Poster # 3231

The chimeric co-stimulatory receptor PD1-41BB enhances the function of T cell receptor (TCR)-modified T cells targeting solid tumors

Nadja Sailer[1], Melanie Salvermoser[1], Maria Gerget[1], Sarah Thome[1], Angelika J. Fischbeck[2], Svenja Ruehland[2], Luis F. Olguín-Contreras[1], Maja Buerdek[1], Christian Ellinger[1], Elfriede Noessner[2], Dolores J. Schendel[3], Patrik Kehler[1].

[1]Medigene Immunotherapies GmbH, Planegg/Martinsried, Germany; [2]Helmholtz Zentrum München, Munich, Germany; [3]Medigene AG, Planegg/Martinsried, Germany

For further information and questions please contact Nadja Sailer: n.sailer@medigene.com.

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Abstract

The use of cellular immunotherapies has led to impressive complete and durable clinical responses in patients with certain types of hematological cancers. However, positive clinical results in solid tumor indications are still rare and many patients are in urgent need of alternative treatment options for several different indications. It has become clear that expression of inhibitory immune checkpoint molecules as well as harsh metabolic conditions in the tumor microenvironment (TME) are responsible for lack of activity of T cell immunotherapies in several settings, especially solid tumors. Here additional strategies are necessary to efficiently employ cellular immunotherapies. With the aim to further enhance the clinical efficacy of TCR-based immunotherapies under immunosuppressive conditions found in tumors, we analyzed the ability of PD1-41BB, a chimeric co-stimulatory receptor, to reverse the natural inhibitory PD-1/PD-L1 interaction into a supporting co-stimulatory signal in TCR-modified T cells encountering tumor cells. We evaluated the ability of the chimeric co-stimulatory receptor PD1-41BB to improve activity of TCR-modified T cells using 2-dimensional or 3-dimensional *in vitro* assays that model different immuno-suppressive conditions found in tumors. We demonstrate that chronic stimulation as well as several immunosuppressive factors of the TME, such as tumor cell expression of inhibitory immune checkpoint molecules or glucose restriction, impede the ability of TCR-transduced T cells to produce inflammatory cytokines and to efficiently lyse tumor cells. By using a chimeric co-stimulatory receptor consisting of the extracellular part of PD-1 and the co-stimulatory domain of 4-1BB we reversed the naturally occurring inhibitory PD-1/PD-L1 interaction to provide a necessary co-stimulatory signal for improved T cell activity under immunosuppressive conditions or chronic stimulation. Addition of the chimeric co-stimulatory receptor PD1-41BB to TCR-modified T cells led to enhanced release of Interferon-γ, increased tumor cell killing, T cell proliferation and persistence in these T cell-tumor cell models. These preclinical studies support our approach to enhance the clinical efficacy of TCR-T therapies in PD-L1-positive malignancies by reversing naturally occurring inhibitory signals enabling counteraction of checkpoint-mediated dysfunction and metabolic insufficiency. The chimeric co-stimulatory PD1-41BB receptor has the potential to further enhance the clinical efficacy of TCR-modified T cells in patients with PD-L1-positive malignancies. Further preclinical *in vitro* and *in vivo* studies are ongoing to investigate the safety and efficacy of PD1-41BB in combination with multiple TCR candidates to explore its feasibility for the treatment of various cancers.

Principle of the chimeric switch receptor PD1-41BB: overcoming inhibitory signals in the TME

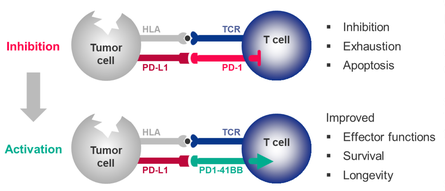

Figure 1: Interaction of wildtype PD-1 on T cells with PD-L1 expressed by tumor cells, results in inhibition of T cell activity und can lead to exhaustion and apoptosis. By incorporation of the chimeric switch receptor PD1-41BB into TCR-transgenic effector cells, this inhibition can be turned into activation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

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

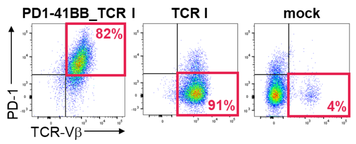

Figure 2: Effector T cells were generated by retroviral transduction of PBL from healthy donors with constructs coding for TCR I or PD1-41BB coupled to TCR I via P2A element. Untransduced cells were used as control (mock). The transduced cells were enriched via FACS for expression of TCR I only or simultaneous expression of PD1-41BB and TCR I, expanded and analyzed by flow cytometry.

PD-L1 expression on tumor cells is induced by IFNγ levels secreted by T cells upon tumor cell recognition

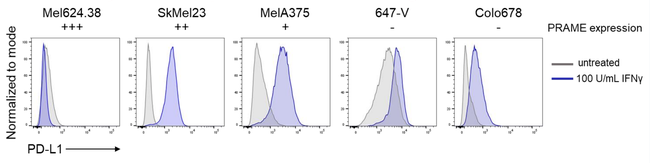

Figure 3: Tumor cell lines expressing high (+++), medium (++), low (+) and no (-) levels of the tumor antigen PRAME according to TRON database (Scholtalbers et al., 2015) and qPCR data (not shown). PD-L1 expression was induced by addition of 100 U/ml IFNγ, which corresponds to IFNγ levels observed in *in vitro* co-cultures of tumor cells with antigen-specific T cells. While 647-V continuously expressed PD-L1, Mel624.38 did not express PD-L1.

Efficacy: Expression of PD1-41BB increases tumor cell recognition, proliferation and tumor cell killing capacity of TCR-transgenic effector T cells

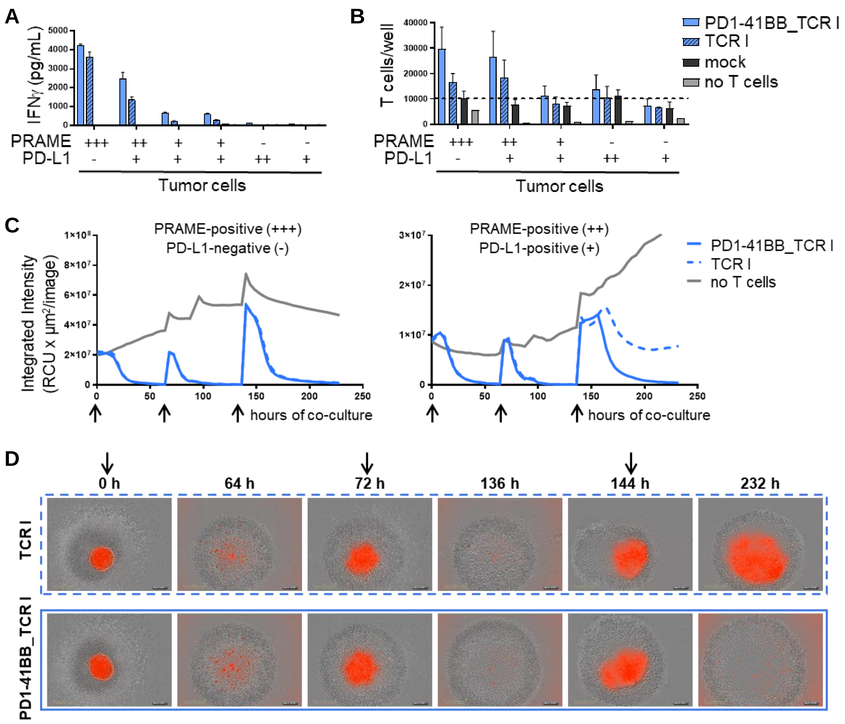

Figure 4: Effector T cells expressing PD1-41BB_TCR I or TCR I only were co-cultured with several tumor cell lines expressing different levels of PRAME and PD-L1. **(A)** IFNγ release of T cells was assessed by ELISA 24 h after co-culture. **(B)** T cell count was determined 7 d after co-culture setup. **(C)** NucLight-Red-labelled tumor cell lines were grown as spheroids and co-cultured with effector T cells expressing PD1-41BB_TCR I or TCR I only. 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 decrease of red fluorescence intensity indicates tumor cell killing mediated by TCR-transgenic T cells. The arrows indicate addition of a new tumor cell spheroid to achieve repetitive challenge of TCR-transgenic T cells in the co-culture. **(D)** 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: PD1-41BB-expressing effectors show antigen-dependent killing of tumor cells without unspecific killing mediated via PD-L1 interaction

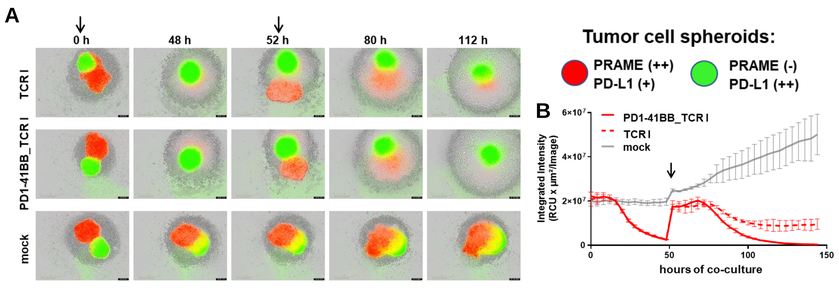

Figure 5: Effector T cells expressing PD1-41BB_TCR I or TCR I alone were co-cultured with two PD-L1-positive tumor cell spheroids exhibiting either medium PRAME expression levels (red) or no PRAME expression (green), respectively. **(A)** Tumor cell growth was monitored over 148 h using a live-cell imaging 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. **(B)** Integrated red fluorescence intensity in the co-culture over a period of 148 h. The arrow indicates addition of a new red tumor cell spheroid.

Control of tumor cell growth under repressive tumor milieu conditions is enhanced when TCR-transgenic effector cells co-express PD1-41BB

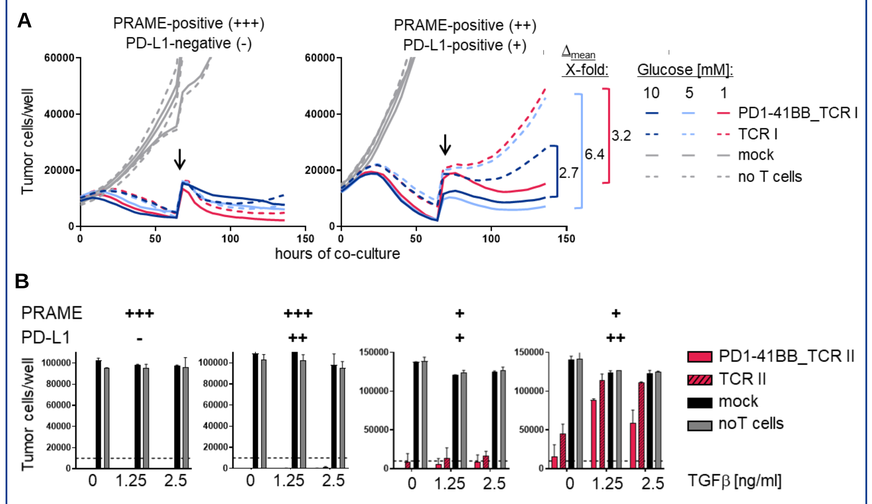

Figure 7: Tumor cell killing capacity of TCR-transgenic effector T cells under conditions mimicking the tumor milieu. **(A)** Effector T cells expressing PD1-41BB_TCR I or TCR I only were co-cultured with tumor cell lines exhibiting high (+++) and medium (++) PRAME expression levels in the presence of decreasing glucose levels (10, 5 and 1 mM). Tumor cells without addition of T cells (no T cells) and untransduced T cells (mock) were used as control. 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)** 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 nM). Tumor cells exhibited either no (-), low (+) or medium (++) PD-L1 expression levels. Tumor cell count was determined after 7 d using a live-cell imaging system.

Presence of PD1-41BB enhances cytokine release, killing capacity and proliferation in effector cells expressing different transgenic TCRs

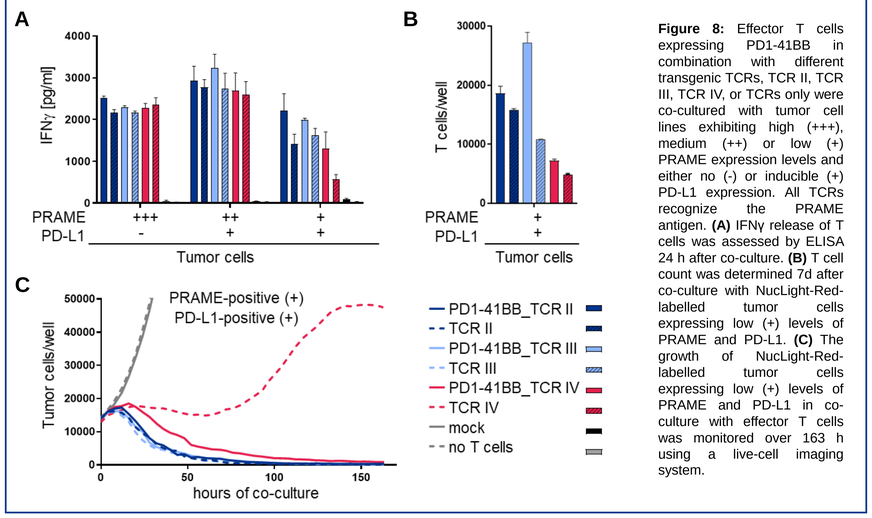

Figure 8: Effector T cells expressing PD1-41BB in combination with different transgenic TCRs, TCR II, TCR III, TCR IV, or TCRs only were co-cultured with tumor cell lines exhibiting high (+++), medium (++) or low (+) PRAME expression levels and either no (-) or inducible (+) PD-L1 expression. All TCRs recognize the PRAME antigen. **(A)** IFNγ release of T cells was assessed by ELISA 24 h after co-culture. **(B)** T cell count was determined 7d after co-culture with NucLight-Red-labelled tumor cells expressing low (+) levels of PRAME and PD-L1. **(C)** The growth of NucLight-Red-labelled tumor cells expressing low (+) levels of PRAME and PD-L1 in co-culture with effector T cells was monitored over 163 h using a live-cell imaging system.

Summary

The PD1-41BB switch receptor represents a promising tool for the use in cellular immunotherapies to prevent inhibitory signaling 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 in PD-L1-positive malignancies using the co-stimulatory PD1-41BB switch receptor.