AAV-Meganuclease-Mediated Gene Targeting Achieves Efficient and Sustained Transduction in Newborn and Infant Macaque Liver

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ASGCT 25th Annual Meeting

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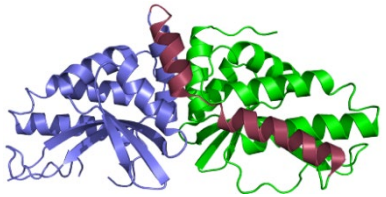
GENE THERAPY PROGRAM

Disclosures

- Lili Wang is an inventor on patents that have been licensed to biopharmaceutical companies
- J.M. Wilson is a paid advisor to and holds equity in iECURE, Scout Bio, Passage Bio, and the Center for Breakthrough Medicines (CBM). He also holds equity in the G2 Bio-associated asset companies. He has sponsored research agreements with Amicus Therapeutics, Biogen, CBM, Elaaj Bio, FA212, G2 Bio, G2 Bio-associated asset companies, iECURE, Janssen, Passage Bio, and Scout Bio, which are licensees of Penn technology. JMW is an inventor on patents that have been licensed to various biopharmaceutical companies and for which he may receive payments.
- This work is funded by 

Meganucleases Targeting the *PCSK9* Gene

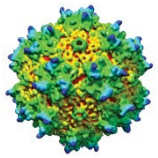
PRECISION BIOSCIENCES



self-guided, engineered meganuclease (ARCUS)

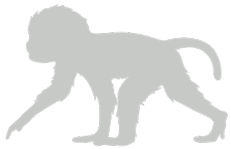
TGGACCTCTTTGCCCCAGGGGA
ACCTGGAGAAACGGGGTCCCCT

In exon 7 of *PCSK9* (conserved between human and rhesus)



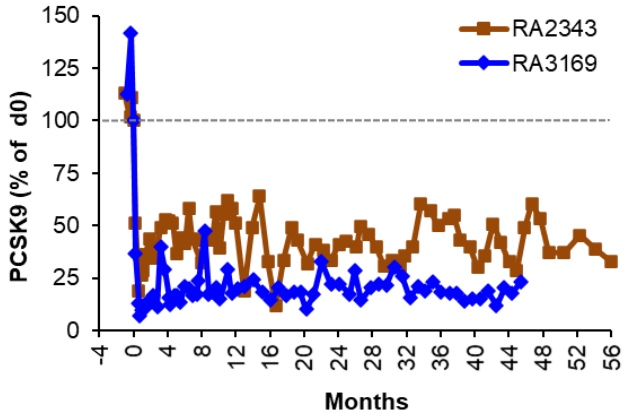
AAV8.TBG.ARCUS

$6x10^{12}$ GC/kg ⇩ IV

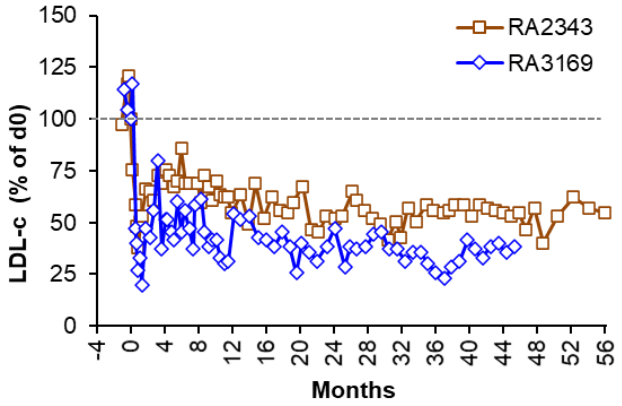


Stable & long-term reduction of PCSK9 & LDL-c

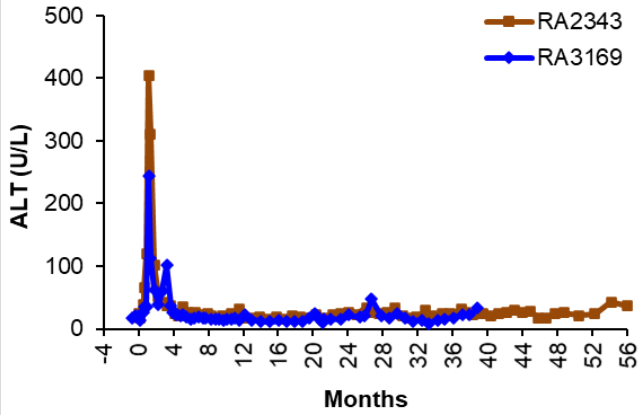
Serum PCSK9



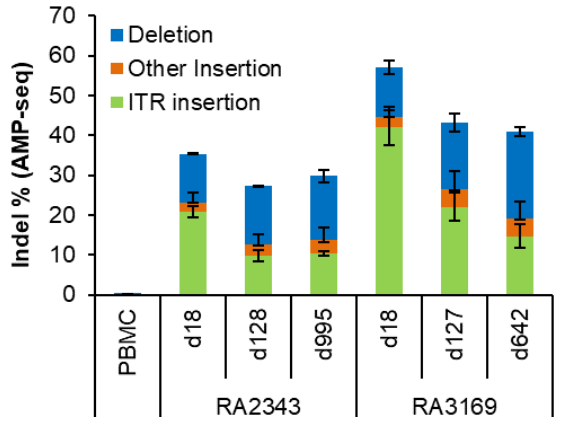
LDL-c



ALT



Indels in liver



Wang L, et al., Nat Biotechnol (2018)
Wang L, et al., Mol Ther (2021)

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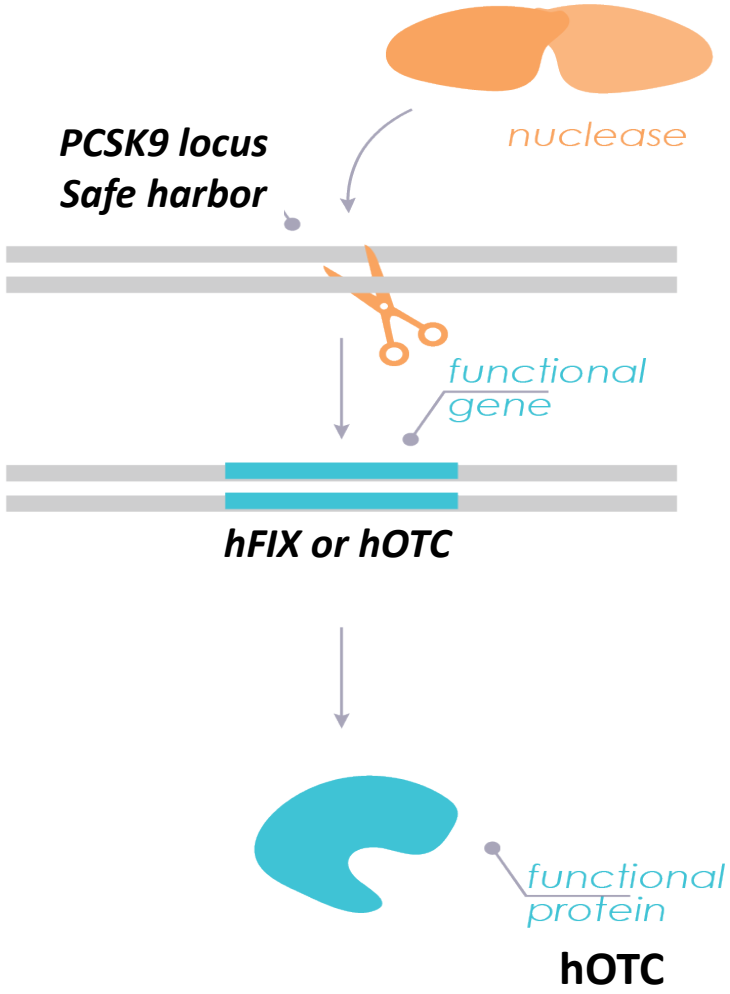
Loss of Function Inborn Errors of Liver Metabolism

Substantial unmet need

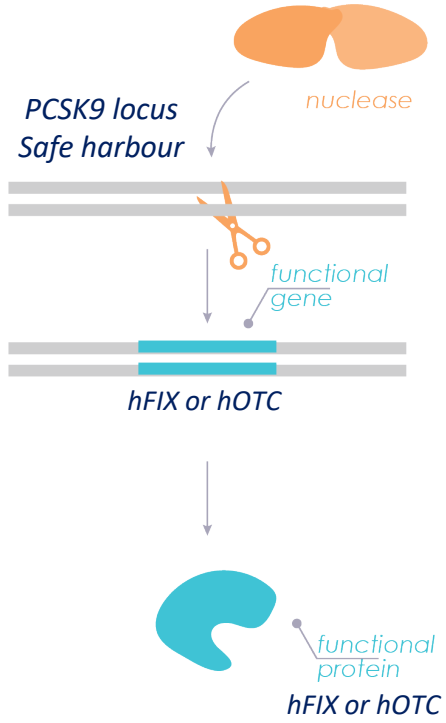
- Examples
  - Urea cycle disorders (e.g., OTC deficiency)
  - Organic acidurias
- Complete deficiency associated with life-threatening metabolic crisis as newborns
- High mortality during first crisis
- Liver transplantation is the only long-term solution
- AAV gene therapy will not work because vector genomes are diluted during liver growth
- Clinical and animal model data suggest 5% corrected cells will be transformative

A potential solution

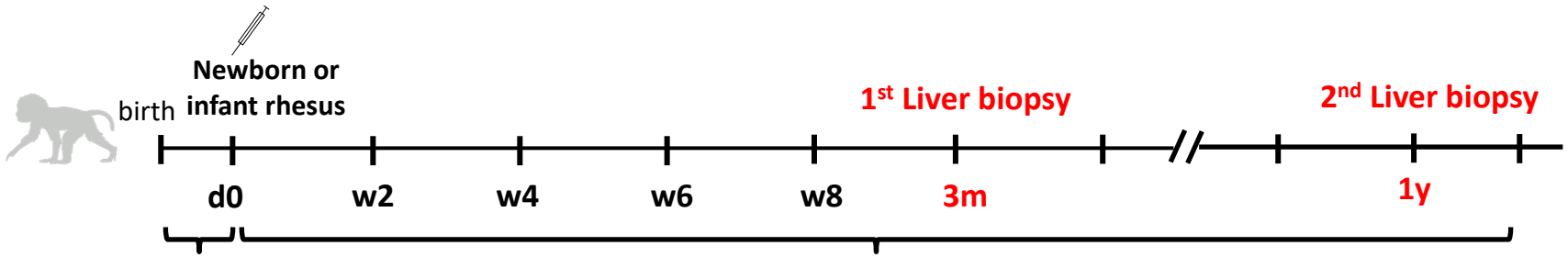


Evaluation of Meganuclease-mediated Targeted Gene Insertion in Newborn and Infant Rhesus Macaques

Gene knock-in



IV (ARCUS + *hFIX or hOTC* donor)

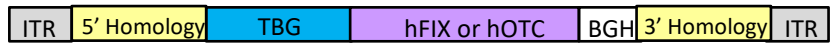


Nuclease Vector

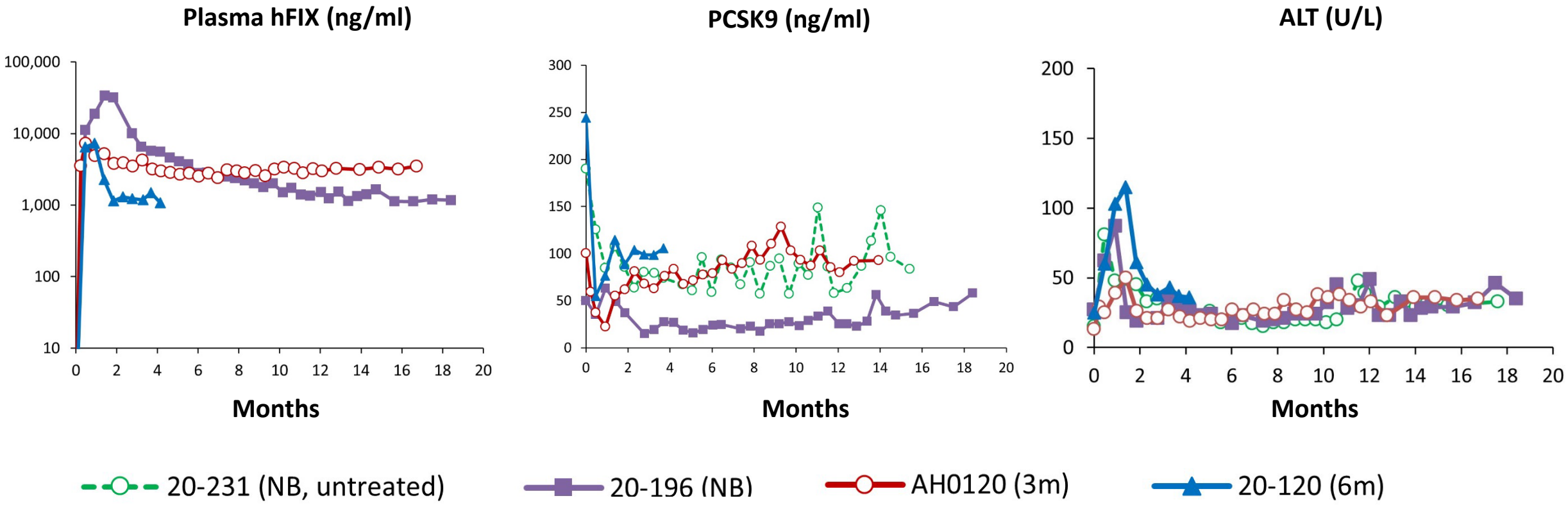
1 x 10^{13} GC/kg

Donor Vector

3 x 10^{13} GC/kg



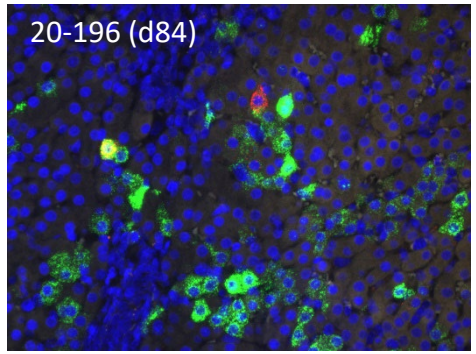
Gene Targeting of Human Factor IX in Newborn and Infant Macaques



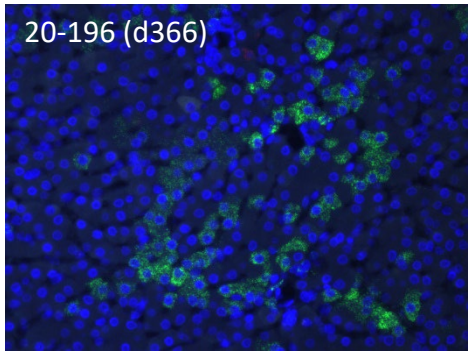
Stable Transduction in Newborn and Infant NHP Liver

Newborn

3-month biopsy

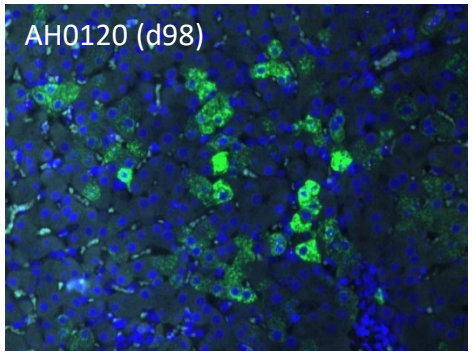


1-year biopsy

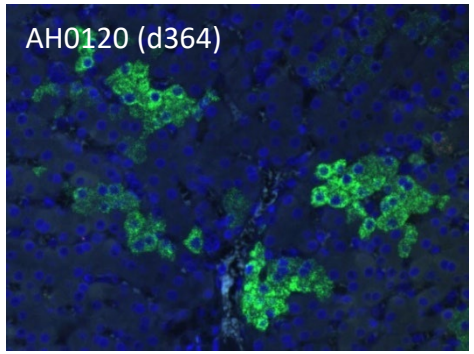


3-mo infant

3-month biopsy

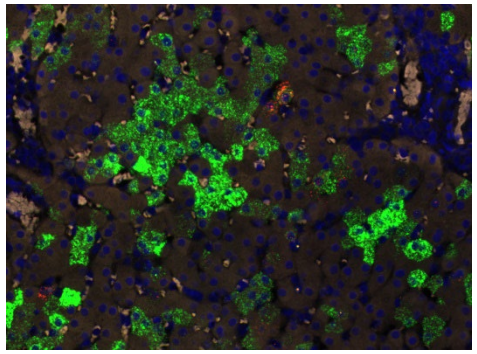


1-year biopsy

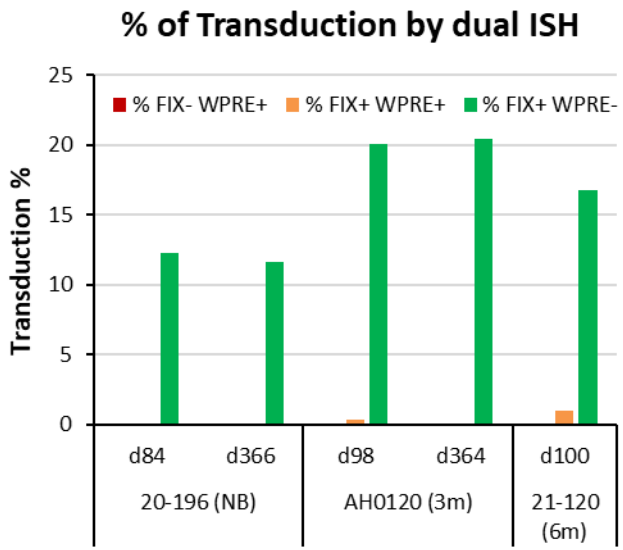


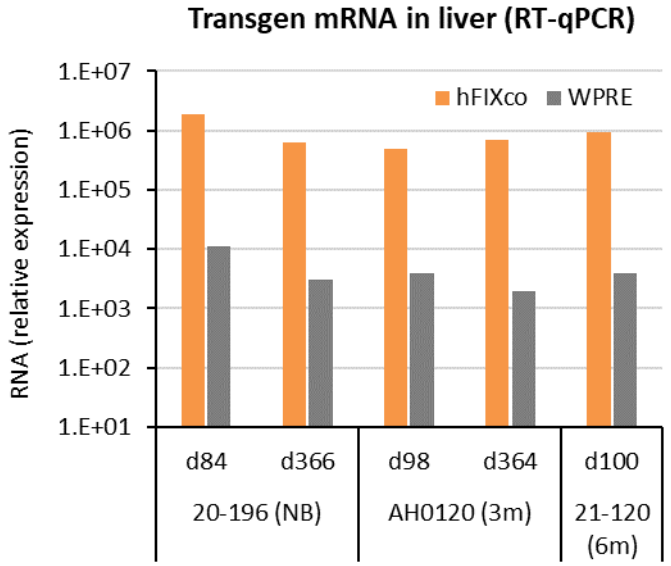
6-mo infant

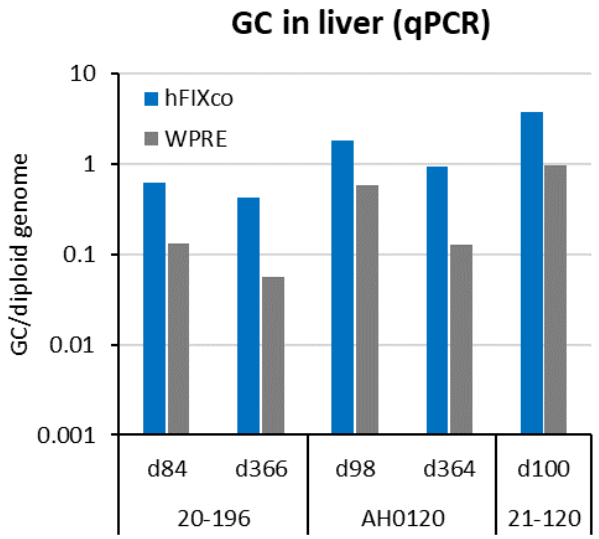
3-month biopsy



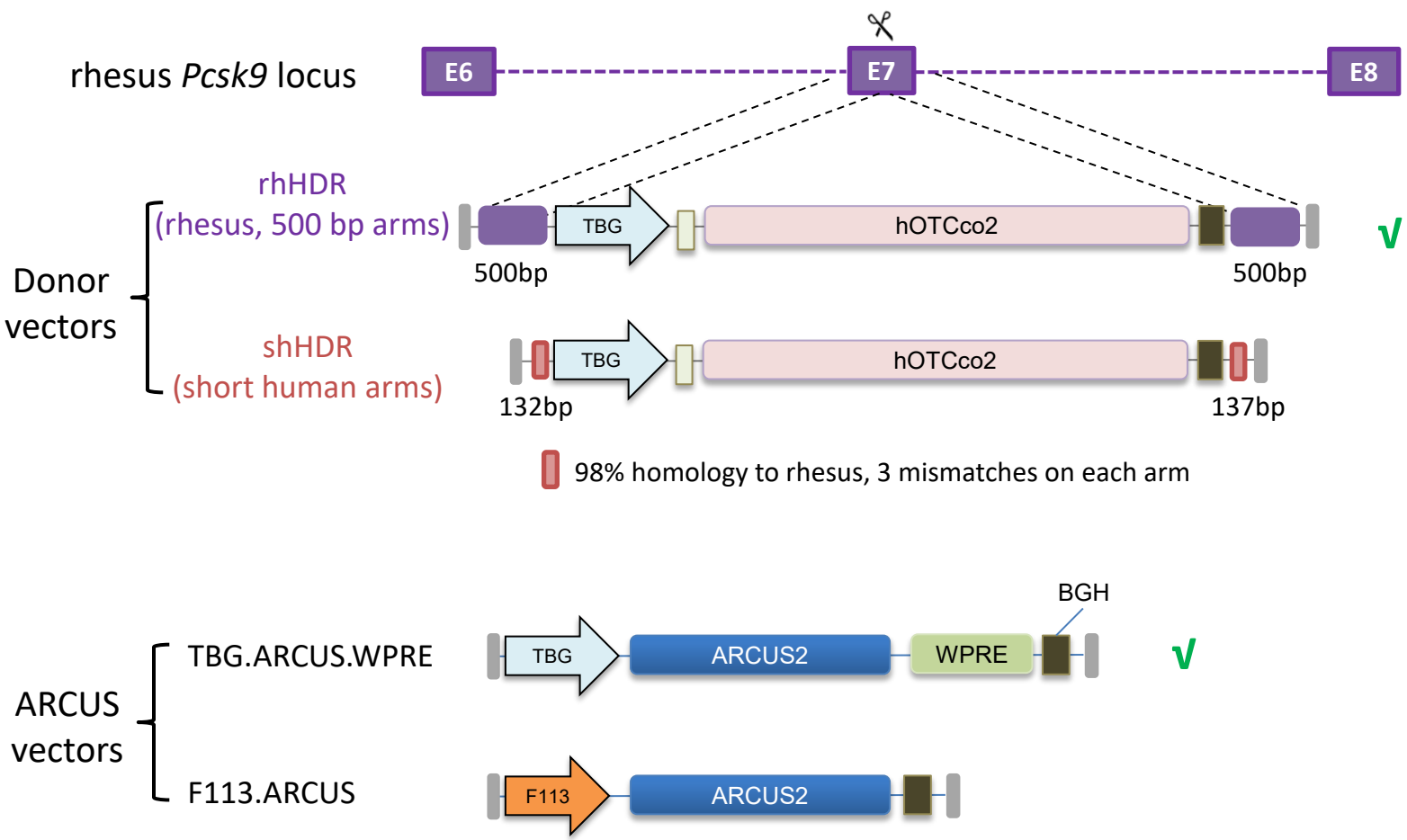
Two-plex ISH
green=hFIXco
red=WPRE=ARCUS

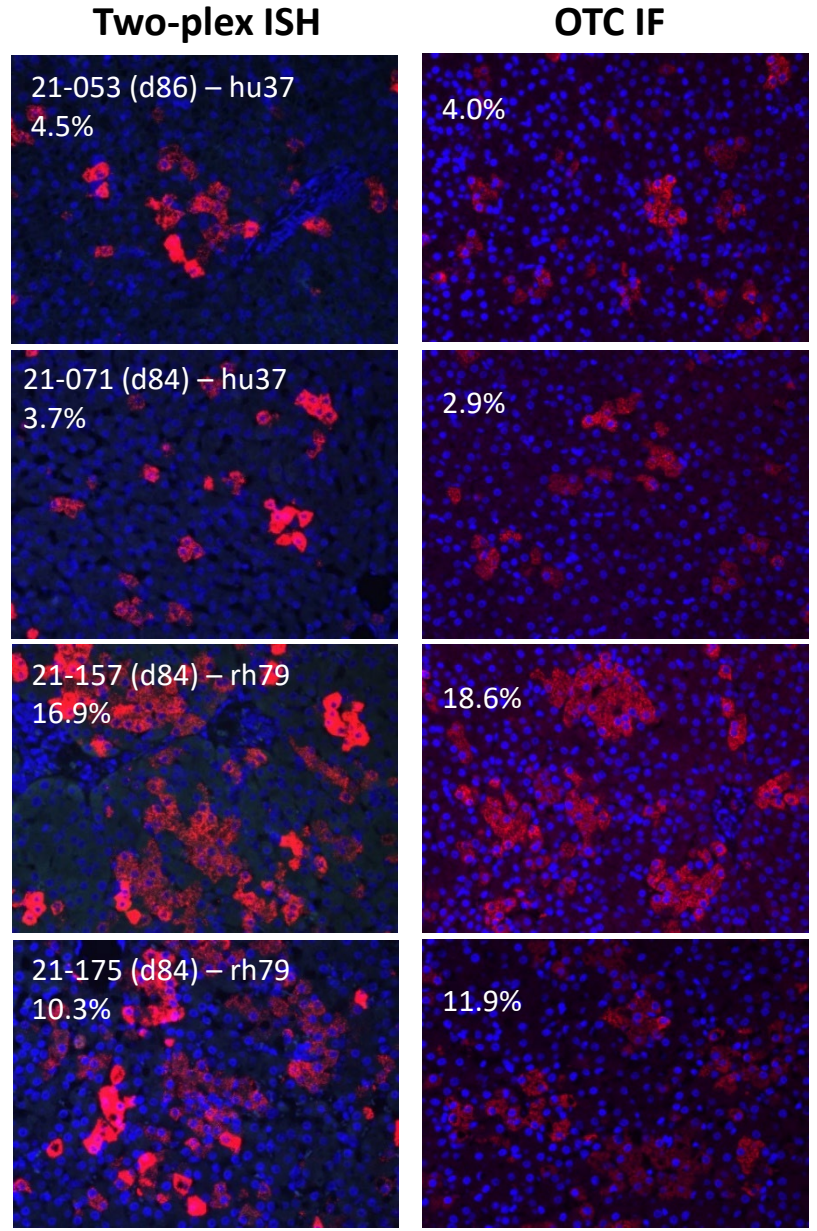






Selection of OTC Vectors for Efficient Targeting in Newborn NHPs





Two-plex ISH
Red = hOTCco
green = ARCUS

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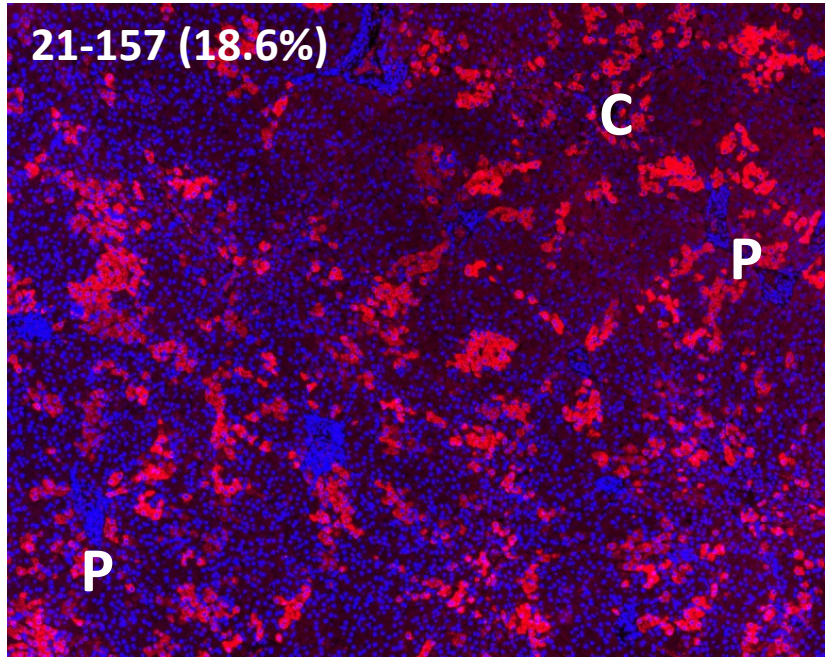
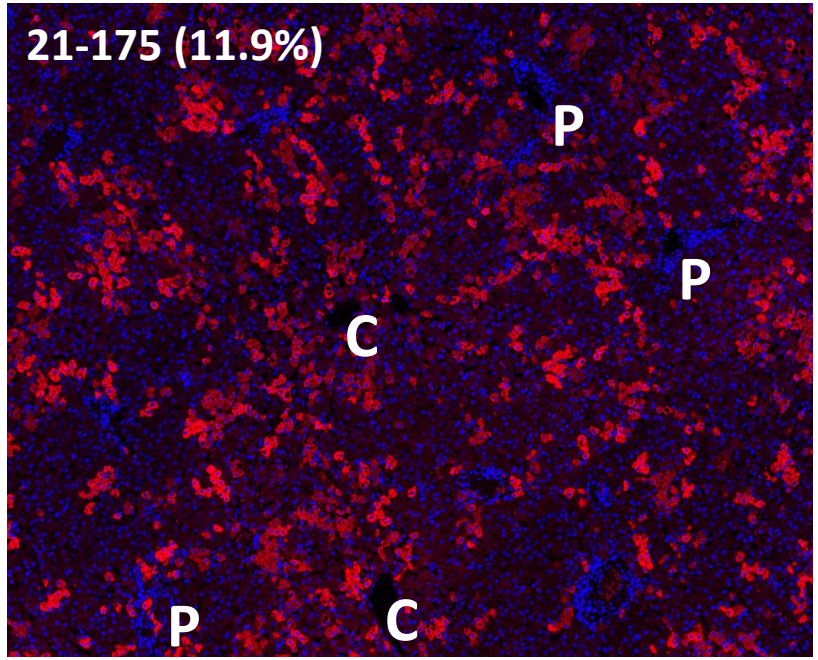
Wide Distribution of Edited Hepatocytes Throughout Portal Axis in Newborn NHP

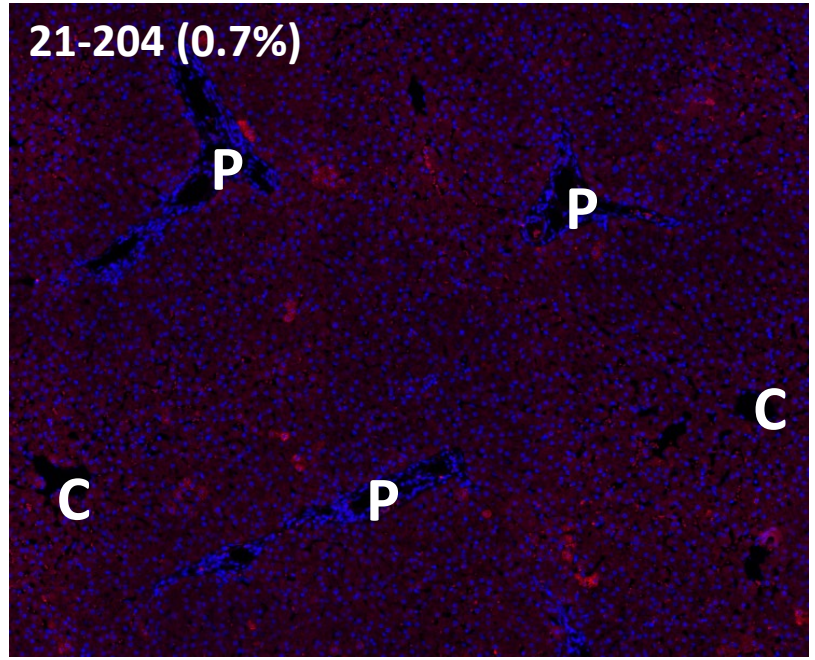
3-month liver biopsy – OTC immunostaining

rhHDR + TBG.ARCUS.W





rhHDR donor only



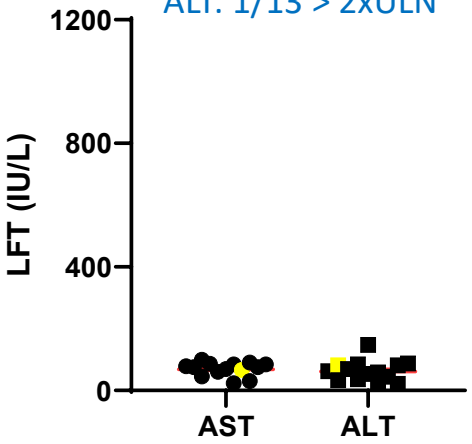
P: portal; C: central

Liver Inflammation is Substantially Reduced in Newborn vs Adult NHPs

Total AAV dose = 4.0 x 10^{13} GC/kg

Newborns, day 14

ALT: 1/13 > 2xULN

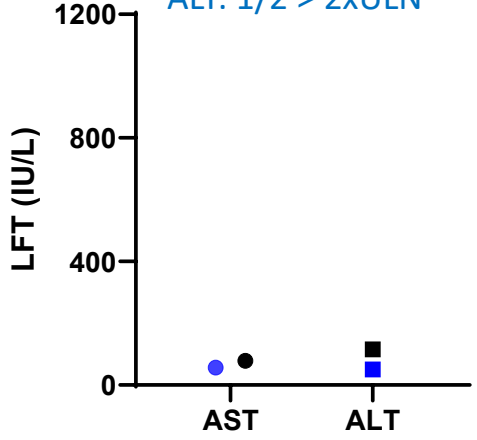


yellow symbol: untreated

black symbols: ARCUS + donor (n=13)

Infants, day 42

ALT: 1/2 > 2xULN



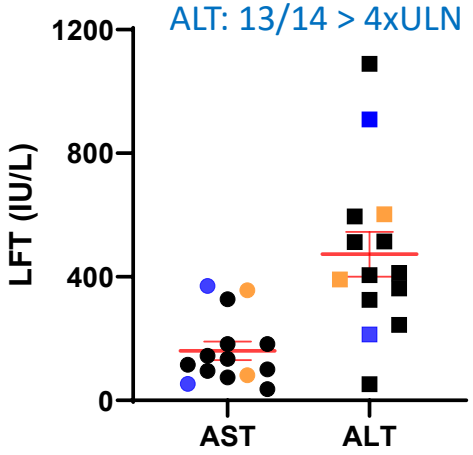
Blue symbol: 3 mo-old

black symbol: 6 mo-old

AAV.ARCUS only

Adults, day 28 ~ 42

ALT: 13/14 > 4xULN



blue symbols: 3x10^{13} GC/kg (n=2)

black symbols: 6x10^{12} GC/kg (n=10)

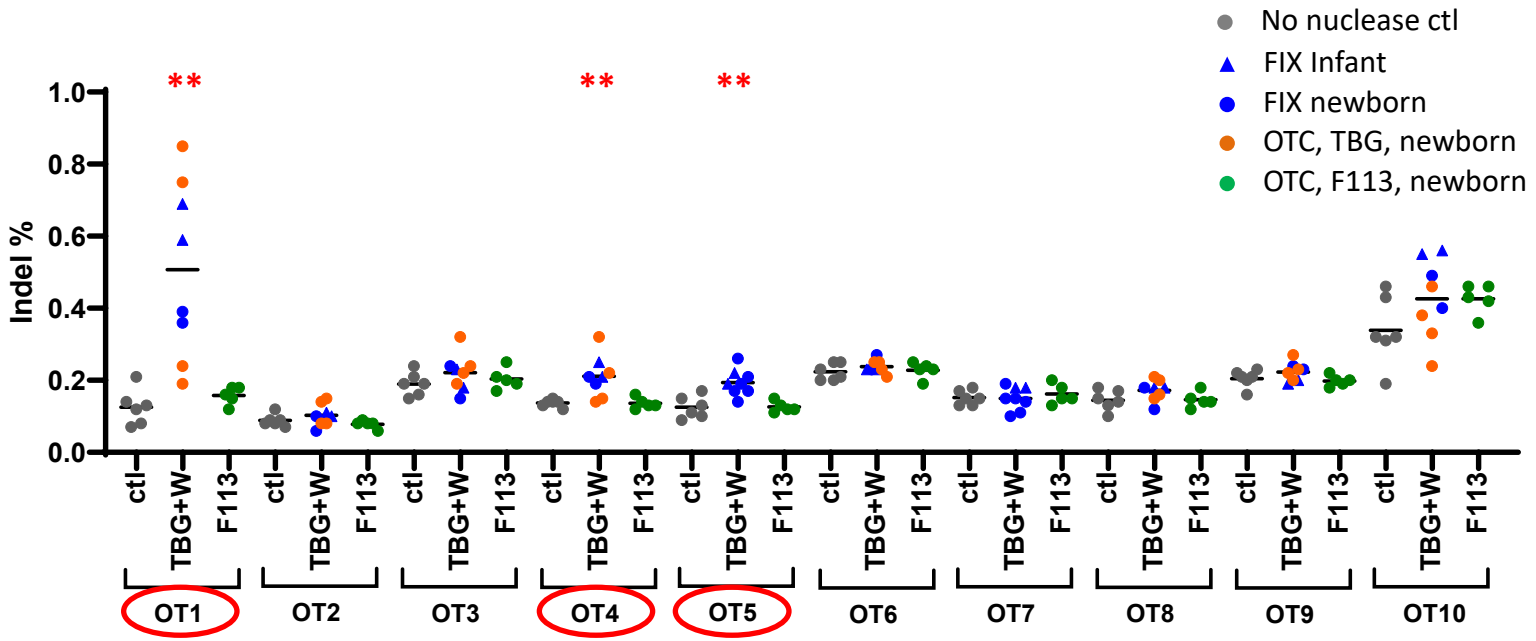
orange symbols: 2x10^{12} GC/kg (n=2)

Normal ranges of ULN for AST is 37 U/L and ALT 47 U/L

Off-Target (OT) Validation (Amplicon-seq)

ITR-seq to identify OT sites *in vivo* → **OT sites detected in multiple samples & animals** → **Validation by Amplicon-seq**

OT#	ITR-seq site	Best match to off-target consensus sequence
	on-target chr1	TGGACCTCTTTGCCCCAGGGGA
OT1	chr10:21153025	TGGCCCTTGGTGCCACAGGGAA
OT2	chr16:54719552	AGGACCTTGGTGAGCCCGGGAA
OT3	chr16:53659419	GGGCCCTCCTTGCCCCAGGGAA
OT4	chr9:58529853	TGGTCTGTGGTACCCCAGGGGA
OT5	chr19:17139526	TGGCCCCTTTTACAGGAGGGCC
OT6	chr7:86142563	TGGCCCTCTTTTCCAGAGGCCA
OT7	chr14:11607123	TCGACCCTGGTACCCCAGGGCG
OT8	chr20:68895219	TAGACCTCTTTTCCCCAGGGTC
OT9	chr19:35457309	TGTCCCTCTGTGCCCCAGGCCA
OT10	chr2:74738162	TGTCCCTCTGTCCCCCAGGCTA



- Editing on OT1, OT4, and OT5 were significantly higher in ARCUS + donor animals than in non-nuclease controls
- Off-target editing are lower in newborn/infants than in adult NHPs

Summary

- Growing livers of newborn/infant primates are highly receptive to site-directed insertion of donor genes
  - Consistently exceed the 5% therapeutic threshold
  - Appears highly durable
- Newborn/infant primates are tolerant to toxicity of systemic AAV
- Excellent correlation of dosing and site-specific integration when comparing NHPs and a mouse model of OTCD
- The long-term risks of off-target editing are unclear although we are encouraged by the over **42** years of event free follow-up in **33** NHPs we have studied with this nuclease
- The aggregate of our pre-clinical data suggest a favorable risk-benefit consideration for neonatal onset OTCD

Acknowledgements

James Wilson

Claude Warzecha
Ilayaraja Muthuramu
Camilo Breton
John White
Zhenning He
Hong Zhang

Cecilia Dyer
Peter Bell
Jessica Chichester
Qi Zheng
Kenton Woodard

Sissy Chikazawa
Yanqing Zhu
Kirsten Copren
Xin Huang
Shu-Jen Chen

Penn Vector Core
Histology Core
Immunology Core
Nucleic Acid Technologies Core
Nonhuman Primate Research Program
Program in Comparative Medicine

Keeling Center for Comparative Medicine and Research

Katrina Ralph
Rick Finch
Kathryn Shelton
Sarah Kezar
Luke Segura
Carolyn Hodo
Leqin Yan
Kathryn Meuth
Laura Zapalac

Funding

SRA from iECURE Inc.