be used as a monotherapy or in combination with tumor-targeting monoclonal antibodies to trigger induction of anti-cancer immunity. DSP107 is currently tested in IND-enabling studies and clinical development is planned to commence in 2020.

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P01.10 IFN γ SECRETION OF ADAPTIVE AND INNATE IMMUNE CELLS AS A PARAMETER TO DISPLAY LEUKAEMIA DERIVED DENDRITIC CELL (DC_{Leu}) MEDIATED IMMUNE RESPONSES IN AML

¹LK Klauer*, ¹O Schutti, ¹S Ugur, ¹F Dorane-Gard, ¹N Rogers, ¹M Weinmann, ²D Krämer, ³A Rank, ³C Schmid, ⁴B Eiz-Vesper, ¹HM Schmetzer. ¹University Hospital Munich, Munich, Germany; ²University Hospital Oldenburg, Oldenburg, Germany; ³University Hospital Augsburg, Augsburg, Germany; ⁴Hannover Medical School, Hannover, Germany

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Background Myeloid leukaemic blasts can be converted into leukaemia derived dendritic cells (DC_{Leu}) with blastmodulatory Kit-I and Kit-M, which have the competence to regularly activate T and immunoreactive cells to gain anti-leukaemic activity or rather cytotoxicity. As innate and adaptive immune responses are notably promoted by the cytokine interferon gamma (IFN γ), we hypothesised that the IFN γ secretion could be a suitable parameter to display DC/DC_{Leu} mediated immunologic activity and even anti-leukaemic cytotoxicity.

Materials and Methods DC/DC_{Leu} were generated from leukaemic WB with Kit-I (GM-CSF + OK-432) and Kit-M (GM-CSF + PGE₁) and used to stimulate T cell enriched immunoreactive cells. Initiated anti-leukaemic cytotoxicity was investigated with a cytotoxicity fluorolysis assay (CTX). Initiated IFN γ secretion of innate and adaptive immune cells (T cells, T^{CD4+} cells, T^{CD8+} cells, NK^{CD56+} cells, NK^{CD161+} cells, CIK^{CD56+} cells, CIK^{CD161+} cells and iNKT) was investigated with a cytokine secretion assay (CSA). In some cases IFN γ production was additionally evaluated with an intracellular cytokine assay (ICA). Conclusively, the IFN γ secretion of immunoreactive cells was correlated with the anti-leukaemic cytotoxicity.

Results Significant amounts of DC and DC_{Leu} as well as migratory DC and DC_{Leu} could be generated with Kit-I and Kit-M without induction of blast proliferation. T cell enriched immunoreactive cells stimulated with DC/DC_{Leu} showed an increased anti-leukaemic cytotoxicity and an increased IFN γ secretion of T, NK and CIK cells compared to control. Both the CSA and ICA yielded comparable amounts of IFN γ positive innate and adaptive immune cells. The correlation between the IFN γ secretion of immunoreactive cells and the anti-leukaemic cytotoxicity showed a positive relationship in T cells, T^{CD4+} cells, T^{CD8+} cells and NK^{CD56+} cells.

Conclusions We found blastmodulatory Kit-I and Kit-M competent to generate DC/DC_{Leu} from leukaemic WB. Stimulation of T cell enriched immunoreactive cells with DC/DC_{Leu} regularly resulted in an increased anti-leukaemic cytotoxicity and an increased IFN γ dependent immunological activity of T, NK and CIK cells compared to control. Moreover the anti-

leukaemic cytotoxicity positively correlated with the IFN γ secretion in T cells, T^{CD4+} cells, T^{CD8+} cells, NK^{CD56+} cells. We therefore consider the IFN γ secretion of innate and adaptive immune cells to be a suitable parameter to assess the efficacy of in vitro and potentially in vivo AML immunotherapy. The CSA in this regard proved to be a convenient and reproducible technique to detect and phenotypically characterise IFN γ secreting cells of the innate and adaptive immune system.

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P01.11 ROLE OF EXOSOMES AS PROMOTORS OR BIOMARKERS TO STUDY ACTIVATION OF LEUKEMIA-DERIVED DENDRITIC CELLS (DC_{Leu})-MEDIATED ANTILEUKEMIC ACTIVATION OF ADAPTIVE AND INNATE IMMUNE-REACTIVE CELLS AGAINST AML-BLASTS

¹L Li*, ²V Mussack, ¹E Pepeldjyska, ¹A Hartz, ³A Rank, ³C Schmid, ¹E Özkeya, ¹S Ugur, ²M Pfaffl, ¹H Schmetzer. ¹Department for Hematopoietic transplantations, Med III, University Hospital of Munich, Munich, Germany; ²Animal Physiology and Immunology, School of Life Sciences Weihenstephan, Technical University of Munich, Munich, Germany; ³Department of Haematology and Oncology, University Hospital of Augsburg, Augsburg, Germany

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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 (DC_{Leu}), that effectively activate immune-cells against leukemic blasts. Exosomes are small (30–150 nm) membranous vesicles of endocytic origin produced by all cells under physiological and pathological conditions. Their involvement in nearly all aspects of malignant transformation has generated much interest in their biology, mechanisms responsible for information transfer and their role in immune-surveillance as well as -escape. Exosomes secreted by dendritic cells (DCs) have been shown to allow efficient activation of T lymphocytes, displaying potential as promoters of adaptive immune responses.

Materials and Methods 1) DC/DC_{Leu}-culture of blast containing AML patients' whole blood (WB) (n=10) and of healthy volunteers (n=8) with kits, T-cell enriched mixed lymphocyte culture (MLC) with kit- vs un-treated WB, functional blast-cytotoxicity and, leukemia-specificity assays (Degranulation/intracellular cytokine-assays), Flowcytometric evaluation of blast-, DC- and lymphocyte composition before or after cultures. 2) Exosomes were isolated by immunoaffinity from serum, DC- and MLC-culture supernatants of 3 AML patients and 3 healthy volunteers. Exosomes were negatively stained and characterized by transmission electron microscopy (TEM). Fluorescence nanoparticle tracking analysis (fNTA) was performed to determine exosomal size and -concentration. Obtained results were compared in AML and healthy volunteers.

Results Addition of kitM to blast-containing WB significantly increased frequencies of mature DC/DC_{Leu} and their subtypes compared to untreated WB without induction of blasts' proliferation. Immune monitoring showed a continuous increase of activated/proliferating cells of the adaptive and innate immune system after T-cell-enriched MLC using kitM

pretreated vs -untreated WB, suggesting a production/activation of (potentially leukemia-specific) cells after kit-stimulation. Moreover kit-pretreated WB regularly and significantly improved provision, activation as well as antileukemic and leukemia-specifically directed immune reactive cells after MLC. TEM showed exosome-like structures with a typically cup-shaped appearance without any differences between healthy and AML samples. fNTA revealed average vesicle sizes of 177 ± 23 nm (healthy) and 178 ± 17 nm (AML). Higher levels of EVs were detectable in AML samples compared to healthy controls in serum and after DC-culture, but lower levels after MLC independent of culture conditions. Interestingly, the number of EVs increased during cultivation of DC of AML and healthy samples, but not in AML-derived MLC samples.

Conclusions We will provide data in AML patients and healthy volunteers about a potential role of DCs- and MLC-derived exosomes as biomarkers in immune responses, malignant progression or as potential therapeutic targets for AML patients.

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P01.12 IMPACT OF COMPLEMENTARY SUBSTANCES ON IMMUNE CELL ACTIVITY

M Luzbetak*, N Süßenguth, E Kronemeyer, H Widera, J Werner, B Mayer. *Department of General, Visceral and Transplant Surgery, LMU, Munich, Germany*

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Background Natural substances and micronutrients are more and more included in anti-cancer therapy. However, benefit and harm both are reported from one and the same substance. This emphasizes the urgent need for the systematic analysis of a personalized approach which patient will profit from which substance.

Materials and Methods Risk analysis was performed using PBMCs isolated from patients diagnosed with advanced solid cancer. Benefit was analyzed using 3D-microtumors directly prepared from individual patient tumors. Blood cells and cancer cells were treated with different natural substances, namely curcumin, artesunate and vitamine C, as single agents and in combination therapy with guideline-directed drugs for 72h. Impact on cell metabolic activity was measured with the Cell-Titer Glo assay. The cell phenotype was described by FACS analysis.

Results In 80% of the patients natural substances induced a slight (mean: 10.7%, range: 2.3–17.7%) metabolic inhibition of the immune cells, which was minor in comparison to the strong immunotoxicity of chemotherapeutic drugs (e.g. 5-FU, mean: 33.5%; Gemcitabine: 67.2%). Contrary, 20% of the patients revealed a stimulatory effect on PBMC depending on the basic activity and the exhaustion of the immune cells. Combination therapy revealed that natural substances were able to reduce (mean: 16.4%, range: 5.2–42.8%) immunotoxicity mediated by chemotherapy. Analysis of the 3D-microtumors indicated that natural substances can mediate an anti-cancer effect, which was most obvious in relapsed tumors heavily pretreated with chemotherapeutic drugs. In addition, natural substances were identified as chemosensitizer. For example, curcumin was found to increase efficacy of

Mitomycin C in breast cancer, Bicalutamid in prostate cancer and 5-FU combined with Cisplatin in gastric cancer.

Conclusions Complementary substances have a different effect depending on dosing, timing, cell type and cell characteristics. Therefore preclinical testing is required to identify the most effective complementary substances for the individual cancer patient analyzing both immune cells and cancer cells.

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P01.13 MERTK SIGNALING IS CRITICAL FOR T CELL PROLIFERATION AND MEMORY

RM Powell*, MJW Peeters, A Rachbech, PT Straten. *National Center for Cancer Immune Therapy, Herlev, Denmark*

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Background Overexpression of TAM receptors, including MERTK, in some cancers are integral for chemoresistance, proliferation and metastasis.¹ Our group has previously demonstrated that T cells also express MERTK and engagement of MERTK signaling is responsible for increased proliferation, functional capacity and metabolic fitness.² It is therefore important to further study the effect of MERTK inhibition on T cell function in the context of cancer treatments where MERTK inhibitors may play a role. Here we provide evidence that MERTK inhibition impacts greatly on T cell proliferation, specifically reducing phosphorylated mTOR. We have also demonstrated that MERTK expression is increased on CD8 central memory subsets during longterm expansion providing evidence that this signaling pathway may be important for sustaining T memory responses.

Materials and Methods Flow cytometric analysis was used to investigate the effect of titration of MERTK small molecule inhibitor UNC2025 on healthy donor T cells activated with CD3/CD28 dynabeads. Cell trace dye was used to track proliferation of CD4 and CD8 T cells along with markers of memory differentiation (CCR7 and CD45RO), activation (CD137) and function (IFN γ , Tnf α and IL-2). MERTK signaling was assessed using phospho flow cytometric methodology of phosphorylated mTOR, AKT, ERK1/2, p38-MAPK and STAT5. Long term cultures of donor T cells of up to 28 days were investigated for MERTK expression alongside memory differentiation.

Results We demonstrated that moderate concentrations of MERTK inhibitor reduced proliferation of activated T cells. Despite inhibition of cell division, cell size still increased 2 fold compared to resting cells and cell viability remained unchanged. Additionally, the proportion of central memory to effector memory populations and intracellular cytokine production was not impacted. Analysis of molecules involved in MERTK signaling revealed that phosphorylated mTOR was significantly modulated following the addition of MERTK inhibitor. Long term culture of CD8 T cells demonstrated MERTK was significantly increased following early and late re-stimulation, and expression of MERTK was strongly associated with central memory subsets.

Conclusions Our results demonstrate that inhibition of MERTK signaling on T cells reduces cell division where mTOR is significantly impacted. Despite this, other functional aspects, such as intracellular cytokine production remain