# Highly efficient editing with CRISPR-Cpf1 in primary T cells and HSCs

John Zuris, Ramya Viswanathan, Katherine Loveluck, Elise Keston-Smith, Justin Fang, Aditi Chalishazar, Jack Heath, Jen DaSilva, Eric Tillotson, Derek Cerchione, Barrett Steinberg, Fred Harbinski, Grant Welstead, Jennifer Gori, Hari Jayaram, Vic Myer

## Abstract

**The CRISPR-Cpf1 (Cas12a) system (1) offers several potential advantages over other nucleases for ex vivo genome editing therapies, including a smaller single crRNA that can be readily synthesized, the ability to target T- and C-rich PAMs with the wild-type protein and engineered PAM variants (2), and a 5'-staggered cut which may lead to different repair outcomes.**

**For ex vivo delivery, the use of ribonucleoprotein (RNP) complexes may be preferable, in many instances, to nucleic acid based delivery such as plasmid DNA. Here we show that several Cpf1 orthologs can be made as RNPs and edit robustly at multiple genomic loci that are also targetable by SpCas9 (3) in multiple cell types. We demonstrate editing over 90% in T cells and over 90% in HSCs with AsCpf1 and its engineered RR and RVR PAM variants.**

**We also demonstrate optimization of the Cpf1 RNP complex, both at the protein and guide level, which improve efficacy across cell types. Collectively, these findings underscore the promise of RNP delivery for Cpf1 nucleases for genome editing therapeutics.**

## Cpf1 variants expand targeting space for gene editing




## Robust pipeline for RNP evaluation




**References:**

1. Zetsche et al. *Cell* 2015
2. Gao et al. *Nat Biotech* 2017
3. Kleinstiver et al. *Nat Biotech* 2016

## AsCpf1 selected from several tested Cpf1 orthologs

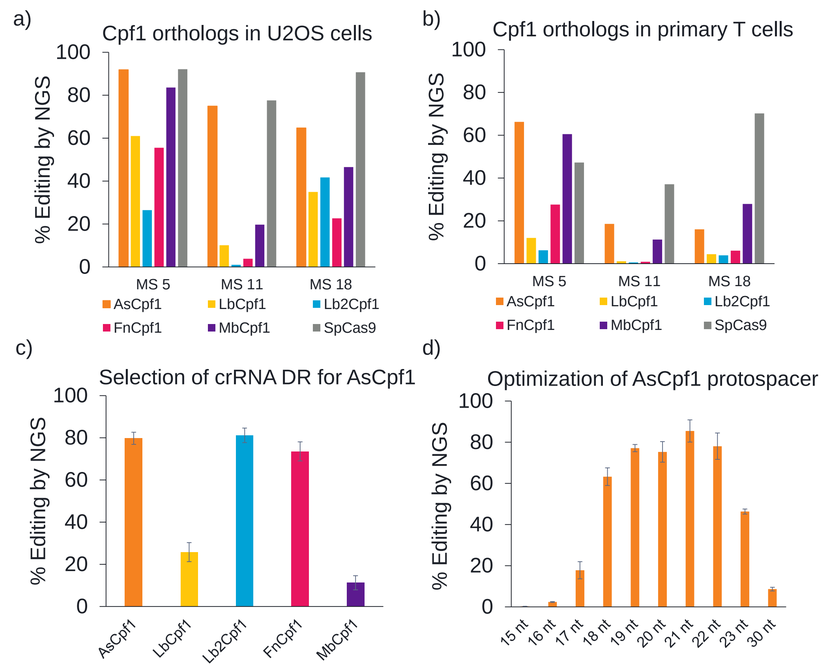


**Figure 1.** AsCpf1 editing efficacy is superior to other orthologs and comparable to SpCas9 at matched sites in a) U2OS cells and b) primary T cells. c) The AsCpf1 crRNA direct repeat (DR) shows best efficacy in the RNP format but can be substituted with ortholog crRNAs. d) AsCpf1 RNP-based cell editing is optimal with 20-nt protospacer.

## AsCpf1 screen in primary T cells yields several hits

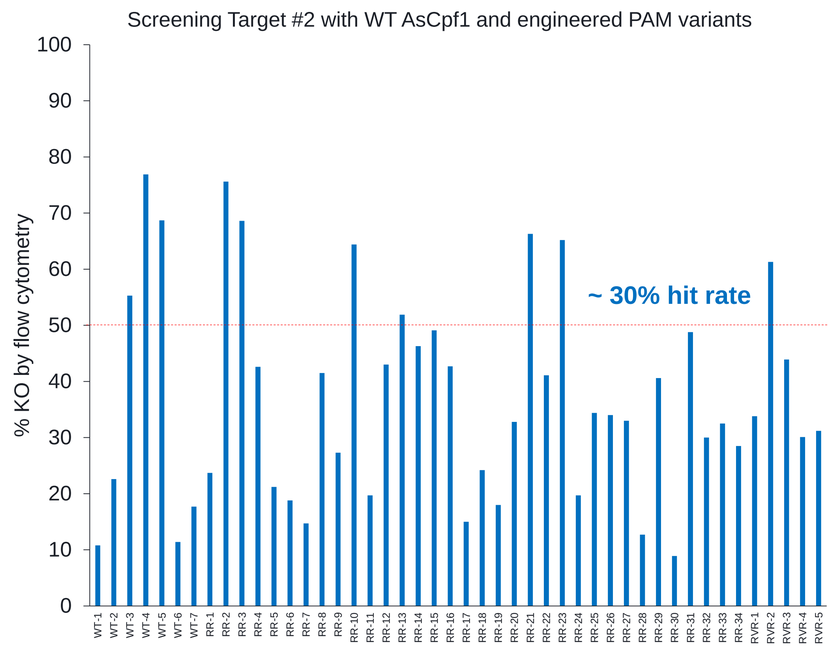


**Figure 2.** Screening a T cell therapeutic target with AsCpf1 and its RR and RVR PAM variants. ~30% of gRNAs show >50% editing in our preliminary screen which is on par with generally observed SpCas9 hit rate, showing that Cpf1 can potentially be used for gene editing on a patient's T cells at a key therapeutic locus.

## Efficient editing in HSCs and T cells by optimization of NLS configuration and nucleofection conditions

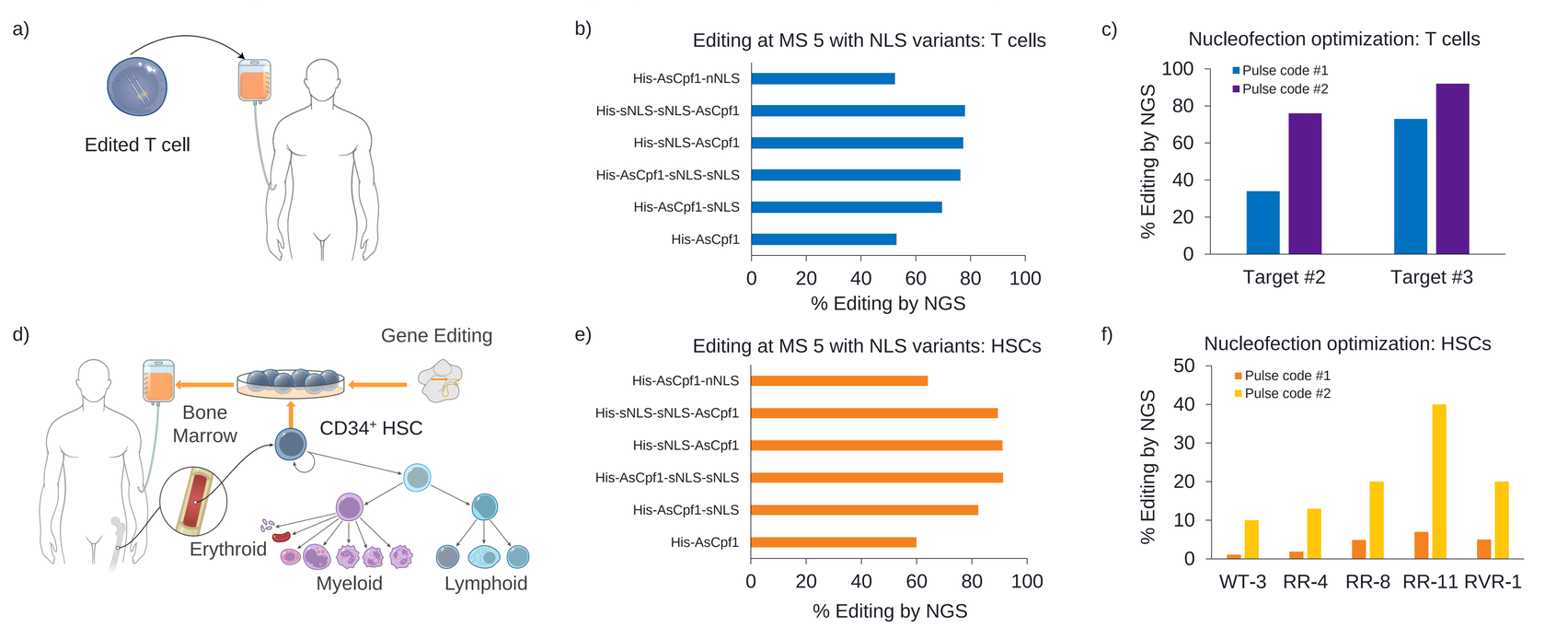


**Figure 3.** a) CAR and TCR engineered T cell therapies have the potential to be transformative additions to the immuno-oncology landscape. b) Optimization of NLS configuration for Cpf1 RNP delivery to primary T cells. c) Changes in the nucleofection pulse code improve maximal editing significantly in T cells at multiple therapeutic target loci d) Edited HSCs have the potential to provide a durable therapy for patients with β-hemoglobinopathies. (e) Optimization of NLS configuration for Cpf1 RNP delivery to HSCs. f) Changes in the nucleofection pulse code improve maximal editing significantly in HSCs when editing at a hereditary persistence of fetal-hemoglobin inducing site in HSCs.

## Efficient single and multiple knockout editing in primary T cells at disease relevant loci with Cpf1 RNPs

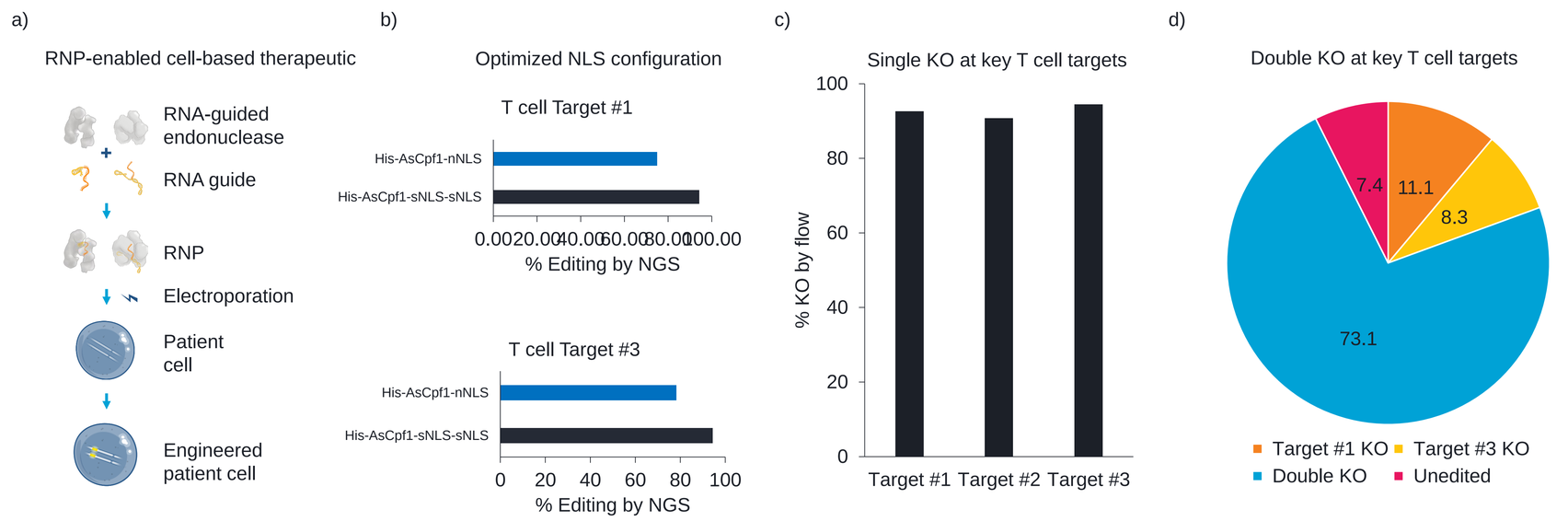


**Figure 4.** a) RNP workflow for an ex-vivo cellular therapy. b) NLS optimization work with MS 5 guide yields NLS variant that is shown to improve editing for multiple T cell targets. c) Efficient single KO at multiple therapeutically relevant T cell loci using AsCpf1 or an engineered PAM variant. d) Highly efficient double KO of two therapeutic targets in T cells treated with Cpf1 RNP as measured by flow cytometry.



11 Hurley Street | Cambridge, MA 02141