to target multiple antigens is required. We propose a controlled ACT approach, where T cells are armed with synthetic agonistic receptors (SARs) that are conditionally activated only in the presence of a target AML-associated antigen, and a cross-linking bispecific T cell engager (BiTE) specific for both (SAR) T cell and tumour cell.

Materials and Methods A SAR composed of an extracellular EGFRvIII, trans-membrane CD28, and intracellular CD28 and CD3z domains was fused via overlap-extension PCR cloning. T cells were retrovirally transduced to stably express our SAR construct. SAR-specific bispecific T cell engagers (BiTE) that target AML-associated antigens were designed and expressed in Expi293FTM cells and purified by nickel affinity and size exclusion chromatography (SEC). We validated our approach in three human cancer models and patient-derived AML blasts expressing our AML-associated target antigen CD33.

Results CD33-EGFRvIII BiTE, monovalently selective for our SAR, induced conditional antigen-dependent activation, proliferation and differentiation of SAR-T cells. Further, SAR T cells bridged to their target cells by BiTE could form functional immunological synapses, resulting in efficient tumor cell lysis with specificity towards CD33-expressing AML cells. SAR-BiTE combination could also mediate specific cytotoxicity against patient-derived AML blasts whilst driving SAR T cell activation. *In vivo*, treatment with SAR-BiTE combination could efficiently eradicate leukemia and enhance survival in an AML xenograft model. Furthermore, we could show selective activation of SAR T cells, as well as a controllable reversibility of said activation upon depletion of the T cell engaging molecule.

Conclusions Here we apply the SAR x BiAb approach in efforts to deliver specific and conditional activation of agonistic receptor-transduced T cells, and targeted tumour cell lysis. The modularity of our platform will allow for a multi-targeting ACT approach with the potential to translate the ACT successes of B cell malignancies to AML. With a lack of truly specific AML antigens, it is invaluable that this approach possesses an intrinsic safety switch via its BiTE facet. Moreover, we are able to circumvent pan-T cell activation due to the specific targeting and activation of SAR T cells.

Disclosure Information M. Benmebarek: None. B.L. Cadilha: None. M. Hermann: None. S. Lesch: None. C. Augsburg: None. B. Brauchle: None. S. Stoiber: None. A. Darwich: None. F. Rataj: None. C. Klein: A. Employment (full or part-time); Significant; Roche. K. Hopfner: None. M. Subklewe: None. S. Endres: None. S. Kobold: None.

L5

RIG-I ACTIVATION ENHANCES MELANOMA IMMUNOGENICITY AND IMPROVES ANTI-TUMOR T CELL RESPONSES IN COMBINATION WITH ANTI-PD-1 IMMUNE CHECKPOINT BLOCKING ANTIBODIES

^{1,2}B Thier*, ^{1,2}L Such, ^{1,2}M Schwamborn, ^{1,2}A Sucker, ³C Coch, ^{1,2}D Schadendorf, ^{1,2}K Griewank, ⁴M Trilling, ^{1,2}F Zhao, ^{1,2}A Paschen. ¹Dermatology, University Hospital Essen, Essen, Germany; ²German Cancer Consortium (DKTK), Partner Site Essen/Düsseldorf, Essen, Germany; ³Institute of Clinical Chemistry and Clinical Pharmacology, University Hospital Bonn, Bonn, Germany; ⁴Institute of Virology, University Hospital Essen, Essen, Germany

10.1136/jitc-2020-ITOC7.5

Background Clinical efficacy of immune checkpoint blocking (ICB) therapy critically relies on the killing of melanoma cells by CD8⁺ T cells, becoming activated upon recognition of tumor antigens presented by HLA class I (HLA-I) surface

molecules. Patient-derived melanoma cells can escape from cytotoxic T cell effector functions by loss of HLA-I surface expression due to the silencing of HLA-I antigen processing and presentation machinery (APM) genes.

Material and Methods Seeking for a strategy to restore HLA-I expression, we transfected melanoma cells obtained from distinct patient metastasis with synthetic short double stranded RNA (3pRNA), an activating ligand of the cytosolic innate pattern recognition receptor RIG-I. 3pRNA-transfected melanoma cells were analyzed for HLA-I surface expression by FACS analysis and gene expression of HLA-I APM components by qPCR. *In vivo* 3pRNA-transfected tumors were analyzed for HLA-I expression by immunohistochemistry staining. Furthermore, T cell activation after coinubation with 3pRNA-transfected melanoma cells was determined by IFN γ -ELISpot assay. The effect of combined 3pRNA and blocking anti-PD-1 antibody treatment on T cell activation was measured by intracellular cytokine staining and FACS analysis.

Results Activation of RIG-I by 3pRNA increased the expression of HLA-I APM components and strongly enhanced recognition of melanoma cells by autologous CD8⁺ T cells. Based on these findings, we asked whether the combination of 3pRNA and blocking anti-PD-1 antibodies could improve anti-melanoma T cell responses in an anti-PD-1 non-responder patient model. Indeed, T cell activation by 3pRNA-transfected melanoma cells was significantly increased in the presence of anti-PD-1 antibodies. In line with the enhancement of anti-tumor T cell responses, we found an association of elevated RIG-I mRNA levels with prolonged patient survival in TCGA melanoma samples.

Conclusions In summary, this study demonstrates a beneficial effect of RIG-I activation on antigen presentation and T cell recognition of melanoma cells. Improved T cell responses by combined 3pRNA and anti-PD-1 treatment suggests that combinational therapy could be a strategy to overcome T cell resistance in melanoma.

Disclosure Information B. Thier: None. L. Such: None. M. Schwamborn: None. A. Sucker: None. C. Coch: None. D. Schadendorf: None. K. Griewank: None. M. Trilling: None. F. Zhao: None. A. Paschen: None.

On Demand Talks

On Demand Talks: Tumor Microenvironment

01

TUMOR LACTIC ACIDOSIS ALTERS DECISIVE T CELL ACTIVITIES

¹AJ Fischbeck*, ¹AN Mendler, ²M Balles, ²J Schwarz, ²R Zantl, ¹E Noessner. ¹Helmholtz Zentrum München, Immunoanalytics, Munich, Germany; ²IBIDI GmbH, Gräfelfing, Germany

10.1136/jitc-2020-ITOC7.6

Background Adoptive T cell therapy is a promising treatment strategy for tumor patients. However, when entering the tumor microenvironment (TME), T cells lose their effector function showing reduced degranulation and cytokine secretion. Besides T cell inhibition through checkpoint pathways (i.e. PD-1/L1, CTLA-4), suppressor cells (i.e. TAM, T_{reg}) and cytokines (i.e. IL-10, TGF, VEGF), various metabolites of the TME also counteract antitumoral activities. Among the latter, lactate and extracellular acidosis are byproducts of the cancer metabolism and commonly observed in high concentrations in