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

E Cendrowicz, UJ Jacob*, SG Greenland, GIT Huls, MD Dranitzki-Elhalel, AY Pereg, A Chajut, E Bremer. University of Groningen, University Medical Center Groningen, Department of Hematology, Groningen, Netherlands; KAHR Medical, biotechnology company, Jerusalem, Israel; Nephrology and Hypertension Department, Hadassah Medical Center, Jerusalem, Israel; collaborators of the I-Direct Marie Curie Innovative Training Network (ITN), Groningen, Netherlands

Background Dual signalling protein 107 (DSP107) is a trimeric fusion protein consisting of the extracellular domains of human SIRPα and 4-1BBL. SIRPα binds to CD47, frequently overexpressed on cancer cells, and 41BBL binds to 41BB on activated T-cells. The SIRPα domain triggers the innate immune response by inhibiting the CD47/SIRPα ‘don’t eat me’ signalling. It thus promotes phagocytosis of cancer cells by granulocytes, macrophages and dendritic cells. With its other side, 41BBL 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 αCD3/α 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 41BB/41BBL 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 αCD3 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/SIRPα checkpoint resulting in enhanced tumour cell phagocytosis and stimulates the 41BBL/41BBL 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

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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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### IFNy Secretion of Adaptive and Innate Immune Cells as a Parameter to Display Leukemia Derived Dendritic Cell (DCleu) Mediated Immune Responses in AML

#### Materials and Methods

DC/DCleu were generated from leukemic 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 IFNy secretion of 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 (IFNy), we hypothesised that the IFNy secretion could be a suitable parameter to display T cell mediated immune activity and even anti-leukaemic cytotoxicity.

#### Results

Significant amounts of DC and DCleu as well as migratory DC and DCleu could be generated with Kit-I and Kit-M without induction of blast proliferation. T cell enriched immunoreactive cells stimulated with DC/DCleu showed an increased anti-leukaemic cytotoxicity and an increased IFNy secretion of T, NK and CIK cells compared to control. Both the CSA and ICA yielded comparable amounts of IFNy positive innate and adaptive immune cells. The correlation between the IFNy secretion of immunoreactive cells and the anti-leukaemic cytotoxicity showed a positive relationship in T cells, NK and CIK cells compared to control. Moreover the anti-leukaemic cytotoxicity positively correlated with the IFNy secretion in T cells, NK and CIK cells.

### Role of Exosomes as Promoters or Biomarkers to Study Activation of Leukemia-Derived Dendritic Cells (DCleu)-Mediated Antileukemic Activation of Adaptive and Innate Immune-Reactive Cells Against AML-Blasts

#### Materials and Methods

Exosomes were negatively stained using a uniform vesicle of dendritic 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.

#### Results

Addition of kitM to blast-containing WB significantly increased frequencies of mature DC/DCleu 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.