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 CD3ζ 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 Exp293F™ 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.

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**TUMOR LACTIC ACIDOSIS ALTERS DECISIVE T CELL ACTIVITIES**

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**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, Treg) 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 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 coinoculation 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.


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