TK-6302, a Supercharged PRAME TCR-T cell therapy containing a high affinity TCR, an activating CD8 coreceptor and a FAS-based switch receptor, demonstrates preclinical safety and efficacy

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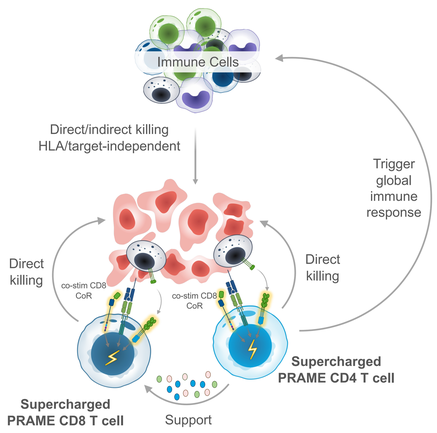
Abstract #329

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, a chimeric CD8 co-receptor that engages CD4 T cells and provides co-stimulation upon TCR engagement (co-stim CD8 CoR), and a FAS switch receptor (SwR) that boosts engraftment and fitness in the periphery and prevents apoptosis in the tumor.



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, driving tumor control through antigen spreading, **beyond HLA and target constraints**
- 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

Preclinical efficacy and safety of TK-6302 were examined in various in vitro assays testing drug products (DPs) from four healthy donors (M037, M038, M039, M042). The evaluation of anti-tumor responses included the measurement of cytokine secretion by ELISA, LEGENDplex and flow cytometry, and the assessment of cytotoxicity upon repeated antigen stimulation in 2D cultures against a panel of cancer cell lines from multiple indications and in a 3D multi-cellular spheroid model, both using IncuCyte live-cell imaging. Potential off-target reactivity of TK-6302 was evaluated by X-scan peptide screening, alloreactivity against a panel of lymphoblastoid cell lines and co-culture with primary healthy tissue cells. Antigen and cytokine independent activation and proliferation were also examined.

TK-6302 shows robust anti-tumor activity against complex tumor spheroids mimicking solid tumor microenvironment challenges

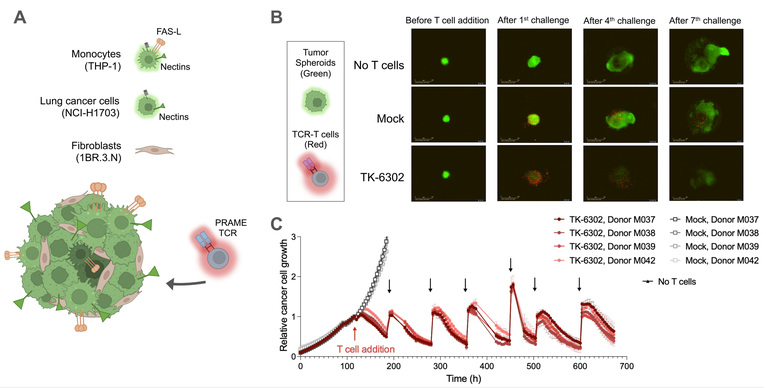


Fig. 1: TK-6302 shows robust and sustained anti-tumor activity upon serial challenge with multi-cellular tumor spheroids.

(A) Schematic representation of the 3D spheroid tumor model. **(B+C)** TK-6302 DPs from 4 healthy donors were co-cultured with 3D tumor spheroids at an effector-to-target (E:T) ratio of 1:20 and monitored by IncuCyte live-cell imaging. When at least 50% of GFP-expressing cancer cells within the spheroids were eliminated, new spheroids were added (time points indicated by black arrows). Spheroids cultured with Mock-T cells from the same donors or without T cells served as negative controls. **(B)** Live-cell imaging pictures showing indicated time points of M037, as a representative donor. **(C)** Relative cancer cell growth defined as GFP expression at each time point normalized to the time point of T cell addition (116h).

TK-6302 exhibits high efficacy towards a panel of cancer cell lines

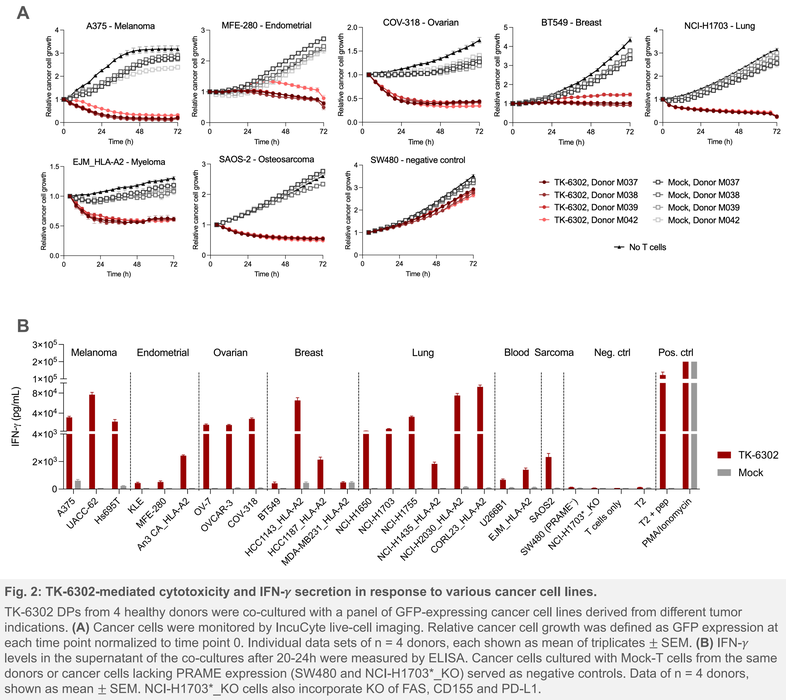


Fig. 2: TK-6302-mediated cytotoxicity and IFN-γ secretion in response to various cancer cell lines.

TK-6302 DPs from 4 healthy donors were co-cultured with a panel of GFP-expressing cancer cell lines derived from different tumor indications. **(A)** Cancer cells were monitored by IncuCyte live-cell imaging. Relative cancer cell growth was defined as GFP expression at each time point normalized to time point 0. Individual data sets of n = 4 donors, each shown as mean of triplicates ± SEM. **(B)** IFN-γ levels in the supernatant of the co-cultures after 20-24h were measured by ELISA. Cancer cells cultured with Mock-T cells from the same donors or cancer cells lacking PRAME expression (SW480 and NCI-H1703*_KO) served as negative controls. Data of n = 4 donors, shown as mean ± SEM. NCI-H1703*_KO cells also incorporate KO of FAS, CD155 and PD-L1.

Integration of FAS-SwR enhances potency and protects from FAS-L induced apoptosis within and outside the tumor

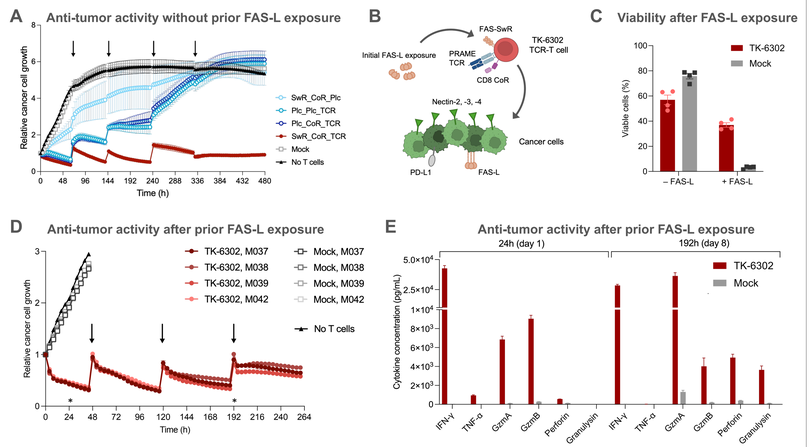


Fig. 3: FAS-SwR protects TK-6302 cells from FAS-L induced apoptosis and enhances cytotoxicity.

(A) PRAME TCR-T cells with or without FAS-SwR and CD8CoR (Plc = placeholder) were co-cultured with GFP transduced NCI-H1703 cells naturally expressing PRAME and HLA-A*02 (50% FAS-L$^+$) and monitored by IncuCyte. Data of n = 4 donors, shown as mean ± SEM. **(B-E)** TK-6302 DPs from 4 healthy donors were incubated with 10 ng/mL soluble recombinant FAS-L for 3 days (refreshed daily) to mirror T cell exposure to FAS-L in the periphery, before tumor homing. T cells were then co-cultured with GFP transduced NCI-H1703 cells naturally expressing PRAME and HLA-A*02 (20% FAS-L$^+$, 20% PD-L1$^+$). **(B)** Schematic representation of the experiment. **(C)** Viability of T cells after 3-day FAS-L exposure. **(D)** Live-cell imaging data of the subsequent co-culture with cancer cells. **(E)** Cytokine concentrations in supernatants collected after 24h and 192h (marked by * in panel D) determined by LEGENDplex. Data of n = 4 donors, shown as mean ± SEM. **(A, D)** Arrows indicate addition of cancer cells. Relative cancer cell growth defined as GFP expression at each time point normalized to time point 0.

TK-6302 shows high level of polyfunctionality upon engagement with PRAME-expressing cancer cells

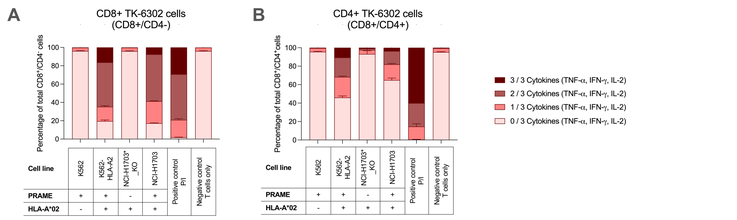


Fig. 4: Polyfunctional profiling demonstrates that the majority of TK-6302 cells secrete multiple cytokines upon activation with various cancer cell lines.

TK-6302 DPs from 4 healthy donors were co-cultured with cancer cell lines expressing PRAME and/or HLA-A*02 for 15 hours. The cell lines lacking either PRAME (NCI-H1703*_PRAME KO) or HLA-A*02 (K562) and T cells cultured in medium alone were used as negative controls. IFN-γ, TNF-α and IL-2 expression was measured by flow cytometry. Frequency of **(A)** CD8+/PRAME+/CD4- and **(B)** CD8+/PRAME+/CD4+ cells that co-express between 0 to 3 cytokines, calculated as a mean ± SEM for all four DPs.

TK-6302 TCR-T cells activated solely by the FAS-SwR do not exhibit functional activity in the absence of TCR engagement

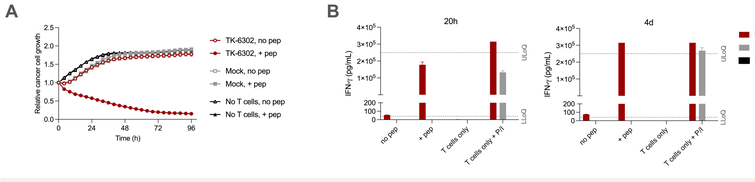
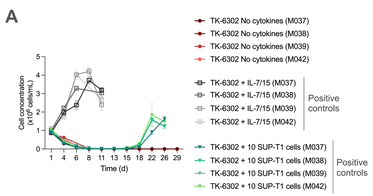


Fig. 5: TK-6302 TCR-T cells activated solely by the FAS-SwR neither kill cancer cells nor produce IFN-γ in the absence of TCR engagement.

TK-6302 DPs were co-cultured with GFP-expressing cells (100% PRAME KO, 75% FAS-L KO) at an E:T ratio of 1:2 and monitored by IncuCyte live-cell imaging for 4 days. **(A)** Relative cancer cell growth defined as GFP expression at each time point normalized to time point 0. **(B)** Supernatants collected at 20h and 4d were analyzed for IFN-γ by ELISA. Mock-T cells from the same donors or no T cell conditions served as negative controls; PRAME peptide-loaded or PMA/Ionomycin-stimulated TCR-T cells served as positive controls. Presented is M037, as a representative donor, shown as mean ± SEM of triplicates. ULoQ: 2.5 × 10^5 pg/mL; LLoQ: 40 pg/mL.

TK-6302 demonstrates a favorable preclinical safety profile without any off-target reactivity or antigen/cytokine independent proliferation



B

TK-6302 cleared nonclinical safety assays

- ✓ No off-target reactivity as tested with X-scan
- ✓ No alloreactivity tested with 59 LCL covering >97% of HLA types
- ✓ Anticipated recognition of renal cells, no reactivity to other healthy tissue
- ✓ No TCR-independent activation
- ✓ No cytokine- or target-independent growth or activation

Fig. 6: TK-6302 preclinical safety profile was demonstrated across comprehensive nonclinical assays.

(A) TK-6302 demonstrates no detectable antigen- or cytokine-independent proliferation. TK-6302 DPs from 4 healthy donors show no proliferation after 29 days of culture in cytokine free medium. TK-6302 DPs cultured with IL-7/IL-15 or with 10 SUP-T1 cells (human T cell lymphoma cells) spiked in each TK-6302 DP were used as a positive control for culture condition and assay sensitivity. The data is represented as mean of cells/mL ± SEM of 24 replicates measured by Cellaca MX. **(B)** Nonclinical safety assays conducted with no relevant findings observed.

Conclusions

- **TK-6302, a Supercharged PRAME TCR-T therapy, demonstrates robust nonclinical anti-tumor activity, polyfunctionality and T cell fitness with at-scale manufactured drug product**
- **TK-6302 cleared all nonclinical safety assays**
- **TK-6302 manufacturing process has been locked, and Clinical Trial Application has been submitted with first patient enrollment planned in 2026**