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

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Abstract
The use of cellular immunotherapies has led to impressive and durable clinical responses in patients with certain types of hematological malignancies. 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, we evaluated strategies 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 enhance T cell-mediated immunity. Using 2-dimensional or 3-dimensional in vitro assays, we demonstrated that PD1-41BB enhances cell proliferation and the secretion of cytokines, as well as tumor cell killing capacity of TCR-transgenic effector T cells. We have used PD1-41BB in combination with multiple TCR candidates to explore its feasibility for the treatment of various cancers.

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

Figure 1: Interaction of various T cell lines cultured with PD1-41BB-expressing tumor cells, results in inhibition of T cell activity and can be abolished by blocking of the PD1-41BB interaction. By incorporation of the chimeric PD1-41BB receptor into TCR-transgenic T cells, we were able to employ this approach for enhanced effector functions, survival and longevity.

Figure 2: Effector T cells were generated by transduction of tumor-specific T cells with PD1-41BB via FACS (see Methods). The transduced T cells were analyzed for expression of PD1-41BB by flow cytometry.

Figure 3: Tumor cell lines expressing high (+++), medium (++), low (+) or no (-) levels of the tumor antigen PRAME, according to TCR database (Schwab et al., 2015) and PD1-41BB transduction (i.e., controlmock). T cell proliferation was determined after 10−4, 24 and 48 h by FACS and by IFN-γ ELISA (see Methods). T cell proliferation was only modestly reduced in the presence of PRAME-expressing tumor cells. The tumor cell lines were cultured with PD1-41BB-expressing effector T cells at a ratio of 3:1. T cell proliferation was significantly reduced compared to the controlmock.

Figure 4: Effector T cells expressing different transgenic TCRs and cultured with tumor cell lines expressing different levels of PRAME and PD1-41BB. (A) PHA release of T cells was assessed by ELISA 24 h after co-culture. (B) The cell count was determined by counting the cell suspension of tumor cell lines using a Nanopore DNA (Nanodrop) device. The tumor cell lines were grown and cultured co-cultured with effector T cells expressing different PD1-41BB TCRs. T cell proliferation was only modestly reduced in the presence of PD1-41BB-expressing T cells. The tumor cell lines were cultured with PD1-41BB-expressing effector T cells at a ratio of 3:1. T cell proliferation was significantly reduced compared to the controlmock.

Figure 5: Tumor cell lines expressing different levels of PRAME and PD1-41BB. The tumor cell lines were cultured with PD1-41BB-expressing effector T cells at a ratio of 3:1. T cell proliferation was only modestly reduced in the presence of PRAME-expressing tumor cells. The tumor cell lines were cultured with PD1-41BB-expressing effector T cells at a ratio of 3:1. T cell proliferation was significantly reduced compared to the controlmock.

Safety: PD1-41BB-expressing effectors show antigen-dependent killing of tumor cells without unspecific killing mediated via PD1-L1 interaction

Figure 6: Effector T cells expressing different transgenic TCRs and cultured with tumor cell lines expressing different levels of PRAME and PD1-41BB. The tumor cell lines were grown and cultured co-cultured with effector T cells expressing different PD1-41BB TCRs. T cell proliferation was only modestly reduced in the presence of PD1-41BB-expressing T cells. The tumor cell lines were cultured with PD1-41BB-expressing effector T cells at a ratio of 3:1. T cell proliferation was significantly reduced compared to the controlmock.

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 II only were co-cultured with tumor cell lines expressing high (+++), medium (++), low (+) or no (-) levels of tumor antigens PRAME and PD1-41BB. T cell proliferation was determined after 10−4, 24 and 48 h by FACS and by IFN-γ ELISA (see Methods). T cell proliferation was only modestly reduced in the presence of PRAME-expressing tumor cells. The tumor cell lines were cultured with PD1-41BB-expressing effector T cells at a ratio of 3:1. T cell proliferation was significantly reduced compared to the controlmock.

Figure 8: Effector T cells expressing different transgenic TCRs and cultured with tumor cell lines expressing different levels of PRAME and PD1-41BB. The tumor cell lines were grown and cultured co-cultured with effector T cells expressing different PD1-41BB TCRs. T cell proliferation was only modestly reduced in the presence of PD1-41BB-expressing T cells. The tumor cell lines were cultured with PD1-41BB-expressing effector T cells at a ratio of 3:1. T cell proliferation was significantly reduced compared to the controlmock.