Mitigation of Tumor Microenvironment-Mediated Immunosuppression Using a PD1-41BB Switch Protein with Optimal Affinity TCRs for First-In-Class, 3rd Generation TCR-T Therapies

Kirsty Cramer†, Giulia Longinotti†, Maria Catanevitola†, Petra U. Prinz†, Stefanie Tippner†, Kathrin Mutzel†, Andrea Coluccio†, Melanie Salvermoser‡, Julia Bittmann‡, Maja Buerdek·, Barbara Loeschin·, Christine Geiger†, Kathrin Davari†, and Dolores J Schendel†* Medigene Immunotherapies GmbH, a subsidiary of Medigene AG, Planegg, Germany

Background

- Despite substantially improved efficacy, TCR-T therapies face several challenges to optimize outcomes in the clinic, one of which is to sustain tumor-cell function in solid tumor immunotherapies (TCR-Ts) (Fig. 1).
- Tumor antigen loads and PD1 expression strongly influence T-cell function. PD1 engagement (i.e. TCR-T recognition of target cell) increases antigen load, thereby reducing the expression of PD1. T-cell exhaustion is induced by repetitive TCR signaling in the absence of T-cell stimulation.
- By improving TCRs on the basis of the patient’s tumor antigen profile, off-target TCR engagement with PD1 (TCR+B), can be reduced (black vs red, respectively) (Fig. 2).
- Here we present data from two in-vivo, 3rd generation TCR-T approaches, Medigene’s MDG015 and MDG017, which were developed to express optimal Affinity TCRs specific for a cancer target antigen (CTA) in NY-ESO-1/LAGE-1 and a non-relevant, specific-negative CTA (CSP) (UT), respectively, with MDG017 being the most advanced program.

Results

PD1-41BB CSP improves CD8+ TCR-T cell polyfunctionality by increasing production of effector, stimulatory and chemotactic cytokines

- Increased NY-ESO-1/LAGE-1- or mKRAS (G12V) specific toxins (rTCRs) were expressed in MDA501, and PD1 expression was confirmed (Fig. 3).
- T cells producing multiple cytokines, so-called “polyfunctional” T cells, are known to provide more effective immune responses compared to single cytokine producing T cells.
- CD8+ TCR-T cells were examined for cytokine polyfunctionality by flow cytometry (Fig. 3).
- Superior polyfunctionality in NY-ESO-1/LAGE-1-expressing T cells was observed by flow cytometry (Fig. 4).

PD1-41BB enhances activation and prolongs cytotoxic activity of CD8+ rTCR-T cells upon repeated stimulation

- Increased NY-ESO-1/LAGE-1- or mKRAS (G12V) specific toxins (rTCRs) were expressed in MDA501, and PD1 expression was confirmed.
- CD8+ T cells co-expressing PD1-41BB CSP display a 3 fold increase of IFNγ production upon culture with target-positive and PD1+ positive tumor cell lines (Fig. 5).
- CD8+ T cells co-expressing PD1-41BB CSP show prolonged and enhanced cytotoxic responses upon serial rechallenge with target and PD1+ positive tumor cell lines (Fig. 6).

Figure 3: Both NY-ESO-1/LAGE-1a and mKRAS G12V rTCR-T cells prominently co-express the TCR and PD1-41BB CSP at the cell surface

Figure 4: Enhanced polyfunctionality of both CD8+ rTCR-T cells specific for NY-ESO-1/LAGE-1a or mKRAS G12V through co-expression of TCR and PD1-41BB CSP co-expressed to CD8+ rTCR-T cells alone

Conclusions

- The PD1-41BB CSP significantly increases the activity and proliferation of tumor antigen specific TCR-T cells upon serial rechallenge with target and tumor cell lines (i.e. NY-ESO-1/LAGE-1a and mKRAS G12V).
- Superior polyfunctionality in NY-ESO-1/LAGE-1-expressing T cells was observed by flow cytometry (Fig. 4).
- Enhanced polyfunctionality of both CD8+ rTCR-T cells specific for NY-ESO-1/LAGE-1a or mKRAS G12V through co-expression of TCR and PD1-41BB CSP co-expressed to CD8+ rTCR-T cells alone

- Superior polyfunctionality is mainly related to proteins associated with effector, stimulatory and regulatory polyfunctionality (% of Sample).
- In addition, the mKRAS + UT, + 41BB, - 41BB CSP show prolonged and enhanced cytotoxic responses upon serial rechallenge with target and PD1+ positive tumor cell lines (Fig. 6).

Figure 5: CD8+ T cells co-expressing the TCR and PD1-41BB CSP showed enhanced IFNγ secretion compared to CD8+ T cells expressing only the TCR

Figure 6: Superior splenoid cytokicity of CD8+ T cells co-expressing the TCR and PD1-41BB CSP compared to CD8+ T cells expressing only the TCR observed upon serial tumor cell rechallenge

*Presenting author has no conflicts of interest to disclose.