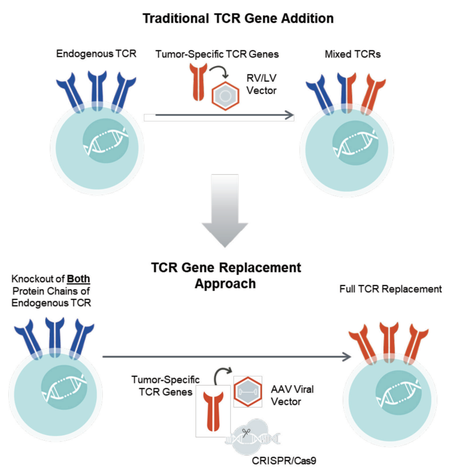
Engineering of Highly Functional and Specific Transgenic T Cell Receptor (tg-TCR) T Cells Using CRISPR-Mediated In-Locus Insertion Combined with Endogenous TCR Knockout

A. Prodeus*, S. Yazinski*, I. Dutta, P. Vinay, S. Koristka, H-R Huang, D. Cook, S. Kumar, J. Jones, A. Forget, M.S. Arredouani, **B.C. Schultes**
Intellia Therapeutics, Inc., Cambridge, MA, USA
*Authors contributed equally

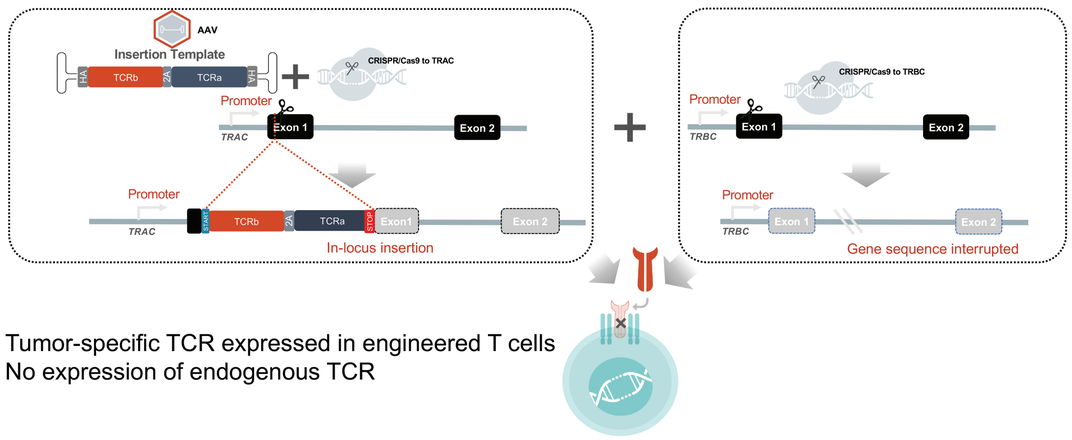
INTRODUCTION



Adoptive transfer of T cells genetically modified to express tumor-specific TCRs for the treatment of cancer has garnered considerable attention in recent years. However, current approaches have certain limitations and safety concerns.

- Insertion of transgenes into T cells has been historically accomplished using retroviral or lentiviral vectors
  - T cells express both endogenous and transferred TCR α/β chains
  - Mispairing of endogenous and transferred chains can occur
  - Diminished expression of tumor-specific TCR
  - Reduced function
  - Unknown specificity
- TCR replacement generates engineered T cells with tumor-specific receptors only

CRISPR/Cas9 mediated cut at the TRAC locus allows for TCR α chain knockout and efficient targeted integration of the transferred TCR using an AAV template. A simultaneously delivered second guide knocks out the TCR β chain at TRBC1/2 loci.



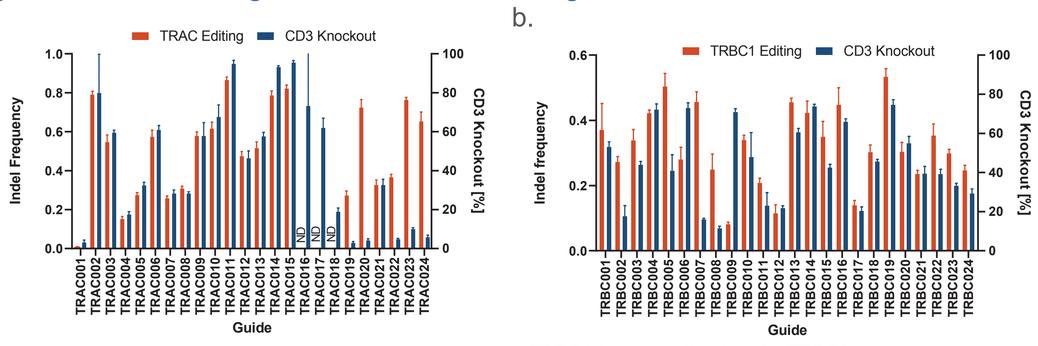
Tumor-specific TCR expressed in engineered T cells
No expression of endogenous TCR

RESULTS

CRISPR/Cas9 GUIDE SELECTION

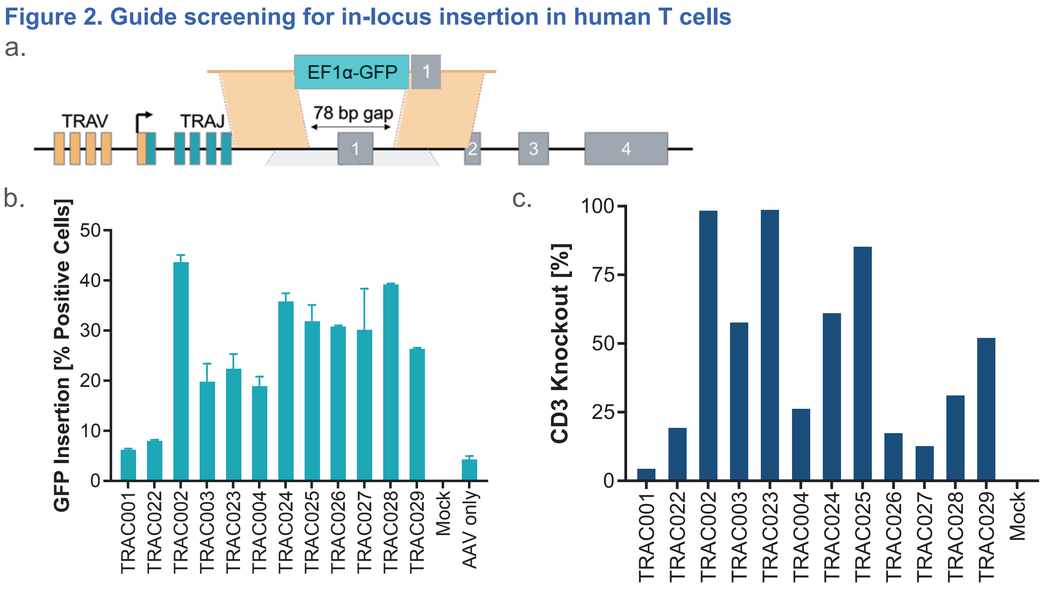
We identified guide RNAs targeting TRAC (Fig. 1 a) and TRBC1/2 (Fig. 1 b) loci with high indel frequency and specificity. Additionally, we identified TRAC guides RNA that yielded high insertion rates for AAV templates encoding a fluorescent marker or different TCRs (Fig. 2).

Figure 1. Guide screening for TRAC and TRBC editing in human T cells



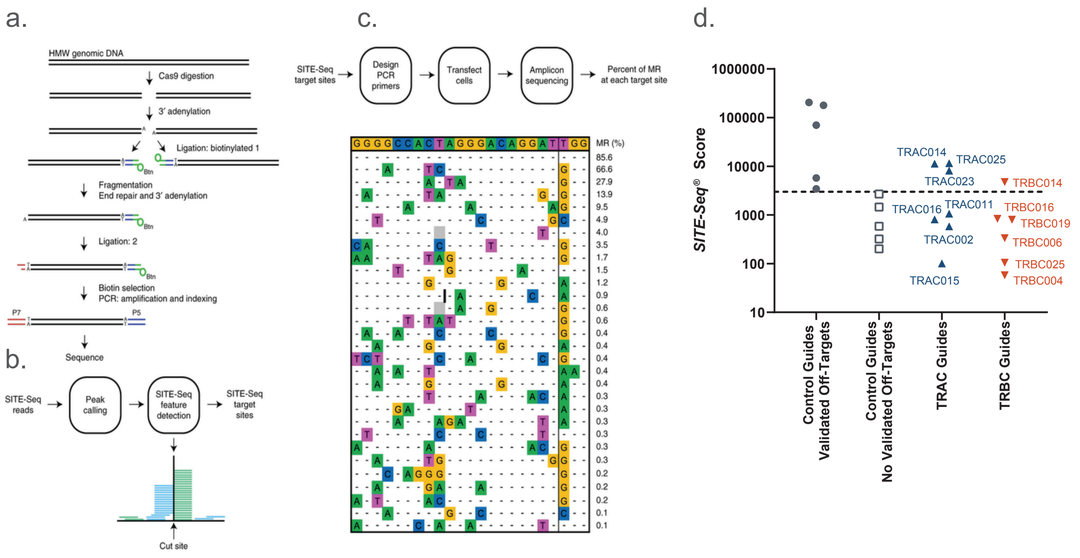
TRBC1 locus, similar data for TRBC2 locus

Figure 2. Guide screening for in-locus insertion in human T cells



a. GAP AAV templates enable screening of multiple guides with a single repair template; b. GFP insertion into TRAC locus across multiple guides; c. Knockout of endogenous TCR by TRAC guides

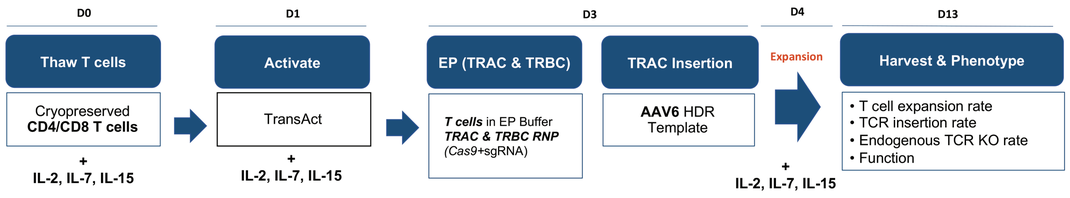
Figure 3. Screening guides for off-target



a. *SITE-Seq*® biochemical method overview; b. NGS sequencing evaluation to identify cut-sites; c. Example of a *SITE-Seq*® editing pattern; d. *SITE-Seq*® guide ranking for lead TRAC and TRBC guides.

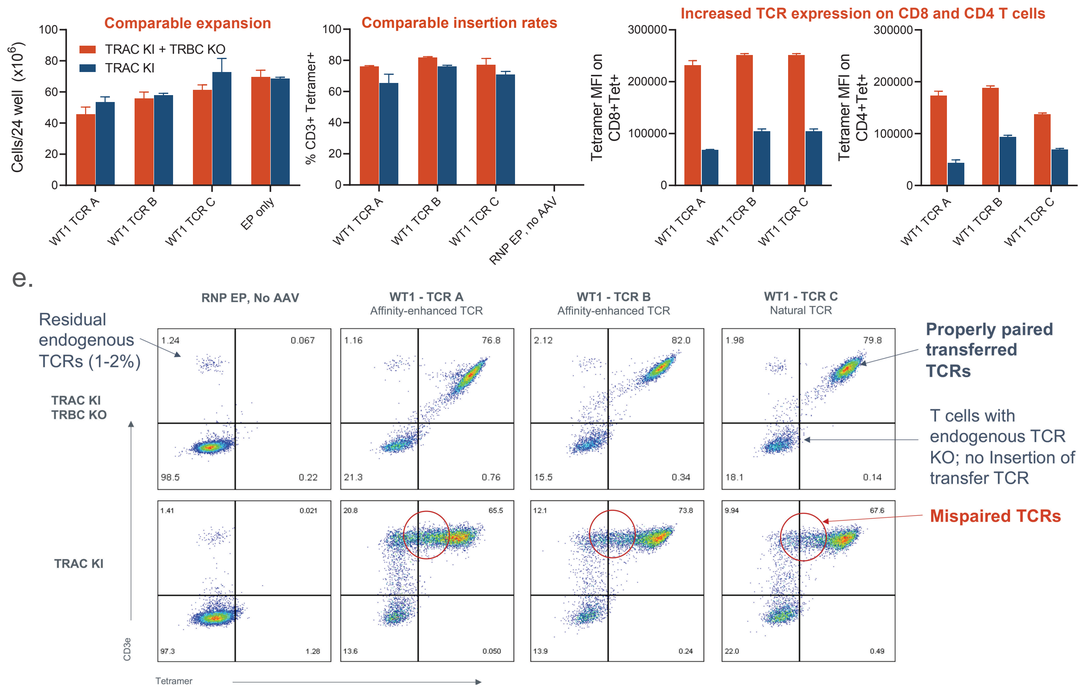
T CELL PRODUCTION AND CHARACTERIZATION

Figure 4. T cell production method for highly efficient in-locus insertion



In this study, the edited tg-TCR T cells expanded comparably to non-edited T cells (Fig. 5 a) and exhibited low levels of exhaustion. Transgenic T cells with edited endogenous TRAC and TRBC loci showed significantly improved tg-TCR expression levels (Fig. 5 c, d) and greatly reduced mispairing (Fig. 5 e) as compared to cells that were edited only for TRAC.

Figure 5. Highly efficient in-locus insertion of different Wilms Tumor 1 (WT1)-specific TCRs with high T cell viability and expansion

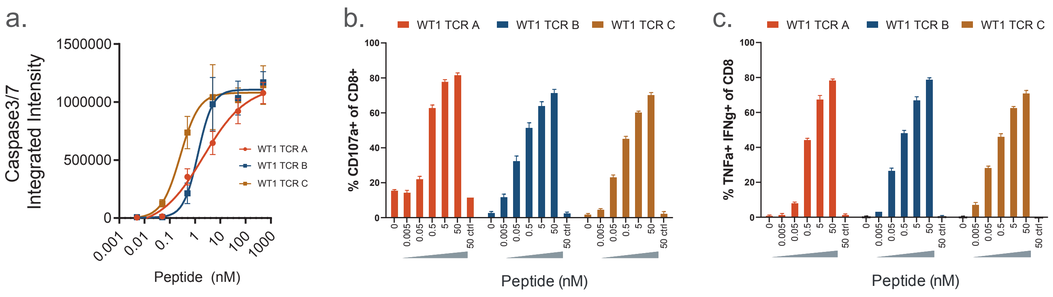


a. T cell expansion in a 24-well G-Rex; b. TCR insertion rates as measured by tetramer staining; c.d. TCR expression levels as measured by tetramer staining in CD8 (c) and CD4 (d) T cells. e. FACS plots showing CD3 and tetramer staining of control T cells and T cells with three different WT1-specific TCRs inserted into the TRAC locus, either with or without simultaneous TRBC KO. Mispairing is evident with all TCRs; however, different TCRs are affected to different degrees.

T CELL FUNCTIONAL ANALYSIS

Functional characterization of Wilm's Tumor 1-specific tg-TCR T cells shows that TCR insertion into the TRAC locus results in functionally reactive T cells with robust on target activity (Fig. 6).

Figure 6. Highly functional WT1-specific TCRs using targeted integration plus endogenous TCR Knockout



a. Killing of peptide pulsed T2 cells in an Incucyte assay. b. T cell degranulation in response to peptide pulsed T2 cells. c. Cytokine production in response to peptide pulsed T2 cells (intracellular staining for TNF-α and IFN-γ).

CONCLUSIONS

TCR replacement using CRISPR/Cas9-mediated genome editing showed:

- Loss of >98% endogenous TCRα and β chains
- 70-80% in-locus insertion rates of tg-TCRs without further purification
- Comparable expansion and memory phenotype to non-edited T cells
- Generation of functionally reactive T cells with robust on-target activity
- Significantly improved tg-TCR expression levels and reduced mispairing for TRAC/TRBC edited T cells as compared to cells that were edited only for TRAC
- TCR replacement versus TCR addition may lead to improved tg-TCR-T cell therapies for the treatment of hematological and solid cancers