whole blood (WB) with kit M; 2) T-cell enriched mixed lymphocyte culture (MLC) with kit- vs un-treated WB; 3) antileukemic 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.

**Disclosure Information**


**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 whole-blood (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 leukemically 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,Tβ7cm)-, NKβ7, CIKβ7-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 leukemically 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.

**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. iNK- , 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 system (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.

**Disclosure Information**


**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 a heterogenous 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 Antibody-dependent Cellular Cytotoxicity (ADCC) when combined with monoclonal antibodies (mAbs). 1,2 In the present study, we evaluated the enhancement of CIK cell killing capacity due to the combination with either the clinical used mAb Trastuzumab (TRS) 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
Liposomal doxorubicin enhances the radiation-induced abscopal effect by promoting the release of mitochondrial DNA

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Background Localized radiotherapy (RT) can cause a T cell-mediated abscopal effect on non-irradiated tumor lesions, particularly in combination with immune checkpoint blockade (ICB). By using syngeneic tumor models, we studied whether adding low-dose doxorubicin to RT and αPD-1 can enhance the RT-induced abscopal effect.

Materials and Methods In mice bearing bilateral subcutaneous tumors, the primary tumor was irradiated with 2 × 12 Gy (B16-CD133 melanoma model) or 3 × 8 Gy (MC38 colon carcinoma model). Liposomal doxorubicin (4 mg/kg) was given i.v. once together with RT; αPD1 was given weekly. Tumor growth and survival of mice were determined (5–9 mice per group). Depleting antibodies were used to elucidate whether the abscopal effect depended on CD8\(^+\) T cells. Tumor-specific CD8\(^+\) T cells were determined flow cytometrically using MHC tetramers and various antibodies. Mitochondrial DNA (mtDNA) was depleted in tumor cells with Zalcitabine. In vitro, extracellular (e)ATP release by tumor cells was determined with CellTiter-Glo\textsuperscript{®} 2.0. Tumor cell production of type I Interferon (IFN\(\beta\)) was measured by ELISA with/without incubation with cGAS-STING pathway inhibitors. CXCL10, cytosolic genomic DNA (gDNA), and cytosolic mtDNA were measured by qPCR.

Results Abscopal tumor control was as follows: RT/αPD-1/doxorubicin > doxorubicin/αPD-1 (p < 0.01) > RT/doxorubicin (p < 0.01) > RT/αPD-1 (p < 0.05) (B16 melanoma model); RT/αPD-1/doxorubicin > RT/αPD-1 (p < 0.01) > RT/doxorubicin (p < 0.001) > doxorubicin/αPD-1 (p < 0.01) (MC38 colon carcinoma model). Experiments with various inhibitors of the cGAS/STING pathway showed that liposomal doxorubicin induced type I IFN through the cGAS/STING pathway (p < 0.05 with vs. without inhibitors). In mtDNA-depleted tumor cells, doxorubicin induced less cytosolic mtDNA (p < 0.001) (but not less cytosolic genomic DNA), less IFN\(\beta\) secretion (p < 0.05), less eATP release (p < 0.0001), and less CXCL10 (p < 0.0001) than in non-mtDNA-depleted tumor cells. Triple therapy with RT, αPD-1, and liposomal doxorubicin induced more mature dendritic cells (p < 0.05) and more tumor-specific CD8\(^+\) T cells (p < 0.01) compared to RT/αPD-1 and doxorubicin/αPD-1 therapy. When CD8\(^+\) T cells were depleted or mtDNA-depleted tumor cells were implanted, the doxorubicin-induced enhancement of the abscopal effect was abolished (p < 0.05).

Conclusions Single low-dose liposomal doxorubicin can substantially enhance the RT-induced abscopal effect in conjunction with αPD-1. mtDNA leakage induced by doxorubicin appears crucial for the doxorubicin-enhanced RT-induced abscopal effect. These findings may be helpful for the planning of clinical radiochemoinmunotherapy trials in (oligo) metastatic patients.

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**P08.03 INTERLEUKIN-12 GENE ELECTROTRANSFER AS AN ADJUVANT IMMUNOTHERAPY TO ELECTROCHEMOTHERAPY**

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