T cells equipped with both a highly potent PRAME-specific T cell receptor and a chimeric PD-141BB co-stimulatory receptor show superior in vitro and in vivo tumor reactivity

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Abstract

To develop successful immunotherapies for cancer patients using T cell receptor (TCR)-modified T cells (TCR-T) several challenges must be addressed. The first is the identification of a target antigen that is highly expressed in tumors with limited expression in normal tissues. The second is the TCR that carries high antigen specificity and induces potent anti-tumor responses without cross-reactivity to healthy cells. A further particular challenge for the treatment of solid tumors is the hostile tumor microenvironment (TME) that negatively influences T cell activity. Besides cytokine dysregulation and immunosuppressive cells, the inhibitory checkpoint PD1-PD-L1 axis reduces T cell effector function and negatively influences efficacy, fitness and persistence of TCR-Ts. New strategies are needed to overcome the inhibitory TME to prevent exhaustion of transferred TCR-Ts.

The cancer-testis antigen PRAME is a relevant antigen for TCR-T immunotherapies since it is well expressed in several cancers while its expression in normal tissues is mainly restricted to the testes.

We screened non-tumor-specific T cell clones from healthy volunteers using high-throughput screening processes for T cell cloning, TCR isolation and characterization. TCRs with high specificity and sensitivity for HLA-A2-restricted PRAME-derived peptides were isolated and among 39 unique TCR sequences the most potent TCR was selected for use. To circumvent the T cell immunity against the CD28 costimulatory molecule in the TCR-T the leader envelope of a chimeric costimulatory domain of 4-1BB (41BB) was inserted into the TCR. The resulting PD-141BB co-stimulatory receptor on TCR-Ts did not change the favorable predictive safety profile but led to enhanced proliferation and increased cytokine release. Under conditions of chronic antigen stimulation via repeated exposure to HLA-A2-PRAME-PD1-41BB-positive tumor cells, TCR-Ts expressing a TCR with 4-1BB showed increased fitness compared to TCR-Ts with the TCR alone. Furthermore, this effect could be confirmed in vivo in a melanoma syngeneic mouse model with tumor cells expressing PRAME and high PD-1 levels, reconstituting the TME of local solid tumor. In this model, tumors were eradicated by a single administration of TCR-Ts expressing a TCR-PD-141BB co-stimulatory receptor further enhanced in vitro and in vivo function of TCR-Ts expressing a PRAME-specific TCR with high natural and tumor reactivity. This is a promising strategy to develop more effective TCRs as immunotherapies for the treatment of solid cancers.

Co-stimulation via PD-141BB enhances function of PRAME-TCR-T cells

PRAME as target antigen for adoptive T cell therapy and TCR isolation

Selection of a PRAME-specific lead T cell receptor

TCR-4 shows anti-tumor effects

Figure 1. Isolation of tumor associated antigen specific TCRs for adoptive T cell therapy. (A) PRAME mRNA expression levels for various cancer cell lines. PRAME mRNA expression most strongly was detected in the carcinoma lines K562, CMK, THP-1, A549+A2, and NCI-H1755. (B) Schematic outline of the protocol for adoptive transfer of PRAME-TCR-T modified T cells. Patient T cells are isolated by leukapheresis and activated (1). PRAME-specific TCR is introduced using a virus vector (2). Modified T cells are expanded in 9-11 days (3). TCR-modified T cells are re-injected into the patient (4). Modified T cells eliminate the tumor (5). (C) TCR candidates with high specificity for a PRAME-derived peptide presented on HLA-A2 were isolated from a non-stimulated T cell repertoire using our well-established high-throughput TCR generation process.

Figure 2. High throughput TCR generation process. (A) Promising TCRs were characterized and transferred into effectors (C) by transient transfection. Based on multiparameter screening (large process) a lead TCR candidate (TCR-4) was selected from 25 antigen specific TCRs. (B) Functional reactivity of TCR-4 was determined in an ELISA setting with graded concentrations of PRAME peptides (9.5-161.6 pg/ml). (C) To show the safety of a TCR T cells to inoculate PRAME-expressing cells on target cells. T cells were cocultured with tumor cell lines, that were either PRAME-PRAME-negative or PRAME-positive.

Figure 3. Anti-tumor efficacy of TCR-4 transgenic T cells. (A) Effectors cells transfected with lead TCRs showing the highest functional activity was tested in a broader tumor cell panel including both hematological malignancies and solid tumors. (B) PRAME-specific TCR-T lines were incubated with TCR-4 transgenic T cells or T cells from naive donors and tumor cells at a ratio of 5:1 (EMT6+/PD-141BB200 MPM/30% E2A). Green fluorescence intensity showing tumor cell killing mediated by TCR-4 transgenic T cells. Data for four exemplary tumor cell lines representing different tumor indications are shown.

Figure 4. Co-stimulation via PD-141BB enhances function of PRAME-TCR-T cells

Expression of PD1-41BB leads to tumor rejection in vivo

Summary

Medigene’s well-established high-throughput TCR generation process enabled the identification of a TCR lead candidate (TCR-4) specific for an HLA-A2-restricted PRAME epitope, demonstrating high natural affinity and potent anti-tumor efficacy.

Combining this potent TCR with our chimeric PD1-41BB co-stimulatory receptor results in a very promising T cell product with a favorable predictive in vitro safety profile and enhanced in vitro and in vivo anti-tumor efficacy.