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703.CELLULAR IMMUNOTHERAPIES: BASIC AND TRANSLATIONAL

Lipid Nanoparticle-Mediated Gene Editing of Human Primary T Cells and Off-Target Analysis of the CRISPR-Cas9 Indels

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Background and Aims: The CRISPR/Cas9 system has emerged as a powerful tool for gene editing of primary cells. In our previous work, we demonstrated a novel lipid nanoparticle (LNP) reagent for the multi-step engineering of gene-edited CAR T cells. We showed high cell viabilities and potent CAR-T mediated killing, even after multiple genetic manipulations. Here, we extend this work by assessing potential off-target editing effects in both LNP-treated T cells and T-cells where the CRISPR reagents were delivered by electroporation. Further, we evaluated multiple Cas9 variants and guide RNA targets.
Methods: LNPs encapsulating wild type or high fidelity S.p. Cas9 mRNA and various *TRAC* and *CD52* targeted guide RNAs (sgRNAs) were produced using our scalable NanoAssemblrTM microfluidics platform. Concurrently, electroporation was performed to deliver equivalent cargoes. Purified primary T cells were cultured, activated, and expanded in serum-free media in plates, flasks, or small bioreactors. The LNPs were added to cells by direct addition for gene editing. Gene expression and cell viability were measured using flow cytometry or colorimetric assays. Multi-target performance of CRISPR-Cas9 editing was evaluated through rhAmpSeq-based targeted next-generation sequencing (NGS) and indels analyzed for with CRISPRAltRations.
Results: TCR or CD52 targeted Cas9 mRNA-LNP addition or electroporation yielded high single and double knockout efficiencies. High-throughput NGS analysis showed strong agreeance to flow cytometry for on-target analysis. We tested a range of sgRNA targets, wild-type and high-fidelity Cas9 mRNAs, and determined off-target editing for all targets and variants investigated. Similar results were obtained when comparing different LNP batch sizes (micro to milligram RNA) and cell culture vessels (0.1 to 45 million cells), demonstrating scalability of both LNP production and cell treatment.
Conclusions: The results from this study further support the utility RNA-LNPs for the genetic engineering of primary T cells. The simple and gentle nature of LNP cell treatment allows for multiple genetic engineering steps for simultaneous expression and deletion of proteins for the next cell therapies. These LNPs can be easily manufactured from small-scale screening of RNA libraries to rapid scale-up for clinical translation.

Disclosures Geczy: *Precision NanoSystems:* Current Employment. **Thommandru:** *Integrated DNA Technologies:* Current Employment. **Swaminathan:** *Precision NanoSystems:* Current Employment. **Zhao:** *Precision NanoSystems:* Current Employment. **Tan:** *Precision NanoSystems:* Current Employment. **Low:** *Precision NanoSystems:* Current Employment. **Balgi:** *Precision NanoSystems:* Current Employment. **Park:** *Precision NanoSystems:* Current Employment. **Watt:** *Precision NanoSystems:* Current Employment. **Thada:** *Integrated DNA Technologies:* Current Employment. **Rettig:** *Integrated DNA Technologies:* Current Employment. **Zhang:** *Precision NanoSystems:* Current Employment. **Turk:** *Integrated DNA Technologies:* Current Employment. **Clarke:** *Precision NanoSystems:* Current Employment.

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