Background Cytokine-Induced Killer (CIK) cells are a population of effector cells that represents a promising tool for adoptive cell therapy. They are easily expandable *ex-vivo*, safe, and exert cytotoxicity against a broad range of tumor histotypes.¹ We recently reported that they have a relevant expression of FcγRIIIa (CD16a), which can be exploited in combination with clinical-grade monoclonal antibodies (mAbs) to redirect their cytotoxicity in an antigen-specific manner, to improve their antitumor activity.² Indeed, the engagement of CD16a on CIK cells leads to a potent antibody-dependent cell-mediated cytotoxicity (ADCC) against ovarian cancer both *in vitro* and *in vivo*. Based on this observation, we investigated whether CIK cells can be specifically retargeted against B-cell malignancies by combination with anti-CD20 mAbs, namely Rituximab® (RTX) and Obinutuzumab® (OBI).

Materials and Methods CIK cells were obtained from peripheral blood mononuclear cells of healthy donors, and stimulated *in vitro* with IFN-γ, CD3 mAb and IL-2 for 14 days; fresh IL-2 was provided every 3–4 days. CIK cell phenotype was analyzed by multicolor flow cytometry; cytotoxic activity was assessed by calcein AM-release assay against B-cell lines, primary samples and patient-derived xenografts (PDX) obtained from B-cell lymphoma patients after written informed consent.

Results The combination with both RTX and OBI significantly increased specific CIK cells lysis against several CD20-expressing lymphoma B cell lines, primary tumors from B-cell lymphoma patients and an established PDX, compared to the combination with a control mAb (cetuximab, CTX). NK-depletion demonstrated that the mAb-mediated cytotoxicity is accountable to the CIK cells fraction within the bulk population since no difference in the lytic activity was detected in the absence of NK cells. In addition, these results are further supported by *in vivo* preliminary experiments where the treatment with CIK cells in combination with OBI extensively reduced the growth of PDX and increased mice survival, compared to CIK cells or OBI administered alone.

Conclusions Here we proved that CIK cells can be retargeted with clinical-grade mAbs against CD20-expressing lymphomas. These data indicate that the combination of CIK cells with mAbs can represent a novel approach for the treatment of haematological malignancies.

REFERENCES

1. Franceschetti M, Pievani A, Borleri G, Vago L, Fleischhauer K, Golay J, *et al.* Cytokine-induced killer cells are terminally differentiated activated CD8 cytotoxic T-EMRA lymphocytes. *Exp Hematol* 2009;**37**:616–28.
2. Cappuzzello E, Tosi A, Zanovello P, Sommaggio R, Rosato A. Retargeting cytokine-induced killer cell activity by CD16 engagement with clinical-grade antibodies. *Oncoimmunology* 2016 Aug;**5**(8):e1199311.

The research leading to these results has received funding from Fondazione AIRC under IG 2018 - ID. 21354 project - P.I. Rosato Antonio

Disclosure Information A. Dalla Pietà: None. E. Cappuzzello: None. P. Palmerini: None. R. Sommaggio: None. G. Astori: None. K. Chiericato: None. O. Perbellini: None. M. Tisi: None. C. Visco: None. M. Ruggeri: None. A. Rosato: None.

P09.02 MAPPING AND TACKLING TUMOR AND CHEMOTHERAPY-INDUCED IMMUNE SUPPRESSION IN BREAST CANCER SENTINEL LYMPH NODES

¹N Prokopi*, ¹M Heeren, ¹I Milenova, ¹K van Pul, ¹T Muijlwijk, ¹M Arends, ¹S van der Velde, ²K Vrijland, ²A van Weverwijk, ²K de Visser, ¹R van de Ven, ¹T de Gruij. ¹Amsterdam UMC, VUMC, Amsterdam, Netherlands; ²Dutch cancer institute, Amsterdam, Netherlands

10.1136/jitc-2020-ITOC7.102

Background Breast cancer (BrC) is the most prevalent cancer in women worldwide. Unfortunately, still limited treatment options are available for the most aggressive subtypes (i.e. hormone receptor [HR] negative). The response to neoadjuvant chemotherapy (NACT) in patients with HR-negative BrC can in part be influenced by an effective anti-tumor immune response. The sentinel lymph node (SLN) is the first site where BrC-specific T cell priming will occur but unfortunately it is also a major target of BrC-induced immune suppression. Lymph-node resident dendritic cells (LNR-DC) were found to be suppressed in metastatic SLN.¹ In addition, this tumor-mediated immune suppression of LNR-DC is related to high-risk triple-negative BrC and may be a negative predictor for prognosis¹. Preliminary data showed that NACT further reduced the activation status of LNR-DC. The goal of this study is to identify immune-enhancing agents that can counteract the tumor-mediated immune suppression of LNR-DC and promote tumor-specific T cell responses in order to improve therapy outcome in BrC patients upon NACT.

Materials and Methods Phenotypic analyses were performed on immune-cell subsets in human BrC SLN using multi-color flow cytometry. In addition, *ex-vivo* cultures with human BrC SLN-derived cells and *in vivo* mouse experiments were performed to study the therapeutic efficacy of Toll-like receptor (TLR)-ligands (R848 and CpG) and a STING-ligand (STING-L; 2'3'-c-di-AM(PS)₂(Rp,Rp)).

Results Higher rates of LNR-DCs, but with an apparently reduced activation state, were found in SLN of NACT-treated patients compared to patients treated with surgery only. A comparative *ex-vivo* study with SLN cultures on the effects of R848, CpG-B and STING-L showed R848 to be superior in terms of LNR-DC activation. In a *Krt14* (*K14*)-*cre*; *Cdh11F/F*; *Trp53F/F* (KEP) BrC mouse model, the effects of intratumoral administration of TLR- and STING-L were determined in combination with doxorubicin. STING-L outperformed R848 and CpG-B in terms of controlling primary tumor growth. Of note, in human *ex-vivo* cultures CpG-B proved effective in LNR-DC activation when combined with a STAT3 inhibitor, leading to the boosting of mammary-specific T cell responses, Th1 skewing, and a drop in CpG-induced Treg levels.

Conclusions In summary, intratumoral delivery of TLR- and STING-ligands in combination with NACT might be an interesting therapeutic approach in patients with high-risk HR-negative BrC, leading to SLN potentiation and enhanced antitumor T cell immunity. Future clinical studies should demonstrate the therapeutic benefit of this approach.

REFERENCE

1. van Pul, *et al.* 2019, *Journal for Immunotherapy of Cancer*.

Disclosure Information N. Prokopi: None. M. Heeren: None. I. Milenova: None. K. van Pul: None. T. Muijlwijk: None. M. Arends: None. S. van der Velde: None. K. Vrijland: None. A. van Weverwijk: None. K. de Visser: None. R. van de Ven: None. T. de Gruijl: None.

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, ⁵S Eustermann, ⁶K Hopfner, ⁷W Hiddemann, ⁷M Bergwelt, ⁸M Schmidt-Suppran, ⁹MB Sárosi, ¹⁰M Rudelius, ¹V 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; ⁴Immunoanalytics: 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

10.1136/jitc-2020-ITOC7.103

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.

REFERENCES

1. Riese, R.J., et al., Essential role for cathepsin S in MHC class II-associated invariant chain processing and peptide loading. *Immunity* 1996; **4**(4): p. 357–66.
2. Kim, K.J., et al., Establishment and characterization of BALB/c lymphoma lines with B cell properties. *J Immunol* 1979; **122**(2): p. 549–54.

Disclosure Information J.A. Hildebrand: None. D. Bararia: None. S. Stolz: None. S. Häbe: None. F. Osorio-Barrios: None. M.D. Bartoschek: None. E. Gaitzsch: None. V. Jurinovic: None. K. Rautter: None. C. Ludwig: None. S. Bultmann: None. H. Leonhardt: None. S. Eustermann: None. K. Hopfner: None. W. Hiddemann: None. M. Bergwelt: None. M. Schmidt-Suppran: None. M.B. Sárosi: None. M. Rudelius: None. V. Passerini: None. J. Mautner: None. O. Weigert: None.

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

10.1136/jitc-2020-ITOC7.104

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