CATHESPIS S ALTERATIONS INDUCE A TUMOR-PROMOTING IMMUNE MICROENVIRONMENT IN FOLLICULAR LYMPHOMA

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. 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 Karpass422, 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 which we slightly modified to reflect FL-like conditions, and in primary patient samples.

Results Single-cell derived Y132D mutant Karpass422 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 Y132D 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.

REFERENCES

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 Tregs, 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.

Disclosure Information J. Kabiljo: None. I. Kuznetsova: None. J. Homola: None. S. Prodinger: None. J. Laengle: None. M. Sachet: None. A. Egorov: A. Employment (full or part-time); Modest; Vacthera Bio Tech GmbH M. Bergmann: A. Employment (full or part-time); Modest; Vacthera Bio Tech GmbH.

P09.05

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

1^1^ N. Klein-González*, 1^1^ EA. González-Navarro; 1^1^A. Bartoló-Ibars; 1^1^ Ortíz-Maldonado, 1^M^ Torrebadell, 5^M^ M. Castellá, 1^D^ Benítez, 1^1^M^ Caballero-Baños, 1^1^R^ R. Cabezón, 1^1^M^ Español, 1^IT^ Baumann, 1^E^ Giné, 1^7^P^ Castro, 1^3^J^ Esteve, 2^5^J^ Yagüe, 1^R^ Síves, 3^5^J^ Álvaro Urbano-Ispuiza, 3^5^J^ Delgado, 1^1^D^G^ J. de Juan; 1^1^Immunotherapy Unit, HSD-Hospital Clínico Barcelona, Barcelona, Spain; 2^Immunology, CDB, Hospital Clinic Barcelona, Barcelona, Spain; 3^Hematology, ICMHO, Hospital Clinic Barcelona, Barcelona, Spain; 4^IMHO, Hospital Clinic Barcelona, Barcelona, Spain; 4^Hospital Sant Joan de Déu (HSJD), Barcelona, Spain; 5^Institut d’Investigacions Biomièdiques August Pi i Sunyer – IDIBAPS, Barcelona, Spain; 5^Banc de Sang i Teixits (BST), Barcelona, Spain; 6^Medical Intensive Care Unit, Hospital Clinic Barcelona, Barcelona, Spain; 7^University of Barcelona, Barcelona, Spain

**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 x10^6 ARI-0001 cells/kg was infused. Autologous T-cells from peripheral blood were expanded and transduced with a lentivirus to express a CART 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 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.

**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.


P09.06

**AN ENHANCED CRISPR TOOL FOR TREATING CHRONIC MYELOGENOUS LEUKEMIA**

1^D^ Lainšček*, 1^V^ Forstneri, 1^S^ Mälenšček, 1^M^ Stršbek, 1^M^ Sever, 1^R^ Jerina. 1^National Institute of Chemistry, Ljubljana, Slovenia; 1^University medical centre Ljubljana, Ljubljana, Slovenia; 1^EN-FIST Centre of Excellence, Ljubljana, Slovenia

**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 of BCR-ABL1 protein, which activates the downstream signaling pathways, leading to cell proliferation and survival. The current standard of care includes tyrosine kinase inhibitors (TKIs) such as imatinib, nilotinib, and dasatinib, which target the BCR-ABL1 kinase domain. However, resistance to TKIs can develop over time, and the emergence of BCR-ABL1 mutations can lead to treatment failure. Therefore, there is a need for novel therapeutic approaches that can effectively target the BCR-ABL1 kinase and overcome resistance mechanisms.

**Methods** In this work, we developed an enhanced CRISPR tool for treating chronic myelogenous leukemia (CML) that targets the BCR-ABL1 kinase domain. We used a Cas9 guided RNAi approach to knock down the expression of the BCR-ABL1 gene and thereby inhibit its activity. We identified a set of small interfering RNAs (siRNAs) that effectively silenced the BCR-ABL1 expression in CML cell lines. The siRNAs were designed to target conserved regions within the BCR-ABL1 gene to ensure high specificity and efficacy. We then tested the siRNAs in CML cell lines to evaluate their ability to inhibit the BCR-ABL1 expression and downstream signaling pathways.

**Results** We found that the siRNAs significantly reduced the BCR-ABL1 expression in CML cell lines compared to the control cells. The knockdown of BCR-ABL1 expression was accompanied by a decrease in the downstream signaling pathways, such as the MAPK and PI3K-Akt pathways. Importantly, the siRNAs also demonstrated their ability to reverse the resistance to TKIs in CML cells. We observed a significant increase in the sensitivity of CML cells to imatinib, nilotinib, and dasatinib after the siRNA-mediated knockdown of BCR-ABL1 expression.

**Conclusions** Our results indicate that the enhanced CRISPR tool we developed can effectively target the BCR-ABL1 kinase domain and inhibit its activity in CML cells. This approach can be a promising therapeutic strategy for the treatment of CML, especially in patients with resistance to TKIs. Further studies are needed to optimize the siRNA design and delivery methods to achieve maximal efficacy and minimal toxicity.