CellFE | syenex

CellFE CAR-T Cell Editing

A "Hybrid" Approach for Complex Gene Editing: CAR T Editing via Nonviral Knockout and Viral Knockin

In the rapidly evolving field of cellular engineering, achieving complex gene edits with high efficiency and minimal workflow disruption remains a significant challenge. CellFE has developed a novel hybrid workflow combined with the use of Syenex virus that leverages the strengths of nonviral gene editing and viral transduction to overcome these challenges. This approach leverages CellFE's delivery technology to complement Syenex's next-generation T cell vector system ("SNX-T1"). The integrated approach couples nonviral knockout (KO) with viral knockin (KI) to create a highly efficient, single-day workflow for complex gene editing.

The hybrid workflow begins with nonviral KO using CellFE's microfluidic platform. This step is followed by Syenex's SNX-T1 KI transduction, both performed on the same day to streamline the process. By combining these processes, researchers can achieve complex edits, including simultaneous KO and KI, with a shortened overall prrocess time and improved workflow efficiency.

Key Benefits of the CellFE Hybrid Workflow

Enhanced Complexity of Edits: CellFE nonviral KO provides a safer, scalable, and precise method for T cell receptor (TCR) and immune checkpoint knockouts. Coupled with SNX-T1 KI, this enables advanced multi-gene editing strategies and improved CAR positivity.

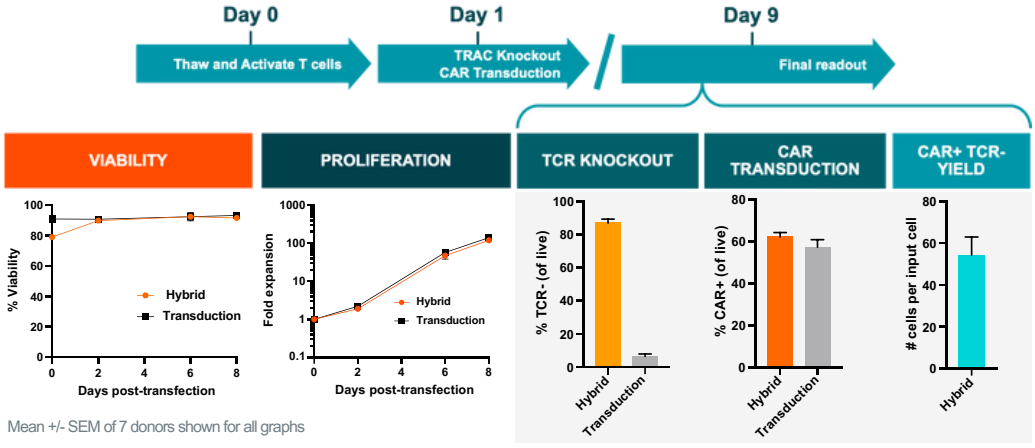
Optimized Workflow: By enabling nonviral transfection and viral transduction on the same day, this method shortens the overall production timelines of the editing process, while improving cell recovery.

Improved Transduction Efficiency: The combination of nonviral KO with SNX-T1 KI can enhance transduction to outperform traditional workflows.

Traditional workflows that use activated T cells for CAR T cell manufacturing rely heavily on electroporation and viral vectors for KO and KI, respectively. Electroporation techniques require longer cell recovery, limiting workflow options. **CellFE and Syenex's coupled CAR-T cell editing allows a congruence of KO and KI methods to circumvent these limitations, offering a broader editing scope and reducing overall workflow duration.**

TCR KO/CAR KI WORKFLOW PROCESS

Hybrid workflow enables high efficiency, high yield CAR-T cell production



Mean +/- SEM of 7 donors shown for all graphs

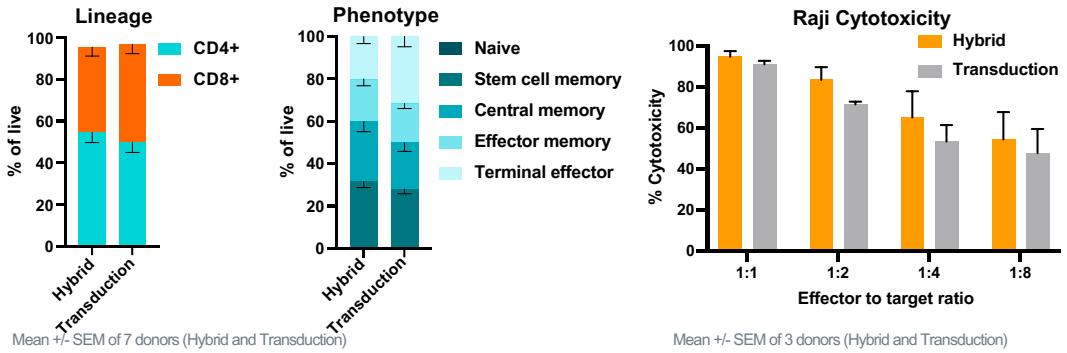
Elevating the Standard in CAR-T Cell Editing

Comparison of Hybrid Process vs. Viral Transduction Alone

Incorporating nonviral KO into a hybrid gene editing workflow accomplishes equivalent phenotypic outcomes compared to viral transduction alone, without any sacrifice to cell functionality.

CAR T PHENOTYPE AND TOXICITY: HYBRID VS. VIRAL TRANSDUCTION

Hybrid process with nonviral TCR KO parallels outcomes using viral transduction methods



Mean +/- SEM of 7 donors (Hybrid and Transduction)

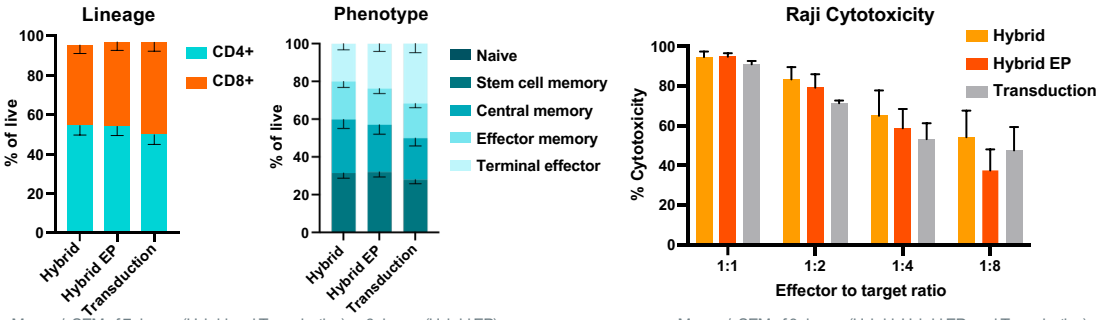
Mean +/- SEM of 3 donors (Hybrid and Transduction)

Comparison with Leading Workflows Using Electroporation

The hybrid workflow using microfluidic transfection and SNX-T1 matches the performance of comparative best-in-class workflows that leverage electroporation (EP) for complex KO/KI edits. The microfluidic-based, nonviral transfection method used for TCR knockout in the hybrid process maintains high stem memory phenotype and preserves the optimal CD4+/CD8+ ratio, which are correlated with improved cell fitness and higher activity products, respectively. Overall cell health as indicated by functional cytotoxicity compares favorably to electroporative technology for CAR T cell editing.

CAR T PHENOTYPE AND TOXICITY: TCR KO VS. EP

Hybrid process with TCR KO performs alongside industry-leading electroporation



Mean +/- SEM of 7 donors (Hybrid and Transduction) or 6 donors (Hybrid EP)

Mean +/- SEM of 3 donors (Hybrid, Hybrid EP, and Transduction)

Unlocking the Future of CAR-T Cell Therapies with a Hybrid Approach

Early CAR-T cell therapies mainly relied on **knockin** strategies—introducing the CAR gene into T cells—enabling them to identify and destroy cancer cells, which underpinned early clinical success. Today, **knockout** approaches and other complex gene edits have emerged to enhance autologous therapies and enable allogeneic therapies.

In autologous CAR T, while inserting the CAR gene remains central, knocking out immune checkpoints can further enhance anti-tumor activity. In allogeneic CAR T, in which healthy donor T cells are used, TCR knockout is crucial to avert severe graft-versus-host disease.

Integrated workflows combing both knockin and knockout techniques are vital for improving both the safety and effectiveness of CAR-T cell therapies, paving the way for more powerful and broadly applicable cancer treatments.

Resting T Cells Unlock Key Advantages in CAR T Cell Editing

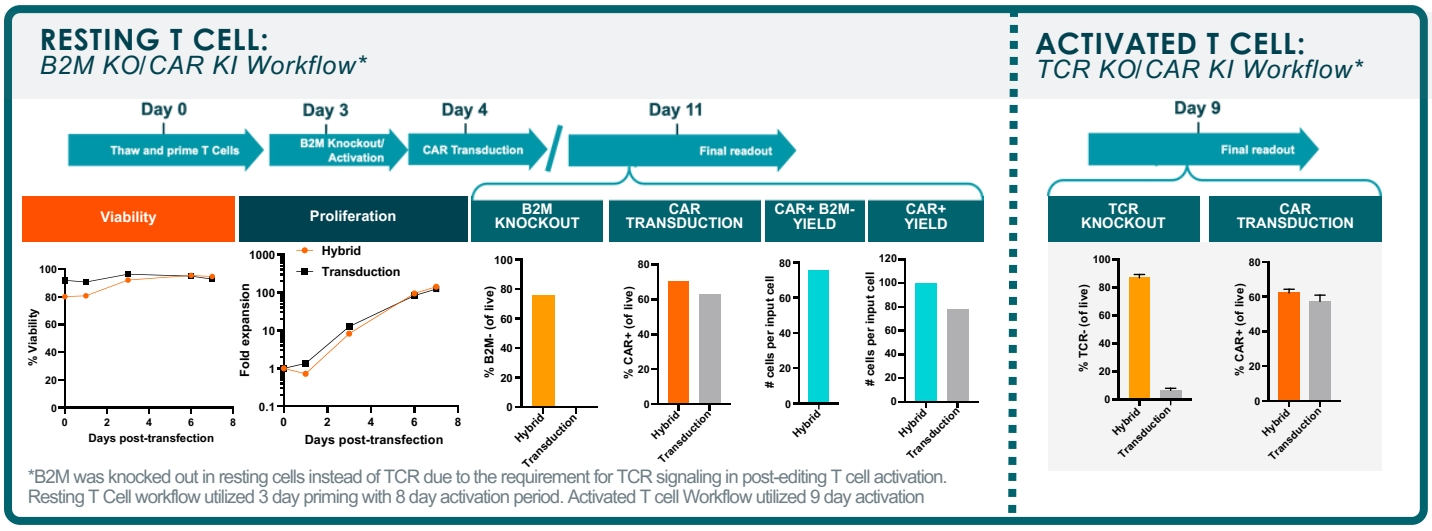
Quiescent Cells as a Starting Material in CAR T Cell Editing: Maximizing Stem Memory and Cell Functionality

Resting (quiescent) T cells differ from most immune cell products by remaining in a non-activated state. This property shields the cells from stress-related damage during manufacturing and minimizes undesired differentiation prior to editing. This inherent stability enables improved functional outcomes in gene editing processes, making these cells an ideal material for durable and potent cell therapies.

When used as a starting material for the CellFE nonviral knockout process, resting T cells offer enhanced editing efficiency and retention of stemness. When combined with the viral knockin using Syenex SNX-T1, the cells improve functionality. The quiescent property adds flexibility to the activation step in the hybrid workflow to deliver a less exhausted product compared to traditional approaches requiring longer activation times.

Resting T Cells Enhance Transduction Efficiency and Yield

CellFE nonviral KO with resting T cells improves cell yield compared to transduction alone. When combined with a downstream activation, the resting T cell process also delivers transduction efficiency and viability in alignment with current state-of-the-art activated workflows.



*B2M was knocked out in resting cells instead of TCR due to the requirement for TCR signaling in post-editing T cell activation. Resting T Cell workflow utilized 3 day priming with 8 day activation period. Activated T cell Workflow utilized 9 day activation

Resting T Cells Preserve Stem Memory Phenotype

In the hybrid workflow, resting T cells can retain the upper bound of stem cell memory when used as a starting material for CAR T gene editing. Furthermore, gene editing in non-dividing cells like resting T cells can reduce the risk of chromosomal abnormalities caused by nuclease activity.

The preserved stem memory is crucial to developing more durable CAR T cell treatments. A hybrid workflow utilizing resting T cells enables the highest preserved stemness in edited cells for superior cell therapy production.