An all-Prime Editing one-step approach for non-viral generation of a multiplex-edited CAR-T cell product

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Background

Multiplex editing may be able to address limitations of CAR-T cell therapy:

- Manufacturing time, costs, and yield for autologous cell therapy cell quantity and quality issues could be addressed by using allogeneic T cells
- Limited efficacy against solid tumors

Current strategies for delivery and expression of CAR transgenes are limited by:

- Semi-random integration via lentivirus or transposons risks unintended gene disruption or activation of proto-oncogenes
- Targeted integration using nuclease + template for HDR limited by low efficiency and risks associated with DSB induction (e.g., chromothripsis, p53 activation)

Limitations of current strategies for multiplex editing:

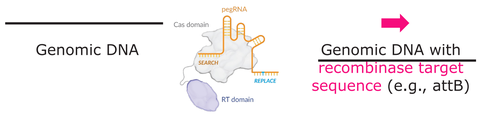
- Targeted gene disruption at multiple loci simultaneously with nucleases carries a risk of chromosomal rearrangements
- Base editing to disrupt splicing or introduce pmSTOP codons is limited in scope, risks pmSTOP readthrough, and cannot support targeted integration

PASSIGE™ in combination with multiplex Prime Editing (PE) may be able to overcome these challenges to create a potentially best-in-class allogeneic CAR-T cell product

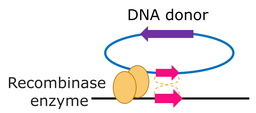
Methods

Prime Editing Assisted Site-Specific Integrase Gene Editing (PASSIGE): Prime Editing in combination with recombinases for targeted integration of gene-sized DNA

Prime Editing to install a recombinase target sequence



Site-specific recombination

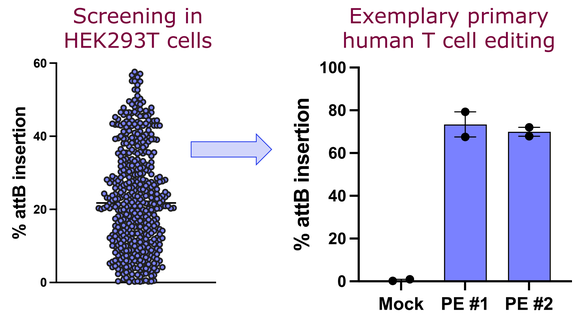


Gene-size DNA inserted at precise genomic location

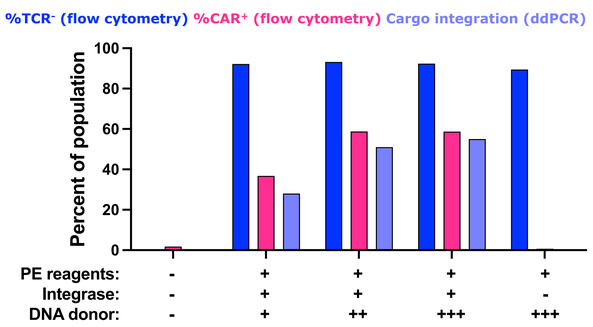
- ✓ Targeted integration of DNA in a **single delivery step**
- ✓ **No double strand break (DSB)** as integrase catalyzes recombination directly
- ✓ **Integration can be irreversible**: e.g., attL and attR products are distinct from initial attB and attP sequences

Results

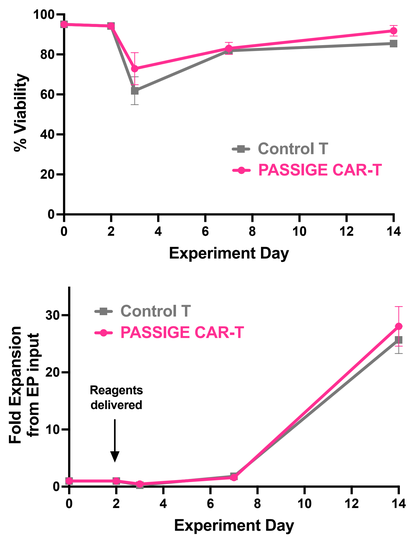
Dual-flap Prime Editing supports efficient attB insertion at *TRAC* locus



Single-step PASSIGE mediates insertion of CD19-CAR at *TRAC* locus, with >90% TCR loss and CD19-CAR expression in 60% of cells

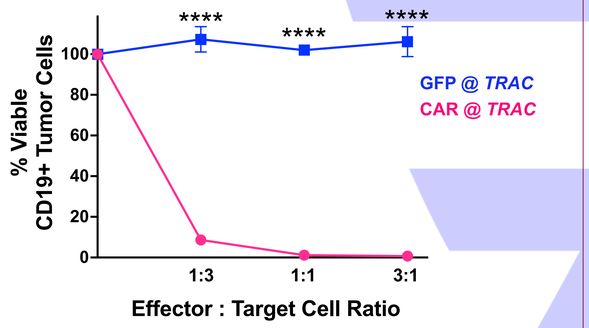


PASSIGE mediated integration of CD19-CAR does not reduce T cell viability or expansion

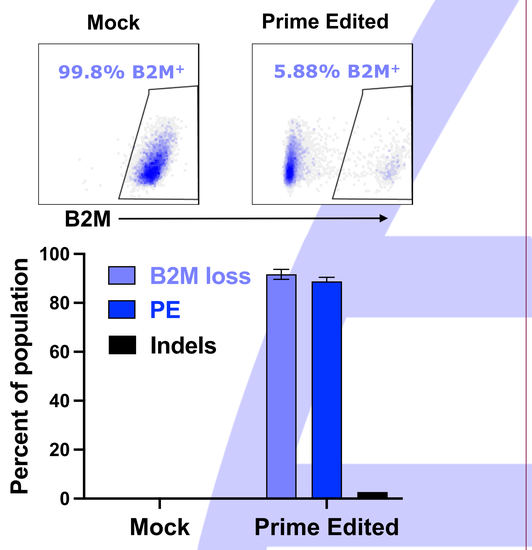


Results

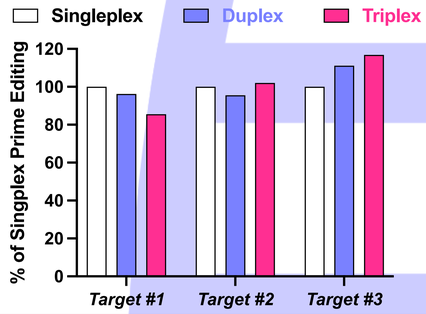
PASSIGE-generated CAR-T cells show robust killing of CD19⁺ Luciferase⁺ Nalm-6 tumor cells



Prime Editing supports efficient knockout of B2M expression in T cells with low indels

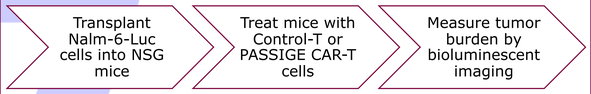


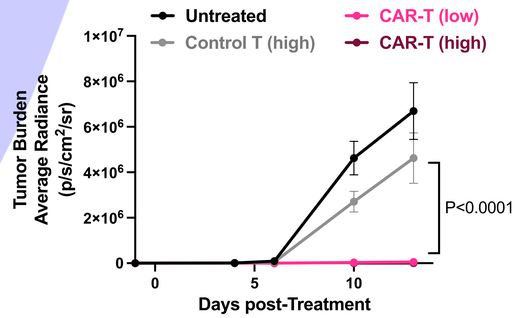
Efficiency of Prime Editing at individual target sites is maintained in multiplex context in primary human T cells



Results

PASSIGE-generated CD19 CAR-T cells reduce tumor burden *in vivo*





Two-way ANOVA with Tukey post-test

Conclusions & Future Directions

Demonstrated robust disruption of relevant target genes (*TRAC* and *B2M*) via Prime Editing in primary human T cells

Efficient Prime Editing is demonstrated in multiplex context at three genomic target sites in T cells

PASSIGE-generated CD19 CAR-T cells show potent anti-tumor activity *in vitro* and *in vivo*

Beyond Proof-of-Concept: PASSIGE and Prime Editing provide a modular, one-step system that has the potential to create a best-in-class CAR-T cell product:

- ✓ **Allogeneic / off-the-shelf**
- ✓ **Targeted specifically to the tumor**
- ✓ **Potent and persistent**