Abstract #3483

MyT™ platform-identified PRAME TCRs for T cell therapy demonstrate superior efficacy and best-in-class potential compared to clinical benchmarks

Lia Walcher, Lena Immisch, Mira Heichenko, Alexei Leliavski, Robert Höltzel, Sharesta Khoenkhoen, Niklas Fellmer, Elisa Kieback, Lorenz Knackstedt*, Lucia Poncette*

T-knife Therapeutics Inc., San Francisco, USA

*shared last authors

Background

PRAME is a clinically validated target for T cell receptor-modified T cell (TCR-T) therapy with high prevalence in multiple solid tumor indications. A best-in-class, high-affinity and high-specificity PRAME-directed TCR was generated using T-knife's MyT platform, for use in next-generation TCR-T therapy targeting PRAME expressing indications.

Methods

We identified PRAME-directed TCRs using the MyT platform, a TCR discovery engine based on transgenic mice expressing the human TCR repertoire with the ability to overcome central tolerance. Reactive TCR candidates were identified by rapid throughput screening using a reporter cell line. Selected TCR candidates were subsequently expressed in primary CD4 and CD8 T cells in combination with a WT CD8 co-receptor (CoR) via retroviral transduction. The T cells were characterized for peptide dose-response, cytokine release and cytotoxicity. We performed in-depth TCR safety profiling by rapid alloreactivity screening of single-HLA-transfected cells and by positional mutagenesis scanning (X-scans) to assess potential cross-reactivity. Finally, we supercharged TCR-modified T cells by co-expression with a single-chain CD8 CoR with built-in co-stimulation (Co-stim CD8 CoR, see poster #4868) and a FAS-TNFR switch receptor (see poster #4867) using CRISPR-Cas9 gene editing combined with knockout of the endogenous TCR (see poster #3198).

The MyT platform delivers a large number of TCRs reactive against the tumor antigen PRAME

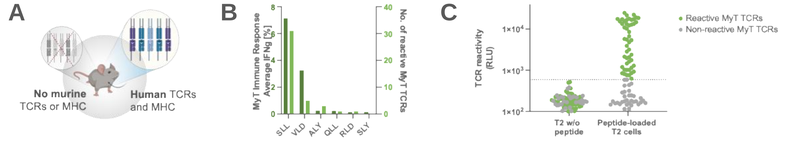


Fig. 1: TCR generation by immunizing MyT platform mice and rapid throughput screening for TCR reactivity. (A) MyT platform-derived mice, carrying the entire human TCRα/β gene loci as well as HLA-A*02:01 and lacking expression of murine TCRα, TCRβ and MHC class I were immunized with the full-length PRAME antigen using an adenoviral vector or with $SLL_{425-433}$ peptide. PRAME was highly immunogenic in MyT platform mice (n = 40/40 responder mice, data not shown). **(B)** In vitro restimulation of splenocytes from mice immunized with full-length PRAME (n = 6) with specific PRAME peptides resulted in immune responses against several HLA-A*02:01-restricted epitopes of PRAME as detected by IFN-γ secretion, wherein $SLL_{425-433}$ was the most dominant epitope. Screening of TCR candidates from the 6 mice confirmed that most TCRs were specific for the SLL epitope. **(C)** Using a reporter cell-based assay, we screened the 158 most frequent TCR clonotypes isolated from responder mice (n = 33) for their reactivity towards T2 cells loaded with 10^{-5} M SLL peptide at an E:T-ratio of 1:1. The threshold for reactivity was defined as the average background signal (T2 w/o peptide) + 3xSD (dashed line). We identified 85 reactive TCRs (green) with a broad reactivity range towards the SLL epitope of PRAME. We selected the most reactive TCRs for in-depth characterization in vitro.

MyT TCRs show high avidity, high cytokine secretion, and high cytotoxicity against PRAME-expressing cancer cell lines

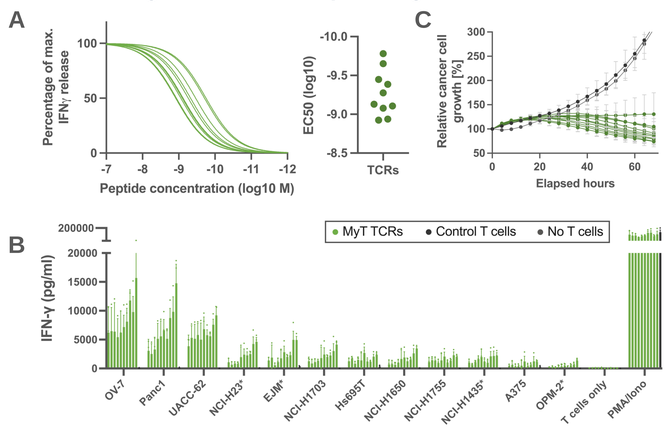


Fig. 2: High efficacy of MyT TCRs in peptide dose-response assays, IFN-γ release and cytotoxicity against cancer cells. Ten selected TCR candidates were co-expressed with WT CD8 CoR in T cells from healthy donors (mix of CD4 and CD8 T cells). **(A**, **B)** IFN-γ production of TCR transduced T cells was assessed upon stimulation with SLL-peptide-loaded T2 cells **(A)** or a range of tumor cell lines with natural PRAME expression **(B)** at an E:T-ratio of 1:1. **(C)** TCR-T cells were co-cultured with GFP-expressing NCI-H1703 cells at an E:T-ratio of 1:1 and relative target cell growth was quantified by monitoring green fluorescence area on an IncuCyte live imaging microscope. Target cells cultured with control-transduced T cells or T cells cultured without target cells or target cells cultured without T cells served as negative controls. PMA/Ionomycin stimulation served as positive control. Data are shown as means of n = 3 donors ± SEM. Asterisks indicate cell lines transduced with HLA-A*02:01.

Higher in vitro efficacy by MyT lead TCR candidates compared to clinical and preclinical-stage benchmark TCRs

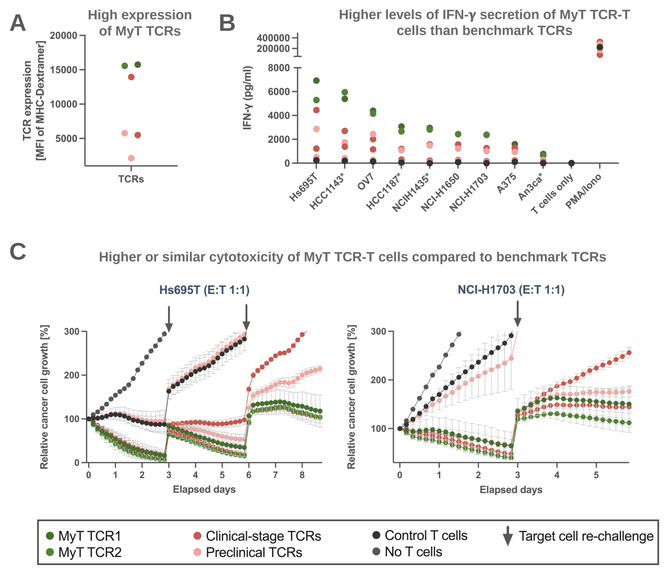


Fig. 3: Benchmarking of MyT TCRs with peer TCRs shows higher TCR expression and IFN-γ release and similar or superior cytotoxicity against cancer cells. MyT TCRs 1 and 2, as well as clinical- and preclinical-stage peer TCRs were co-expressed with WT CD8 CoR in T cells from healthy donors (mix of CD4 and CD8 T cells). Peer TCRs were selected from publicly disclosed sequences. **(A)** Functional TCR expression was quantified by flow cytometric analysis of MHC-Dextramer binding. **(B)** IFN-γ production of TCR transduced T cells was assessed upon stimulation with a range of tumor cell lines with natural PRAME expression at an E:T-ratio of 1:1. **(C)** TCR-T cells were co-cultured with GFP-expressing NCI-H1703 or Hs695T cells at an E:T-ratio of 1:1, and green fluorescence area was monitored on an IncuCyte live imaging microscope. At indicated time points, fresh target cells were added to re-challenge TCR-T cells. Data are shown as means of n = 2-5 donors ± SEM. Asterisks indicate cell lines transduced with HLA-A*02:01.

No allo- or off-target-reactivity by lead MyT TCR candidates

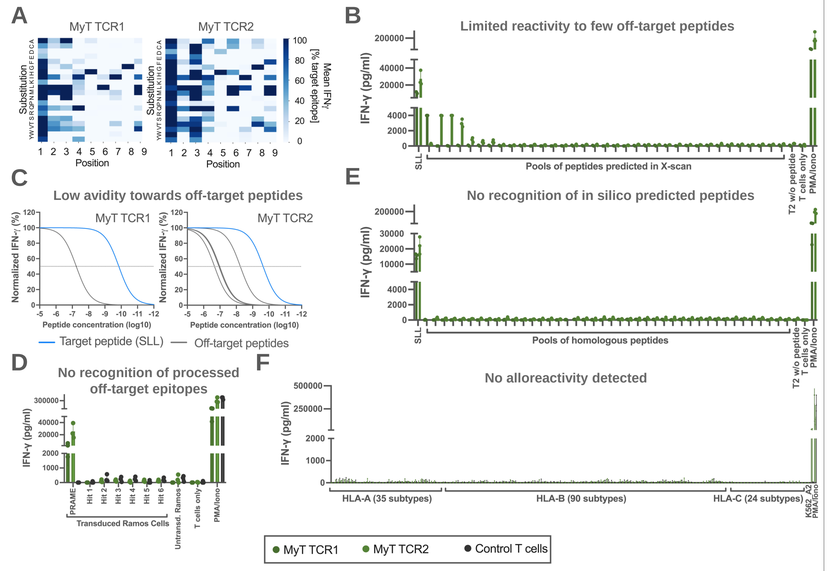
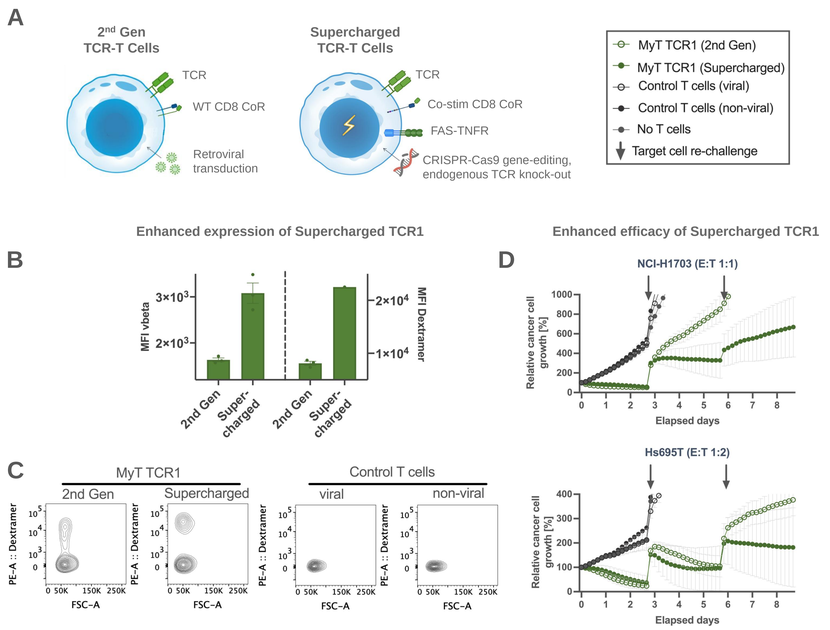


Fig. 4: High on-target specificity of MyT TCRs as assessed by X-scan, homologous epitope testing and allo-reactivity screening. MyT TCRs 1 and 2 were co-expressed with WT CD8 CoR in T cells from healthy donors (mix of CD4 and CD8 T cells). **(A)** IFN-γ production of TCR transduced T cells was assessed upon stimulation with T2 cells loaded with peptides from a mutational cross-reactivity library (X-scan). Heat maps show recognition motifs (threshold was set to 10% relative IFN-γ), which was subsequently screened in a human proteome database. **(B)** Peptides predicted in the X-scans were further filtered according to their predicted HLA-A2-binding. T2 cells were loaded with peptide pools (10^{-7} M per peptide) and recognition was assessed via IFN-γ ELISA. **(C+D)** Recognized off-target peptides were investigated more in-depth in dose-response assays **(C)** and in the B-cell derived cell line Ramos overexpressing the corresponding gene and HLA-A2 **(D)**. **(E)** As a complementary approach, *in silico* predicted homologous peptides were screened by loading T2 cells with peptide pools (10^{-7} M per peptide). **(F)** Alloreactivity against 149 HLA-subtypes covering 98% of class-I HLA alleles was screened using single-HLA-transfected K562 cells. Data are shown as means of n = 3 donors ± SEM.

Supercharging PRAME TCR-T cells by dual armoring using a gene editing process including endogenous TCR knockout further enhances expression and efficacy of lead candidate TCR1 without impacting safety



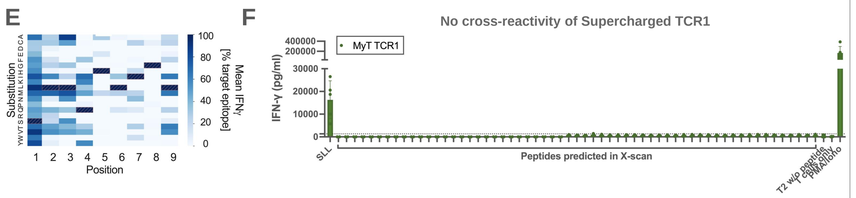


Fig. 5: Supercharged TCR-T product demonstrates higher TCR expression and higher cytotoxicity against cancer cells while retaining specificity. (A) To create a Supercharged PRAME TCR-T product, TCR expression and function was further enhanced by dual T cell armoring (Co-stim CD8 CoR and FAS-TNFR) and using gene editing combined with endogenous TCR knockout. We selected the less promiscuous TCR1 as lead candidate and compared the Supercharged TCR-T product to the previously used 2nd generation product (virally transduced T cells co-expressing TCR and WT CD8 CoR). In both cases a mix of CD4 and CD8 T cells was analyzed. **(B)** pMHC-Dextramer binding and TCR vbeta surface expression of Supercharged product compared to 2nd Gen product measured by median fluorescence intensity (MFI). Representative contour plots of Dextramer stainings are displayed in **(C)**. **(D)** TCR-T cells were co-cultured with GFP-expressing NCI-H1703 or Hs695T cells at an E:T-ratio of 1:1 or 1:2, respectively, and green fluorescence area was monitored on an IncuCyte live imaging microscope. At indicated time points, fresh target cells were added to re-challenge TCR-T cells. **(E+F)** X-scan assay and screening of predicted peptides of MyT TCRs Supercharged product. Data are shown as means of n = 3 donors ± SEM.

Conclusions

- **T-knife's MyT platform delivered best-in-class high-affinity PRAME TCRs**
- **Lead TCR candidate is highly specific and does not demonstrate off-target reactivity**
- **Supercharged PRAME-TCR-T cells incorporating MyT TCR, a single-chain co-stimulatory CD8 CoR and a SwR in a gene editing process, provide potential to induce deep and durable responses in hard-to-treat solid tumor indications**