtype, regardless of IL-2 expression. In primary human PBMC models, the virus based on H1 hemagglutinin led to enhanced CD8 T-cell activation compared to H5. This effect was further enhanced by IL-2 expression, although all viruses led to significant activation. Surprisingly, viruses based on H1 hemagglutinin led to increased expression of the immune checkpoint PD-1. The virus based on H5 hemagglutinin did not lead to upregulation of PD-1, indicating a favorable balance between activation and exhaustion. Infection with the H5 based virus led to both enhanced apoptosis and immunogenic calreticulin exposure in human and murine melanoma cell lines compared to H1.

Conclusions IL-2 does not promote T-regs, when expressed in a viral background. H1 viruses induced PD-1 more potently than H5 viruses. The choice of viral entry protein is more relevant for the therapeutic effect of the virus, than the expression of a T-cell stimulating cytokine such as IL-2. Efficacy of oncolytic viral treatment appears to depend more on viral growth than on virally expressed T-cell promoting cargo.

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P09.05 IMMUNOGENICITY INDUCED BY THE ACADEMIC CHIMERIC ANTIGEN RECEPTOR CAR19 (ARI-0001) IN PATIENTS WITH CD19-POSITIVE RELAPSED/REFRACTORY B-CELL MALIGNANCIES RECRUITED INTO THE CART19-BE-01 CLINICAL TRIAL

^{1,2}N Klein-González*, ^{1,2}EA González-Navarro, ^{1,2}A Bartoló-Ibars, ³V Ortiz-Maldonado, ⁴M Torrealadell, ^{5,6}M Castellà, ^{1,2}D Benítez, ^{1,2}M Caballero-Bañós, ^{1,2}R Cabezon, ^{1,2}M Español, ³T Baumann, ³E Giné, ^{7,8}P Castro, ^{3,5,8}J Esteve, ^{2,5,8}J Yagüe, ⁴S Rives, ^{3,5,8}Álvaro Urbano-Ispizua, ^{3,5}J Delgado, ^{1,2,5,8}M Juan. ¹Immunotherapy Unit, HSJD-Hospital Clínic Barcelona, Barcelona, Spain; ²Immunology, CDB, Hospital Clínic Barcelona, Barcelona, Spain; ³Hematology, ICMHO, Hospital Clínic Barcelona, Barcelona, Spain; ⁴Hematology, Hospital Sant Joan de Déu (HSJD), Barcelona, Spain; ⁵Institut d'Investigacions Biomèdiques August Pi i Sunyer – IDIBAPS, Barcelona, Spain; ⁶Banc de Sang i Teixits (BST), Barcelona, Spain; ⁷Medical Intensive Care Unit, Hospital Clínic Barcelona, Barcelona, Spain; ⁸University of Barcelona, Barcelona, Spain

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Background Chimeric Antigen Receptor (CAR)-T cells directed against CD19 have induced high rates of response in patients with relapsed/refractory (R/R) B-cell malignancies. Two CD19-targeting constructs have been approved by the FDA and EMA (Yescarta[®], Kymriah[®]) for B lymphoblastic leukemia (B-ALL) and aggressive lymphoma. Despite deep remissions, there are still major challenges and disparate data are reported about the immunogenicity induced by CART-cell therapy. On May/2017, the Spanish Agency of Medicines approved our first clinical trial (clinicaltrials.gov NCT03144583) with a fully academic CART-19.

Materials and Methods Eligibility criteria included R/R B-ALL (adult and pediatric), non-Hodgkin's lymphoma (NHL) and

chronic lymphocytic leukemia (CLL) who failed standard therapy. The primary objective of the study was safety and secondary objectives were response rate and its duration. The humoral anti-CART response was assessed by a (cell-based) fluorescence assay to detect human anti-murine antibodies (HAMA) in patients sera. Assessment was conducted at different time points: 1) at baseline (pre-dose), 2) on day 14 after the administration of ARI-0001 cells, 3) on day 28, 4) on day 100, and 5) every 3 months thereafter.

Results Forty-seven patients (37 adults/10 pediatrics) received ARI-0001 cells. Thirty-eight patients had a diagnosis of R/R B-ALL (28 adults and 10 children); all but 5 had relapsed after allogeneic hematopoietic stem cell transplant (HCT). Seven patients had a diagnosis of NHL, four of them (57%) had relapsed after HCT, and 2 patients had a diagnosis of CLL (2). Median age was 27 years (3–68). After conditioning with fludarabine (90 mg/m²) and cyclophosphamide (900 mg/m²), a total dose of 0.5–5 x10⁶ ARI-0001 cells/kg was infused. Autologous T-cells from peripheral blood were expanded and transduced with a lentivirus to express a CAR with a single-chain variable fragment (scFv) with anti-CD19 specificity, conjugated with the co-stimulatory regions 4-1BB and CD3z. The scFv was originated from a mouse monoclonal antibody A3B1. Twenty-five per cent of the patients tested positive for the presence of anti-CAR antibodies, all of them post-dose, in contrast to previous data reported on Kymriah[®] with a significant presence of pre-dose anti-murine CAR19 antibody. Of these 12 patients, 8 patients presented with a weak, and 4 patients with a strong presence of HAMA. The last 4 patients had lost the effectiveness of the CART- therapy at that time point, reflected by simultaneous B-cell recovery in the periphery. Moreover, three of them received a second dose of CART-19, which did not revert the relapse.

Conclusions To conclude, these data suggest the importance of the immunogenicity induced by CART-cell therapies. Immune monitoring should include the assessment of humoral response, especially before considering a second dose after relapse.

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P09.06 'AN ENHANCED CRISPR TOOL FOR TREATING CHRONIC MYELOGENOUS LEUKEMIA'

¹D Lainšček*, ¹V Forstnerič, ¹S Malenšek, ¹M Skrbinek, ²M Sever, ^{1,3}R Jerala. ¹National Institute of Chemistry, Ljubljana, Slovenia; ²University medical centre Ljubljana, Ljubljana, Slovenia; ³EN-FIST Centre of Excellence, Ljubljana, Slovenia

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Background Chronic myeloid leukemia (CML) is a myeloproliferative neoplastic disease, occurring in 1 to 2 cases per 100.000 adults, which accounts this type of cancer for approximately 15% of newly diagnosed leukemia in adult patients. The diagnosis is based upon the genetic translocation between the t(9;22)(q34;q11.2), resulting in formation