Multiplex Prime Editing and PASSIGE™ for Non-Viral Generation of an Allogeneic CAR-T Cell Product

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Background

Multiplex Prime 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
- Safety risks associated with semi-random integration and double strand breaks at multiple genomic loci

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

- Semi-random integration via lentivirus or transposons risks unintended gene disruption of 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

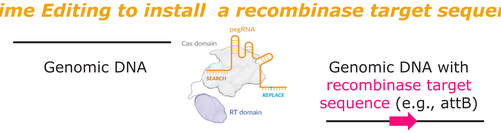
- Targeted gene disruption at multiple loci simultaneously with nucleases carries a risk of chromosomal rearrangements
- Base editing to disrupt splicing or introduce pmSTOP codons is limited in scope, risks pmSTOP readthrough, and cannot support targeted integration

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

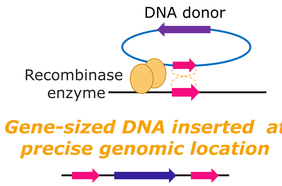
Methods

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

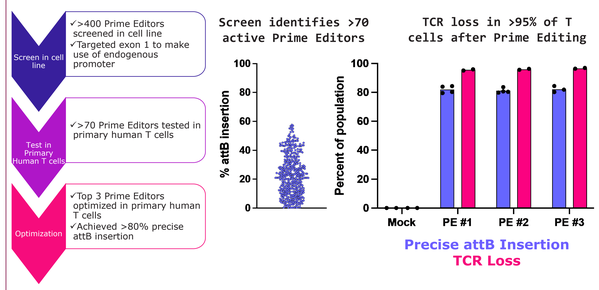


Gene-sized DNA inserted at precise genomic location

- ✓ 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

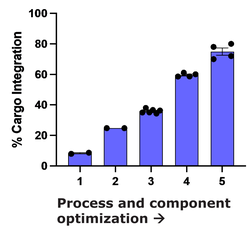
High performing Prime Editors precisely insert attB sequence at TRAC locus with >80% efficiency



Non-viral PASSIGE delivery supports integration of >3.5 kb CD19 CAR construct at TRAC locus in >70% of T cells

- ✓ Loss of endogenous TCR with attB insertion in *TRAC* exon 1
- ✓ Use of endogenous *TRAC* promoter allows for tuned regulation of expression
- ✓ Promoter-less cargo will not express if integrated elsewhere in genome

Ongoing PASSIGE component and process optimization leads to higher cargo integration efficiency

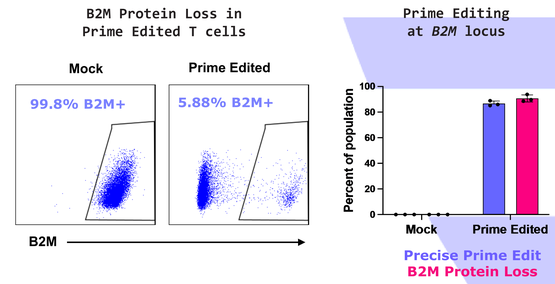


Achieved up to 80% integration of 3.5-kb cargo

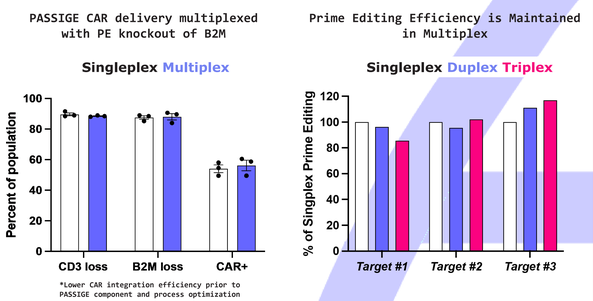
- ✓ Prime Editor configuration (protein and pegRNAs)
- ✓ Recombinase
- ✓ DNA donor
- ✓ Dose
- ✓ Timing of delivery

Results

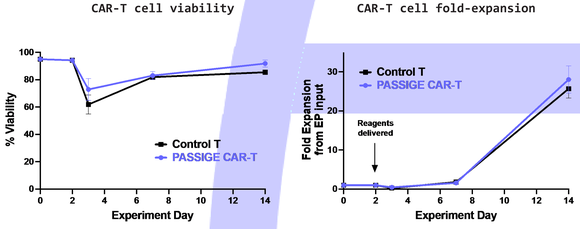
High performing Prime Editors disrupt B2M expression with >90% efficiency



Prime Editing and PASSIGE efficiency are maintained in multiplex context

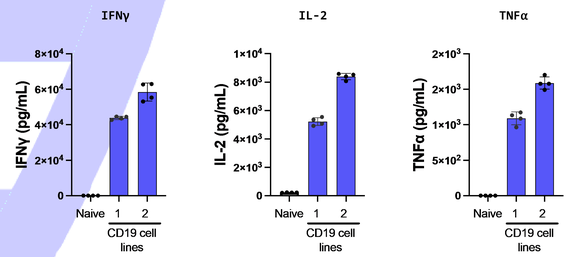


T cell health is maintained: PASSIGE-mediated integration of CD19 CAR does not reduce T cell viability or fold-expansion

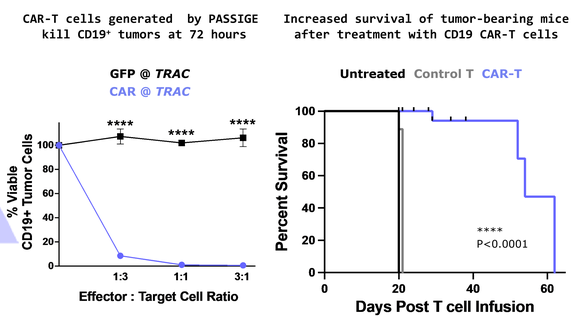


Results

PASSIGE-generated CD19 CAR-T cells produce pro-inflammatory cytokines after exposure to CD19+ cell lines



CAR-T cells generated via PASSIGE have potent cytotoxicity against CD19+ cells in vitro and in vivo



Summary & Next Steps

- **PASSIGE is efficient for non-viral, site-specific delivery of large cargo to primary human T cells**
- **Achieved >80% site-specific integration of CD19 CAR through systematic PASSIGE component and process optimization**
- **PASSIGE can be multiplexed with Prime Editing at other target sites by non-viral one-step delivery with no loss of efficiency**
- **PASSIGE-generated CAR-T cells are healthy and show potent antigen-specific function and cytotoxicity**
- **Next: apply Prime Medicine's comprehensive suite of assays to CAR-T product for off-target discovery**
- **Next: potential for additional multiplex Prime Edits to improve CAR-T properties**