Abstract #347

In-depth characterization of TK-6302, a supercharged PRAME TCR-T therapy, manufactured at-scale from healthy donors and patients

Ronan O'Riordan[1], Melanie Manzke[1], Vida Farsam[1], Dana Hoser[2], Jara J Joedicke[1], Albert Vallejo-Gracia[1], Katja Lindner[1], Martina Defendi[1], Mira Heichenko[1], Pranvera Tanushaj[1], Mario Bunse[1], Kathrin Skibbe[1], Mikhail Steklov[2], Panagiota A. Sotiropoulou[2], Ute Brassat[2#] and **Marleen M. Van Loenen**[2#]

[#]Authors contributed equally, [1]T-knife GmbH, Berlin, Germany, [2]T-knife Therapeutics Inc., San Francisco, CA, USA

Background

PRAME-targeting T cell receptor (TCR) T cell therapy induces deep and durable responses in melanoma and sarcoma but has shown limited efficacy in other solid tumor indications, potentially due to suboptimal T cell engraftment and fitness, and immune suppression by the tumor microenvironment (TME). To tackle these challenges, TK-6302 was developed as a TCR-T candidate with best-in-class potential, incorporating a high-affinity TCR, a co-stimulatory CD8 co-receptor (co-stim CD8-CoR), and a FAS switch receptor (FAS-SwR). TK-6302 is manufactured using a non-viral, GMP-compliant, genome-editing process, that reliably produces pure and potent PRAME TCR-T cells, characterized by gene expression profiles associated with favorable clinical outcomes, including broad immune activation, proliferation, persistence, T cell fitness and tumor homing capacity. Tech transfer of the manufacturing process to a CDMO has been completed.

Methods

TK-6302 drug product (DP) was characterized using (a) 5 at-scale products manufactured in-house from healthy donors, (b) 1 at-scale product manufactured in-house from a melanoma patient, (c) 3 verification runs from healthy donors at CDMO after process lock, (d) 6 small-scale R&D products from NSCLC patients, and (e) 4 small-scale R&D products from healthy donors. Isolated CD4/CD8 T cells were non-virally genome edited to express PRAME-TCR, co-stim CD8-CoR and FAS-SwR with simultaneous knock-out of the endogenous TCR. TK-6302 was analyzed by flow cytometry for PRAME TCR expression, cell count, viability, knock-in (KI) rate, T cell purity, memory phenotype and activation/exhaustion. Deep characterization was performed by bulk RNA-sequencing. Functional activity was assessed by cytokine secretion and serial killing upon co-culture with cancer cells and advanced cancer spheroid models designed to mimic the immunosuppressive TME.

Robust manufacturing of TK-6302 with high yield and purity, and favorable phenotype

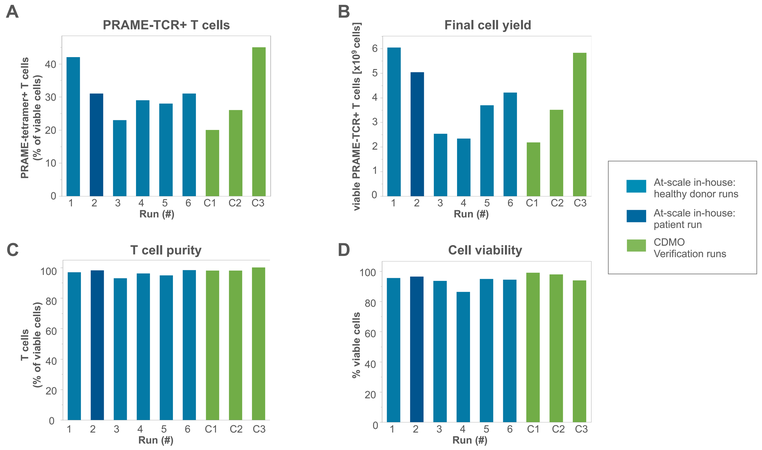


Figure 1. Manufacturing consistently results in high yield of pure TK-6302 products.
At harvest, cells were analyzed by flow cytometry using antibody panels and PRAME-tetramer to evaluate knock-in and T cell purity, and to calculate PRAME-TCR T cell number. **(A)** KI percentage measured by PRAME-TCR expression. **(B)** Final yield of therapeutic PRAME TCR-T cells. **(C)** Analysis of T cell purity, depicted as percentage of T cells in all viable cells. **(D)** Total viable cells. Notes: In-house run 1 and 2 were generated using R&D-grade sgRNAs, all other runs used GMP-grade sgRNAs.

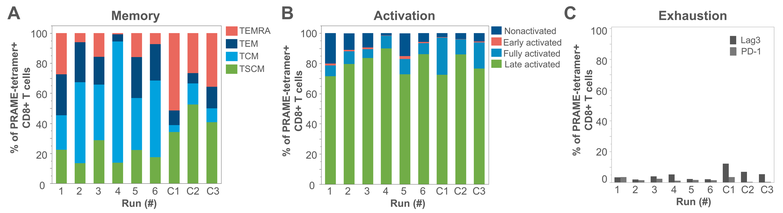


Figure 2. TK-6302 has a favorable memory / activation phenotype.
Memory, activation and exhaustion phenotype were assessed at harvest by flow cytometry. **(A)** Percentages of memory T cell populations based on CD197 and CD45RA expression. Terminally differentiated effector memory T cells (TEMRA) are CD197$^-$ CD45RA$^+$, effector memory T cells (TEM) are CD197$^-$ CD45RA$^-$, central memory T cells (TCM) are CD197$^+$ CD45RA$^-$, stem cell memory T cells (TSCM) are CD197$^+$ CD45RA$^+$. **(B)** T cell activation status was based on CD69 and CD25 expression. Nonactivated T cells are CD69$^-$ CD25$^-$, early activated are CD69$^+$CD25$^-$, fully activated are CD69$^+$CD25$^+$, and late activated are CD69$^-$CD25$^+$. **(C)** Minimal expression of Lag3 and PD-1 detected across drug products.

TK-6302 demonstrates robust and sustained functional activity

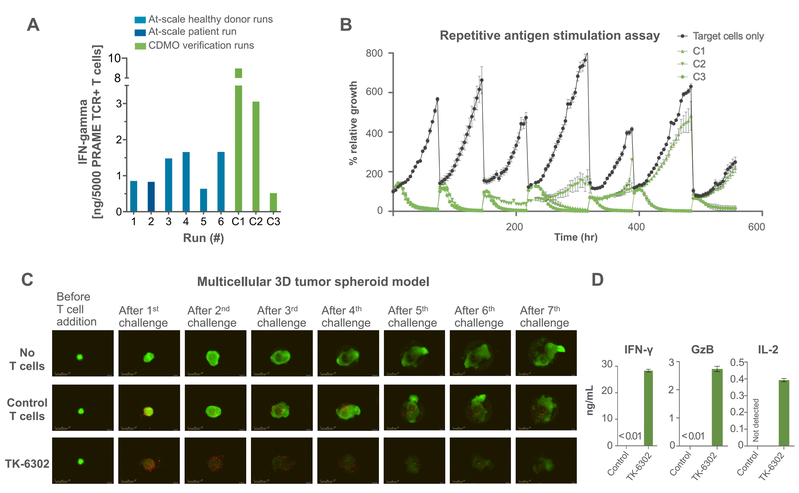
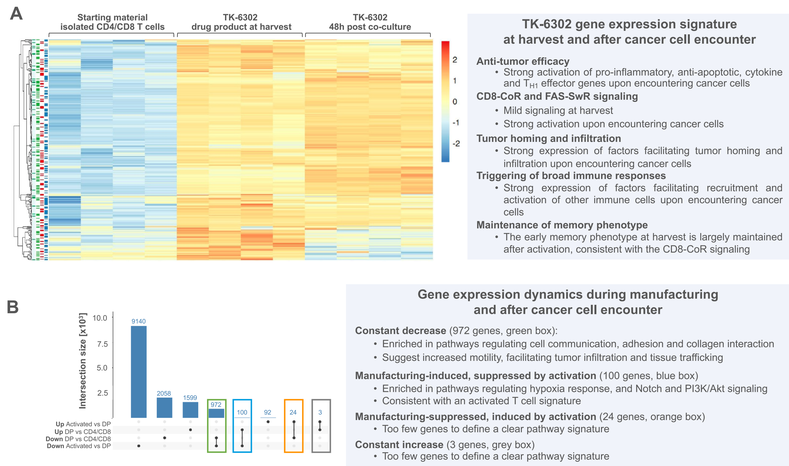


Figure 3. TK-6302 drug products manufactured at-scale secrete high levels of IFN-γ and exhibit durable cytotoxicity against cancer cells and in complex three-dimensional (3D) tumor spheroids.
(A) IFN-γ produced after 24hr activation with the PRAME peptide SLLQHLIGL presented on HLA-A*02:01. **(B)** Serial killing assay of TK-6302 DP against the melanoma cancer cell line Hs695T. Cytotoxicity was monitored with live-cell microscopy. Sequential panels depict re-challenge with freshly added cancer cells. TK-6302 DPs from at-scale runs (not shown) and verification runs were capable of 3 to 8 rounds of serial killing. **(C-D)** Anti-tumor efficacy of TK-6302 products 2, 3, 4, 5, and 6 was assessed in a complex 3D tumor spheroid model comprised of 2 types of lung cancer cells (NCI-H1703 cell line expressing either only TIGIT-ligands or TIGIT-ligands and PD-L1), monocytes (THP-1 cell line) expressing FAS-ligand and TIGIT-ligands, and fibroblasts (1BR.3.N). Panels (C) show representative microscopic pictures at the co-culture initiation and the end of each spheroid rechallenge (green: cancer cells; red: TK-6302). Depicted in (D) is mean cytokine secretion ± SEM of all 5 DPs measured after the 1st challenge. All evaluated TK-6302 products secreted cytokines and effector molecules, with reduced levels at advanced re-challenges.

TK-6302 gene expression signature is consistent with broad immune responses, T cell fitness, and tumor homing



TK-6302 gene expression signature at harvest and after cancer cell encounter

Anti-tumor efficacy
- Strong activation of pro-inflammatory, anti-apoptotic, cytokine and T_{H1} effector genes upon encountering cancer cells

CD8-CoR and FAS-SwR signaling
- Mild signaling at harvest
- Strong activation upon encountering cancer cells

Tumor homing and infiltration
- Strong expression of factors facilitating tumor homing and infiltration upon encountering cancer cells

Triggering of broad immune responses
- Strong expression of factors facilitating recruitment and activation of other immune cells upon encountering cancer cells

Maintenance of memory phenotype
- The early memory phenotype at harvest is largely maintained after activation, consistent with the CD8-CoR signaling

Gene expression dynamics during manufacturing and after cancer cell encounter

Constant decrease (972 genes, green box):
- Enriched in pathways regulating cell communication, adhesion and collagen interaction
- Suggest increased motility, facilitating tumor infiltration and tissue trafficking

Manufacturing-induced, suppressed by activation (100 genes, blue box)
- Enriched in pathways regulating hypoxia response, and Notch and PI3K/Akt signaling
- Consistent with an activated T cell signature

Manufacturing-suppressed, induced by activation (24 genes, orange box)
- Too few genes to define a clear pathway signature

Constant increase (3 genes, grey box)
- Too few genes to define a clear pathway signature

Figure 4. Gene expression analysis of TK-6302 drug products at harvest and upon co-culture with cancer cells indicates potential for high anti-tumor activity and broad immune cell activation.
Bulk RNA sequencing was performed on RNA isolated from starting material (isolated CD4/CD8 T cells from healthy donor apheresis), TK-6302 drug products manufactured at-scale with the clinical process ("DP") and TK-6302 48h after co-culture with the NSCLC cell line A549 ("Activated"). Gene expression and pathway activation analysis was performed by BioLizard. **(A)** Heatmap of T cell pathway related GO term genes: regulation of T cell activation (blue), TCR signaling (red), lymphocyte mediated immunity (green), regulation of Ig production (dark green). **(B)** Differential expression analysis using the Linear Mixed Model with Log2 fold-change threshold 2.5.
Manufacturing led to a transition toward a highly proliferative, metabolically active state, with strong activation of DNA repair and mitochondrial function, coupled with suppression of inflammatory responses. Co-culture with cancer cells was characterized by a marked functional shift with robust activation of T cell functions, DNA replication and repair, and mitochondrial biogenesis, alongside suppression of select adhesion processes.

Comparable phenotype and function of TK-6302 manufactured at small-scale from patients and healthy donors

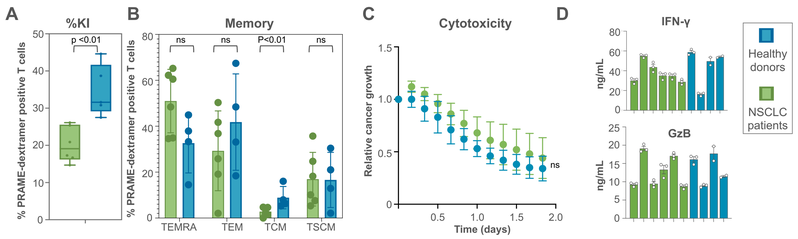


Figure 5. Comparable KI rate, memory phenotype, cytokine secretion and cytotoxicity of TK-6302 derived from non small cell lung cancer (NSCLC) patients and healthy donors.
A small-scale R&D process mirroring TK-6302 clinical manufacturing was used to generate TK-6302 from PBMCs of 6 NSCLC patients and 4 healthy donors. TK-6302 cells were analyzed at harvest and upon co-culture with cancer cells. **(A)** Percentage of KI as measured by PRAME-dextramer staining. **(B)** Percentages of memory T cell types based on CD197 and CD45RA expression, as indicated in Fig. 2A. **(C, D)** TK-6302 were co-cultured with GFP$^+$ HLA-A*02$^+$ PRAME$^+$ NSCLC cell line A549. (C) Cytotoxicity was assessed by time-lapse live-cell microscopy (D) Culture supernatants were collected after 48hrs for the assessment of cytokines. ns = not significant.

Encounter with cancer cells drives convergent transcriptional programs in TK-6302 derived from patients and healthy donors

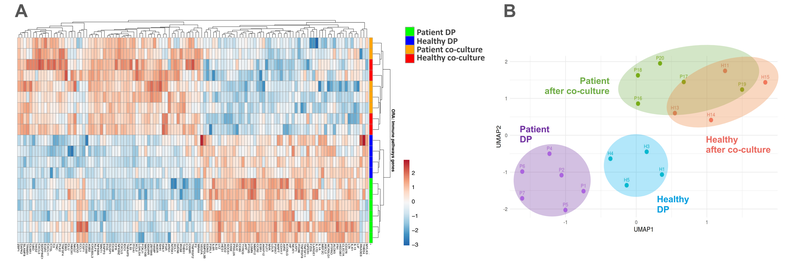


Figure 6. Gene expression profiles of TK-6302 derived from patients and healthy donors converge upon encountering cancer cells, despite initial differences at harvest.
Bulk RNA sequencing was performed on TK-6302 samples derived from 6 NSCLC patients and 4 healthy donors using a small-scale R&D process, both at harvest and after 48h co-culture with the NSCLC cancer cell line A549. Gene expression and pathway activation analysis was performed by BioLizard. **(A)** Over-representation analysis (ORA) and **(B)** UMAP analysis demonstrate that at harvest healthy donor- and patient-derived DP cluster separately. Upon co-culture with cancer cells, however, transcriptional profiles converge, reflecting activation of shared pathways. ORA revealed enrichment of biologically relevant pathways in both healthy donor- and patient-derived DPs, including leukocyte migration and chemotaxis, with stronger representation in patient-derived cells, suggesting that these are more primed for tumor trafficking. Upon co-culture with cancer cells, activated pathway repertoire is similar, but healthy donor-derived cells show stronger activation signatures, consistent with higher proliferative and metabolic fitness.

Conclusions

- The non-viral genome-editing manufacturing process consistently achieves high yield of pure TK-6302 drug products with a favorable phenotype
- All at-scale drug products demonstrate potent anti-tumor activity across multiple assays, including physiologically relevant 3D tumor models that mimic solid tumor barriers
- Transcriptomic profiling at harvest and following co-culture with cancer cells reveals a TK-6302 gene expression signature consistent with broad immune activation, enhanced tumor homing and sustained T cell fitness
- TK-6302 manufactured at small-scale from NSCLC patients and healthy donors exhibits comparable phenotype and functional potency. Despite initial transcriptomic differences at harvest, gene expression profiles converge after cancer cell encounter, indicating the beneficial effects of the high affinity PRAME-TCR, CD8-CoR and FAS-SwR
- TK-6302 manufacturing process has been locked, the tech transfer has been completed and a Clinical Trial Application has been submitted. First patient enrollment is planned for Q2 2026