Poster #614

Co-stimulation via PD1-41BB chimeric switch receptor enhances function of TCR-T cells in an immune-suppressive milieu and under chronic antigen stimulation

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Abstract

The immunosuppressive tumor microenvironment (TME) of solid tumors negatively influences the efficacy and fitness of tumor-specific T cells and can render them non-functional. In this repressive tumor milieu, expression of inhibitory immune checkpoint molecules and cytokines as well as deprivation of essential metabolic factors contribute to T cell exhaustion and reduced T cell infiltration. Due to these harsh conditions found in the TME of solid tumors, successful treatment of non-hematological cancer indications with T cell-based immunotherapies remains challenging. New strategies are required to equip therapeutic tumor-specific T cells with the necessary traits to overcome inhibitory signals in the TME and increase T cell persistence in an environment lacking essential metabolic nutrients, like oxygen or glucose. To enhance the clinical efficacy of TCR-T cells in treatment of solid tumors, we generated a chimeric receptor that combines the co-stimulatory domain of 4-1BB with the extracellular domain of PD-1. Expression of this chimeric PD1-41BB switch receptor in TCR-T cells should reverse the inhibitory signal induced by the PD-1/PD-L1 interaction and provide additional co-stimulation to increase functionality and persistence.

Using 2D and 3D in vitro model systems we mimic immunosuppressive conditions in the TME of solid tumors, including low glucose and high TGFβ levels as well as repeated tumor cell challenge. We evaluate the ability of the chimeric PD1-41BB switch receptor to enhance TCR-T cell activity and functionality under these repressive conditions.

Our results demonstrate that TCR-T cells expressing the chimeric PD1-41BB switch receptor show an increased capacity to recognize and kill tumor cells during chronic stimulation with antigen. The enhanced functionality of PD1-41BB-TCR-T cells allows them to eradicate tumor cells even in the presence of additional immunosuppressive factors, including nutrient starvation and expression of inhibitory PD-L1 checkpoint molecules. Furthermore, PD1-41BB-expressing TCR-T cells show a higher persistency and proliferation rate in these challenging co-culture model systems.

Equipping therapeutic T cells with the chimeric PD1-41BB switch receptor enhances T cell functionality under immunosuppressive conditions and counteracts checkpoint-mediated dysfunction. For the treatment of PD-L1-poitive malignancies, expression of PD1-41BB by TCR-T cells has the potential to greatly improve the targeting of solid tumors using T cell-based immunotherapies. These preclinical studies support our approach to enhance the clinical efficacy of TCR-T therapies of solid tumors using the chimeric PD1-41BB switch receptor. Subsequent in vivo studies and safety evaluations will pave the way for clinical use of PD1-41BB in adoptive T cell therapy.

Efficacy: Expression of PD1-41BB increases killing capacity of TCR-transgenic effector T cells

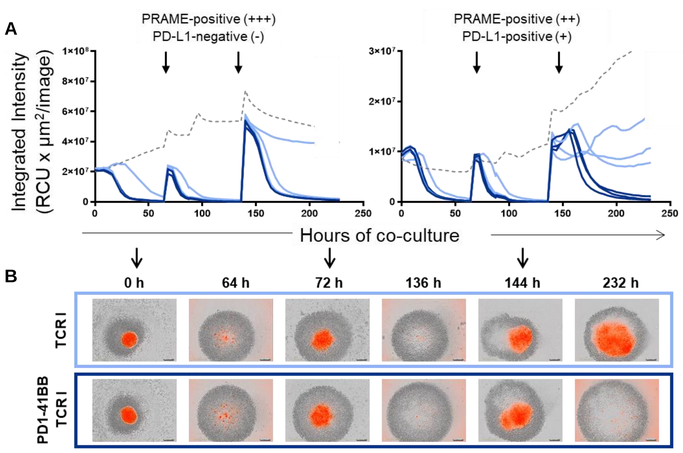


Figure 3: Effector T cells expressing PD1-41BB_TCR I or TCR I only were co-cultured with Mel624.38 tumor cells showing high PRAME (+++) expression and no PD-L1 (-) expression or SkMel23 tumor cells exhibiting medium PRAME (++) expression and inducible PD-L1 (+) expression upon IFNγ exposure. **(A)** NucLight-Red-labelled tumor cell lines were grown as spheroids and co-cultured with effector T cells. Tumor cells without addition of T cells (no T cells) were used as control. Tumor cell growth was monitored over 228 h using a live-cell imaging system (IncuCyte® ZOOM, Essen BioScience). The arrows indicate addition of a new tumor cell spheroid to achieve repetitive challenge of TCR-transgenic T cells in the co-culture. **(B)** Images of TCR-transgenic effector T cells (transmission) in co-culture with PRAME-positive (++) and PD-L1-positive (+) tumor cells (red) recorded at indicated time points. The arrows indicate the addition of a new tumor cell spheroid.

Safety: Effector functions of TCR-transgenic T cells remain strictly antigen-dependent with no off-target recognition mediated via PD-L1 interaction

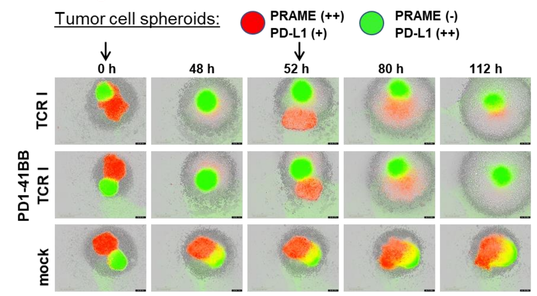


Figure 4: Effector T cells expressing PD1-41BB_TCR I or TCR I only were co-cultured with two PD-L1-positive tumor cell spheroids exhibiting either medium PRAME (++) expression levels (red) or no PRAME (-) expression (green), respectively. Tumor cell growth was monitored over 148 h using a live-cell imaging system. A decrease in red (PRAME-positive) or green (PRAME-negative) fluorescence intensity indicates killing of the respective tumor cell lines. The arrows indicate addition of a new red tumor cell spheroid with medium PRAME expression levels. No tumor cell spheroids were added after 52 h to untransduced T cells (mock) that were used as control.

Overcoming inhibitory signals in the TME with the help of the chimeric switch receptor PD1-41BB

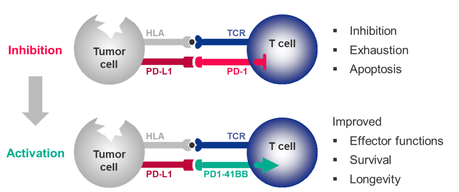


Figure 1: Interaction of wildtype PD-1 expressed by T cells with PD-L1 on tumor cells results in inhibition of T cell activity und can lead to exhaustion and apoptosis. In contrast, the presence of the chimeric switch receptor PD1-41BB on TCR-transgenic effector cells, can turn this inhibition into activation leading to improved effector functions, survival and longevity.

Fitness: PD1-41BB expression enhances cytotoxicity and proliferative capacity of TCR-transgenic T cells under glucose deprivation

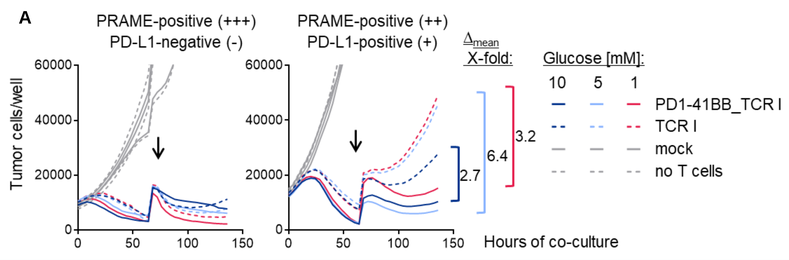
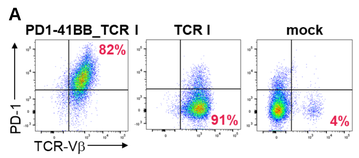


Figure 5: Effector T cells expressing PD1-41BB_TCR I or TCR I only were co-cultured with tumor cell lines exhibiting high (+++) and medium (++) PRAME expression levels in the presence of decreasing glucose levels (10, 5 and 1 mM). PD-L1 expression levels of tumor cells were either low (+), high (+++) or not present (-). Tumor cells without addition of T cells (no T cells) and untransduced T cells (mock) were used as control. **(A)** Tumor cell growth was monitored over 136 h using a live-cell imaging system. Differences in tumor cell count after 136 h for the respective glucose concentrations are indicated as Δ_{mean} X-fold. **(B)** X-fold expansion of T cells was determined by cell count 7 days after co-culture.

T cells stably express the transgenic TCR and PD1-41BB switch receptor



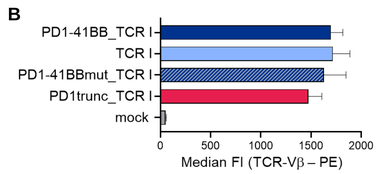
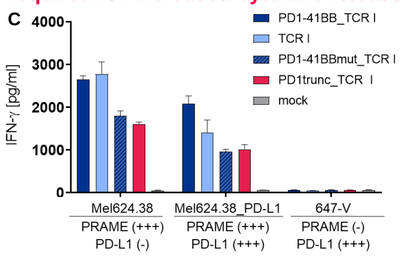


Figure 2: Effector T cells were generated by retroviral transduction of PBL from healthy donors with constructs coding for TCR I or PD1-41BB coupled to TCR I via P2A element. Untransduced cells were used as control (mock). **(A)** The transduced cells were enriched via FACS for expression of TCR I only or simultaneous expression of PD1-41BB and TCR I, expanded and analyzed by flow cytometry. **(B)** Enriched effector T cells expressed TCR I only or TCR I in combination with variants of PD1-41BB, including a version with mutations in critical signaling residues in the 4-1BB domain (PD1-41BBmut) or a version lacking the signaling domain of 4-1BB completely (PD1trunc). TCR expression levels of different effector T cell preparations were analyzed after staining with a TCR-Vb antibody. Median Fluorescence Intensity (FI) values are shown. **(C)** Effector cells from (B) were co-cultured with tumor cells lines (Mel624.38, Mel624.38_PD-L1, 647-V) with different PRAME and PD-L1 expression levels. IFNγ release of effector T cells was assessed by ELISA 24 h after co-culture.

4-1BB signaling via PD1-41BB is required for increased cytokine release



Fitness: Cytokine release and proliferation of PD1-41BB-expressing TCR-transgenic T cells is increased even in the presence of immune-suppressive TGFβ

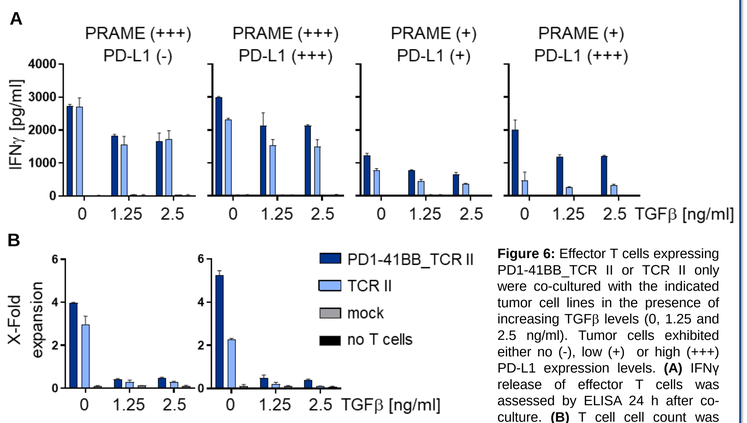


Figure 6: Effector T cells expressing PD1-41BB_TCR II or TCR II only were co-cultured with the indicated tumor cell lines in the presence of increasing TGFβ levels (0, 1.25 and 2.5 ng/ml). Tumor cells exhibited either no (-), low (+) or high (+++) PD-L1 expression levels. **(A)** IFNγ release of effector T cells was assessed by ELISA 24 h after co-culture. **(B)** T cell cell count was determined after 7 d of co-culture.

Fitness: PD1-41BB-expressing TCR-transgenic effector T cells show increased infiltration into tumor cell spheroids embedded in collagen matrix

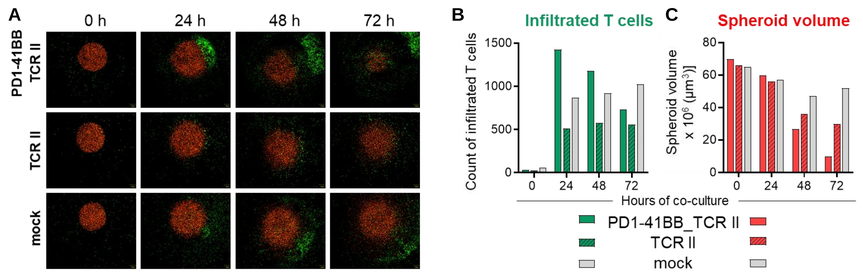


Figure 7: Tumor cell spheroids exhibiting low PRAME expression levels and inducible PD-L1 expression upon IFNγ exposure were embedded in collagen matrix and effector T cells expressing PD1-41BB_TCR II or TCR II only were added. Effector T cell infiltration into tumor cell spheroids was analyzed using confocal miscroscopy. **(A)** Images of TCR-transgenic effector T cells (green) in co-culture with tumor cell spheroids (red) embedded in collagen matrix recorded at indicated time points. **(B)** The number of infiltrated T cells into the tumor cell spheroid at indicated time points was quantified using the CQ1 analysis software (Yokogawa). **(C)** Analysis of the spheroid volume at indicated time points was performed using the same software.

Summary

For cellular immunotherapies, the PD1-41BB switch receptor represents a promising tool to prevent inhibitory signals in T cells through the PD1/PD-L1 axis. Reversing this inhibitory checkpoint while providing additional co-stimulation increases T cell effector functions under immunosuppressive conditions and chronic stimulation, characteristic for the tumor milieu of solid tumors. These preclinical studies support our approach to enhance the clinical efficacy of TCR T-cell therapies using the co-stimulatory PD1-41BB switch receptor for the treatment of PD-L1-positve solid tumors.