ENABLING AUTOMATED MANUFACTURING OF GENE-MODIFIED T CELLS WITH LIPID NANOPARTICLES

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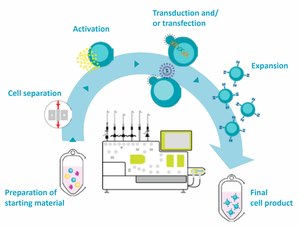
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Introduction

Chimeric antigen receptor (CAR) engineered T cells are a promising therapy for oncological and autoimmune diseases, with lentiviral (LV) particle–mediated modification remaining the state-of-the-art approach for generating adoptive T cell products. To enable more sophisticated cell engineering, lipid nanoparticles (LNPs) are emerging as a promising technology. They combine simple usage and time-effectiveness with a safe transfection profile and flexible administration schedule. Here we show, that LNPs can be easily implemented into existing workflows on the CliniMACS Prodigy® platform allowing to readily perform automated, closed, GMP-compliant processing of gene-engineered T cell therapies at a clinical scale.

Methods

Figure 1

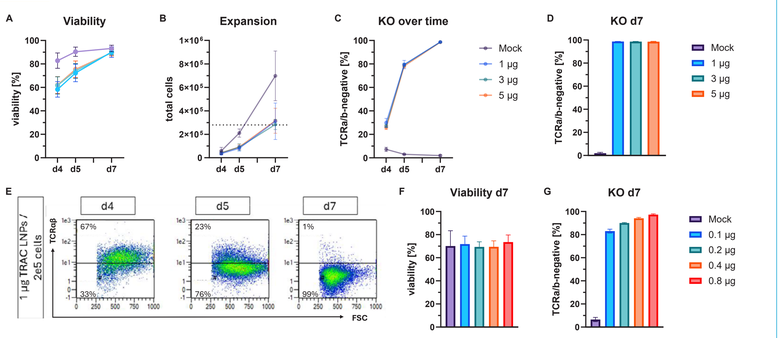


This study explores scalable LNP-mediated gene-editing of CAR-engineered T cells. Initially, a *TRAC* knock-out (KO) was established by titrating the amount of LNPs (S01-eSpCas9 mRNA(m1)-TRAC sgRNA, GenScript) packaging the gene-editing machinery, administered together with 1 µg/ml ApoE4 on 2 x 10^5 activated primary human T cells. Next, feasibility of CAR-LV and *TRAC*-LNP co-modification was assessed. 2 x 10^5 T cells were transduced at MOI 10 with CD33-CAR LV on d1 and subsequently transfected with *TRAC*-LNPs on d3. Besides evaluation of immune composition, activation and exhaustion, co-culture assays with CD33+ tumor target cells were performed. Next, this process was scaled up starting with 1 x 10^8 CD4/CD8-enriched T cells on the CliniMACS Prodigy® platform as culture set-up (*Fig. 1*). After activation with MACS® GMP T Cell TransAct™ (d0), cells were transduced (d1) and transfected (d3) in TexMACS™ GMP Medium. T cells were characterized regarding their cell composition, phenotype and potency. Finally, we assessed the flexibility of the process by incorporating GFP-LNP transfection at different timepoints during the process (d2, d3, d7) in combination with CAR-LV transduction (d1).

Results

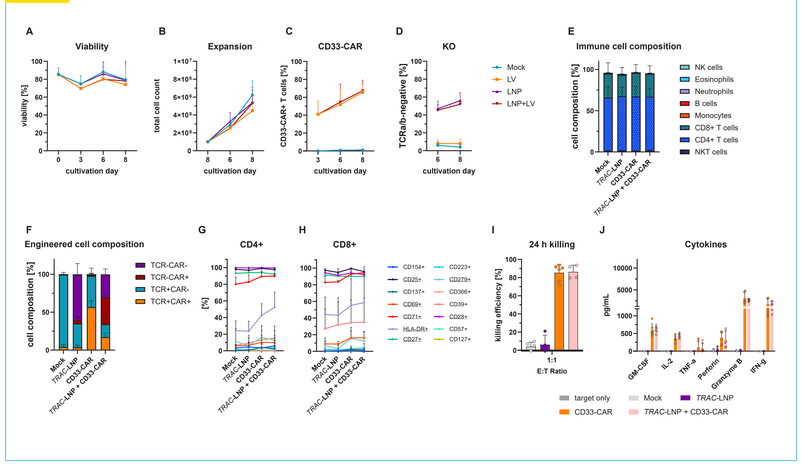
1 Optimizing small-scale transfection of primary T cells using LNPs

Figure 2



To establish an efficient protocol for LNP-mediated *TRAC*-KO, we titrated the LNP amount from 1 to 5 µg. Viability was slightly reduced compared to untreated (Mock) T cells, *yet all* conditions recovered by d7 (*Fig. 2A*). Proliferation showed a similar pattern, with LNP-modified cells expanding 6–9-fold within 7 days, compared to 10–14-fold in Mock samples (*Fig. 2B*). TCR KO was highly efficient (> 98% on d7) and independent of LNP dose (*Fig. 2C–E*). Therefore, the amount was further reduced. At these lower doses, viability was comparable on d7, and KO efficiencies above 80% were achieved with as little as 0.1 µg LNP (*Fig. 2F, 2G*).

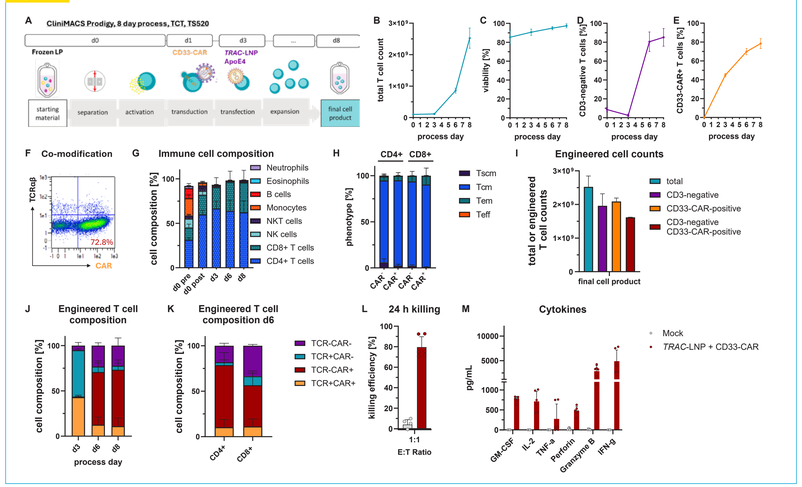
Figure 3



Next, we combined LNP-transfection with LV-CD33-CAR transduction in a small-scale setting. Viability and expansion were comparable between Mock and LNP-modified T cells (*Fig. 3A, 3B*). CAR transduction reached 67.3 ± 9.6% and the KO efficiency was 52.0 ± 3.6% (*Fig. 3C, 3D*), while CD4 and CD8 T cell composition was not influenced by LNP modification (*Fig. 3E*). More than half of each CAR- or LNP-modified condition showed CAR expression or TCRα/β KO, whereas around 36 ± 4.6% of T cells were efficiently co-modified (*Fig. 3F*). LV- and co-modified cells showed a slightly higher expression of activation markers for both CD4+ and CD8+ T cells (*Fig. 3G, 3H*). Despite LNP modification no differences in killing potency was detected as shown in 24 hour killing and cytokine assessment (*Fig. 3I, J*).

2 Development of a scalable manufacturing process for LNP-based transfection using the CliniMACS® Prodigy platform

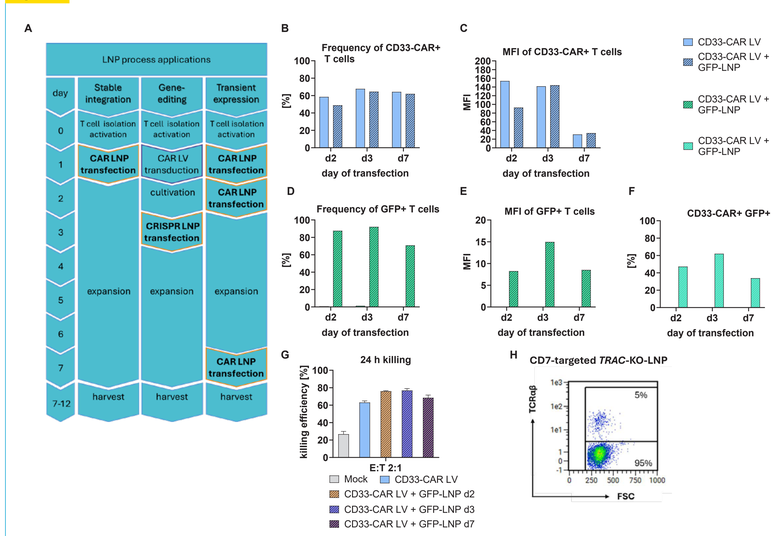
Figure 4



Finally, the transfection protocol was scaled-up using the CliniMACS Prodigy® platform (*Fig. 4A*). After 8 days the process yielded 2.5 x 10^9 ± 2.64 x 10^8 total highly viable T cells (> 96%; *Fig. 4B, 4C*). KO efficiency (85.2 ± 7.7%) and CAR expression (78.4 ± 4.4%) were high. Overall, co-modification reached 62.3 ± 8.8% on process day 8 (*Fig. 4D-F*). Manufactured T cells exhibited a high purity (Fig. 4G) and a favourable central memory (Tcm) phenotype (Fig.4H) at harvest day. Finally, the process yielded clinically relevant co-engineered (TCR-CAR+) T cell numbers with 1.6 × 10^9 ± 2.6 × 10^7 T cells. (Fig. 4I). Within CD4+ T cells a higher modification efficiency was shown (*Fig. 4J, 4K*). Manufactured CAR T cells exhibited a high cytolytic potential (79.6 ± 9.3% killing) and efficient cytokine secretion (*Fig. 4L, 4M*).

3 LNPs enable flexible manufacturing workflows

Figure 5



LNP-mediated cell engineering offers a high flexibility of T cell modification for integrated and automized large-scale manufacturing on the CliniMACS Prodigy® (*Fig. 5A*). One way to integrate LNPs into existing workflows is transfection on different time points as well as implementation of a variety of RNA or DNA cargos for gene-of-interest delivery. Therefore, we tested co-engineering with a CD33-CAR transduction (d1) and GFP-LNP transfection (d2, d3, d7). GFP expression was comparable across all transfection timepoints, while CAR expression was unaffected (Fig. 5B-F). Moreover, all protocols were feasible and showed high efficiency, favorable T central memory phenotype, while transfection time point showed no negative impact on potency (*Fig. 5G, 5H).* In a final step, we tested CD7-tageted LNPs for ApoE4-independent transfection at d3, resulting in a high *TRAC*-KO effciency of 95% and introducing another layer of specificity while maintaining full flexibility (Fig. 5H).

Conclusion

In summary, we showed efficient co-modification of human primary T cells with *TRAC*-KO LNPs and CD33-CAR LV in small- and large-scale. Moreover, we demonstrated the flexibility of LNP administration, offering a promising *ex vivo* manufacturing technology that has the potential to foster a high variety of LNP applications in the future.

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