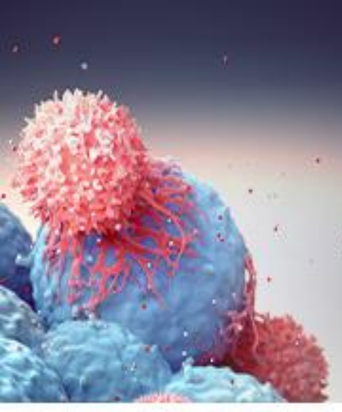

High-affinity PRAME TCRs synergize with tailored CD8 co-receptor to generate potential best-in-class PRAME-targeting TCR-T therapy

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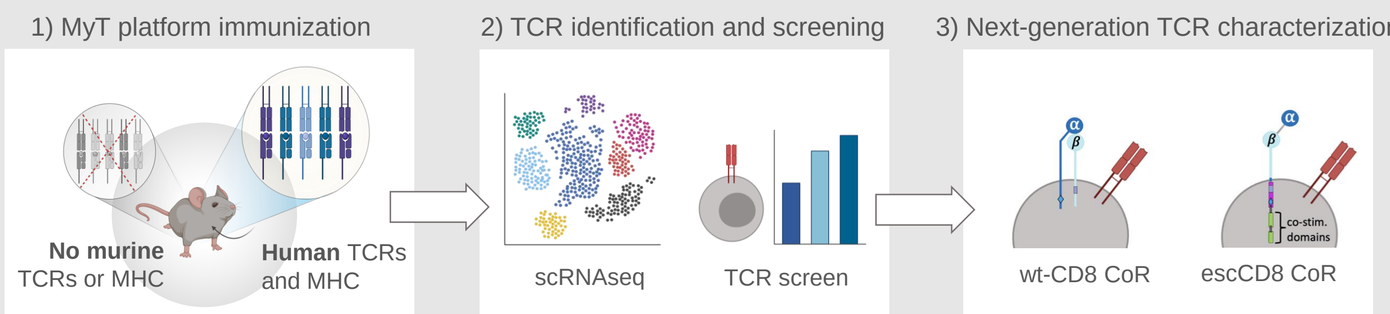
Background

PRAME is a promising target for immunotherapy due to both its high prevalence and expression in multiple tumor indications with high unmet medical need, and its limited expression in healthy tissues. T cell receptor-engineered T cell (TCR-T) therapy targeting PRAME has shown promising early clinical validation. Accumulating clinical evidence suggests that deep and durable clinical responses require additional T-cell engineering, such as incorporating a CD8 co-receptor (CoR) to enable redirection of CD4+ T cells to peptide-MHC class I complexes. Since CD4 T cells play a pivotal role in the anti-tumor response (e.g., cytokine secretion, CD8 T cell cross-activation, memory formation), these next-generation approaches have the potential to improve the efficacy of TCR-based therapeutics.

Methods

We identified highly reactive PRAME-directed TCRs from T-knife's MyT™ platform – a murine-based human TCR discovery engine with the ability to overcome central tolerance. Using the MyT platform, we were able to generate TCRs of high affinity when benchmarked to TCRs from human donors, where tolerance to self-antigens eliminates high-affinity TCRs. We further increased the efficacy of these TCRs by co-expressing them with wildtype (wt) CD8 CoR or enhanced single-chain (esc) CD8 CoRs (for details on CD8 co-receptor design and constructs please visit poster #375).



1) 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 were immunized with the full-length antigen or with antigenic peptides. Booster immunizations were performed as required.

2) Antigen-specific T cells were isolated from spleens of responding mice and the TCR sequences were identified using single cell RNA sequencing. The most frequent TCR clonotypes were screened in a reporter cell line-based assay.

3) Reactive TCRs were cloned in expression vectors together with CD8 CoRs and re-expressed in human T cells for characterization of functionality and specificity.

PRAME is highly immunogenic in the MyT platform with a dominant T-cell response against the epitope SLL

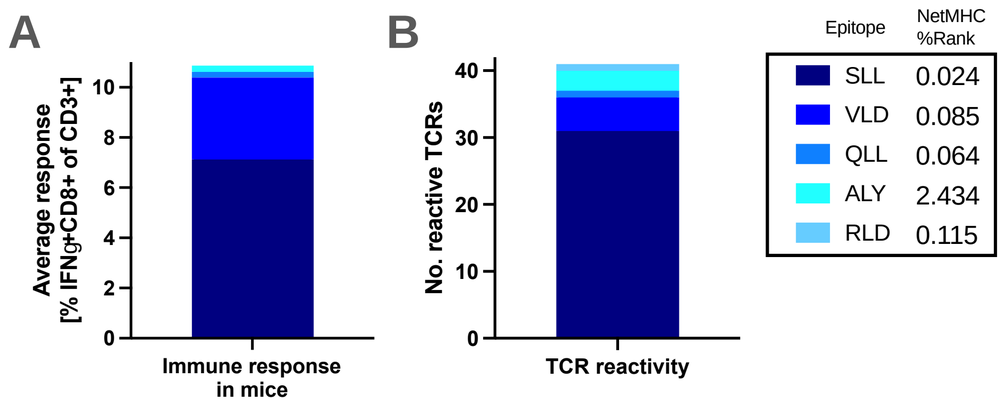


Fig. 1: (A) PRAME was highly immunogenic in MyT platform mice. In vitro stimulation of splenocytes from immunized mice with PRAME peptides resulted in high IFNγ secretion. Shown are splenocyte cultures of n = 6 mice. We observed immune responses against several HLA-A*02:01-restricted epitopes of PRAME, wherein $SLL_{425–433}$ was the most dominant epitope. This epitope has the highest predicted rank for binding HLA-A*02:01 and is highly abundant on PRAME-expressing tumor cells (data not shown), confirming that the MyT platform selected the most suitable target epitope for TCR-T therapy (for more information on epitope selection in the MyT platform please visit poster #367). **(B)** Screening of TCR candidates from the 6 mice shown in (A) confirmed that most TCRs were specific for the SLL epitope. TCR reactivity was screened by co-culturing TCR-expressing reporter cells with peptide-loaded T2 cells. %Rank values for peptide-MHC affinity are derived from NetMHCpan-4.1 (accessed Sep 2023).

85 TCRs directed against the PRAME epitope SLL were identified across a broad reactivity range

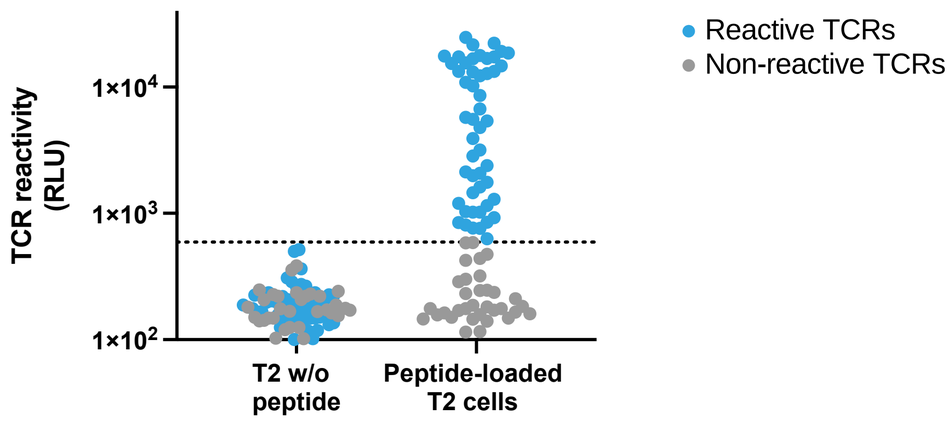


Fig. 2: Using a reporter cell-based assay, we screened the 158 most frequent TCR clonotypes isolated from responder mice (n = 33) for their reactivity towards T2 cells loaded with 10^{-5} M $SLL_{425-433}$ peptide at an E:T-ratio of 1:1. The threshold for reactivity was defined as the average background signal (T2 w/o peptide) + 3xSD (dashed line). We identified 85 reactive TCRs (blue) with a broad reactivity range towards the SLL epitope of PRAME. We selected the 22 most reactive TCRs for in-depth characterization and benchmarking *in vitro*.

Combining PRAME TCRs with escCD8 CoR further increases the competitive advantage of MyT TCRs

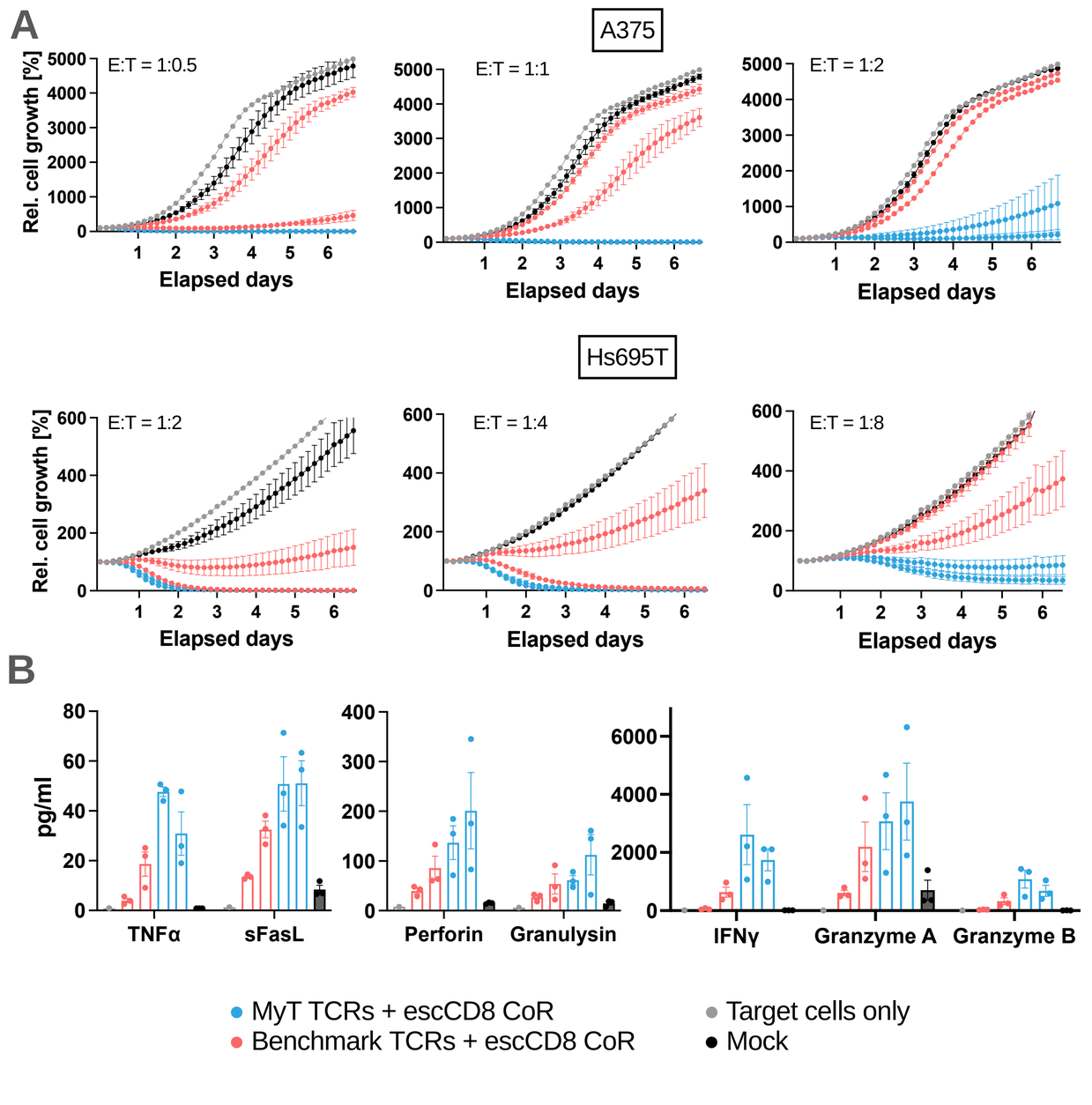


Fig. 4: (A) T cells (mix of CD4/CD8) transduced with TCRs and escCD8 CoR were co-cultured with GFP-expressing target cells and relative target cell growth was quantified by monitoring integrated green fluorescence intensity on an IncuCyte live microscope. Target cells cultured without T cells or with Mock transduced T cells served as negative controls. In contrast to benchmark TCRs, including a clinical-stage TCR, MyT TCRs effectively eliminated cancer cells at very low E:T ratios. (**B)** Culture supernatant was collected at 72h and cytokine secretion was evaluated with LEGENDplex, confirming increased functional activity of MyT TCRs. (E:T-ratio 1:4 of Hs695T target cells is displayed). Data are shown as means of n = 3 donors ± SEM.

MyT TCRs confer higher avidity and cytokine secretion than benchmark TCRs, both combined with wt-CD8 CoR

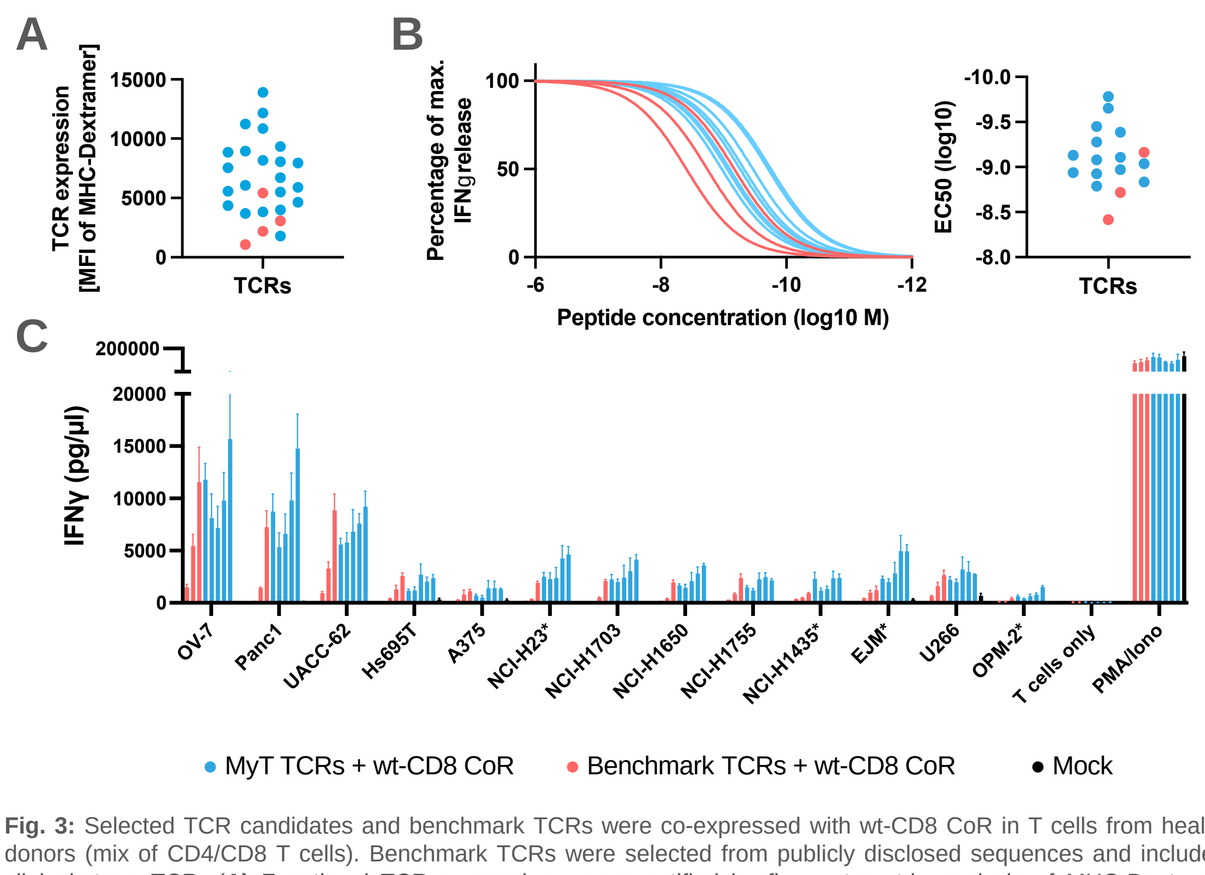


Fig. 3: Selected TCR candidates and benchmark TCRs were co-expressed with wt-CD8 CoR in T cells from healthy donors (mix of CD4/CD8 T cells). Benchmark TCRs were selected from publicly disclosed sequences and include a clinical-stage TCR. (**A)** Functional TCR expression was quantified by flow cytometric analysis of MHC-Dextramer binding. Compared to MyT TCRs, several benchmark TCRs could not be functionally expressed when combined with wt-CD8 CoR, presumably due to limited TCR stability in a construct of large payload. **(B+C)** IFNγ production of TCR transduced T cells was assessed upon stimulation with peptide-loaded T2 cells **(B)** or a range of tumor cell lines with natural PRAME expression **(C)** at an E:T-ratio of 1:1. Displayed are the most functional TCR candidates. Target cells cultured with Mock transduced T cells or T cells cultured without target cells served as negative controls. PMA/Ionomycin stimulation served as positive control. Several MyT TCRs displayed similar or higher reactivity compared to benchmark TCRs. Data are shown as means of n = 3 donors ± SEM. Asterisks indicate cell lines transduced with HLA-A*02:01.

Outlook: T-knife's add-on toolbox to enhance TCR-T function and protect from TME-mediated immunosuppression

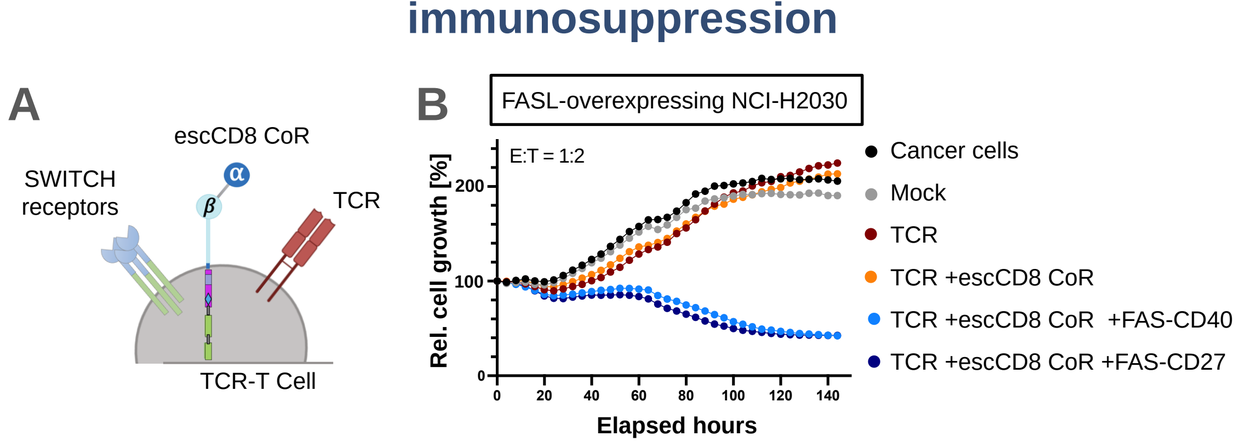


Fig. 5: (A) FAS-based switch receptors combine the extracellular domain of FAS and cytoplasmic domain derived from different co-stimulatory molecules. **(B)** T cells expressing MAGE-A1 TCR and escCD8 CoR together with FAS-CD40 or FAS-CD27 switch receptors efficiently mediate long-term cytotoxicity against FASL- and HLA-A*02:01-expressing NCI-H2030 cells (E:T-ratio 1:2). Representative results of 4 donors are shown. Combinations of PRAME-targeting TCRs with different CD8 co-receptors and switch receptors tailored to indications expressing PRAME are currently under development. For more detail on escCD8 CoR and switch receptors please visit posters #375 and #368 respectively.

Conclusions

- **T-knife's MyT platform delivered high-affinity, potentially best-in-class PRAME TCR candidates for further clinical evaluation.**
- **Combining such high-affinity PRAME TCRs with CD8 CoRs and switch receptors tailored to indications highly expressing PRAME has the potential to induce deep and durable clinical responses.**