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 immune-suppressive 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 preclinical tumor model, 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 tools 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 the chimeric PD1-41BB switch receptor in TCR-T cells should reverse the inhibitory signal induced by the PD-1/ligand interaction and provide additional co-stimulation to improve function and persistence.

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

Safety: Tumor cell spheroids exhibit increased infiltration into tumor cell spheroids embedded in collagen matrix

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

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

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

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

Figure 2: Effect of T cells were generated by retroviral transduction of PD1 from healthy donors with constructs coding for TCR or PD1-41BB coupled to TCR via PD1 transduction (B) and 4-1BB signaling via PD1-41BB is required for increased cytokine release (A) The transduced cells were enriched via FACs to expression of PD1 only or simultaneously of PD1-41BB and PD1-41BB and TCR, expanded and analyzed by flow cytometry (B). (C) Expressed efficacy T cell responses in 4-1BB only or TCR only in combination with variations 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 (PD1mut), TCR expression levels of different T cell populations were analyzed after staining with TCR, PD1 and 41BB by flow cytometry. Median Fluorescence Intensity (MFI) values are shown. (D) Effect from effector T cells were cultured with PD1mut, PD1mut and different PRAME and PD-L1 expression levels. PRAME expression of effector T cells was determined by ELISA 24 h after co-culture.

Figure 3: Effect of T cells expressing PD1-41BB, TCR I or both only were co-cultured with Mel1234b tumor cells exhibiting high PRAME expression and PD-1 (++) expression upon IFNγ exposure. (A) NucLight® labelled tumor cells were grown as spheroids with or without PD1-41BB switch receptor expressing T cells. Tumor cell proliferation was measured as cell viability using NucLight®. (B) Real-time fluorescence imaging was performed to assess the addition of a new tumor cell spheroid. (C) Effector cells and T cells were counted at the indicated time points. The arrow indicates the addition of a new tumor cell spheroid. (D) Time course of tumor cell spheroids with or without PD1-41BB switch receptor expressing T cells (transmission) or co-culture with PRAME-positive (+) and PD1-41BB-positive (+) tumor cells (red) recorded at indicated time points. The arrow indicates the addition of a new tumor cell spheroid. Additional information indicates killing of the respective tumor cell lines. (E) Additional information indicates killing of the respective tumor cell lines. (F) Additional information indicates killing of the respective tumor cell lines.

Figure 4: T cells expressing PD1-41BB, TCR I or both only were co-cultured with PD-L1 positive tumor cell spheroids exhibiting either medium PRAME (+++) expression (red) or no PRAME (-) expression (green), respectively. Tumor cell growth was monitored over 148 hours and compared to control conditions. The Tcell killed tumor cell ratio was calculated as the ratio of tumor cell spheroids with and without effector cells. Relative tumor cell growth was assessed by ELISA 24 h after co-culture. (A) Time course of tumor cell spheroids with or without PD1-41BB switch receptor expressing T cells (transmission) or co-culture with PRAME-positive (+) and PD1-41BB-positive (+) tumor cells (red) recorded at indicated time points. The arrow indicates the addition of a new tumor cell spheroid. (B) Additional information indicates killing of the respective tumor cell lines. (C) Additional information indicates killing of the respective tumor cell lines. (D) Additional information indicates killing of the respective tumor cell lines. (E) Additional information indicates killing of the respective tumor cell lines. (F) Additional information indicates killing of the respective tumor cell lines. (G) Additional information indicates killing of the respective tumor cell lines. (H) Additional information indicates killing of the respective tumor cell lines. (I) Additional information indicates killing of the respective tumor cell lines. (J) Additional information indicates killing of the respective tumor cell lines. (K) Additional information indicates killing of the respective tumor cell lines. (L) Additional information indicates killing of the respective tumor cell lines. (M) Additional information indicates killing of the respective tumor cell lines. (N) Additional information indicates killing of the respective tumor cell lines. (O) Additional information indicates killing of the respective tumor cell lines. (P) Additional information indicates killing of the respective tumor cell lines. (Q) Additional information indicates killing of the respective tumor cell lines. (R) Additional information indicates killing of the respective tumor cell lines. (S) Additional information indicates killing of the respective tumor cell lines. (T) Additional information indicates killing of the respective tumor cell lines. (U) Additional information indicates killing of the respective tumor cell lines. (V) Additional information indicates killing of the respective tumor cell lines. (W) Additional information indicates killing of the respective tumor cell lines. (X) Additional information indicates killing of the respective tumor cell lines. (Y) Additional information indicates killing of the respective tumor cell lines. (Z) Additional information indicates killing of the respective tumor cell lines.

Figure 5: 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 I or both only were added. Effector T cells infiltration into tumor cell spheroids was assessed using confocal microscopy (A) Images of TCR-transgenic T cells (green) in co-culture with tumor cell spheroids (red) embedded in collagen matrix were recorded at indicated time points. The number of infiltrated T cells into the tumor cell spheroid at indicated time points was quantified using the CellSens image analysis software (Nikon). (B) Analysis of the spheroid volume at indicated time points was performed using the same software.

Summary
For cellular immunotherapy, the PD1-41BB switch receptor represents a promising tool to prevent inhibitory signals in T cells through the PD1-41BB switch receptor. Reversing this inhibitory checkpoint while providing additional co-stimulation increases T cell function 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 PD1-41BB-positive solid tumors.

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