Characterization of Guide RNA Site Consistency Across Ancestries and the Potential for Off-Target Editing with the Clinical-Stage Base Editing Medicine, VERVE-101

Joseph Biedenkapp, PhD

Alexandra C Chadwick, Jamie DeNizio, Sara Garcia, Anthony Federico, Manashree Damle, Hui-Ting Hsu, Estela Shabani, Daniel Weiner, Amit V Khera, Joseph Biedenkapp, Sekar Kathiresan, Troy Lister, Andrew M Bellinger

Verve Therapeutics, Boston, MA, USA

Presented at the American Society & Gene and Cell Therapy 27th Annual Meeting
11 May 2024

Speaker Disclosure

Joseph Biedenkapp is an employee and equity holder of Verve Therapeutics.

Investigational Product

VERVE-101 is an investigational agent that is not approved for commercial use in any jurisdiction.

Forward-looking statements

This presentation contains "forward-looking statements" within the meaning of the Private Securities Litigation Reform Act of 1995 that involve substantial risks and uncertainties, including statements regarding the potential advantages and therapeutic potential of the Company's programs, including VERVE-101. All statements, other than statements of historical facts, contained in this presentation, including statements regarding the Company's strategy, future operations, future financial position, prospects, plans and objectives of management, are forward-looking statements. The words "anticipate," "believe," "continue," "could," "estimate," "expect," "intend," "may," "plan," "potential," "predict," "project," "should," "target," "will," "would" and similar expressions are intended to identify forward-looking statements, although not all forward-looking statements contain these identifying words. Any forward-looking statements are based on management's current expectations of future events and are subject to a number of risks and uncertainties that could cause actual results to differ materially and adversely from those set forth in, or implied by, such forward-looking statements. These risks and uncertainties include, but are not limited to, risks associated with the Company's limited operating history; the Company's ability to timely submit and receive approvals of regulatory applications for its product candidates; advance its product candidates in clinical trials; initiate, enroll and complete its ongoing and future clinical trials on the timeline expected or at all; correctly estimate the potential patient population and/or market for the Company's product candidates; replicate in clinical trials positive results found in preclinical studies and/or earlier-stage clinical trials of VERVE-101, VERVE-102 and VERVE-201; advance the development of its product candidates under the timelines it anticipates in current and future clinical trials; obtain, maintain or protect intellectual property rights related to its product candidates; manage expenses; and raise the substantial additional capital needed to achieve its business objectives. For a discussion of other risks and uncertainties, and other important factors, any of which could cause the Company's actual results to differ from those contained in the forward-looking statements, see the "Risk Factors" section, as well as discussions of potential risks, uncertainties and other important factors, in the Company's most recent filings with the Securities and Exchange Commission and in other filings that the Company makes with the Securities and Exchange Commission in the future. In addition, the forward-looking statements included in this presentation represent the Company's views as of the date hereof and should not be relied upon as representing the Company's views as of any date subsequent to the date hereof. The Company anticipates that subsequent events and developments will cause the Company's views to change. However, while the Company may elect to update these forward-looking statements at some point in the future, the Company specifically disclaims any obligation to do so.

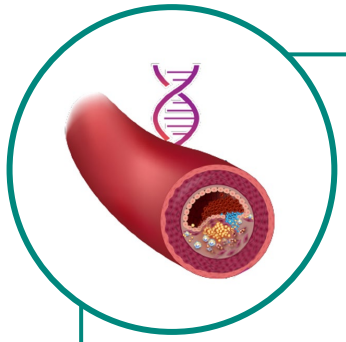
verve™ THERAPEUTICS

Atherosclerotic cardiovascular disease (ASCVD) is the leading cause of death worldwide

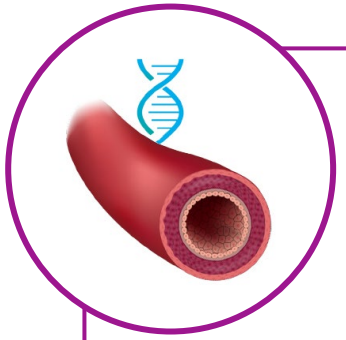
100s of millions
of people affected

One person
dies every 34 seconds
from cardiovascular disease in the U.S.[1]

~800K heart attacks
per year in the U.S.[2]



Cause: Exposure to blood low-density lipoprotein cholesterol (LDL-C) clogs heart arteries



Solution: keep blood LDL-C as low as possible for as long as possible

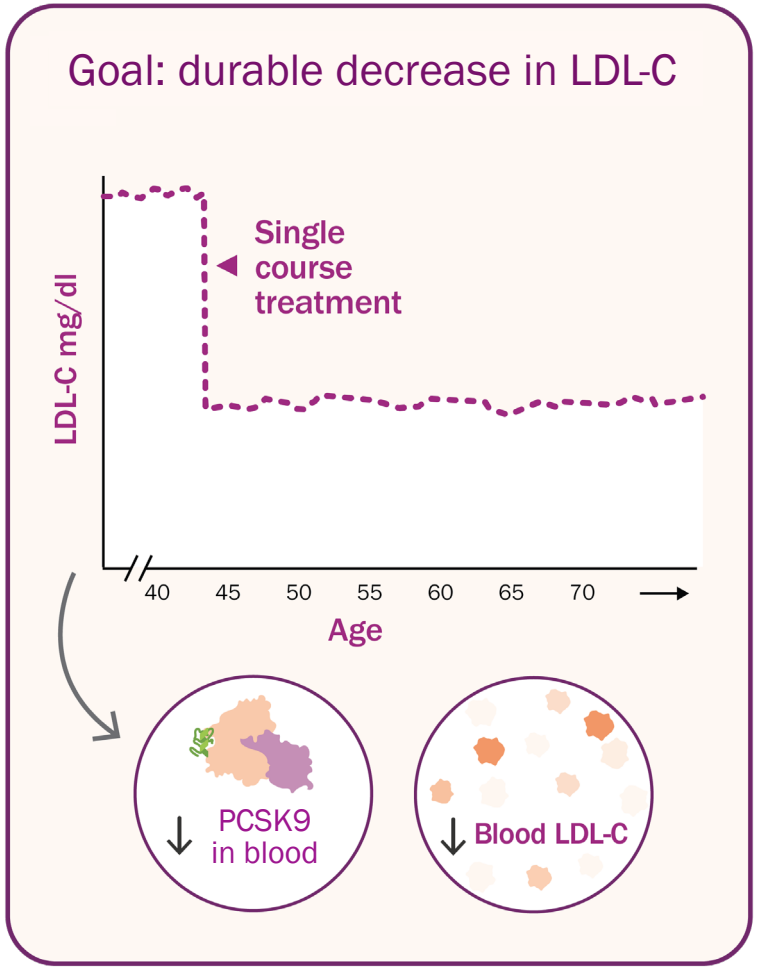
1. Centers for Disease Control and Prevention, National Center for Health Statistics. About Multiple Cause of Death, 1999-2020. CDC WONDER Online Database website. Atlanta, GA: Centers for Disease Control and Prevention; 2022. Accessed February 21, 2022; 2. Tsao CW et al. Circulation. 2022;145(8):e153–e639.

Human genetics provides a potential solution: Inactivate *PCSK9* to permanently reduce LDL-C

Naturally occurring loss-of-function variants in *PCSK9* result in:

- Lifelong LDL-C lowering
- Protection against CV events
- No apparent deleterious effects[1,2,3]

Pharmacologic validation of target



Can we develop a single-course treatment that mimics natural *PCSK9* variants which protect against ASCVD?

1. Zhao Z, et al. Am J Hum Genet. 2006;79:514-523; 2. Cohen JC, et al. N Eng J Med. 2006;354:1264-1272; 3. Rao AS, et al. Circ Genom Prec Med. 2018;11(7):e002162.
CV, cardiovascular; PCSK9, proprotein convertase subtilisin/kexin type 9

VERVE-101 is an investigational base editing medicine with *in vivo* LNP delivery designed to inactivate *PCSK9*

DRUG SUBSTANCES

RNA components encode base editor and a guide targeting *PCSK9* gene

- mRNA for adenine base editor (ABE)
- gRNA localizes editor to *PCSK9* gene

+

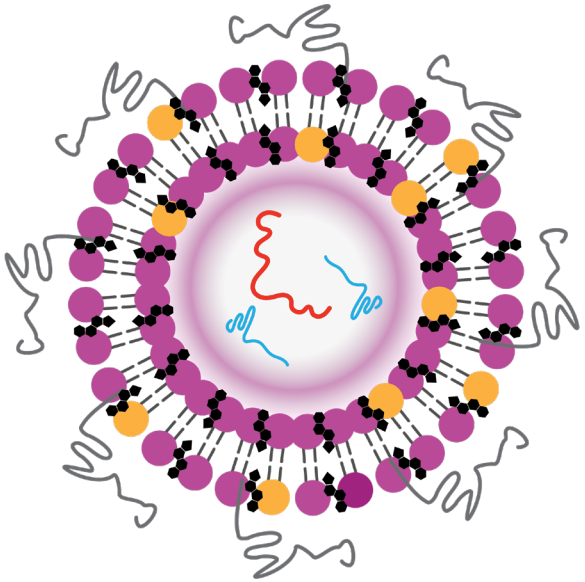
DELIVERY VEHICLE

Lipid nanoparticle (LNP) for delivery to liver cell includes 4 components

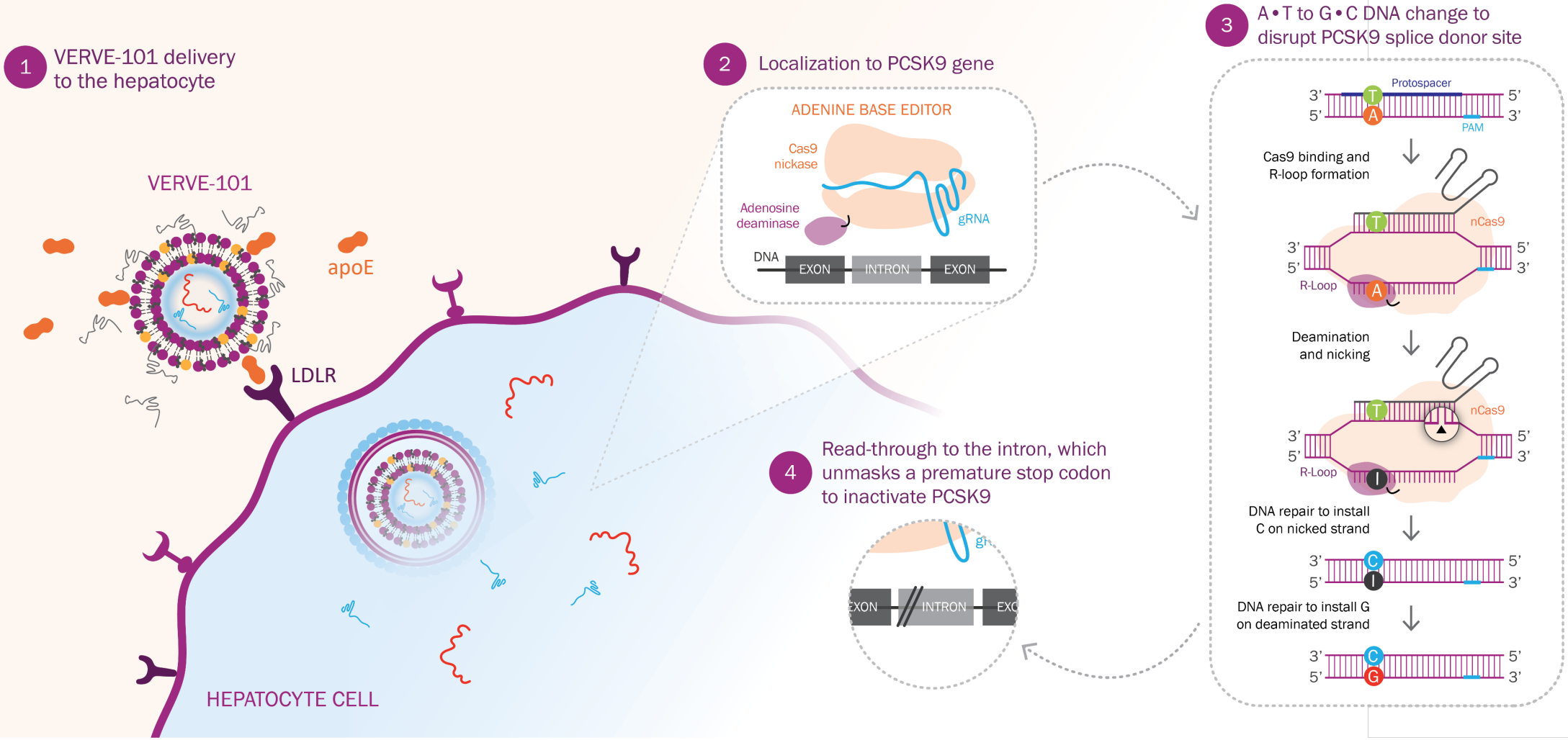
- Ionizable amino lipid
- DSPC
- Cholesterol
- PEG

=

VERVE-101



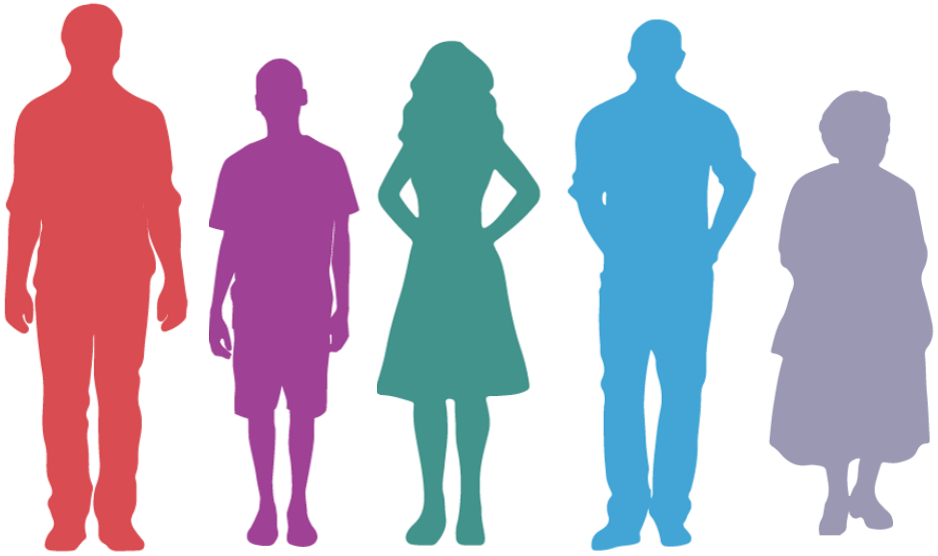
VERVE-101 targets hepatocytes where it inactivates *PCSK9* by unmasking a premature stop codon



Lipid nanoparticle · Ionizable amino lipid · DSPC · LDL receptor (LDLR) · apoE · mRNA · gRNA · PEG Lipid · Cholesterol

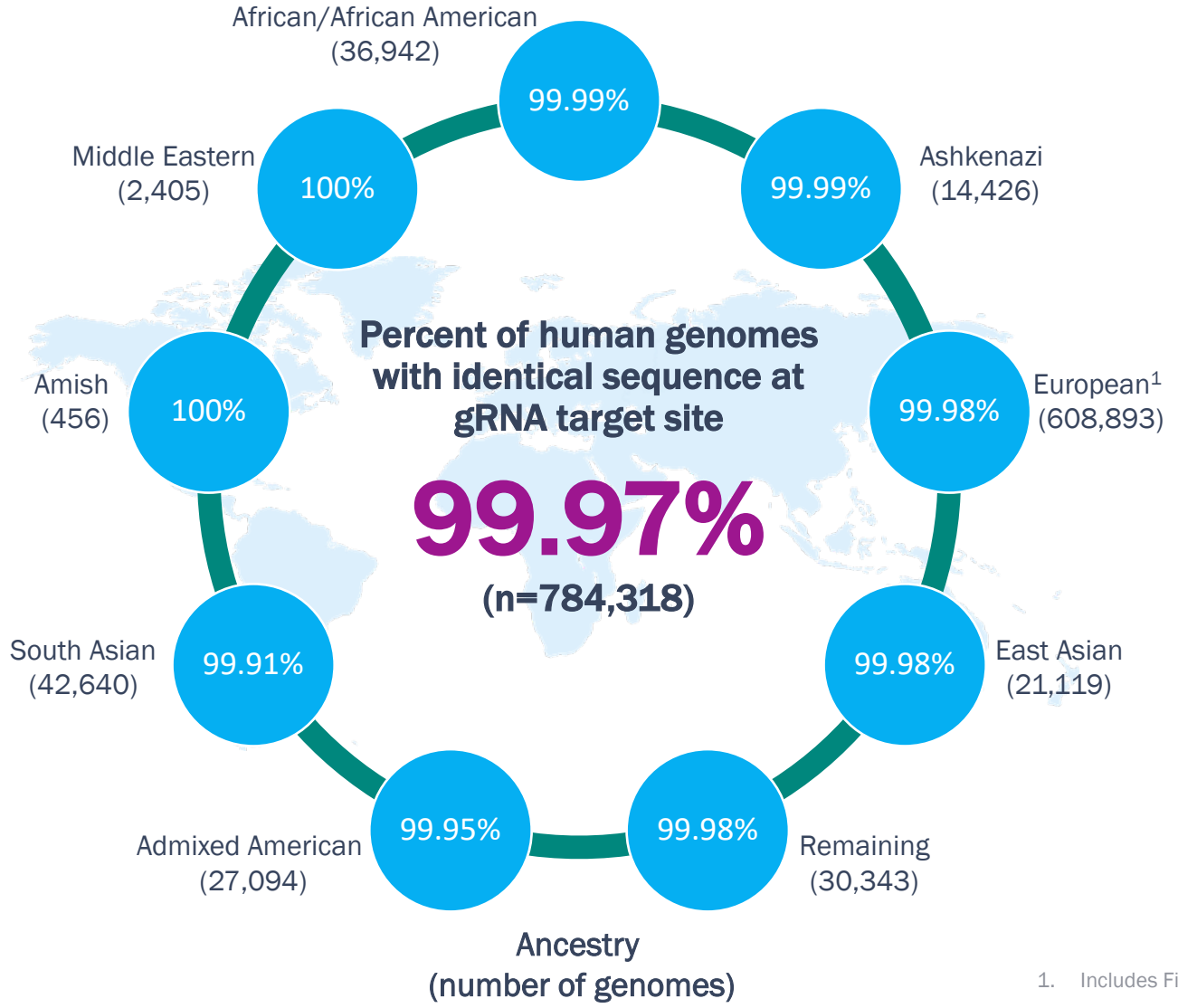
Key questions

How consistently will a gene-targeting therapy work across diverse ancestries?



Are there unintended edits being made at other sites in the genome?

Consistency in *PCSK9* target site indicates potential benefits should apply across diverse ancestries

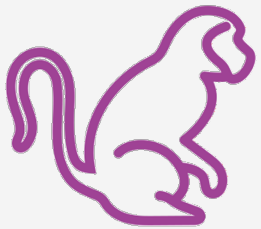


- PCSK9 gRNA site identical in 99.97% of genomes in gnomAD v4.0[2]
- Consistency >99.9% in each genetically assigned ancestry category

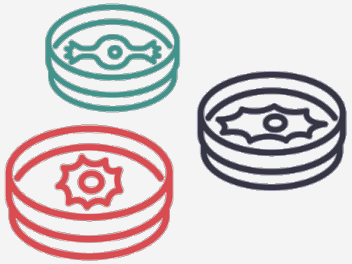
1. Includes Finnish and non-Finnish European populations; genomes include whole genomes and exomes
2. Chen S, et al. Nature. 2024;625:92-100.

Comprehensive and systematic approach to screen for off-target editing with VERVE-101

Select human cell types for off-target assessment



Guided by biodistribution of editing in animal models



Incorporate diverse cellular contexts and genomic backgrounds

Screen for three types of off-target edits in human cells

gRNA-dependent:
Unintended edits driven by gRNA pairing with DNA

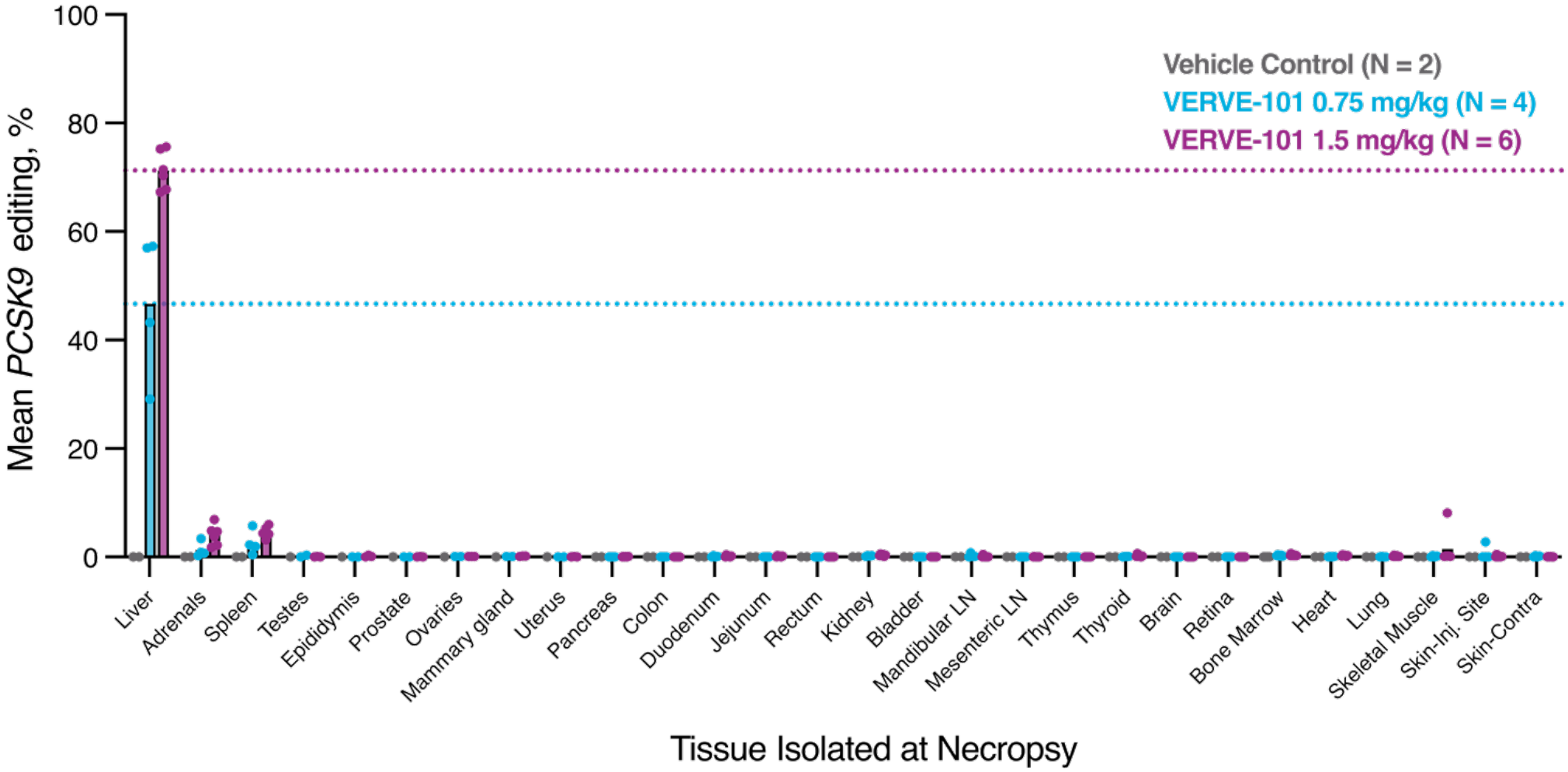
gRNA-independent:
Nonspecific excess adenine editing of DNA

Structural Variant:
Induced large chromosomal rearrangements

gRNA, guide RNA

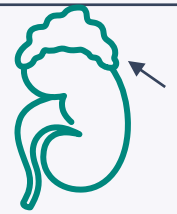
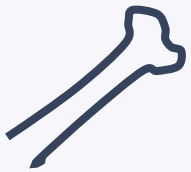
Liver, adrenal, and spleen cells selected for off-target analysis based on biodistribution in non-human primates

On-target editing predominantly occurs in the liver in NHPs treated with VERVE-101



NHP, non-human primates

VERVE-101 off-target analysis incorporated multiple cell types from four tissues and twenty donors

Tissue type	Cell types, n	Donors, n	Reason for inclusion in OT program
 Liver	2 (PHH, HuH-7)	11	Liver is intended tissue for on-target editing
 Adrenal Glands	1	3	NHP biodistribution analysis
 Spleen	2	3	NHP biodistribution analysis
 Bone Marrow	1	3	Provides open chromatin cellular context

Donor ancestries represented: European, African-American, Hispanic/Latino, Japanese

NHP, non-human primate; PHH, primary human hepatocytes

gRNA-dependent off-target editing screen: Multiple orthogonal methods to nominate candidate sites

candidate site nomination methods

Experimental: ABE-digenome-seq[1,2]

Genome-wide analysis of DNA from human liver cells exposed to base editor

Experimental: ONE-Seq[3]

Editing of synthetic library of tens of thousands of DNA sequences with high homology to target site

Bioinformatics:

In silico assessment of human genome

panel of candidates

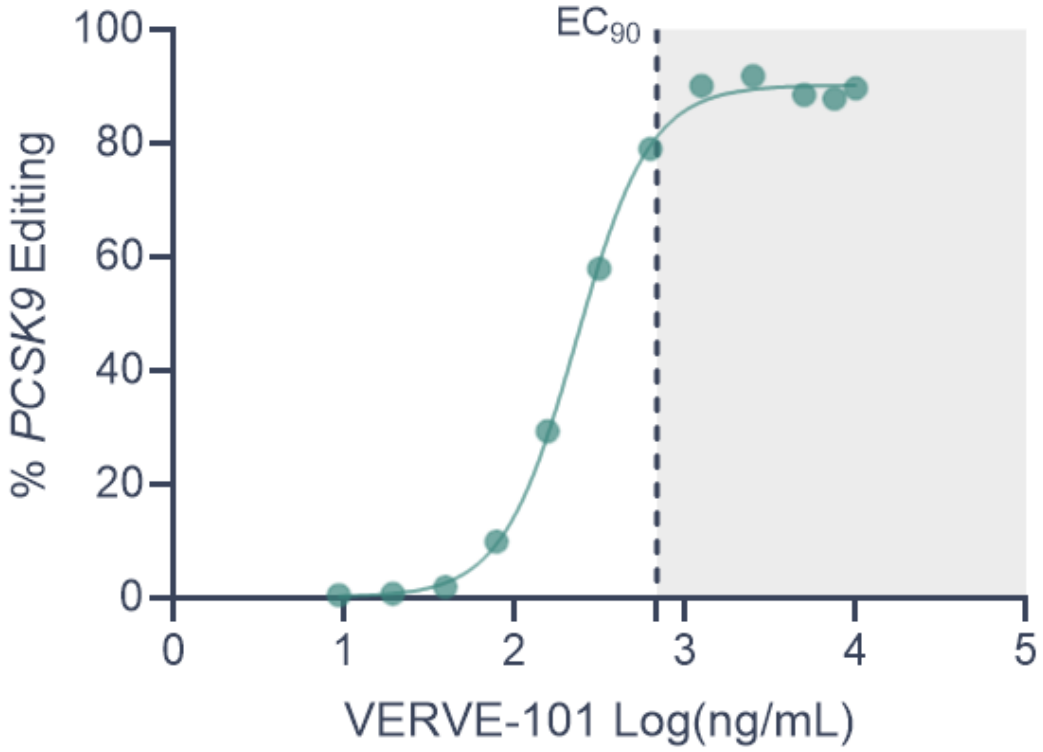
~6000 sites

across the genome with experimental or bioinformatic similarity to the on-target site

1. Kim et al. Nature Biotechnology 2019;37(4):430-435. 2. Liang et al. Nature Communications 2019;10(1):67; 3. Petri et al. bioRxiv 2021;2021.04.05.438458.

Cells treated with VERVE-101 to screen for gRNA-dependent off-target editing at ~6000 candidate sites

Representative dose responsiveness of on-target editing in PHH



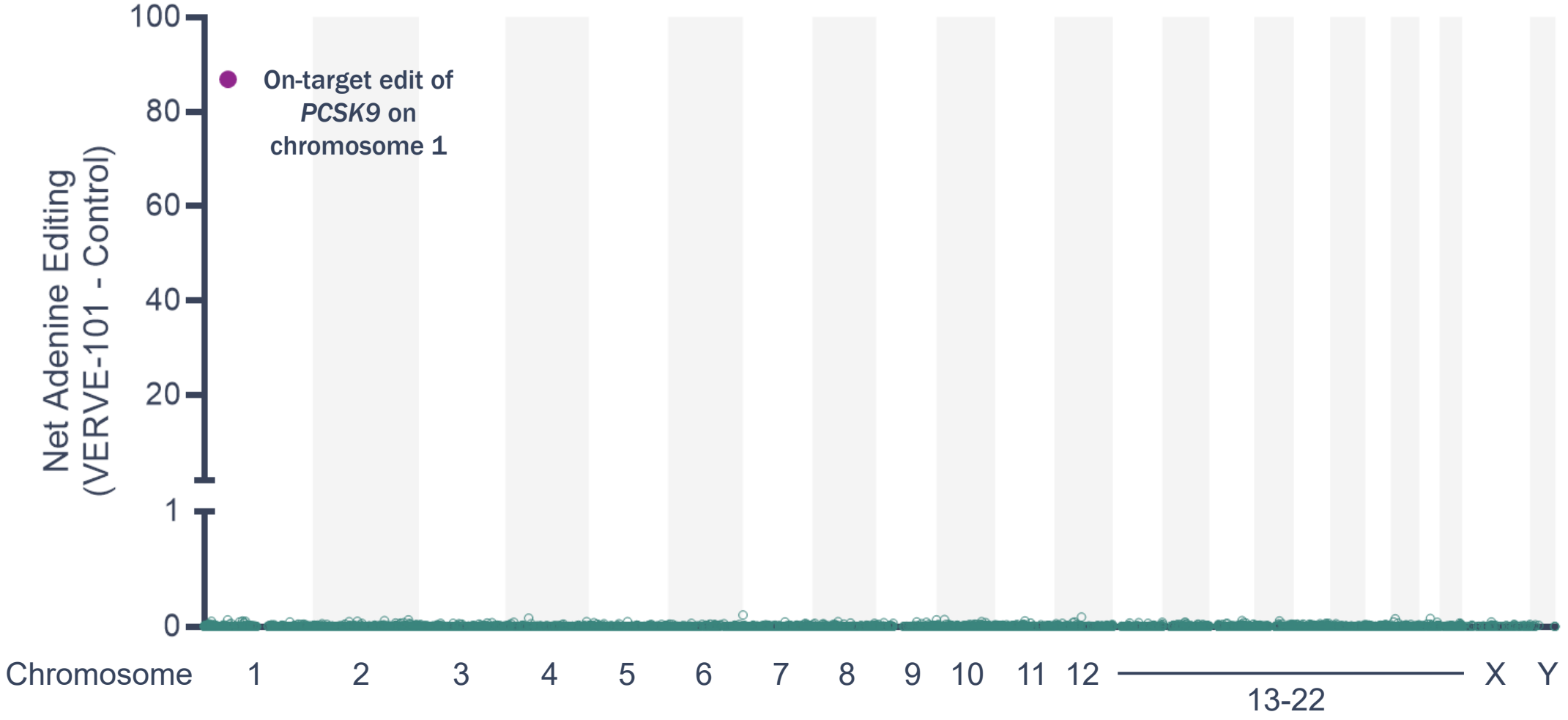
Steps to increase detection sensitivity and quantify off-target editing:

- Use of doses ≥ EC_{90} for on-target editing, exceeding what is pharmacologically achievable *in vivo*
- DNA from treated cells enriched for candidate sites using hybrid capture
- Suspected off-target sites are verified with targeted amplicon sequencing and assessed for dose responsiveness

EC_{90}, 90% maximal effective concentration; PHH, primary human hepatocytes

No off-target gRNA-dependent editing with VERVE-101 in primary human hepatocytes, adrenal, or bone marrow cells

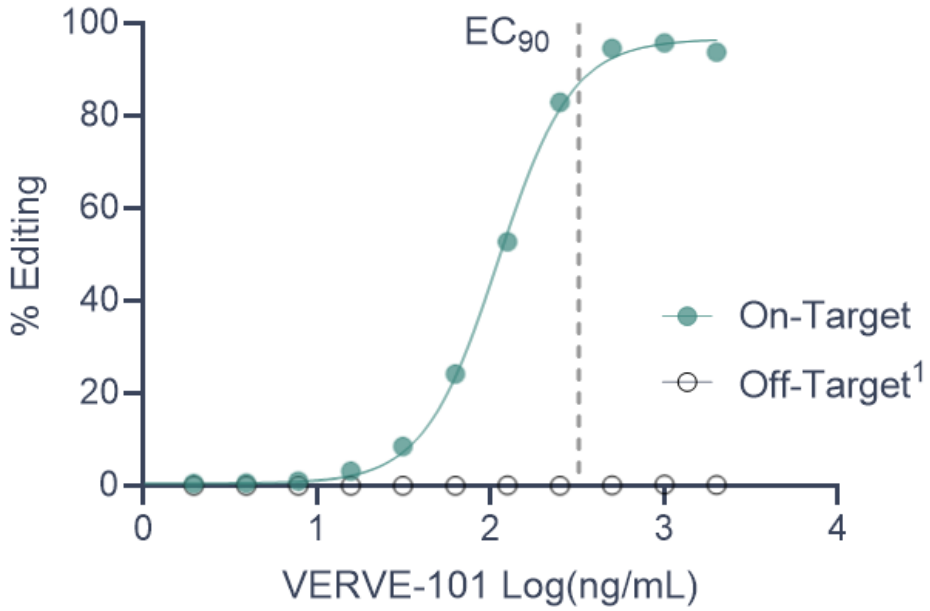
Manhattan style plot of net adenine editing in analysis of ~6000 candidate sites in PHH



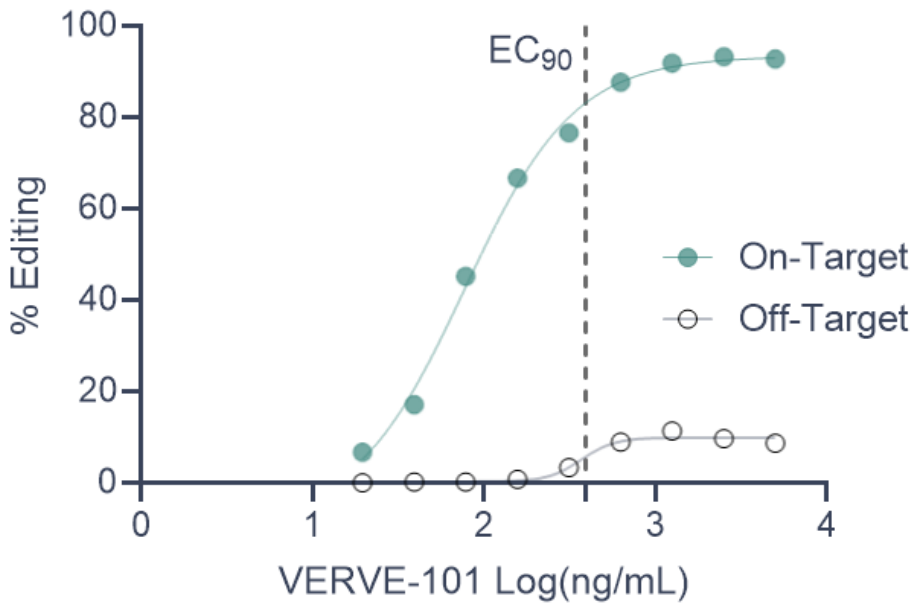
Y axis indicates net editing (alternate allele frequency in treated primary liver cells – matched untreated controls); PHH, primary human hepatocytes

Two gRNA-dependent off-target sites detected with low frequency editing: One in HuH-7 cells and one in splenic cells

HuH-7 Liver Cell Line



Primary Splenic Cells



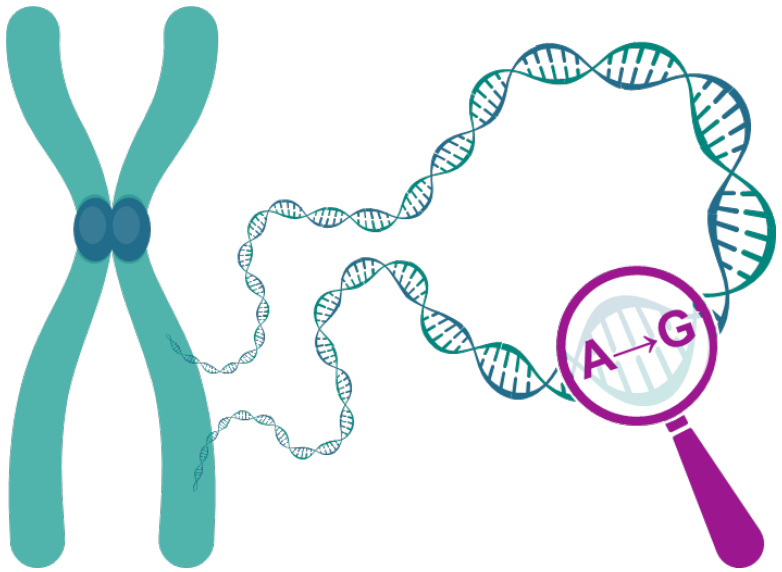
Characterization

- Off-target editing unlikely to occur at pharmacological doses *in vivo*
- Sites not in protein coding regions
- Sites not in or near genes associated with cancer
- Sites not likely to impact nearby gene expression

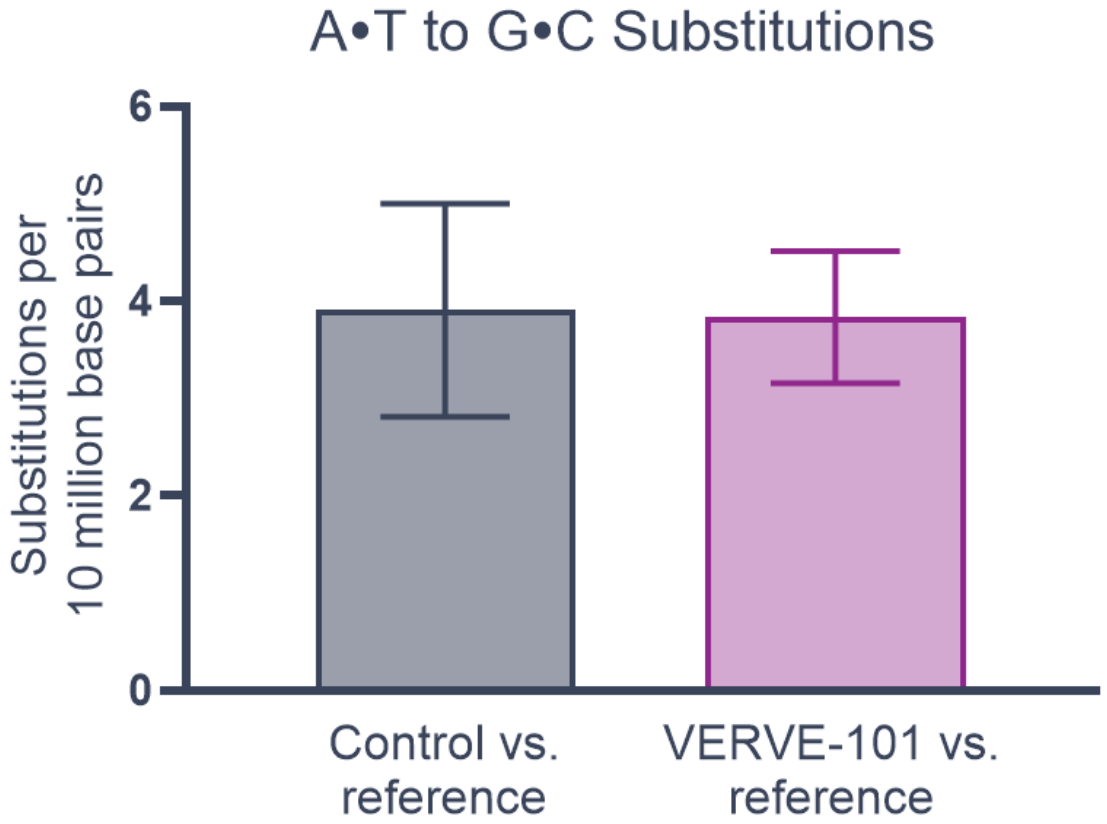
1. Off-target editing of HuH-7 cells was very low frequency (< 1%) but was dose responsive
EC_{90}, 90% maximal effective concentration

gRNA-independent off-target editing: No evidence for excess adenine editing

Whole Genome Sequencing



global search for excess adenine editing using treated and untreated cells sequenced at 500x coverage



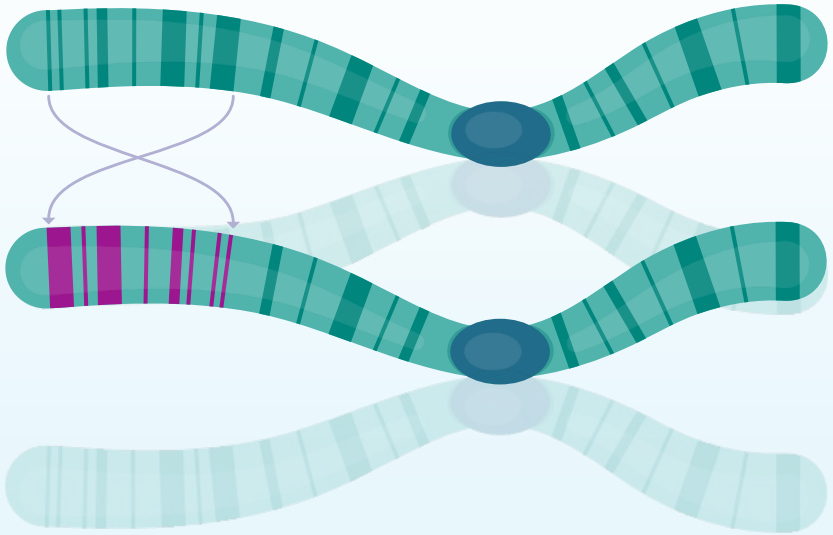
Representative analysis in PHH

PHH, primary human hepatocytes

Structural variant screening: Two orthogonal methods for detecting chromosomal changes

Optical Genome Mapping (OGM)

Long DNA fragments are labeled at a specific sequence motif[1,2,3]



Genome-wide method to detect all SVs at resolution >500 base pairs

Anchored Tn5 Unidirectional PCR-based sequencing (ATUP)

Look outward from the target site[4]

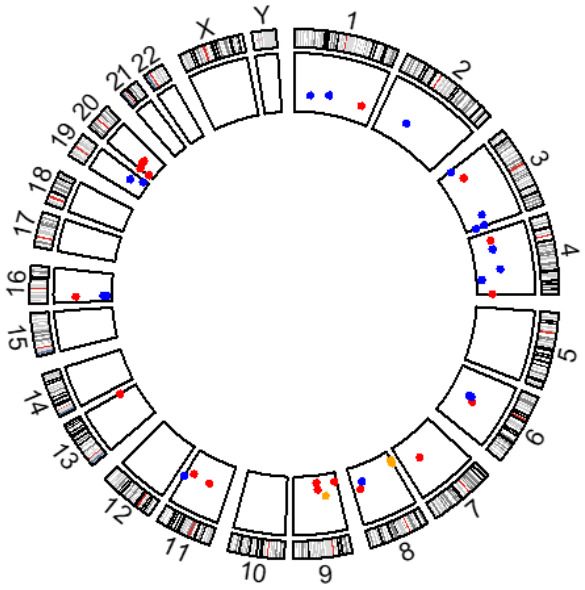
Identifies gRNA-dependent SVs at resolution >20 base pairs

1. Cao et al. Gigascience 2013;3(1):34. 2. Mak et al. Genetics 2015;202(1):351-62. 3. Bionano Genome White Paper Series. 2020. Online available: bionanogenomics.com/wp-content/uploads/2020/02/Bionano_Human-Structural-Variations-White-Paper.pdf; 4. Giannoukos et al. BMC Genomics 2018;19:212.

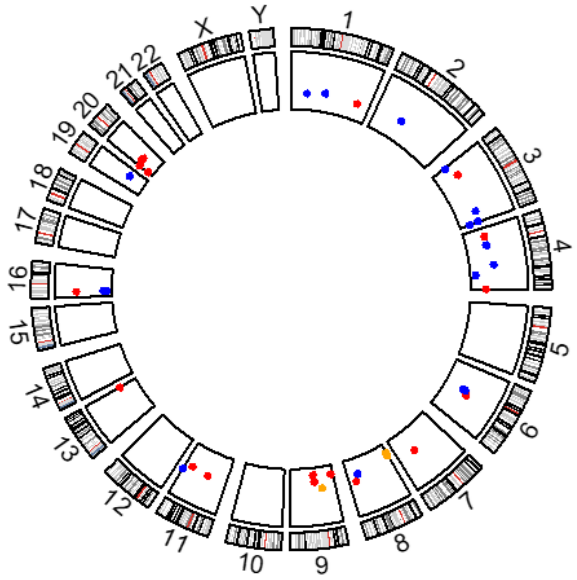
SV, structural variant

No structural variant formation following VERVE-101 treatment

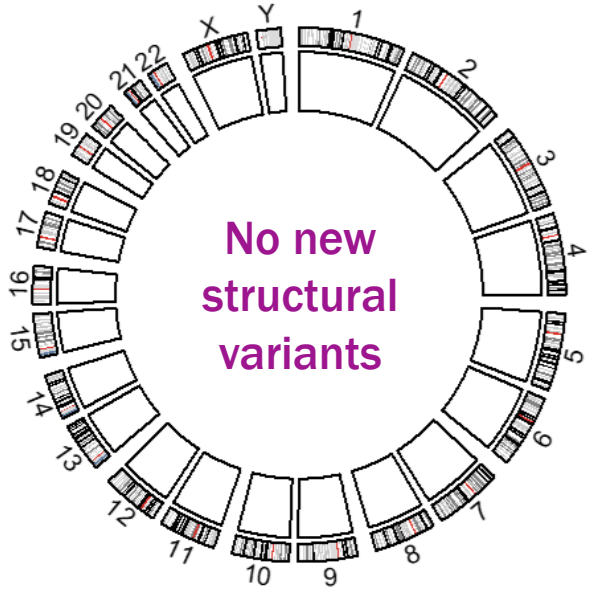
SVs present in untreated control cells vs. reference



SVs present after VERVE-101 treatment



VERVE-101 minus untreated



insertion duplication deletion

Anchored Tn5-mediated unidirectional PCR method also showed no evidence for induced structural variant formation (data not shown)

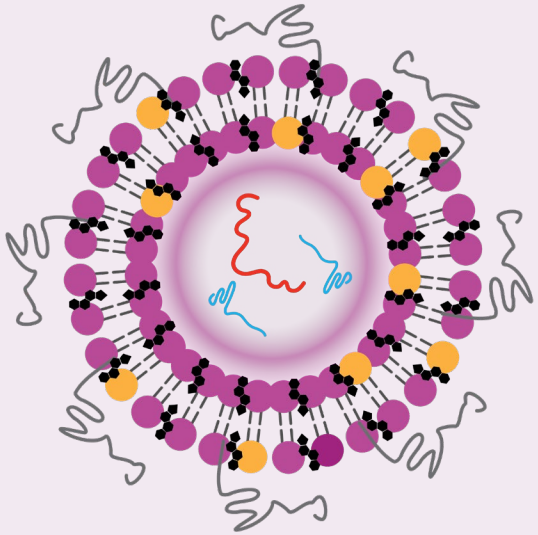
SV, structural variant

Conclusions

- VERVE-101 is the first *in vivo* base editing medicine to demonstrate pharmacodynamic proof-of-concept in humans
- The consistency of the gRNA target site across populations suggests potential therapeutic benefits of editing should apply broadly across individuals with diverse ancestries
- Comprehensive off-target assessment incorporating twenty donors, four tissue types, and different cellular contexts showed VERVE-101 to be highly specific with a low risk for clinically relevant off-target edits
  - Two gRNA-dependent off target edits were detected at low frequency in select cell types and characterized as low risk
  - No evidence for global excess adenine editing or structural variant formation with VERVE-101

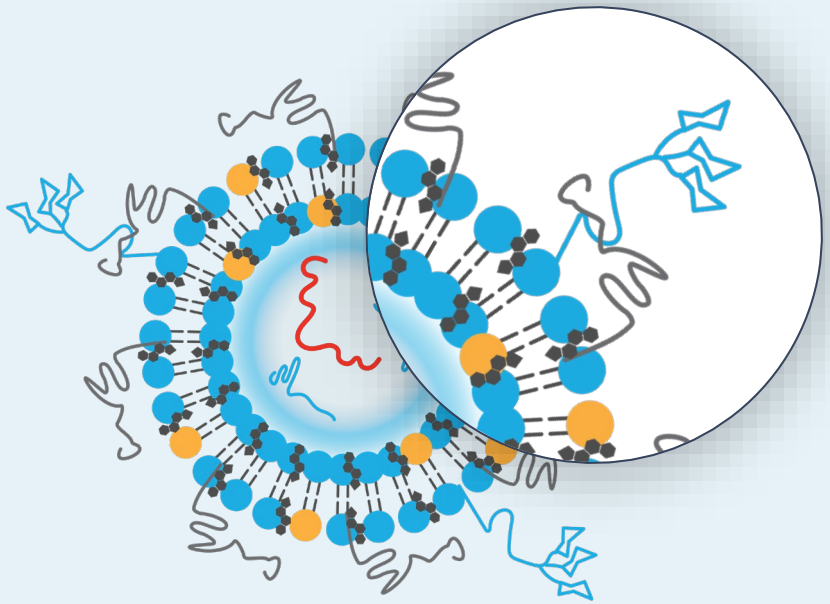
Verve has two *in vivo* CRISPR base editing product candidates that target *PCSK9* with an identical ABE and gRNA but different LNP delivery systems

VERVE-101



First-in-human program

VERVE-102



Dosing ongoing

- Different ionizable lipids
- VERVE-101 enters hepatocytes through the LDL receptor (LDLR)
- VERVE-102 has an added GalNAc targeting ligand – enabling entry by LDLR or asialoglycoprotein receptor

ABE, adenine base editor; gRNA, guide RNA; LNP, lipid nanoparticle