were analysed using the CEL-seq2 platform. Interactions between tumour and immune cells were predicted using an unbiased ligand-receptor interaction analysis. Proteins secreted by tumour cells were analysed by performing mass spectrometry on conditioned medium from patient derived organoid cultures. The conditioned medium was concentrated using a 3kDa Millipore filter and prepared for LC-MS. Mass spectrometry data were acquired in data-dependent acquisition mode. For determining suppression, healthy donor PBMCs were stimulated with anti-CD28/anti-CD3 Dynabeads in the presence of recombinant proteins or concentrated conditioned medium from neuroblastoma organoids. In vitro killing assays were performed with GFP and luciferase transduced organoids and healthy donor PBMCs.

**Results** Analysis of scRNA-seq of 25 neuroblastoma tumours showed a negative correlation between MIF and MDK expression and the cytotoxicity of NK cells, CD8 and γδ-T cells within the tumour. These findings could be confirmed with a previously published SEQC bulk-RNAseq dataset containing 498 patients. Next to that, a higher expression of MIF and MDK correlated with poor survival in the same dataset. In the secretome from cultured neuroblastoma organoids, we have used mass spectrometry to identify MIF and MDK amongst the top 100 most abundant proteins from a total of ~1200 proteins. In vitro, we showed that rMIF and rMDK have a suppressive effect on the activation of T cells and the amount of cytotoxic factors such as granzyme B and Perforin are produced by the T cells. This confirms our hypothesis that MIF and MDK negatively influence the cytotoxicity of T cells.

**Conclusions** Using two different unbiased analyses - scRNA-seq analysis of tumours and mass spectrometry of neuroblastoma organoid secretome-, we have identified MIF and MDK as immunosuppressive factor in neuroblastoma. Currently, we are testing several MIF and MDK inhibitors to test if T cell mediated killing of neuroblastoma can be increased if the immunosuppressive MIF and MDK are blocked.


**P07 Cell therapy in haematologic diseases**

**P07.01** THE POTENTIAL ROLE OF EXTRACELLULAR VESICLE-DERIVED SMALL RNAS IN AML RESEARCH AS NON-INVASIVE BIOMARKER

*1 Li*, 2 Mussack, 3 Görgens, 4 Pepeljuska, 5 AS Hartz, 6 H Aslan, 7 E Radki, 8 A Rank, 9 Schmohl, 10 MW Pfaff, 11 Schmetzer. 1Immune-Modulation, Medical Department III, University Hospital of Munich, München, Germany; 2Department of Animal Physiology and Immunology, TUM School of Life Sciences Weihenstephan, Technical University of Munich, München, Germany; 3Department of Laboratory Medicine, Division of Biomolecular and Cellular Medicine, Karolinska Institute, Stockholm, Sweden; 4Department of Hematology and Oncology, University Hospital of Augsburg, Augsburg, Germany; 5Department of Hematology and Oncology, Hospital of Stuttgart, Stuttgart, Germany

**Background** Antileukemic responses of immune reactive cells in AML-patients need to be improved. Combinations of blast-modulatory kitM (GM-CSF+PGE1) (vs control) convert myeloid blasts into dendritic cells of leukemic origin (DCleu), that effectively activate immune-cells against leukemic blasts. DC-derived EVs express molecules and carry nucleic acids (eg. miRNA) with immune regulatory functions.

**Materials and Methods** 1) DC/DCleu-generation of blast containing AML patients’ (n=5) and of healthy volunteers(n=5) relevant TAAs were determined by Fluorospot and protein-bound bead assays. Digital image analysis was used to evaluate the correlation of TAAs and T-cell abundance. T-cell receptor sequencing, in-vitro expansion with autologous CD40-activated B cells (CD40Bs) and in-vitro cytotoxicity assays were applied to determine specific expansion, clonality and cytotoxic activity of expanded T cells.

**Results** 68.3% of patients expressed ≥5 TAAs simultaneously with co-regulated clusters, which were similar to data from The Cancer Genome Atlas (n=505). Endogenous cellular or humoral responses against ≥1 TAA were detectable in 75.0% and 53.7% of patients, respectively. We found a correlation of T-cell abundance and the expression of TAAs and genes related to antigen-presentation. TAA-specific T-cell responses were polyclonal, could be induced or enhanced using autologous CD40Bs and were cytotoxic in-vitro. Despite the frequent expression of TAAs co-occurrence with immune responses was rare.

**Conclusions** We identified the most relevant TAAs in EGA for monitoring of clinical trials and as therapeutic targets. Antigen-escape rather than missing immune response should be considered as mechanism underlying immunotherapy resistance of EGA.

Disclosure Information M. Thelen: None. D. Keller: None. J. Lehmann: None. K. Wennhold: None. H. Weitz: None. E. Bauer: None. B. Gathof: None. M. Brüggemann: None. M. Kotrova: None. A. Quaas: None. C. Mallmann: None. S. Chon: None. A.M. Hillmer: None. C. Bruns: None. M. von Bergwelt-Baillon: B. Research Grant (principal investigator, collaborator or consultant and pending grants as well as grants already received); Modest; Astellas, Roche, SD. F. Consultant/Advisory Board; Modest; Bristol Myers Squibb. M.A. Garcia-Marquez: None. H.A. Schlößer: B. Research Grant (principal investigator, collaborator or consultant and pending grants as well as grants already received); Modest; Astra Zeneca.