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.


**P06.04 IMMUNE RESPONSES AGAINST SHARED ANTIGENS ARE COMMON IN ESOPHAGO-GASTRIC CANCER AND CAN BE ENHANCED USING CD40-ACTIVATED B CELLS**


Background Immune response is a hallmark of cancer immunotherapy and shared tumor-associated antigens (TAAs) are important targets. Recent advances using combined cellular therapy against multiple TAAs renewed the interest in this class of antigens. Our study aims to determine the role of TAAs in esophago-gastric adenocarcinoma (EGA).

**Materials and Methods** RNA expression was assessed by NanoString in tumor samples of 41 treatment-naïve EGA patients. Endogenous T cell and antibody responses against the 10 most 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. Anti-escape rather than missing immune response should be considered as mechanism underlying immunotherapy resistance of EGA.

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

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**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)
whole blood (WB) with kit M; 2) T-cell enriched mixed lymphocyte culture (MLC) with kit- vs un-treated WB; 3) anti-leukemic functional assays; 4) Sequencing of EV-derived miRNA from culture supernatants; 5) Qualitative/quantitative characterization of (leukemia/healthy) supernatant derived EVs and EV derived miRNAs as biomarkers.

**Results** 1) kitM increased significantly frequencies of (mature) DC/DCleu compared to untreated WB without induction of blasts’ proliferation. 2) kitM treated vs untreated WB increased significantly activated (leukemia-specific) cells of the adaptive and innate immune system after T cell-enriched MLC. 3a) EVs were qualitatively and quantitatively different in DC/MLC culture supernatants compared control as well as 3b) in kitM treated vs untreated healthy and AML samples. 3c) Different EV derived miRNAs were up- or downregulated in DC/MLC culture supernatants.

**Conclusions** These findings contribute to understand the unique/functional role of (leukemic derived) DCs to improve antileukemic immune responses, with respect to the role of EVs/EV derived miRNA and to potentially deduce new EV derived biomarkers.


**P07.02 CONTROL LEUKEMIA BY INDUCING ANTI-CANCER IMMUNE REACTIVITY IN VIVO? – POTENTIAL OF A DC-TRIGGERED MECHANISM**

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**Background** Kit-M, the combination of response-modifiers GM-CSF+ Prostaglandine (PGE)1, converts myeloid blasts into dendritic cells (DC) of leukemic origin (DCleu), that activate immune-cells against leukemia. Kit M treatment may be an attractive tool for immunotherapy in myeloid leukemia.

**Materials and methods EX-VIVO:** Culture of leukemic wholeblood (WB) with Kit-M, followed by ‘mixed lymphocyte culture’ (MLC) with patients’ T-cells and (functional) assays (flowcytometry, degranulation-, intracellular cytokine- and cytotoxicity assays. Correlation-analyses.

**IN VIVO:** treatment of leukeimically diseased rats or therapy-refractory patient with Kit M, followed by immune-, hematological and clinical monitoring

**Results 1 ex vivo:** Treatment of 65 leukemic WB-samples with KIT-M increases frequencies of mature DC/DCleu, reduces tolerogenic DC. Kit-M treated WB increases frequencies of (leukemia-specific) cells of the adaptive and innate immunosystem (incl. (TCRγδ,Tβ7), memory cells (Tcm,Tβγ7cm), NKβ7, CIKβ) cells, decreases immune-suppressive T-cells (Treg8/8) and improves blast lysis after MLC. Blast-lysis correlates with frequencies of DC and leukemia-specific cells, but not with patients’ age/sex/ELN-risk/response to chemotherapy

**2 In vivo - rats** Kit-M treatment of 3 leukeimically diseased (vs control) rats (followed by sacrifice after treatment) leads to reduced blasts and Tregs in blood and spleen and increased DCleu and memory-like T cells.

**3 In vivo - human** Kit-M salvage treatment of a 72 yo refractory AML-patient was well tolerated and the patient improved clinically (PB-blasts were below detection threshold, Neutrophils increased from 10% to 50%, thrombocytes normalized). Immune-monitoring showed a continuous activation of adaptive and innate (IFNγ producing) cells increase (incl. iNKT-, TH1/17-, Bmem- and DC in PB). The patient was discharged in good clinical after the 4-weeks-therapy, however relapsed 2 weeks later.

**Conclusions** Kit-M Treatment of leukemic WB ex vivo or of leukemically diseased rats or a patient in vivo leads to (leukemia specific) activation of the adaptive and innate immune reactive cells and downregulated suppressive mechanisms) via a DC/DCleu triggered mechanism - resulting in significantly improved blast-lysis compared to controls (independent of patients’ characteristics). In-vivo treatment was well tolerated, led to an increase of platelets and granulocytes and stable (low) blast counts in PB - probably mediated by a (leukemia specifically) DC/DCleu activated immune system. A dose defining clinical trial in carefully selected patients to confirm clinical safety and efficacy is being prepared.


**P08 Combination therapy**

**P08.01 COMBINED APPROACH OF ADOPTIVE CELL THERAPY WITH CYTOKINE-INDUCED KILLER CELLS RETARGETED WITH IMMUNOTOOLS AGAINST HER-2-EXPRESSING BREAST CANCER**

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**Background** Cytokine-Induced Killer (CIK) cells are heteroge- neous effector cells CD3+ CD56+ easily to expand from PBMC and in clinically relevant numbers with phenotypic and functional properties between T and NK cells. They show MHC-unrestricted cytotoxicity against tumors and exert Antitumor- dependent Cellular Cytotoxicity (ADCC) when combined with monoclonal antibodies (mAbs). In the present study, we evaluated the enhancement of CIK cell killing capacity due to the combination with either the clinical used mAb Trastuzumab (TR) and its optimized form TRS V90Lec13, or with the bispecific antibody (bsAb) HER2xCD3, against HER-2+ breast cancer cells.

**Materials and Methods** CIK cells were expanded from PBMCs of healthy donors by the addition of IFNγ, OKT-3 and IL-2. The immunotools binding proprieties on target and CIK cells were determined by flow cytometry. The CIK cell cytotoxicity and the dose-dependent activity of HER2xCD3 and TRS