Abstract #330

# Preclinical assessment of genome editing safety in CRISPR-engineered PRAME-targeting TK-6302 TCR-T product demonstrates editing precision and safety

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## 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. To overcome these challenges, we have developed TK-6302, a PRAME targeting TCR-T cell therapy 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 (SwR). TK-6302 is manufactured with a non-viral, GMP-compliant, gene editing process (see Poster #347) that increases PRAME TCR expression on the cell surface enhancing efficacy and prevents TCR mispairing, ensuring safety. Comprehensive analysis of TK-6302 drug products manufactured at-scale with the clinical process showed high levels of knock-in and knock-out editing, without concerning off-targets and chromosomal aberrations.

## TK-6302 genome editing process

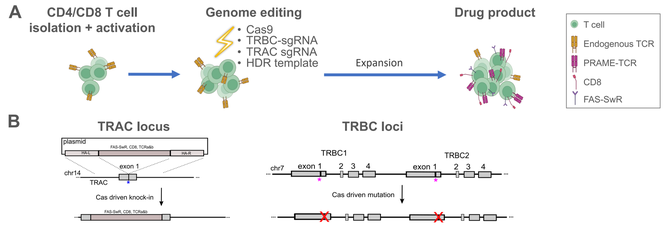


**Figure 1. TK-6302 editing process results in simultaneous endogenous TCR knock-out (KO) with knock-in of (KI) of PRAME-TCR, co-stim CD8-CoR and FAS-SwR.**
(**A**) Schematic of TK-6302 manufacturing process: Isolated CD4+ and CD8+ T cells are activated, electroporated with a recombinant Cas9, two sgRNAs targeting TRAC and TRBC loci, and a plasmid functioning as a template for homologous DNA repair (HDR). (**B**) Left panel shows TRAC targeting: A Cas9 induced double strand break (DSB) on TRAC exon 1 is repaired by HDR copying-in coding sequence for the PRAME TCR, CD8-CoR, and FAS-SwR. Small mutations KO endogenous TCRα chain expression, avoiding mispairing with the introduced PRAME-TCR chains. Right panel shows TRBC targeting: A single gRNA drives Cas9 breaks on both TRBC1 and TRBC2 due to sequence homology. KO of endogenous TCRβ chain expression avoids mispairing with the introduced PRAME-TCR chains and drastically increases TCR expression rendering TK-6302 a highly efficient and safe product.

## TK-6302 analysis methods

Four TK-6302 drug products manufactured at-scale were analyzed for on-target and potential off-target editing. **dPCR** was used to measure on-target transgene integration (Fig. 2) and translocations (Fig. 7). **Amplicon sequencing** was used to measure small mutations at on-target and off-target sites (Fig. 2, Fig. 7). **Directional genomic hybridization** (dGH In-Site™) was used to monitor zygosity of knock-in (Fig. 3). Tapestri, a **single cell sequencing** method combining amplicon sequencing with analysis of surface protein expression, was used to determine global editing events on a per cell basis and **target locus amplification** (TLA) was used to confirm integrity and location of integration of the multicistronic transgene (Fig. 4). **ONE-Seq** and **DEUX-Seq** were used to nominate off-target sites, and multiplexed amplicon sequencing (**rhAMP-Seq**) was used to confirm off-target sites (Fig. 5). In addition, ONE-Seq was used to derisk Cas9 sites containing poly-morphisms (Fig. 6). **G-banding** was carried out to understand chromosomal integrity of the product (Fig. 7).

## Conclusions

**TK-6302 demonstrated high editing precision and favorable preclinical safety profile:**

- ✓ **Editing efficiency:** ~40% KI rate, including ~10% homozygous KI
- ✓ **Editing precision:** Edits predominantly observed at all 3 target sites, with full and correct integration of the transgene
- ✓ **Functional safety:** Effective TRBC1 and TRBC2 KO minimizes TCR mispairing, enhancing product safety and promoting robust PRAME TCR expression
- ✓ **Off-target analysis:** A single, previously characterized low frequency off-target site was detected, posing no safety risk
- ✓ **Genomic integrity:** No disruptive or concerning on-target or off-target polymorphisms detected. Expected translocations between on target sites occurred at low frequency, with no chromosomal aberrations of safety concern observed

**TK-6302 manufacturing process has been locked, and Clinical Trial Application has been submitted with first patient enrollment planned in Q2 2026**

## TK-6302 cells demonstrate efficient on-target genome editing

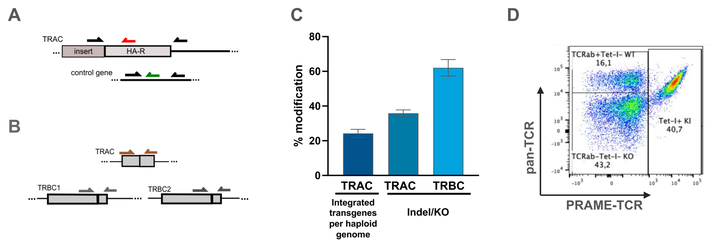


**Figure 2. On-target genome editing analysis of four TK-6302 drug products demonstrate effective KO of TRAC and TCRB and efficient KI of the transgene in the TRAC locus.**
(**A**) TRAC KI is quantified by dPCR using probes flanking the homology arm. (**B**) Small Indel mutations at TRAC and TRBC loci are measured by amplicon deep sequencing. (**C**) On-target editing (given as transgenes per haploid genome) and Indel frequencies for TRAC and TRBC1/2. Data of n = 4 drug products, shown as mean ± SEM. (**D**) Representative flow cytometry plot of TCR surface expression (protein level) demonstrating close alignment with the genetic measurements of gene editing.

## TK-6302 cells exhibit both homozygous and heterozygous knock-in with more frequent heterozygous integration

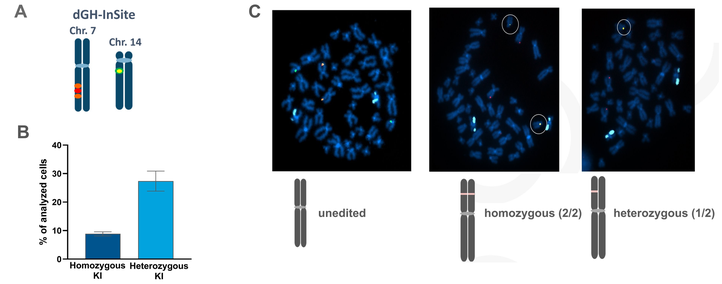


**Figure 3. Homozygosity and heterozygosity KI quantification at single cell level shows efficient KI, with heterozygous alleles being more frequent.**
(**A**) The directional genomic hybridization method shows all chromosomes in a cell and uses probes to label the TRAC site (green: unedited TRAC locus; yellow: integrated transgene; orange: unedited TRBC loci; red: sequence between TRBC cut sites). (**B**) Percentage of cells with homozygous (both alleles edited) and heterozygous (only one allele edited) KI frequency as measured by dGH-InSite. Data of n = 4 drug products, shown as mean ± SEM. (**C**). Representative pictures of dGH InSite™. Data on KI frequency is in alignment with flow cytometry data assessing TCR protein expression at the cell surface.

## Edited TK-6302 cells harbor mutations at all target sites, with complete transgene cassette integration at the TRAC locus

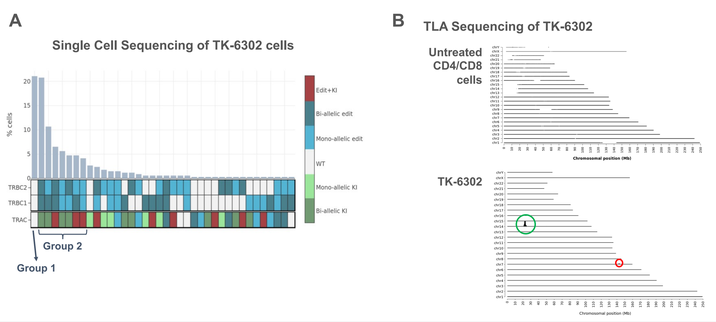


**Figure 4. Single cell sequencing of genome-editing target sites shows that most TK-6302 cells are edited at all target sites.**
(**A**) Single cell analysis (Tapestri®) shows two major cell type groups: Group 1 is completely unedited cells and group 2 is the highly edited cells with a knock-in or mutation at all target sites. One representative out of 4 drug products is displayed. (**B**) NGS sequencing using Targeted Locus Amplification showed correct transgene integration into the expected TRAC site on chromosome 14 (green circle) and a small amount of integration into TRBC on chromosome 7 (red circle). All HDR integrations of the transgene at TRAC site support full integration of the transgene with no partial integration (data not shown).

## Comprehensive off-target analysis identified only one known, low frequency site

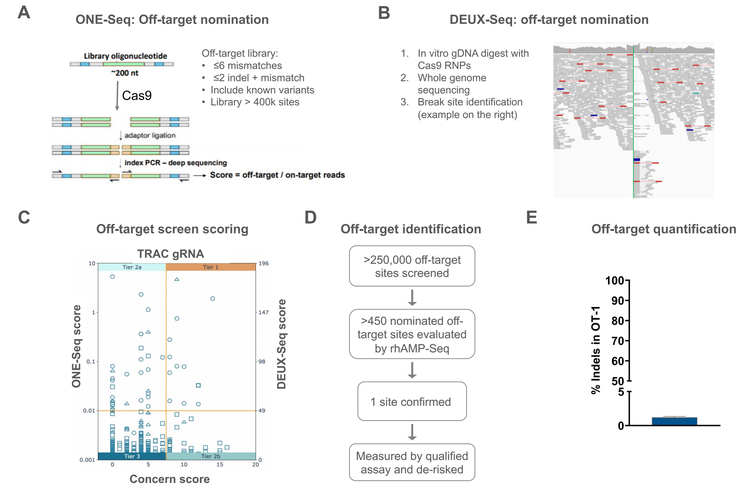


**Figure 5. Comprehensive Cas9 off-target site screening using orthogonal methods did not identify off-targets of concern.**
(**A**) A library comprising >400,000 potential off-target sites nominated by homology-based genomic location enumeration was screened using ONE-Seq. (**B**) DEUX-Seq was used to orthogonally nominate sites using genomic DNA of one healthy donor. (**C**) Cutting frequency of potential editing sites nominated by ONE-Seq or DEUX-Seq were plotted, and a concern score was generated for each site. All sites with moderate to high cutting scores were further tested. Sites with low cutting activity scores are unlikely to be functional off-target sites but were also further tested if located in critical genomic regions. (**D, E**) Multiplexed amplicon sequencing (rhAMP-Seq) confirmed only one off-target site (off-target 1, OT-1) with mutations above threshold (D), and a qualified amplicon sequencing assay was used to quantify OT-1 mutations (E). Data of n = 4 drug products, shown as ± SEM. OT-1 has been de-risked and does not pose a risk to the drug product safety.

## No polymorphisms of concern detected in TK-6302

### No deleterious on-target polymorphisms detected

- 1 SNP in TRAC and 3 SNPs in TRBC target site identified
- All TRAC and TRBC target site SNPs occur in ≤0.1% global population
- SNPs do not inhibit Cas9 cutting based on ONE-seq biochemical data

### No occurrence of concerning off-target sites with polymorphisms

- 92k TRAC and 65k TRBC off-target sites containing polymorphisms identified
- All polymorphic sites synthesized and digested to in-vitro by ONE-Seq
- Off-target sites containing polymorphisms identified and de-risked using cutting frequency, allelic frequency, and location in the genome
- No sites of concern were identified

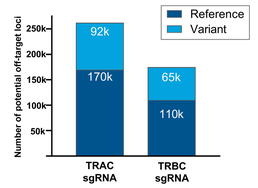


**Figure 6. No polymorphisms affecting on-target and off-target editing identified using ONE-seq with oligos designed against reference and variant genomes.**
ONE-Seq libraries were designed against HG38 and CHM13 human reference genomes, and variant sites were designed against 76,156 globally diverse genomes from population sequencing initiatives.

## Chromosomal integrity analysis reveals rare translocations and other structural aberrations, consistent with a favorable safety profile

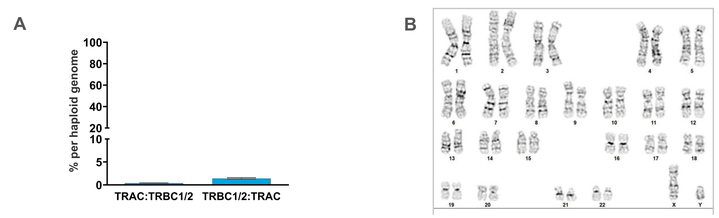


**Figure 7. Chromosomal integrity analysis by dPCR and G-banding showed rare structural aberrations**
(**A**) Expected chromosomal translocations were measured by dPCR using translocation spanning primers and a reference standard. Results are given as percentage of haploid genome. TRBC or TRAC to OT-1 translocations were lower than the detection threshold (<0.1%) (**B**) Representative image of unbiased overall chromosomal integrity assessment using G-banding. Metaphase spreads of TK-6302 showed some aneuploidy in chromosome 14 in one sample out of 4 tested but not in chromosome 7. No chromothripsis, chromosome truncations, nor other large aberration was observed in the drug products at statically significant levels.