lymphocytes was restored *ex vivo*. A correlation of high DGK- α and loss of function was also observed in an experimental mouse model of adoptive therapy where CAR T cells that had lost their activity after infiltrating into solid tumors were found to have increased DGK- α .¹ Blockade of the Programmed cell death protein 1 (PD-1) with monoclonal antibodies is used in the clinic enabling some patients to achieve tumor control. However, not all patients respond. DGK- α activity is positioned downstream of PD-1 and should, if overactive, curb T cell function even if PD-1 inhibition is released. Thus, we hypothesize that dual inhibition of PD-1 and DGK- α might be required to fully unleash the T cell's potential in the TME. Current DGK- α inhibitors are not suitable for clinical application. Therefore, we investigated alternative means using an RNA interference (RNAi) approach to target DGK- α alone as well as in combination with PD-1 in T and NK cells. **Material and Methods** Knockdown is performed by RNAi using INTASYLTM compounds developed by Phio Pharmaceuticals. INTASYLTM compounds incorporate drug-like properties into the siRNA, resulting in enhanced uptake in the presence of serum with no need for further transfection reagents. Knockdown is analyzed by RT-qPCR and flow cytometry. Functional assays include cytotoxicity, degranulation and cytokine production in tumor mimicking environments.

Results A tumor mimicking *in vitro* system was developed which allows for the demonstration of functional restoration or prevention of functional loss of cell activity. Using T cell/tumor cell co-cultures at high tumor cell density, functional suppression could be induced in T and NK cells comparable to those observed in the TME. Testing of DGK- α targeting INTASYLTM compounds, silencing of DGK- α was observed in human U2OS osteosarcoma cells. Using a fluorescently labeled compound, highly efficient transfection of human primary immune cells was seen. Combinations of PD-1 and DGK- α targeting compounds are being tested and evaluated for synergism in experimental models.

Conclusions Strong activity of specific T and NK cells is necessary for tumor control. Dual targeting of PD-1 and DGK- α may be required to fully enable T and NK cell reactivity in the TME. Current DGK- α inhibitors do not exhibit the desirable pharmacokinetic/pharmacodynamic (PK/PD) properties for clinical development. The tested self-delivering RNAi technology represents a promising approach to targeting intracellular immune checkpoints such as DGK- α .

REFERENCE

1. Moon EK, Wang L-C, Dolfi DV, Wilson CB, Ranganathan R, Sun J, *et al.* Multifactorial T-cell hypofunction that is reversible can limit the efficacy of chimeric antigen receptor-transduced human T cells in solid tumors. *Clin Cancer Res* 2014; **20** (16):4262–73

Disclosure Information A.S. Herbstritt: B. Research Grant (principal investigator, collaborator or consultant and pending grants as well as grants already received); Significant; Phio Pharmaceuticals. C. Other Research Support (supplies, equipment, receipt of drugs or other in-kind support); Significant; Phio Pharmaceuticals. P.U. Prinz: None. M. Maxwell: A. Employment (full or part-time); Significant; Phio Pharmaceuticals. M. Kadiyala: A. Employment (full or part-time); Significant; Phio Pharmaceuticals. D. Yan: A. Employment (full or part-time); Significant; Phio Pharmaceuticals. E. Noessner: B. Research Grant (principal investigator, collaborator or consultant and pending grants as well as grants already received); Significant; Phio Pharmaceuticals. C. Other Research Support

(supplies, equipment, receipt of drugs or other in-kind support); Significant; Phio Pharmaceuticals.

P01.09

DUAL SIGNALLING PROTEIN 107 TRIGGERS INNATE AND ADAPTIVE IMMUNE RESPONSE TOWARDS TUMOUR CELLS

¹E Cendrowicz, ¹U Jacob*, ²S Greenwald, ¹G Huls, ³M Dranitzki-Elhalel, ^{2,4}Y Pereg, ^{2,4}A Chajut, ^{1,4}E Bremer. ¹University of Groningen, University Medical Center Groningen, Department of Hematology, Groningen, Netherlands; ²KAHR Medical, biotechnology company, Jerusalem, Israel; ³Nephrology and Hypertension Department, Hadassah Medical Center, Jerusalem, Israel; ⁴collaborators of the I-DireCT Marie Curie Innovative Training Network (ITN), Groningen, Netherlands

10.1136/jitc-2020-ITOC7.22

Background Dual signalling protein 107 (DSP107) is a trimeric fusion protein consisting of the extracellular domains of human SIRP α and 4-1BBL. SIRP α binds to CD47, frequently overexpressed on cancer cells, and 4-1BBL binds to 4-1BB on activated T-cells. The SIRP α domain triggers the innate immune response by inhibiting the CD47/SIRP α 'don't eat me' signalling. It thus promotes phagocytosis of cancer cells by granulocytes, macrophages and dendritic cells. With its other side, 4-1BBL domain binds to pre-activated T cells and stimulates their expansion, cytokine production and cytolytic effector function. Our hypothesis is that augmented phagocytosis and improved co-localization of immune cells will lead to better antigen presentation towards activated T and B cells and the generation of memory T and B cells will be enforced. As result DSP107 might lead to immunity after rechallenge with the same tumour type.

Materials and Methods Primary phagocytes were incubated with stained tumour cells in presence or absence of DSP107 or/and therapeutic antibodies. Fluorescence microscopy measured uptake of tumour cells by macrophages. FACS identified primary granulocytes positive for CD11b staining and membrane dye. HT1080-41BB cells were mixed with HT1080-CD47 or HT1080-wt in presence of DSP107 and IL-8 release to supernatant was measured by ELISA. Further, primary T cells were co-cultured with α CD3Fc and fluorescent protein transduced carcinoma cells at different DSP107 concentrations.

Results The number of granulocytes that phagocyte tumour cells was increased in presence of DSP107. Further, DSP107 not only stimulated more macrophages to engulf tumour cells, but also the number of tumour cells that were taken up per phagocyte rose. Already enhanced phagocytosis of tumour cells by therapeutic antibodies (e.g. Cetuximab, Rituximab and Trastuzumab) was improved even further by DSP107. A model system showed that activation of the 4-1BB/4-1BBL axis by DSP107 was dependent on cross-linking via CD47 domain. This indicates low off-target T cell activation. Apart from the model system, DSP107 stimulated primary T cells in co-culture with carcinoma cells (transduced to express α CD3 and a fluorescent protein). Cytolytic activity against carcinoma cells was improved and outgrowth of tumour cells was reduced in a dose dependant manner.

Conclusions DSP107 blocks the CD47/SIRP α checkpoint resulting in enhanced tumour cell phagocytosis and stimulates the 4-1BB/4-1BBL axis leading to T cell mediated tumour cell killing. DSP107 is a novel bifunctional therapeutic that targets and activates both innate and adaptive anticancer immune responses. DSP107 is a first-in-class drug candidate that can

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.

Disclosure Information E. Cendrowicz: None. L.J. Jacob: A. Employment (full or part-time); Significant; KAHN Medical. S. Greenwald: A. Employment (full or part-time); Significant; KAHN Medical. G. Huls: None. M. Dranitzki-Elhalel: None. Y. Pereg: A. Employment (full or part-time); Significant; KAHN Medical. A. Chajut: A. Employment (full or part-time); Significant; KAHN Medical. E. Bremer: None.

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

10.1136/jitc-2020-ITOC7.23

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.

Disclosure Information L.K. Klauer: None. O. Schutti: None. S. Ugur: None. F. Dorane-Gard: None. N. Rogers: None. M. Weinmann: None. D. Krämer: None. A. Rank: None. C. Schmid: None. B. Eiz-Vesper: None. H.M. Schmetzer: None.

P01.11 ROLE OF EXOSOMES AS PROMOTORS OR BIOMARKERS TO STUDY ACTIVATION OF LEUKEMIA-DERIVED DENDRITIC CELLS (DCLEU)-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

10.1136/jitc-2020-ITOC7.24

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