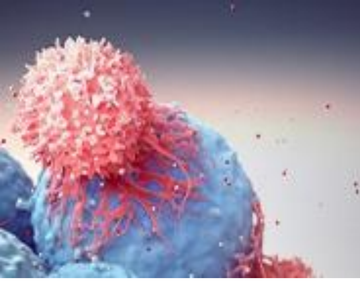

TK-6302, a Supercharged PRAME TCR-T cell therapy containing a high affinity TCR, an activating CD8 coreceptor (co-stimCD8 CoR) and a FAS-based switch receptor, drives sustained anti-tumor responses

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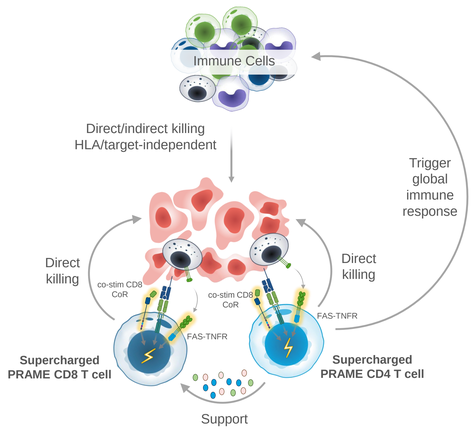
Abstract #3198

Background

T cell receptor (TCR) T cell therapy targeting PRAME has shown durable responses in melanoma and sarcoma but efficacy in other solid tumors has been limited. Suboptimal T cell engraftment and fitness, and immune suppression by the tumor microenvironment (TME) have been pinpointed as main causes of the limited efficacy of T cell therapy in solid tumors. We have developed TK-6302, a PRAME targeting TCR-T cell therapy with best-in-class potential, manufactured with a non-viral gene editing process, incorporating a high-affinity TCR (see poster #3483), a chimeric CD8 co-receptor that engages CD4 T cells and provides co-stimulation upon TCR engagement (co-stim CD8 CoR, see poster #4868), and a FAS switch receptor (SwR) that boosts engraftment and fitness in the periphery and prevents apoptosis in the tumor (see poster #4867).



TK-6302 Mechanism of Action

- Supercharged PRAME CD4 and CD8 T cells **directly kill tumor cells** via the high-affinity TCR and co-stim CD8 CoR
- Supercharged PRAME CD4 T cells **secrete cytokines to support** CD8 T cell function, and trigger global immune responses by recruiting and activating other immune cells, providing the potential for **HLA and target-independent tumor cell killing**
- The co-stim CD8 CoR mediates TCR-T **fitness and durable functional activity** through optimal co-stimulation
- The FAS-TNFR checkpoint converter enhances TCR-T cell **engraftment and persistence** via activation in the lymph nodes and prevention of FAS-L induced cell death in the tumor

Methods

TK-6302 or known clinical and preclinical approaches were generated using a non-viral gene editing process inserting all elements into the TRAC locus and then compared to benchmark products based on, activation/exhaustion phenotype by flow cytometry, cytokine secretion by LEGENDplex™, and cytotoxicity upon chronic or repeated antigen stimulation using Incucyte. Cell lines used: (1) GFP transduced NCI-H1703 cancer cells knocked-out for CD155 (PVR, a TIGIT ligand) and FAS-L genes and transduced with either FAS-L or PD-L1 and (2) NucLight Red transduced HLA-A*02:01-overexpressing COR-L23 cancer cells transduced with either FAS-L or PD-L1.

Supercharged PRAME TCR-T cells (TK-6302)

1 High Affinity MyT TCRs

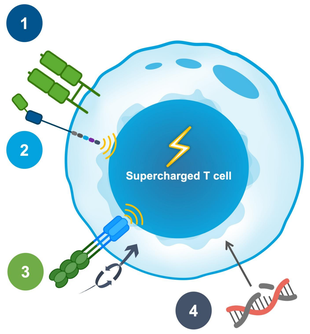
- High affinity and specificity PRAME TCR generated with proprietary MyT platform avoiding central tolerance
- Superior affinity, cytotoxicity and cytokine secretion to clinical-stage PRAME TCRs
- See poster #3483

2 Costimulatory CD8 Coreceptor

- Engages CD4 T cells and enables global immune responses
- Enhances fitness and persistence by providing the costimulatory signaling often missing in tumors
- Mediates enhanced functional activity compared to clinical-stage CD8 co-receptors
- See poster #4868

3 TME Armoring / Checkpoint Converter

- Selected after profiling 4,622 stage III/IV tumors from PRAME-expressing indications
- Blocks death signals in the tumor
- Enhances engraftment and fitness in the periphery
- Mediates higher functional activity compared to peer approaches
- See poster #4867



4 Non-viral Gene Edited Manufacturing

- Greater payload capacity
- Knock out endogenous TCR for improved MyT TCR expression

TK-6302 demonstrate sustained serial killing and cytokine secretion in a model mirroring the inhibitory ligand expression in PRAME+ tumors

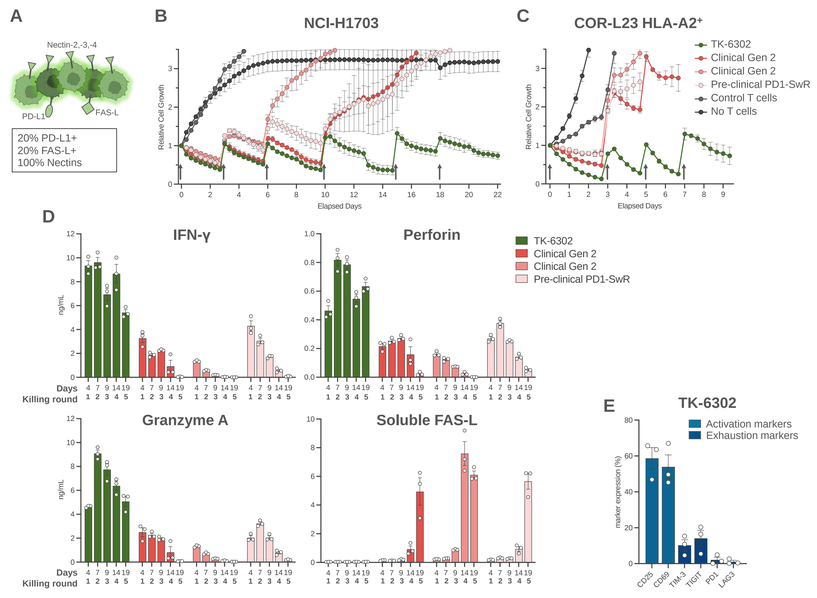


Fig. 1: Cytotoxicity, cytokine secretion and activation/exhaustion profile of PRAME TCR-T cells after repeated antigen stimulation. (**A**) Schematic representation of cancer cell line mixtures. (**B**,**C**) TK-6302 or known clinical and preclinical approaches were co-cultured with either (**B**) NCI-H1703 or (**C**) HLA-A*02:01-overexpressing COR-L23 cancer cells. Arrows indicate addition of cancer cells. (**D**) Cytokine secretion and soluble FAS-L was determined during NCI-H1703 co-culture by LEGENDplex. Data of a representative donor is shown. (**E**) TK-6302 maintains a favorable activation/exhaustion profile after multiple rounds of cancer re-challenge, when T cells from benchmarking approaches were exhausted. Expression levels determined by flow cytometry. Data of $n = 3$ donors ± SEM.

TK-6302 TCR-T cells show increased polyfunctionality

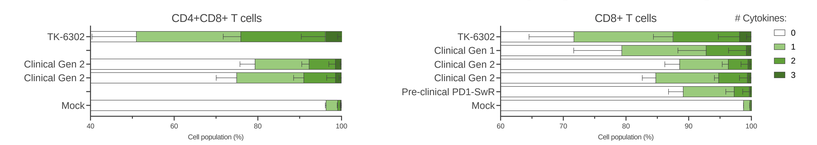


Fig. 2: Simultaneous measurement of IFN-γ, TNF-α and IL-2 production at single cell level of PRAME TCR-T cells after stimulation. TK-6302 or known clinical and preclinical approaches were co-cultured with PRAME peptide-loaded NCI-H1703 cancer cells, of which 50% were FAS-L positive. Brefeldin A was added to the co-culture overnight. Intracellular cytokine staining for CD4+CD8+ T cells or CD8+ T cells was assessed by flow cytometry. Note: Gen1 TCR-T cells do not contain CD4+CD8+ T cells and therefore are not depicted in the left panel. Data of $n = 3$ donors ± SEM.

TK-6302 cells are protected from FAS-L-induced apoptosis

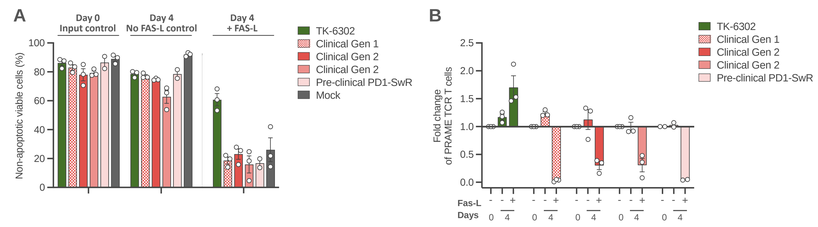
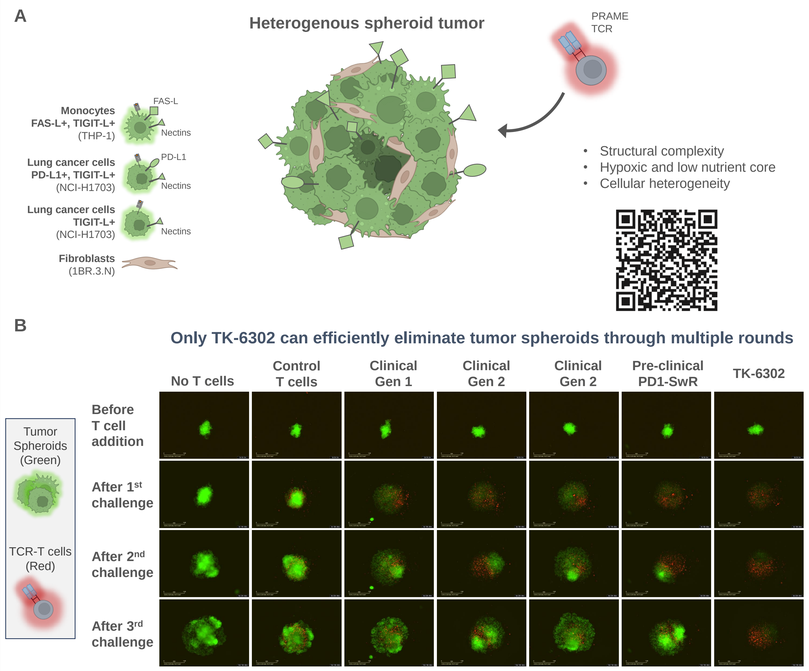


Fig. 3: Viability and expansion of PRAME TCR-T cells upon FAS-L stimulation. TK-6302 or known clinical and preclinical approaches were thawed and cultured in media with IL-7/IL-15 and analyzed by flow cytometry. Soluble FAS-L protein was added daily at 10 ng/ml from Day 1 to Day 4. (**A**) Cell viability as defined by 7–AAD/annexin V staining. (**B**) Fold change of TCR-T cells defined by multimer and CD95 staining. Data of $n = 3$ donors ± SEM.

Superior anti-tumor activity of TK-6302 in a 3D spheroid model mimicking multiple solid tumor barriers



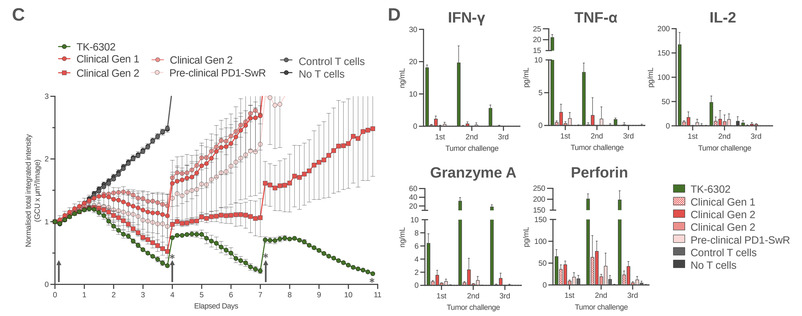


Fig. 4: Cytotoxicity and cytokine secretion profile of PRAME TCR-T cells in an advanced 3D spheroid model. (**A**) Schematic representation of advanced 3D multi-cellular tumor spheroids, TK-6302 or known clinical and preclinical approaches were co-cultured with 3D spheroids and assessed by time-lapse live-cell microscopy. (**B**) Representative microscopic pictures of the spheroids at the co-culture initiation and at the end of multiple spheroid rechallenges. (**C**) Spheroid growth from one representative of $n = 2$ donors. Arrows and asterisks indicate time points at which new spheroids were added, and supernatants were collected for cytokine analysis, respectively. (**D**) Cytokine secretion was measured using a multiplex cytokine bead array from one representative of $n = 2$ donors.

Conclusions

- **Supercharged PRAME TCR-T therapy demonstrates best-in-class preclinical anti-tumor efficacy and T cell fitness compared to current clinical and preclinical stage PRAME TCR-T approaches, providing the potential for deep and durable responses in hard-to-treat indications**
- **TK-6302 manufacturing process has been locked, interactions with regulatory agencies are completed and Clinical Trial Application is planned for Q4 2025 with first patient enrollment planned in Q2 2026**