Docket #: S23-103

Touchless Selection of Gene Modified Cell Therapies Through TRAC Intron Knockins

Genetically modified cell therapies, such as CAR-T therapies, have revolutionized cancer treatments. However, the process of making the necessary genetic modifications to these cells can be inefficient, especially targeted genetic editing using CRISPR/Cas9 systems, leaving a mixed cell population of some edited cells mixed with many unedited or incorrectly edited cells. Prior studies have shown that targeted integration of a chimeric antigen receptor (CAR) or new synthetic T-cell receptor (TCR) at the TCR alpha chain locus (TRAC) yields cells with improved functionality. To date this has always been accomplished by knockin of the new CAR or TCR into one of the exons of the TRAC gene. This yields a population of cells that includes some knockin cells, but also many TCR knockout cells, as the dominant editing outcome in cells that do not receive a knockin is a short indel (small insertion or deletion) that causes a frameshift mutation and subsequent knockout of the TRAC gene. This leads to a population of cells where both the edited and unedited cells do not have a T cell receptor.

The Satpathy Lab at Stanford has developed a technology that enables successfully edited cells to be enriched without cumbersome selection markers or antibody binding - a touchless selection method that enables maximal functional performance of the enriched edited cell population while removing unwanted and potentially unsafe, unedited and incorrectly edited cells. This new technology target CARs into an intron of the TRAC gene, rather than the traditional exon targeting. By knocking in a synthetic exon containing the CAR to a TRAC intron, successfully edited cells will have the CAR spliced into the final TRAC mRNA sequence, resulting in expression of the CAR and knockout of the TCR. In contrast, unsuccessfully edited cells, which predominantly will have an indel at the intronic target site, will still maintain expression of their TCR. The mutated base pairs will all be spliced out during mRNA

processing, unlike the frameshift mutations seen with exonic TRAC targeting. This yields a population of cells where the successfully edited cells do not have a TCR, while the unsuccessfully edited cells all have a TCR. By binding antibodies or magnetic beads to the TCR and performing a negative selection, only the successfully edited TCR negative cells will remain for further research or clinical use without having to bind any reagents to the selected cells. This low-cost, effective, and high throughput method will be advantageous for cell therapy manufacturing.

Stage of Development
Proof of Concept

If interested in this technology, please reach out to us by March 30, 2025.

Applications

- Cell therapy manufacturing
- CAR-T cells
- Genomic and cell editing

Advantages

- High throughput, as compared to Fluorescence Activated Cell Sorting (FACS) and other currently available strategies
- Uses negative selection, eliminates the need for exogeneous gene markers
- Reduces risk of immune response against modified cells, by removing exogenous genes
- Efficient and low-cost method

Publications

- Theodore L. Roth, Johnathan Lu, Alison McClellan, Oliver Takacsi-Nagy, Ansuman T. Satpathy (2024). "Non-viral Intron Knockins Enable Simplified and Flexible Targeting of Endogenous Genes" *bioRxiv*, 2024.03.05.582227.

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