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Efficient Homology-Independent Solution for Precise Targeted DNA Insertions in Human T-cells

Stanford researchers have developed a non-viral, homology-independent method for precise targeted DNA insertions into T-cells using electroporation and CRISPR/Cas9, enabling cost-effective production of CAR T-cells for T-cell therapies.

T-cell therapies, especially chimeric antigen receptor (CAR) T-cell treatments, have shown significant promise in oncology, offering hope to patients with cancers resistant to traditional therapies. However, the current manufacturing processes face significant bottlenecks and inefficiencies. Existing methods primarily rely on viral vectors for genetic modifications, and these methods present drawbacks such as random integration issues, high costs, and stringent safety protocols, limiting their accessibility to a broader patient population. Current methods for CRISPR/Cas9 targeted gene insertion rely on homology directed repair (HDR) for genetic modifications. Homology independent targeted gene insertion provides an alternative innovative approach for T cell engineering. Additionally, our homology independent approach allows for engineering of resting T cells, unlike HDR-mediated approaches. There is an urgent need for innovative methods that perform non-viral targeted DNA insertions for manufacturing T-cell therapeutics.

To address these issues, Stanford researchers have developed a novel approach that directly addresses the limitations of current T-cell therapeutic manufacturing methods by introducing a fully non-viral, homology-independent method for targeted DNA insertions into T-cells to produce therapeutic T-cells. By leveraging electroporation to introduce therapeutic transgenes and delivered, for instance, as nanoplasmid or minicircle DNA along with CRISPR/Cas9 Ribonucleoprotein (RNP), this method ensures precise insertion of transgenes at desired loci using the non-homologous end joining (NHEJ) pathway. Post-electroporation, T-cells are enriched

for the inserted transgenes using either drug-based systems or surface markers, resulting in highly pure CAR T-cells. This innovative technology significantly reduces manufacturing costs compared to viral transduction methods, facilitating broader clinical application and accessibility of novel adoptive T-cell therapies.

Stage of Development:

Pre-clinical. Next steps include genetic safety evaluations and automated scale up of manufacturing process for non-viral targeted CAR T cells. This process will then serve for clinical manufacturing within clinical trials.

Applications

- Production of autologous and allogeneic CAR T-cells for adoptive T-cell therapies

Advantages

- Precise targeted integration, eliminating the risks associated with random integration
- Non-viral and homology-independent approach
- Reduces manufacturing costs and safety concerns, when compared with viral vectors
- Increased efficiency and higher yields of CAR T cells for patient treatments
- Allows for larger genetic payloads without compromising cell viability
- Accessible, effective, and scalable T-cell therapies

Publications

- Balke-Want, H., Keerthi, V., Gkitsas, N. et al. Homology-independent targeted insertion (HITI) enables guided CAR knock-in and efficient clinical scale CAR-T cell manufacturing. Mol Cancer 22, 100 (2023).

Patents

- Published Application: WO2024129984

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