Poster #3

Selection of superior KRAS G12V mutation-specific T cell receptors with unique characteristics for 3rd generation armored and enhanced T cell therapy

Julia Bittmann, Melanie Salvermoser, Dominik Alterauge, Anne-Wiebe Mohr, Doris Brechtefeld, Mario Catarinella, Petra U Prinz, Maja Buerdek, Kathrin Davari, Dolores J Schendel and Giulia Longinotti

Medigene Immunotherapies GmbH, a subsidiary of Medigene AG, Planegg, Germany

Background

- Activating mutations in the Kirsten rat sarcoma (KRAS) gene are highly prevalent oncogenic driver mutations in human cancers associated with tumorigenesis and aggressive tumor growth.
  - Mutant KRAS (mKRAS) is estimated to be present in over 300,000 patients, with high prevalence found in pancreatic (81.72%), colorectal (37.97%) and non-small cell lung cancer patients (21.20%).
  - mKRAS mainly comprises 21 missense mutations, with G12D (29.19%), G12V (22.97%), and G12C (13.43%) being the most common.
- Despite the recent approval for targeted therapies targeting G12C mutations the unmet need for further efficacy improvements remains.

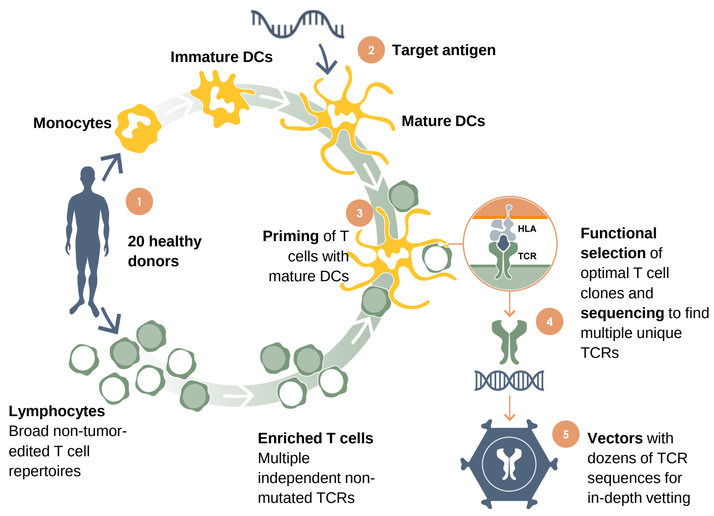
A. Using our T cell receptor (TCR) discovery process, we were able to identify multiple unique mKRAS G12V-specific TCRs. T cells of multiple healthy donors were primed for the mKRAS G12V antigen using dendritic cells (auto-HLA-A*11:01 priming), providing diverse TCR sequences for comparison. High-throughput functional screens rapidly delivered six unique G12V-specific candidate TCRs for in-depth characterization.

B. To extensively compare candidate TCRs and identify lead TCRs that fulfill defined characteristics, a robust and refined workflow was performed for in-depth vetting of specificity, sensitivity and safety (3S) as well as for investigation of additional unique TCR attributes such as CD8 co-receptor independency.

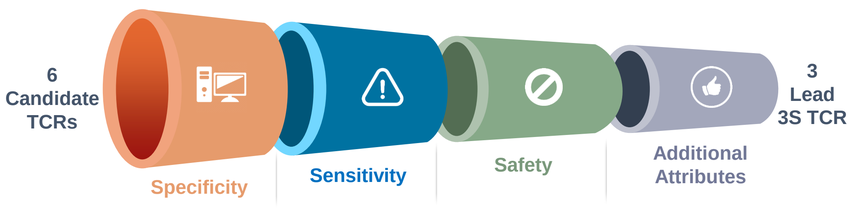
C. Candidate TCRs were combined with PD1-41BB armoring and enhancing co-stimulatory switch protein (CSP) to address the challenges in hostile tumor microenvironments (TMEs).

D. Here, we show preclinical data of the Top-3 candidate TCRs. TCRs combined with PD1-41BB CSP, were individually expressed in CD8+ T cells using our Jovi-Tag technology (WO2022/038115A1). TCR+PD1-41BB-expressing CD8⁺ T cells were enriched by fluorescence-activated cell sorting (FACS) and analyzed by flow cytometry after staining with anti-TCR Cβ1, anti-PD1 and anti-CD8 antibodies. Untransduced cells (UT) were used as controls in all assays

A TCR Discovery process



B TCR vetting algorithm



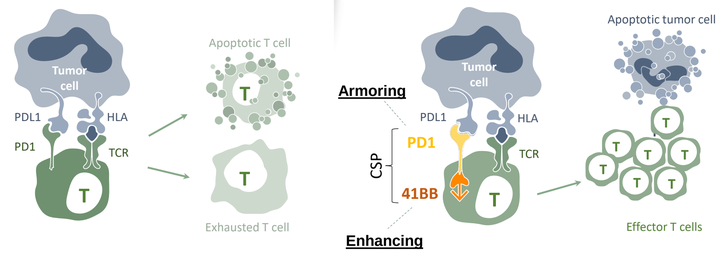
C Armoring & enhancing with the PD1-41BB CSP

TME challenge: PD1-PDL1 signals

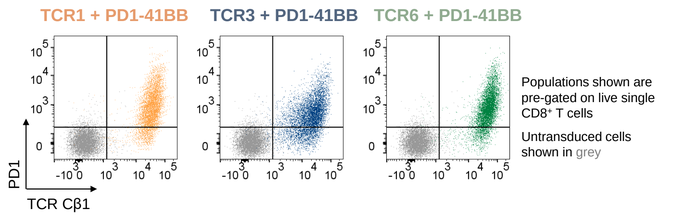
- Inhibits T cell activity
- Induces T cell exhaustion
- Drives T cell apoptosis

Medigene's solution: PD1-41BB CSP

- Blocks PDL1 mediated inhibition
- Provides T cell co-stimulation via 41BB pathway
- Enhances and sustains TCR-T activity and survival

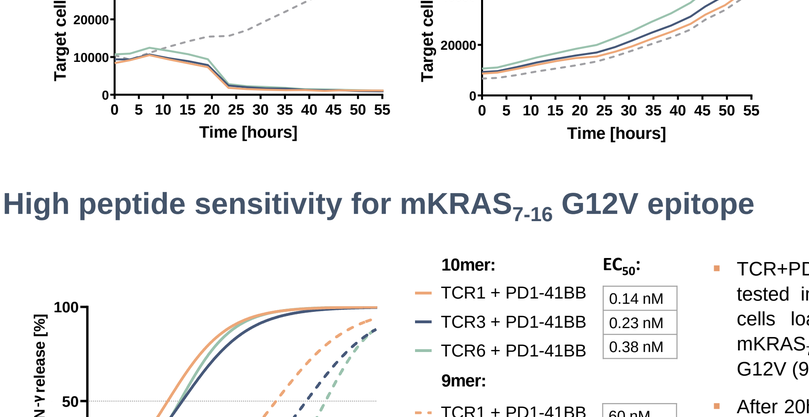


D Top-3 candidate TCRs



Top-3 candidate TCRs show exquisite specificity, high sensitivity and a favorable safety profile (3S TCRs)

Strong and exclusive recognition of mKRAS G12V-positive tumor cells

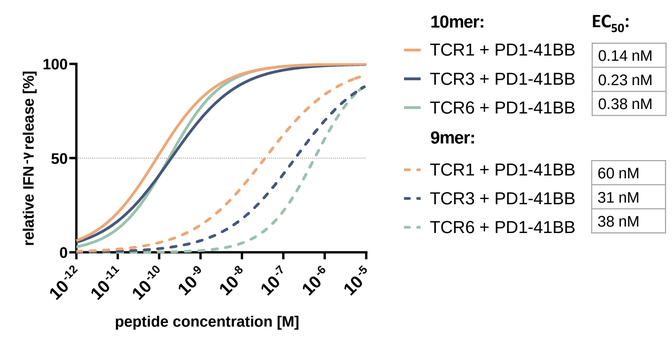


- Tumor cell lines derived from various tumor indications expressing either mKRAS G12V or KRAS wildtype were used as targets in co-culture experiments with TCR+PD1-41BB-transduced CD8⁺ T cells. Tumor cells stably expressed a red fluorescent protein for cell tracking.

A. IFN-γ secretion by CD8⁺ T cells was assessed by ELISA 20 hours (h) after setting up the co-culture.

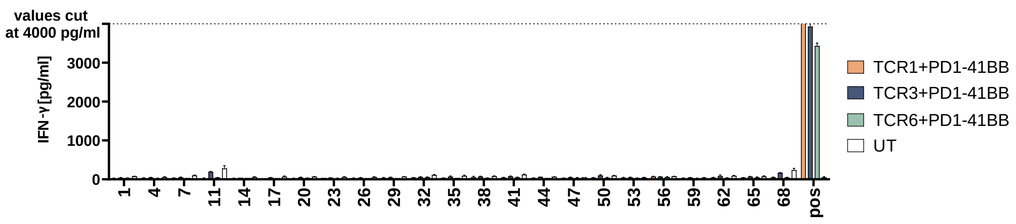
B. Co-cultures were monitored using a live-cell imaging system to evaluate the decrease of red fluorescent tumor cells over time. Data of two exemplary tumor cell lines are shown.

High peptide sensitivity for $mKRAS_{7-16}$ G12V epitope



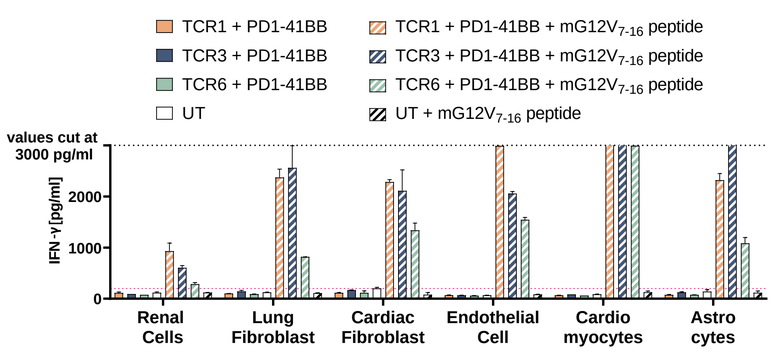
- TCR+PD1-41BB-transduced CD8⁺ T cells were tested in a co-culture experiment using target cells loaded with graded amounts of either $mKRAS_{7-16}$ G12V (10-mer) peptide or $mKRAS_{8-16}$ G12V (9-mer) peptide.
- After 20h of co-culture, an ELISA was performed to evaluate IFN-γ secretion by T cells (shown one exemplary experiment).
- A nonlinear regression analysis was used to determine EC_{50} values. Shown EC_{50} values are averages of three independent experiments.

No signs of target peptide-independent HLA-allo cross-recognition



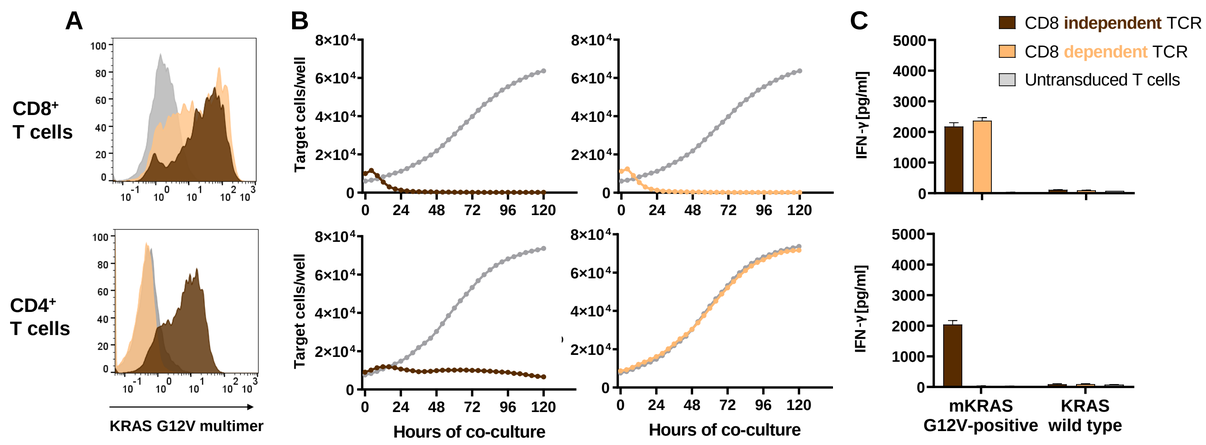
- TCR+PD1-41BB-transduced CD8⁺ T cells were co-cultured with a cellular library of 70 lymphoblastoid cell lines (LCLs) covering the most frequent HLA allotypes worldwide.
- Target cells loaded with $mKRAS_{7-16}$ G12V peptide served as internal positive control (pos).
- IFN-γ secretion was evaluated by ELISA 20h after setting up the co-culture and values for representative LCLs are shown.

No signs of off-target toxicity with a panel of healthy cell types



- Healthy cell types representing essential tissues or organs were tested for recognition by TCR+PD1-41BB-transduced CD8⁺ T cells. Target cells loaded with $mKRAS_{7-16}$ G12V peptide were included as internal positive controls.
- IFN-γ secretion was evaluated by ELISA 20h after setting up the co-culture.

One candidate TCR exhibits full functionality independently of CD8 co-receptor presence



A. TCRs+PD1-41BB CSP were expressed in CD4⁺ and CD8⁺ T cells, respectively, and transduced T cells were enriched by FACS. T cell samples were analyzed for multimer binding by flow cytometry after staining with $mKRAS_{7-16}$ G12V HLA-A*11:01 multimer.

B. TCR+PD1-41BB-transduced CD4⁺ and CD8⁺ T cells were co-cultured with tumor cell lines expressing either mKRAS G12V or KRAS wildtype. In addition, tumor cells stably expressed a red fluorescent protein for cell tracking. Live-cell imaging analysis of the red fluorescent tumor cell count for a representative mKRAS G12V-positive cell line is shown.

C. IFN-γ secretion by T cells was assessed 20h after setting up the co-culture. Data of two exemplary tumor cell lines are shown.

Co-expression of PD1-41BB CSP enhances and sustains TCR-T cell functionality

- mKRAS G12V-specific CD8⁺ T cells with (TCR+PD1-41BB) or without (TCR) co-expression of PD1-41BB CSP were co-cultured with 3-dimensional tumor cell spheroids of mKRAS G12V-positive or KRAS wildtype tumor cells overexpressing PD-L1, and multiple tumor challenges were performed (day 0, 3, 6, 9). Tumor cells stably expressed a red fluorescent protein for cell tracking.

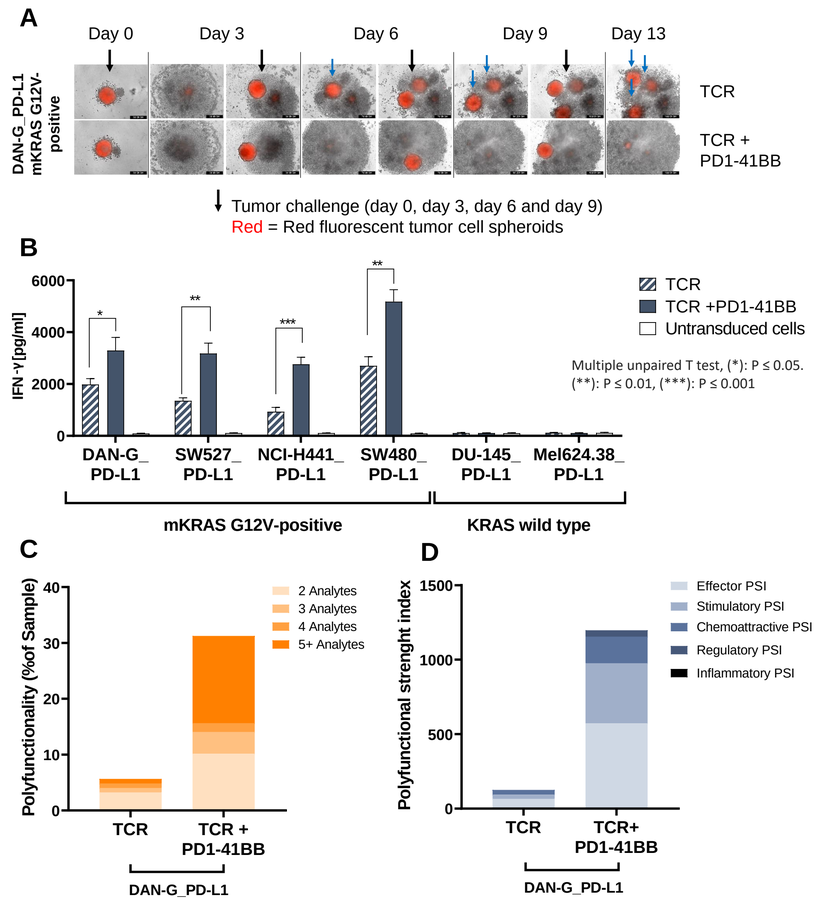
A. Co-cultures were monitored using a live-cell imaging system to evaluate the decrease of red fluorescent tumor cells over time. Results of one exemplary tumor cell line are shown.

B. IFN-γ secretion by CD8⁺ T cells 20h after the first challenge.

- TCR-/+PD1-41BB-transduced CD8⁺ T cells were co-cultured with mKRAS G12V-positive tumor cells overexpressing PD-L1 for 24h and analyzed using single cell proteomic analysis (IsoLight® technology, PhenomeX).

C. Polyfunctionality of single CD8⁺ T cells displayed as % of cells secreting 2, 3, 4 or 5+ analytes at the same time.

D. Polyfunctional Strength Index (PSI) defined as the number of CD8⁺ T cells secreting ≥ 2 effector molecules per cell multiplied by mean fluorescence intensity (MFI) of the proteins secreted by the respective cells.



Conclusions

- Medigene´s TCR discovery process and vetting assay algorithm allow isolation and selection of optimal TCRs with exclusive mKRAS G12V specificity, high peptide sensitivity with strong tumor cell recognition and a favorable safety profile (3S TCRs).
- Top-3 mKRAS G12V-specific TCRs display unique attributes such as CD8 co-receptor independency.
- Combining mKRAS G12V-specific 3S TCRs with PD1-41BB CSP armors and enhances T cell functionality for the development of best-in-class TCR-T therapies to overcome the challenges of a hostile TME and improve patient outcomes.