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Unique features of ARCUS nucleases enable high efficiency, targeted gene insertion in vivo

Cassandra L. Gorsuch[1], Wendy K. Shoop[1], Emma Sevigny[1], Paige Nemec[1], Adam Mischler[1], Janel Lape[1], Ying K. Tam[2], Paulo J.C. Lin[2], Sean C. Semple[2], Derek Jantz[1], Jeff Smith[1]

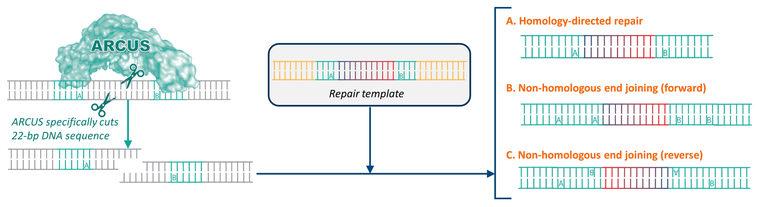
[1]Precision BioSciences, Durham, NC, USA; [2]Acuitas Therapeutics, Vancouver, British Columbia, Canada

INTRODUCTION

- Nuclease-driven, targeted gene insertion into the genome is an appealing therapeutic approach to overcome durability limitations associated with adeno associated virus (AAV) gene therapy.
- ARCUS gene editing nucleases possess attributes that lead to high efficiency gene insertion across a variety of cellular contexts.
- Here we demonstrate the importance of the unique 3', 4 base pair overhang that ARCUS nucleases create in achieving high efficiency gene insertion.

USING ARCUS FOR GENE INSERTION

FIGURE 1. Mechanisms of targeted gene insertion using ARCUS



METHODS

In Vitro Experiments

- Primary Human T Cells: Stimulated human T cells were electroporated with ARCUS mRNA and transduced with AAV carrying a gene insertion template. Gene insertion efficiency was measured by flow cytometry.
- Primary Human Hepatocytes: Cells were transfected with LNP containing ARCUS mRNA and transduced with AAV carrying a gene insertion template. Gene insertion efficiency was measured by dPCR.

In Vivo Experiments

- Newborn/Infant NHPs: NHPs were administered two AAVs carrying a PCSK9-targeting ARCUS nuclease and gene insertion template at $1x10^{13}$ GC/kg and $3x10^{13}$ GC/kg, respectively. Liver biopsies were collected at various timepoints post dosing, and expression was quantitated by in situ hybridization (ISH) or immunofluorescence (IF).
- Adult NHPs: NHPs were administered an LNP containing ARCUS mRNA at 1.75 mg/kg and an AAV carrying the gene insertion template at $3x10^{13}$ GC/kg. Gene insertion efficiency was measured at 1 and 3 months post dosing by dPCR.

RESULTS

ARCUS 3' overhangs drive high efficiency gene insertion compared to blunt cuts

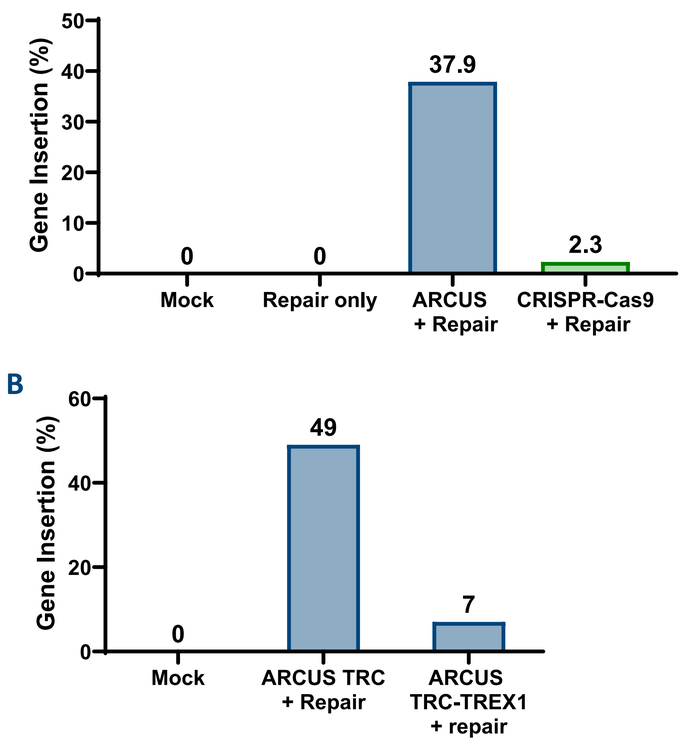


FIGURE 2. Human T cells were electroporated with TRC-targeting ARCUS, spCas9 **(A)**, or ARCUS linked to a TREX1 exonuclease to produce blunt ends **(B)** mRNA and transduced with AAV containing a CAR insertion cassette. Gene insertion was measured by flow cytometry.

RESULTS

ARCUS leads to stable expression of ornithine transcarbamylase (OTC) in newborn NHPs using AAV delivery

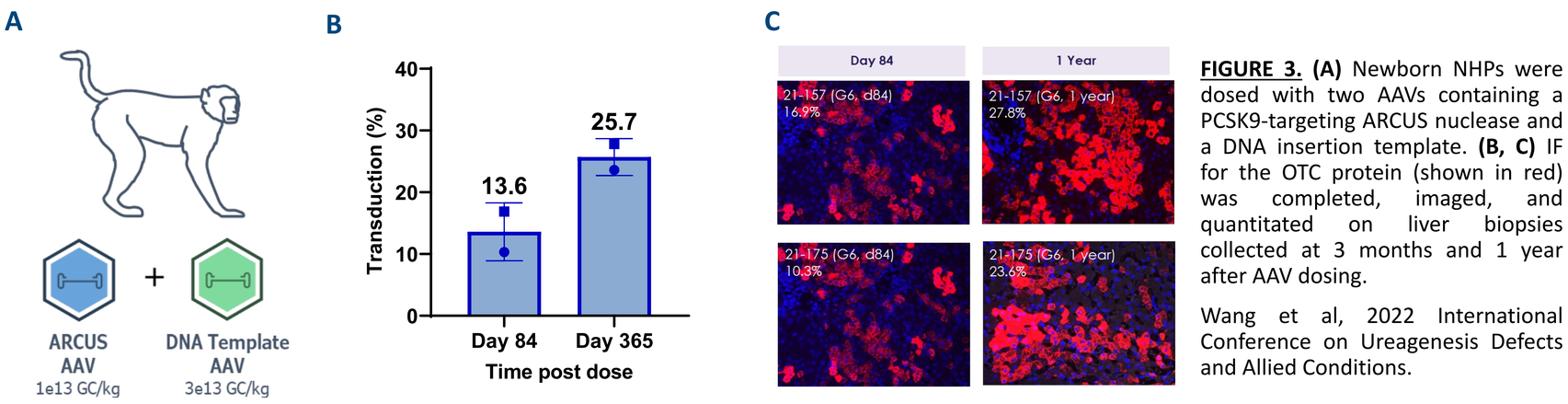


FIGURE 3. (A) Newborn NHPs were dosed with two AAVs containing a PCSK9-targeting ARCUS nuclease and a DNA insertion template. **(B, C)** IF for the OTC protein (shown in red) was completed, imaged, and quantitated on liver biopsies collected at 3 months and 1 year after AAV dosing.

Wang et al, 2022 International Conference on Ureagenesis Defects and Allied Conditions.

ARCUS leads to durable Factor IX (F9) gene expression in newborn, 3-month, and 6-month infant NHPs using AAV delivery

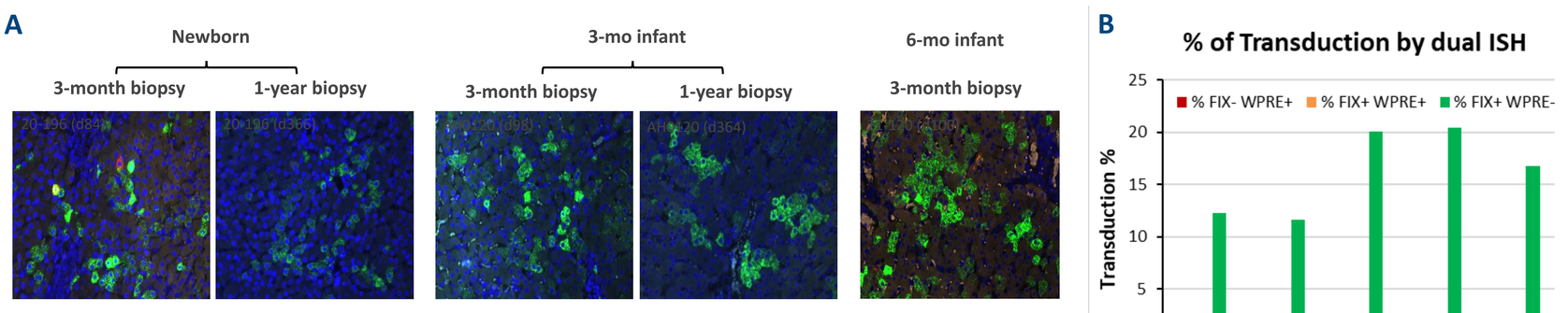


FIGURE 4. (A, B) Newborn, 3-month, and 6-month infant NHPs were dosed with two AAVs containing a PCSK9-targeting ARCUS nuclease and a DNA insertion template. ISH for the F9 mRNA (shown in green) and the ARCUS mRNA (shown in red) was completed, imaged, and quantitated on liver biopsies collected at various timepoints after dosing.

Wang et al, 2022 American Society for Cell and Gene Therapy.

ARCUS inserts with high efficiency by homology-directed repair (HDR) in nondividing cells using LNP + AAV

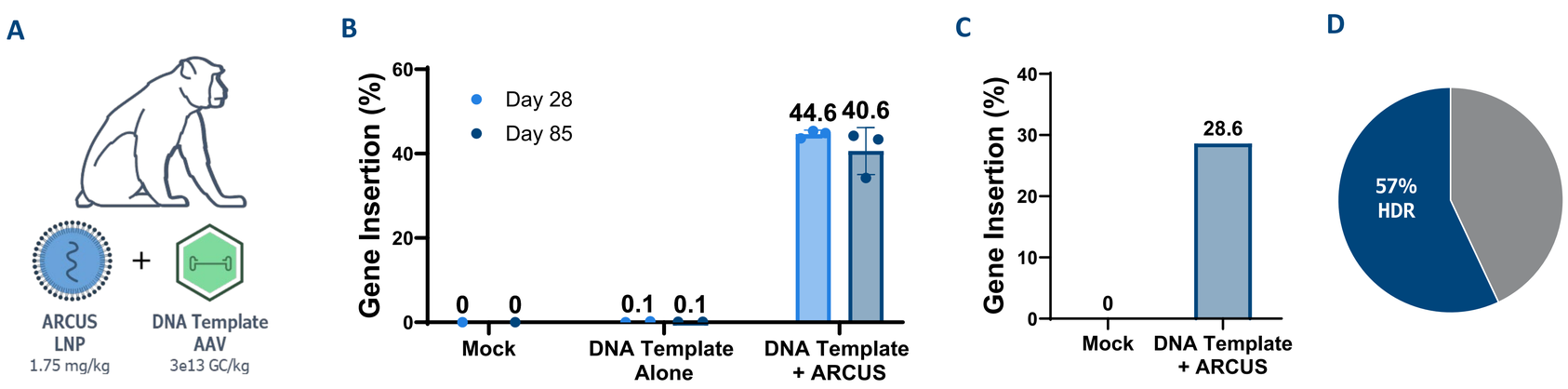


FIGURE 5. (A) Adult NHPs were dosed with an ARCUS LNP and AAV carrying a DNA insertion template. **(B)** Gene insertion was measured by dPCR at 1 and 3 months after dosing. **(C)** PHHs were dosed with ARCUS LNP and an AAV carrying a DNA insertion template. Gene insertion was measured by dPCR. **(D)** Using dPCR, the majority of insertions were determined to be HDR-mediated.

CONCLUSIONS

- **ARCUS achieved 17 times higher gene insertion efficiency compared to CRISPR-Cas9. Removal of the 3' overhang after the ARCUS cut resulted in loss of gene insertion efficiency.**
- **ARCUS demonstrated high efficiency and durable insertion in newborn and infant NHPs when used with OTC and F9 transgenes after AAV + AAV delivery.**
- **ARCUS inserted up to ~45% in adult NHPs administered AAV + LNP.**
- **ARCUS shows high efficiency gene insertion via homology directed repair in nondividing, primary human hepatocytes treated with ARCUS LNP and a DNA template AAV.**

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PRECISION BIOSCIENCES

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