

T cell Knockout via LumiPore™ Platform

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Photoporation provides an efficient and robust approach for gene editing in hard-to-transfect cells such as CD3⁺ T cells. By enabling rapid intracellular delivery of CRISPR-Cas9 ribonucleoprotein complexes, photoporation supports consistent and reproducible gene knockout across multiple donors, detectable as early as 48 hours after delivery. Importantly, this high editing efficiency is achieved without compromising cell health, as treated cells maintain normal proliferation rates following photoporation.

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Summary



By delivering CRISPR-Cas9 ribonucleoprotein (RNP) complexes into the cells, the LumiPore™ system achieved an 80% TRAC gene knockout efficiency across multiple donors while maintaining optimal cell viability and replication kinetics.

The LumiPore™ platform developed by Trince is a versatile intracellular delivery system capable of delivering a wide array of cargo molecules into virtually any cell type with high precision and efficiency. By leveraging a gentle, laser-induced photoporation mechanism, the system creates transient pores via nanoscale photothermal effects, allowing for an optimal balance between high delivery efficiency and maximum cell viability.

Here we demonstrate the platform's efficacy in engineering sensitive primary human cells, specifically purified activated T cells and patient-derived PBMCs (Peripheral Blood Mononuclear Cells). By delivering CRISPR-Cas9 ribonucleoprotein (RNP) complexes into the cells, the LumiPore™ system achieves an 80% TRAC gene knockout efficiency across multiple donors while maintaining optimal cell viability and replication kinetics.

The LumiPore™ platform achieves high gene-editing efficiencies through a system architecture built for true high-throughput operations, including compatibility with high-density formats such as 384- and 1536-well plates. Its rapid parameter-optimization capability substantially reduces reagent consumption, enabling a streamlined workflow in which cell-engineering processes can be scaled without repeated protocol redevelopment. Another major advantage is that cells can be treated directly in their preferred culture vessels, eliminating time-consuming recipient transfers, buffer exchanges, and avoiding the associated stress or toxicity to cells.

2 Introduction



CAR-T therapy has become a cornerstone of modern cancer treatment, yet its clinical impact is still constrained by challenges in gene-delivery efficiency, cell viability, and scalable manufacturing. Current manufacturing workflows rely heavily on viral vectors (e.g., lentivirus or AAV), which introduce substantial limitations, including high production costs, restricted cargo capacity, and safety risks associated with insertional mutagenesis.

A key frontier in this field is the targeted knockout (KO) of the TRAC gene, which is essential for enabling high-throughput manufacturing and large-scale engineering of allogeneic CAR T cells. Furthermore, orthotopic CAR insertion into the TRAC locus enables expression under the endogenous TCR promoter, resulting in more physiological signaling, reduced tonic activation, and significantly lower exhaustion compared with random viral integration.

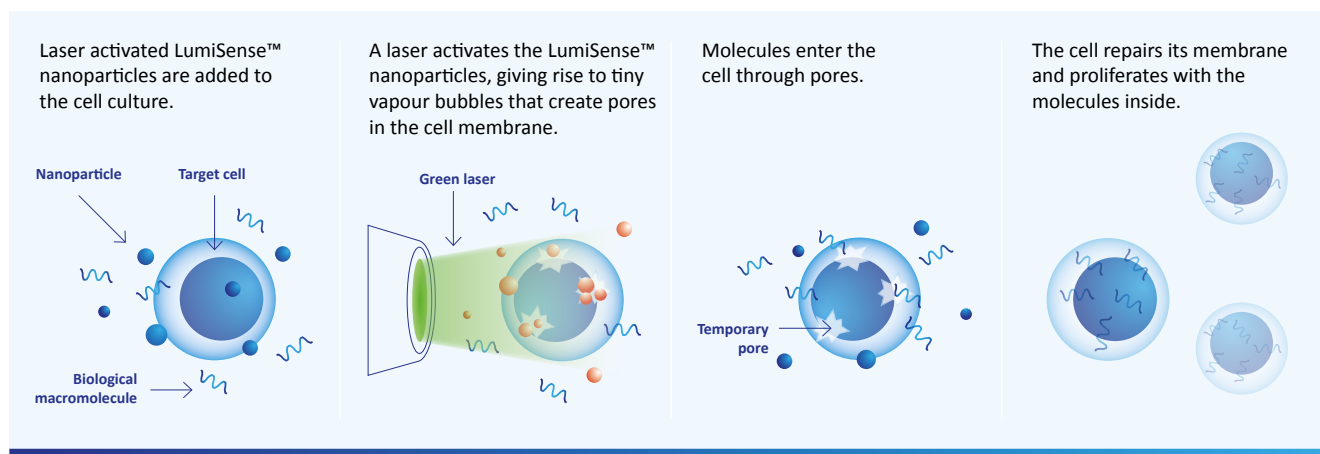
However, achieving these precise genome edits remains challenging because primary human T cells are notoriously sensitive and difficult to transfect using non-viral methods. Conventional physical delivery methods, most notably bulk electroporation, force a trade-off between high editing efficiency and cell viability. These methods impose substantial cellular stress, including prolonged elevated levels of intracellular calcium, increased pro-inflammatory IFN γ transcript levels, and markedly

delayed cell proliferation kinetics. Such perturbations compromise antitumor efficacy and greatly complicate process optimization, especially for patients who have limited numbers of functional T cells.

To address this need, Trince developed the LumiPore™ intracellular delivery platform, which combines laser irradiation with engineered light-sensitive nano-particles (LumiSense™) to transiently open the cell membrane with nanometer precision (Fig. 1). The process begins by adding LumiSense™ nanoparticles to the cell culture, ensuring they are in close proximity to the target cells. When a laser activates these particles, they generate photothermal effects that create temporary pores in the cell membrane, allowing biological macromolecules, such as proteins or nucleic acids, to enter. Because these pores are created at a nanoscale level, cellular stress is minimal, preserving both viability and function.

For targeted T cell knockouts, LumiPore™ enabled efficient intracellular delivery of CRISPR-Cas9 RNPs into both isolated T cells and patient-derived PBMCs from multiple donors. The platform consistently achieved up to 80% knockout efficiency while maintaining high cell viability and robust proliferation, demonstrating that LumiPore™ supports precise gene editing without compromising cellular fitness or function.

▼ FIGURE 1*



*Source: www.nature.com/articles/d41586-024-02167-1, 'A light-touch approach to intracellular delivery' by Tim Vernimmen (July 2024)

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Materials and Methods

PBMCs and purified T cells were isolated from human peripheral blood of three donors and cultured in complete cell culture medium supplemented with 10% heat-inactivated fetal bovine serum (FBS, GIBCO), 2% penicillin (1.000U/ml)-streptomycin (10.000µg/ml) (GIBCO), 1 mM Sodium Pyruvate, 50 µM 2-Mercaptoethanol, 300 IU/mL IL-2, 10 ng/mL IL-7 and IL-15. Cells were activated for 48-72 hours using ImmunoCult (CD3/CD28 activation, Stemcell technologies). To enhance delivery efficiency, cells were incubated with the Trince Delivery Enhancer 1 (TDE1) for 1 hour at 37°C and 5% CO₂. Prior to the procedure, the cell density was adjusted to ensure the formation of a confluent monolayer, typically adding 1.000.000 cells per well in a 96-well flat-bottom plate.

Ribonucleoprotein (RNP) complexes were assembled by mixing single guide RNA (sgRNA) and Cas9 protein at 2.5:1 ratio with final concentrations of 5 µM and 2 µM respectively. The mixture was incubated at 25°C for 10 minutes to allow complex formation.

Intracellular delivery was performed using the LumiPore™ platform. First, T cells were briefly incubated with LumiSense™ nanoparticles in Opti-MEM. Following incubation, excess LumiSense™

was removed through a standardized washing protocol, after which the cells were resuspended and RNP complexes were added to the wells. To promote rapid sedimentation and ensure formation of a uniform cell monolayer at the bottom of each well, plates were subjected to a short-spin at 1.000g. Laser-induced photoporation was then carried out using a laser intensity of 60%. Treated cells were allowed to recover for 5 minutes before the addition of recovery medium. After 2 hours, cells were transferred to 96-well plates containing complete culture medium and incubated for 10 days at 37°C and 5% CO₂.

Cell viability and total cell number were assessed daily for up to 10 days post-treatment. Measurements were performed using a NucleoCounter (ChemoMetec) according to the manufacturer's instructions.

TCR KO efficiency was monitored at multiple time points (days 2, 4, 7, and 10) post-photoporation and quantified by measuring the loss of surface expression of the TCR complex via flow cytometry.

For flow cytometric analysis, cells were resuspended in FACS buffer and stained with a fluorophore-conjugated anti-human TCR antibody (BioLegend).

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Results



The LumiPore™ platform delivers rapid, robust, and donor-independent gene editing, achieving 80% knockout efficiency within 2 days and maintaining stability over time.

The LumiPore™ platform was used to perform targeted gene knockout in human primary cells, including purified activated T cells from healthy donors 1 and 3, and PBMCs from healthy donor 2, in a 96-well plate format. RNP complexes were assembled as described in the Materials and Methods section. To ensure uniform delivery, cells were prepared as a high-density confluent monolayer and subjected to photoporation. Following treatment, cells were allowed to recover for 24 hours to enable membrane stabilization and were subsequently monitored for up to 10 days to assess cell fitness and knockout efficiency.

As shown in Fig. 2 A, the LumiPore™ platform achieved a consistent and reproducible KO efficiency of approximately 80% across all three donors. Notably, this high level of genomic editing was reached as early as 48 hours post-treatment and remained stable through day 10. This underscores the platform's ability to deliver rapid, robust and donor-independent results regardless of whether purified T cells or PBMCs were used.

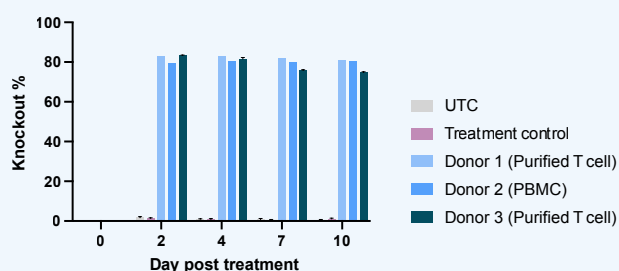
A hallmark of the LumiPore™ technology is how gently it treats cells. Cell viability and total cell number were quantified using a NucleoCounter to monitor both viability and growth kinetics (Fig. 2 B-D). Although a slight decrease in viability was observed 1 day post-treatment, the cells showed rapid and robust recovery. By day 2, viability and proliferation profiles across all treated samples were nearly indistinguishable from the Untreated Control (UTC) and the treatment control, which consisted of cells exposed to LumiSense™ and photoporated in the absence of cargo. This rapid stabilization is critical for preserving the biological integrity of sensitive primary T cells, enabling seamless progression to downstream applications without the prolonged “lag phase” commonly observed with other non-viral methods such as bulk electroporation.

The long-term proliferative capacity of the edited cells was confirmed through a fold expansion analysis (Fig. 2 C). Starting from an initial density of 1 million cells per well in a 96-well plate, the engineered cells exhibited robust growth, reaching a 100-fold expansion by day 10 at the same growth rate as UTCs.

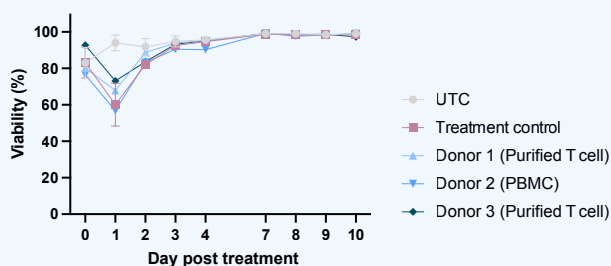
FIGURE 2
Longitudinal assessment
of TRAC knockout efficiency
and T cell growth kinetics.

- A** TCR KO efficiency is expressed as the percentage of KO cells over time. Following RNP delivery via photoporation, KO efficiency reached approximately 80% by day 2 across three healthy donors, which was sustained until day 10. Inter-donor variability was low and not significant, and all control groups (CTRL) maintained a base level of near 0% editing;
- B** Cell viability expressed as percentage of viable cells up to day 10 after photoporation. Samples exhibited a minor, transient decrease in viability on day 1. By day 2, cell viability was indistinguishable from untreated control groups;
- C** Proliferation kinetics expressed as fold expansion over a 10-day period, showed that expansion dynamics remained similar across all samples and controls, confirming the gentle nature of the LumiPore™ platform;
- D** Longitudinal absolute cell counts demonstrated that growth rates remained identical to untreated controls as from day 1 onwards, indicating no long-term impairment of proliferative capacity.

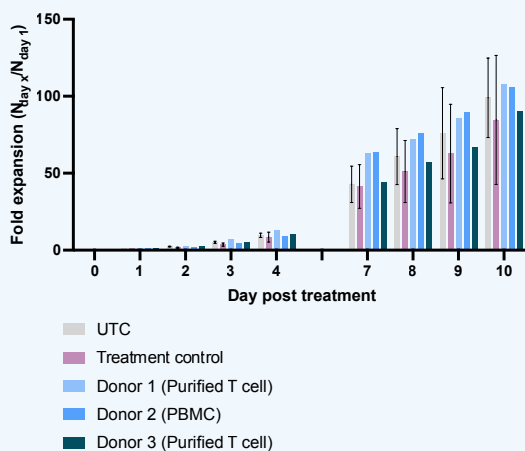
A Knockout efficiency after treatment



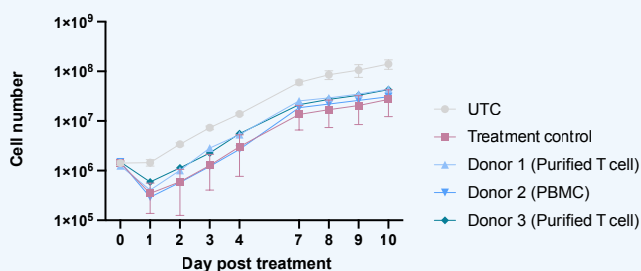
B Viability after knockout



C Fold expansion after photoporation



D Cell number post treatment



5 Conclusions

In conclusion, the LumiPore™ platform demonstrates exceptional efficacy in primary T cell engineering by achieving 80% TRAC knockout while circumventing the biological limitations of traditional delivery methods. Unlike conventional techniques that often induce significant cellular stress and growth delays, this gentle method ensures rapid cell recovery and maintains robust proliferation over time, while providing a fast, scalable, and cost-effective workflow with reduced RNP cargo consumption.





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