Case Study: Checkpoint Inhibitor Knockout Using Paired Cas9 Nickases

High Efficiency Nickase-mediated Gene Knockout in Human Primary Hematopoietic Cells

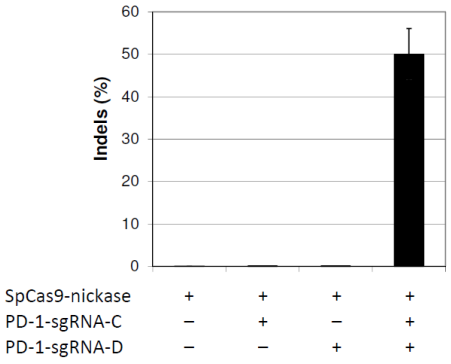
Scientific Brief

Abstract — Gene Editing: Paving the Way for Accelerated Clinical Development of Adoptive Cell Immunotherapies

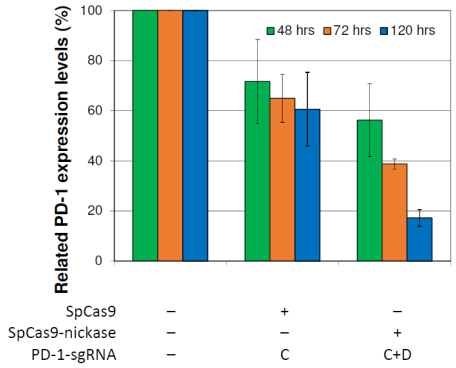
Precision genome engineering requires technologies that allow efficient and reproducible delivery of DNA, mRNA and RNP-based reagents into a range of primary cells and stem cells. In addition, clinical gene editing requires a transfection platform that is GMP-compliant and scalable to accommodate billions of cells in a single transfection. Here we share data on gene deletion in primary T cells following transfection of CRISPR SpCas9 and SpCas9-nickase ribonucleoproteins (RNPs) and PD-1-specific guide RNAs using the clinically validated MaxCyte Flow Electroporation® Technology. FACS analysis confirms reduced PD-1 target gene expression. Finally, we show multiplex editing of both CTLA-4 and TIM-3 gene targets in CD38+ primary T cells.

Case Study MERCK

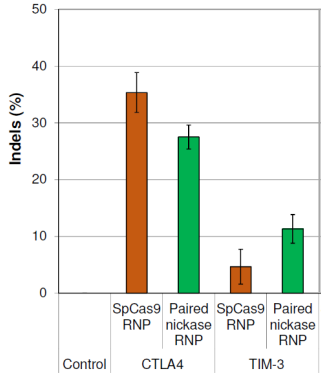
A. Nickase-mediated Knockout of PD-1 in Primary T Cells



B. Gene Knockout Correlates with Lower PD -1 Expression



C. Multiple Target Knockout in Primary CD8⁺ T Cells



Paired Nickase RNPs Outperform spCas9 RNPs for Checkpoint Inhibitor Knockout in Human Primary Hematopoietic Cells.

In a paired nickase approach, Streptococcus pyogenes Cas9 (SpCas9) is modified to inactivate one of its two nuclease domains, causing single-stranded nicks rather than a double stranded break. Paired with a second Cas9 nickase targeting a proximal location, these two single-stranded nicks cause a DSB to occur. Off-target modifications caused by paired gRNA are rare. **(A)** Human primary T cells were electroporated with paired SpCas9 nickase RNP, with or without PD-1-specific sgRNAs. Genomic DNAs were harvested 72 hours post-electroporation for indels analysis. **(B)** Human primary T cells were electroporated with SpCas9 RNP or paired nickase RNP with or without PD-1-specific sgRNA. At various times post electroporation, cells were assessed via FACS for surface PD-1 expression. **(C)** Human primary CD8+ T cells were electroporated with SpCas9 RNP or paired SpCas9 nickase RNP specific for CTLA4 or TIM-3. Genomic DNA was harvested 120 hours post-electroporation for indels analysis. A – C: Averages from three biological replicates are plotted with error bars representing one standard deviation. *bioRxiv Sept. 2019 preprint (https://doi.org/10.1101/766493).*

Summary

- Gene editing using MaxCyte non-viral engineering enables rapid development of next-generation adoptive cell therapies for treatment of a wide variety of diseases.
- MaxCyte Flow Electroporation® Technology efficiently (co)delivers a diversity of payloads including mRNA, sgRNA, and RNPs to difficult-to-engineer primary cells commonly used for adoptive cell therapies including hematopoietic stem cells and T cells.
- The high efficiency and low toxicity of MaxCyte Flow Electroporation provides for high levels of nuclease or nickase-mediated gene editing including:
  - Gene knockout/disruption
  - Gene knock-in
  - Single nucleotide gene mutation correction
- MaxCyte clinical scalability and regulatory compliance provide for streamlined clinical translation of new therapies.

Corresponding Author: James Brady; jamesb@maxcyte.com; MaxCyte, Inc., Tel: (301) 944-1700

© 2020 MaxCyte, Inc. All Rights Reserved. Flow Electroporation, MaxCyte, ExPERT and MaxCyte GTx are trademarks of MaxCyte, Inc.