Comprehensive approach to evaluate off-target editing for an *in vivo* liver base editing medicine targeting the PCSK9 gene

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Disclosure

I am an employee of Verve Therapeutics.

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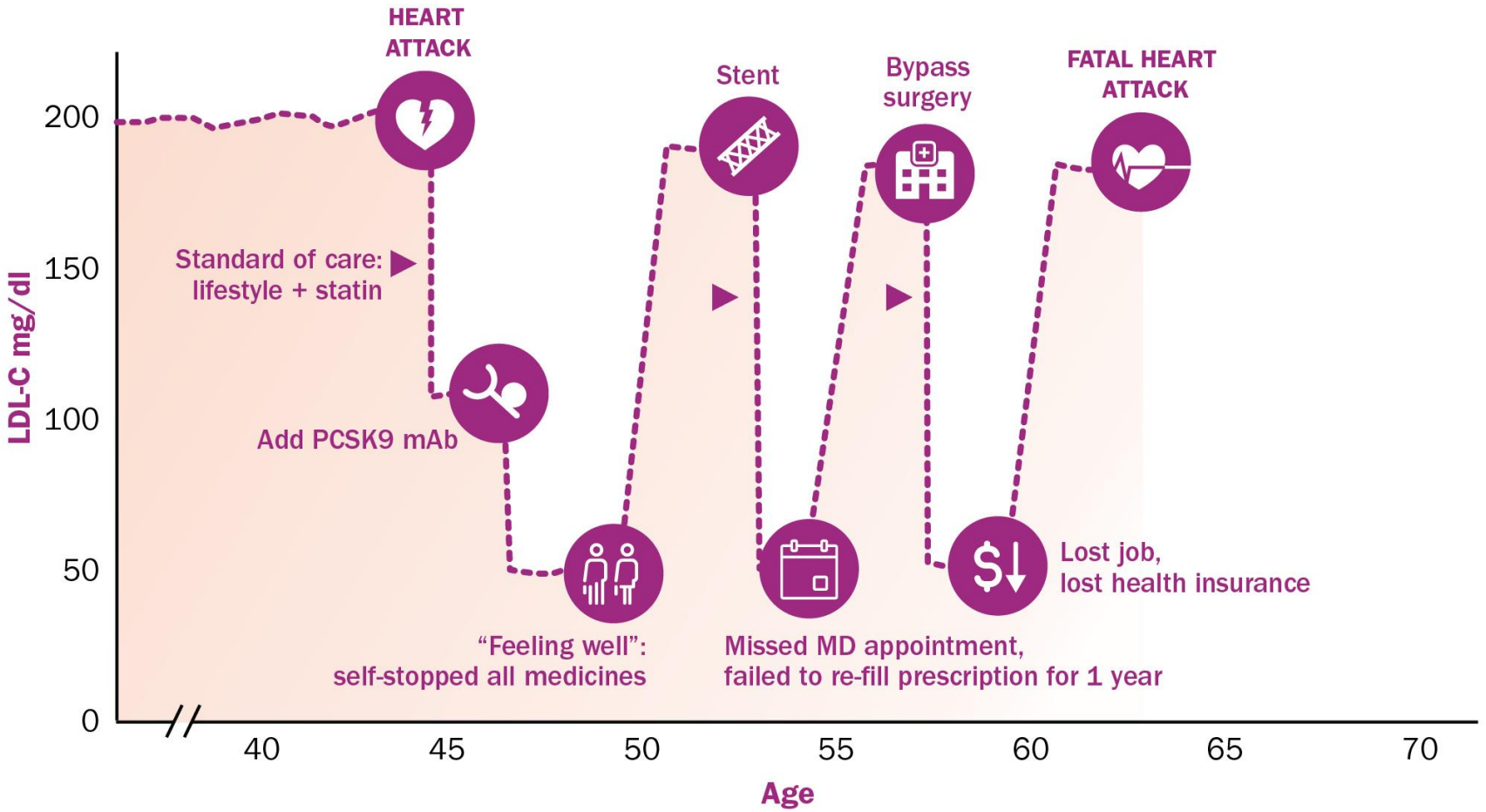
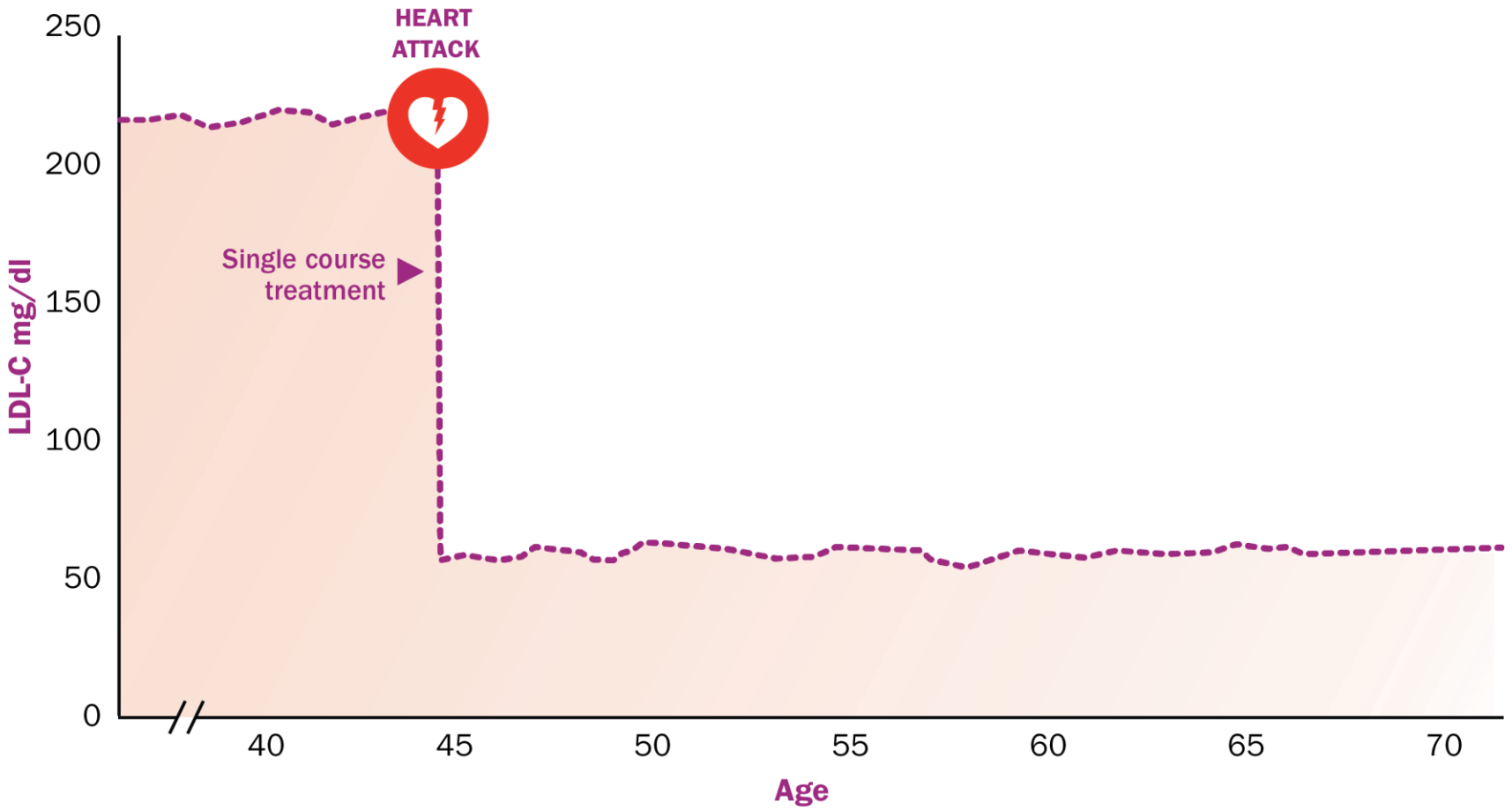
Chronic care model results in poor control of cumulative blood low-density lipoprotein cholesterol (LDL-C) exposure



Illustrative graphic depicts the journey of a hypothetical patient with familial hypercholesterolemia who began standard-of-care treatment after suffering a heart attack at age 44

Single-course treatment for atherosclerotic cardiovascular disease (ASCVD) could address key unmet need: getting LDL-C as low as possible for as long as possible



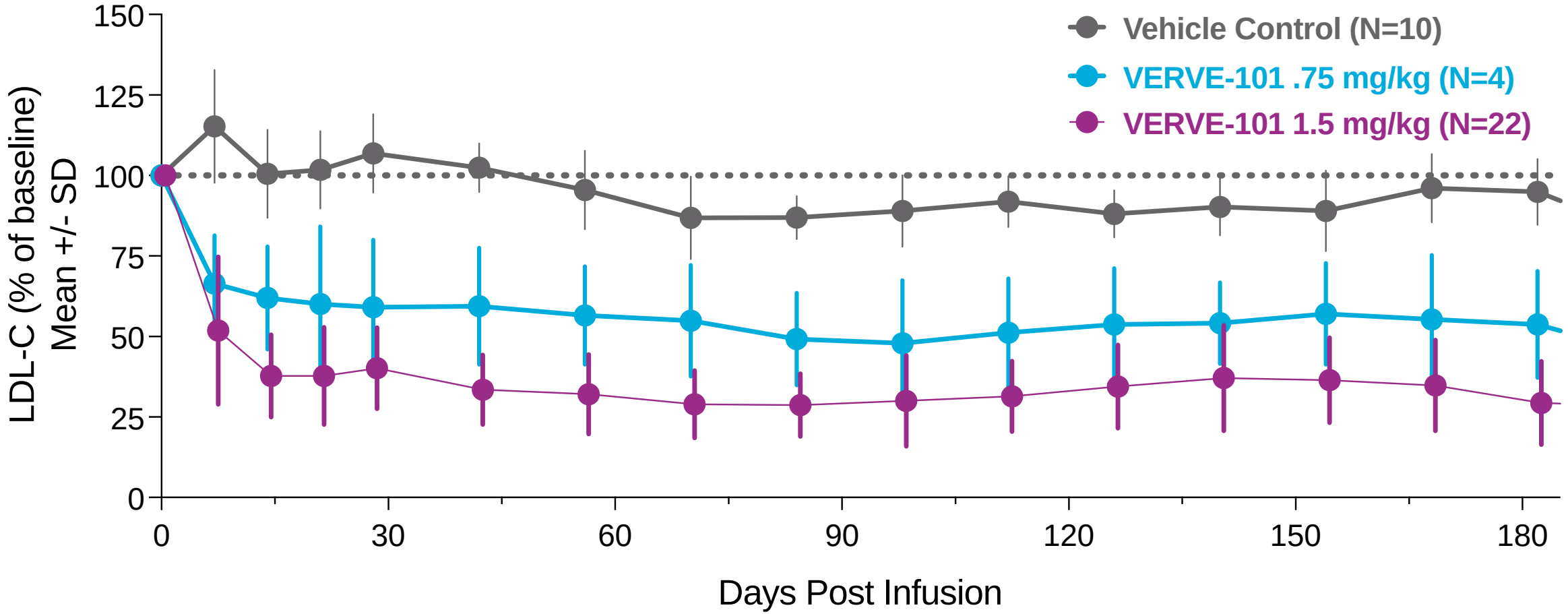
Illustrative graphic depicts the journey of a hypothetical patient with familial hypercholesterolemia who was treated with a single-course treatment after suffering a heart attack at age 44

Advancing a pipeline of single-course *in vivo* gene editing programs to safely and durably lower LDL-C and treat ASCVD

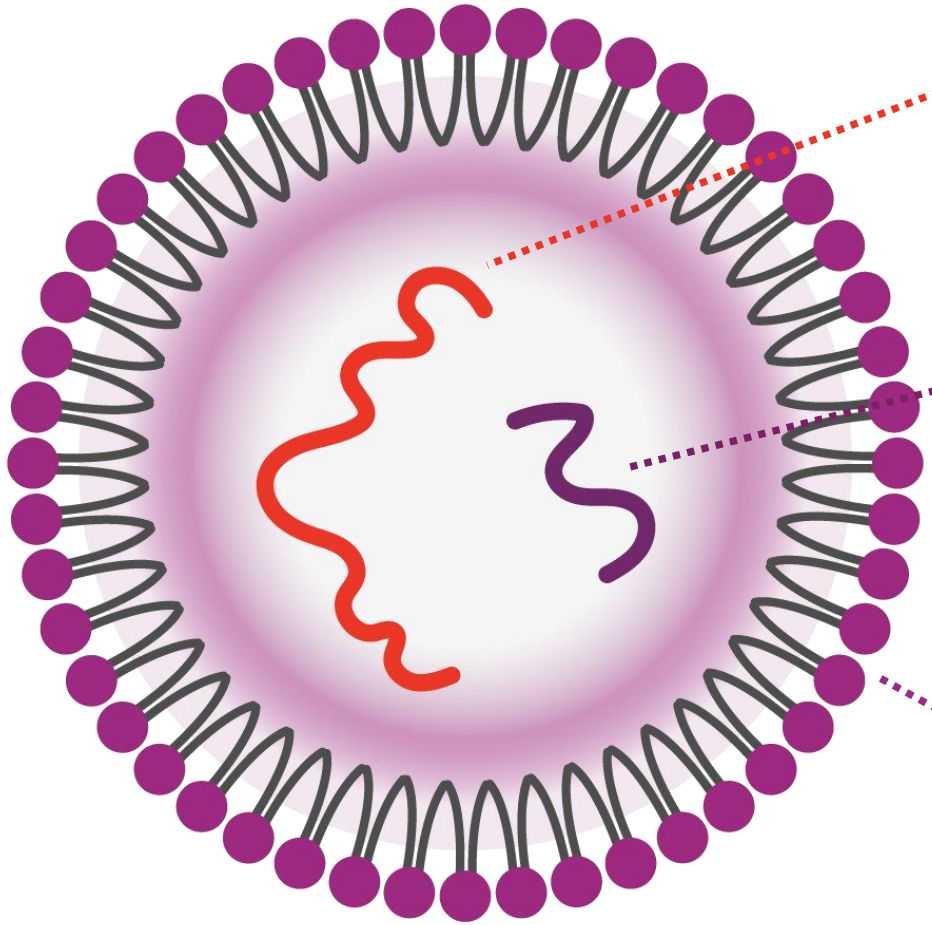
PROGRAM	INDICATIONS	DEVELOPMENT STATUS				
		Research/ Lead optimization	IND-Enabling	Phase 1	Phase 2	Phase 3
Low-density lipoprotein cholesterol (LDL-C)						
VERVE-101 PCSK9	Heterozygous familial hypercholesterolemia					
	ASCVD not at LDL-C goal on oral therapy					
LDL-C & Triglyceride-rich lipoprotein (TRL)						
ANGPTL3	Homozygous familial hypercholesterolemia					
	ASCVD not at LDL-C goal on oral + PCSK9i					

VERVE-101: one-time intravenous infusion in non-human primates, durable lowering of blood LDL-C by >60%

Phase 1 study initiation for VERVE-101 planned for 2nd half of 2022



VERVE-101's three components have been designed to minimize the risk of off-target editing



Adenine base editor

- Single base pair change without double stranded breaks
- delivered as an mRNA

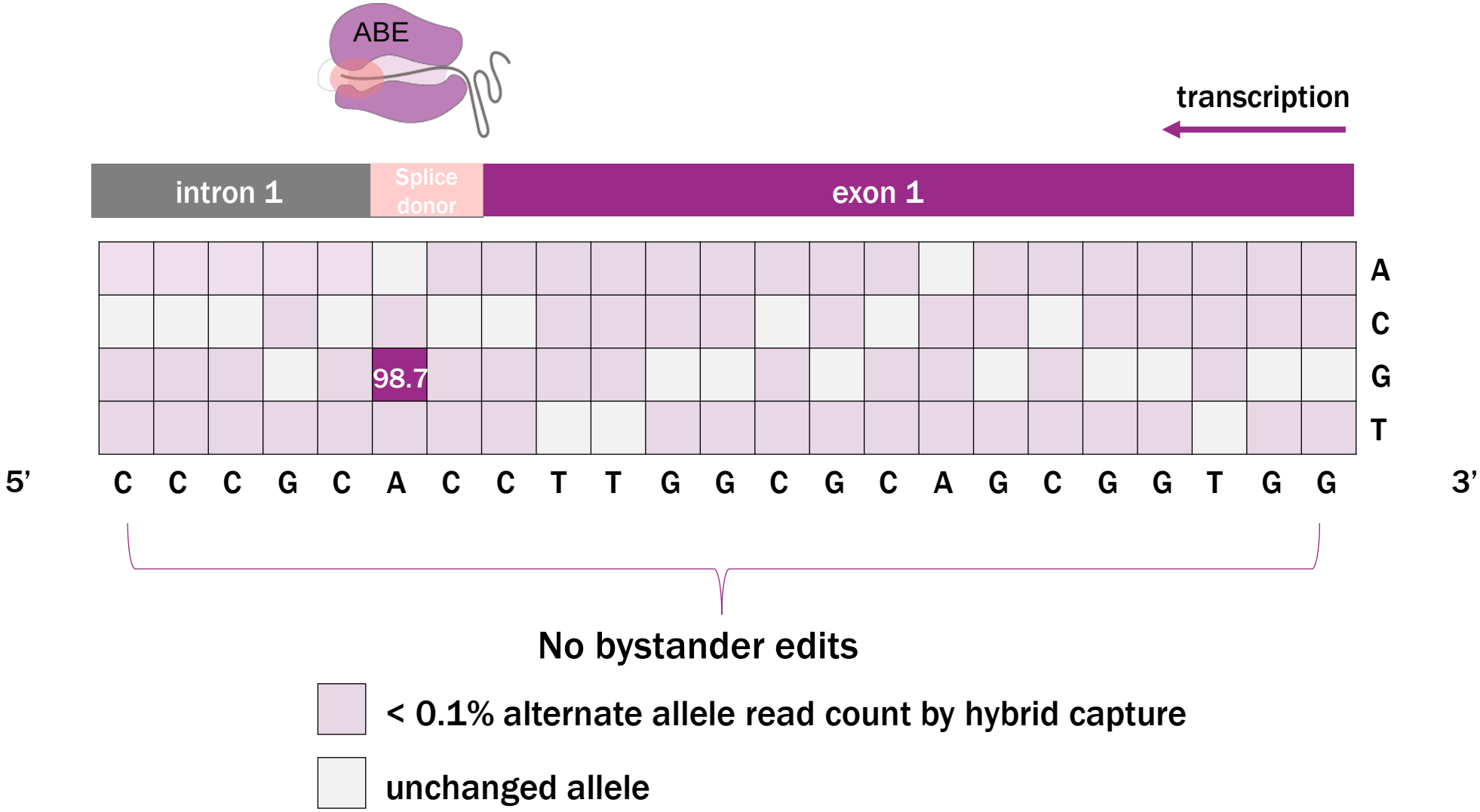
Unique PCSK9 gRNA

- No 0, 1, or 2 mismatch sites in genome
- Conserved site across human population

Non-viral LNP delivery

- Delivery predominantly to liver
- Transient exposure < 7 days

Base editing of the PCSK9 on-target site allows for a precise single base pair change without bystander edits

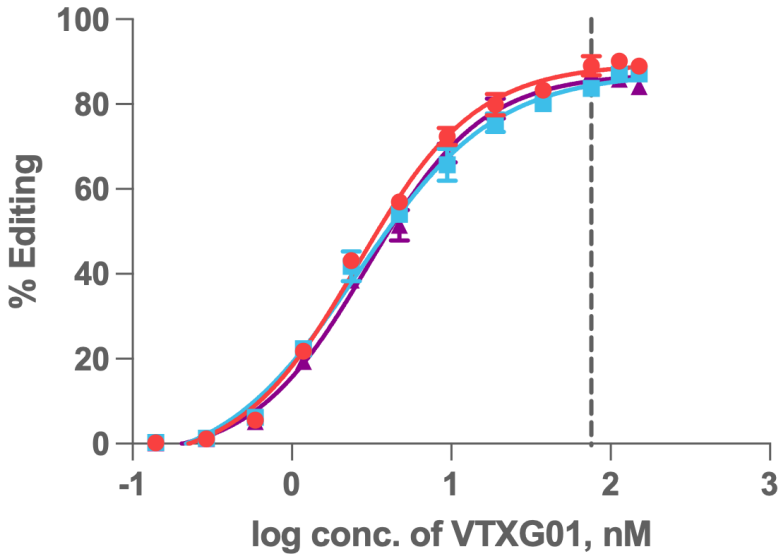


Question:

What is the risk of off-target editing posed by VERVE-101, an intravenously administered *in vivo* base editing medicine, at pharmacologically relevant doses?

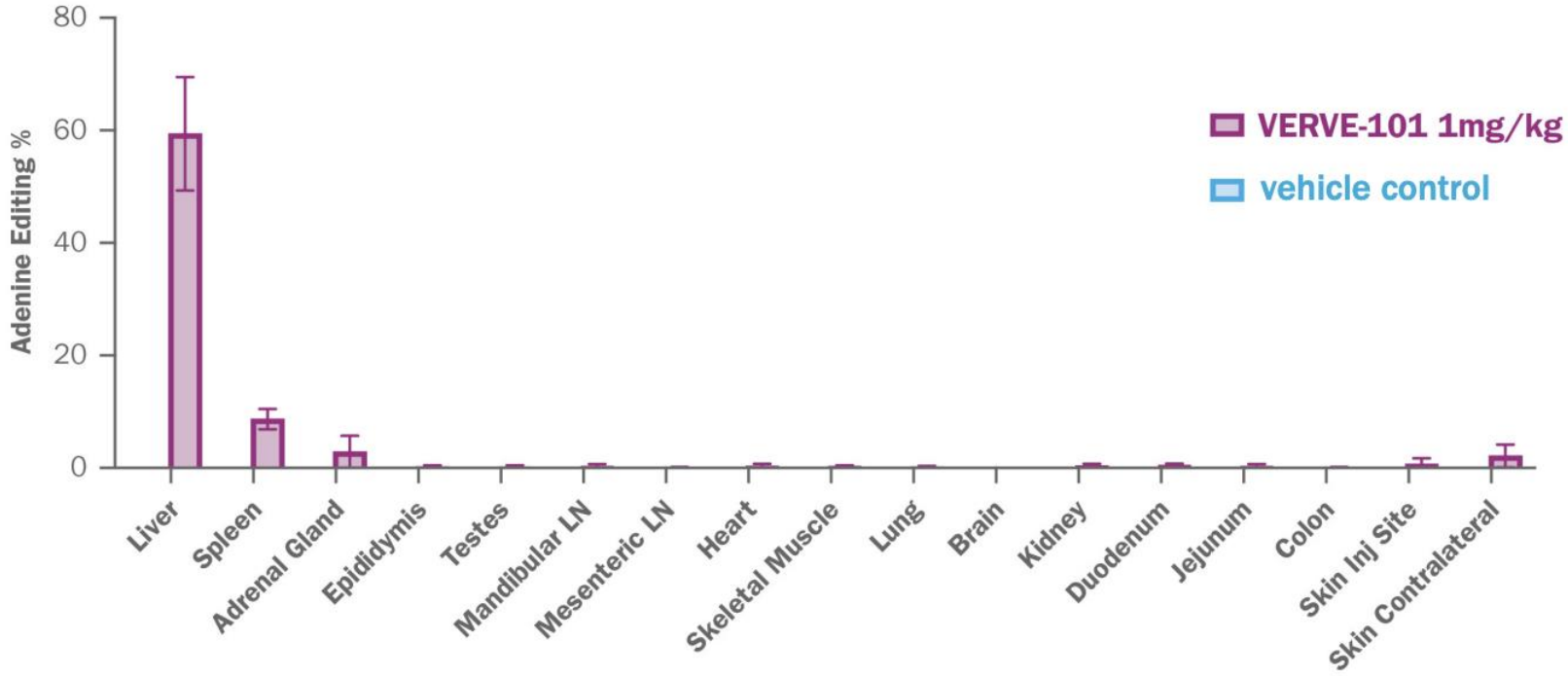
For an *in vivo* gene editing medicine, pharmacologically relevant context means: dose and biodistribution

Dose



- VERVE-101 PD Batch
- VERVE-101 Engineering Batch
- VERVE-101 GMP Batch
- Dose used in off-target assessments

Biodistribution



Multiple orthogonal techniques have been used to nominate ~3000 candidate off-target sites

entire human genome

identification techniques

Experimental: ABE-digenome-seq

Unbiased whole genome sequencing of liver genomic DNA treated with ABE in vitro

Experimental: ONE-Seq

library of ~30,000 barcoded sites with greatest sequence similarity to on-target site treated with ABE in vitro

Bioinformatics:

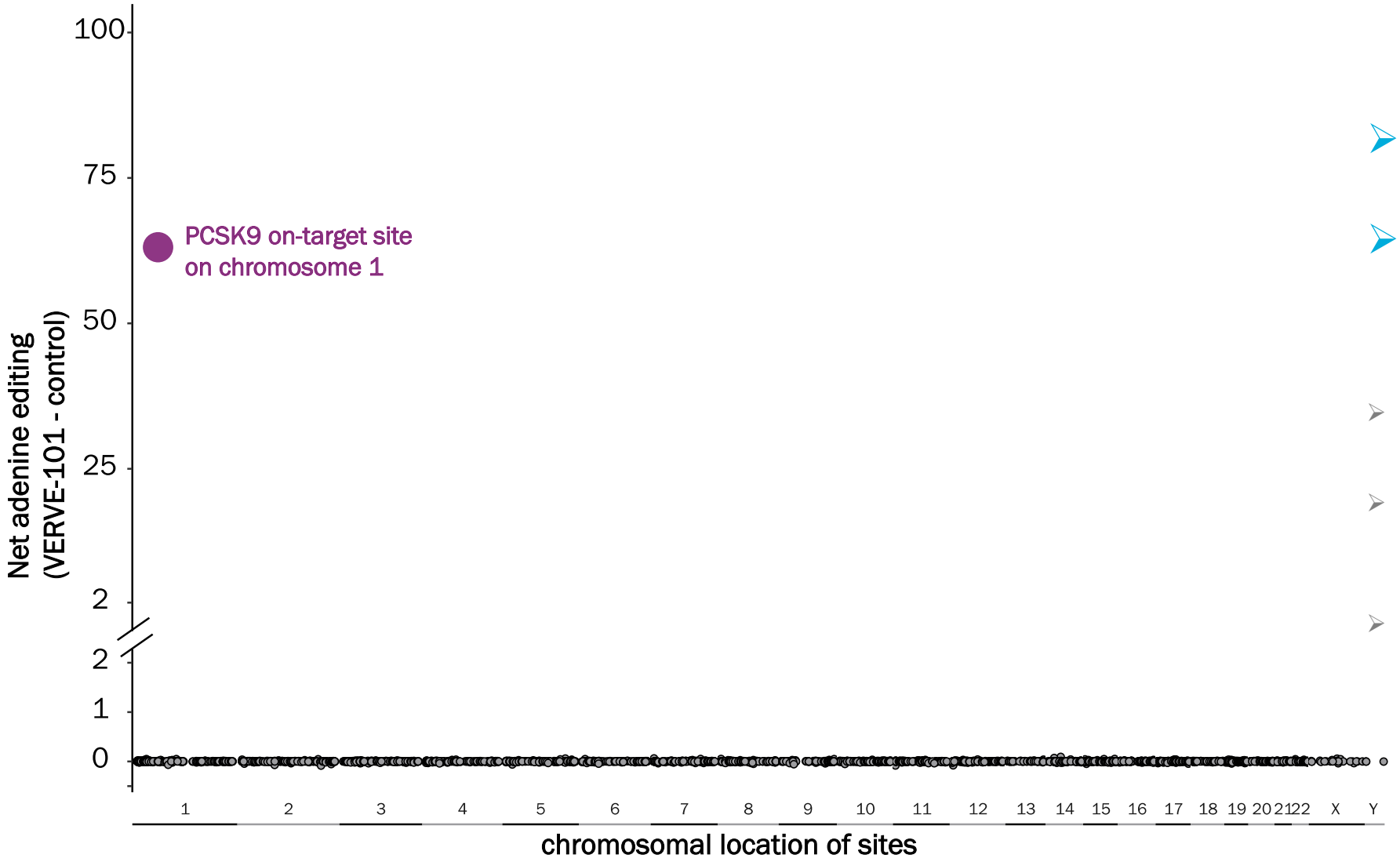
sites of greatest sequence homology

panel of candidates

3166 sites

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

No observed off-target editing at ~3000 candidate sites in primary human liver cells treated with VERVE-101

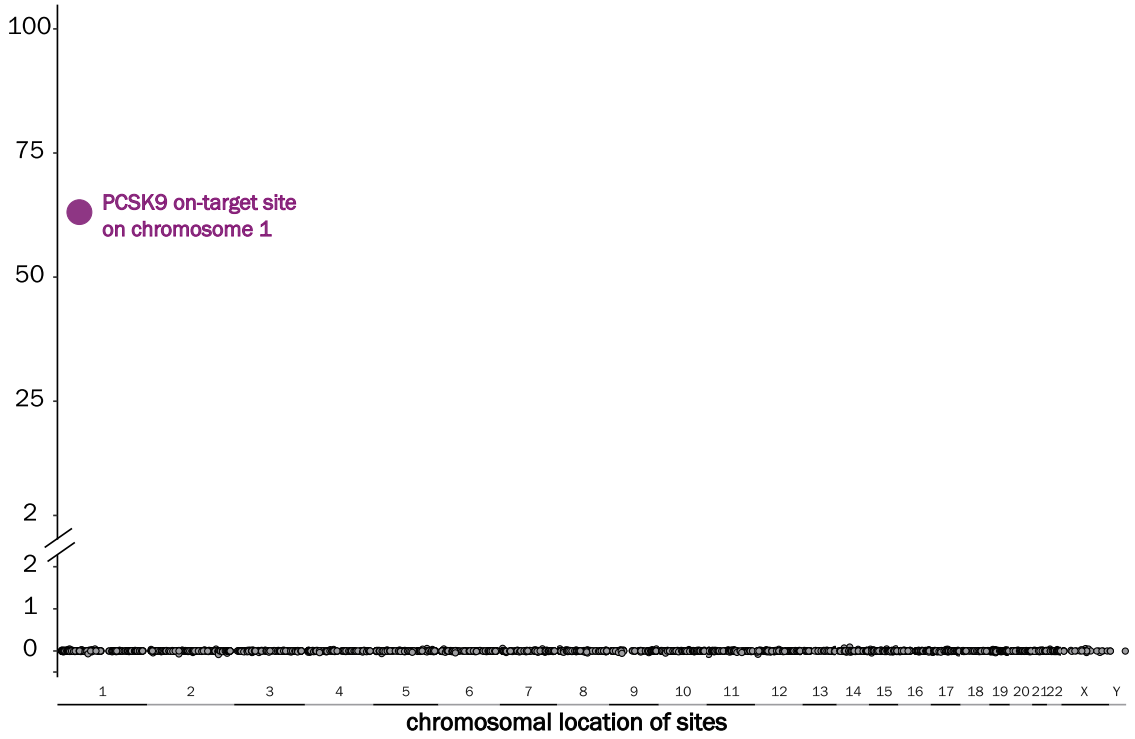


- **Manhattan style plot of ~3000 candidate sites**
- **No candidate sites show statistically significant net editing**

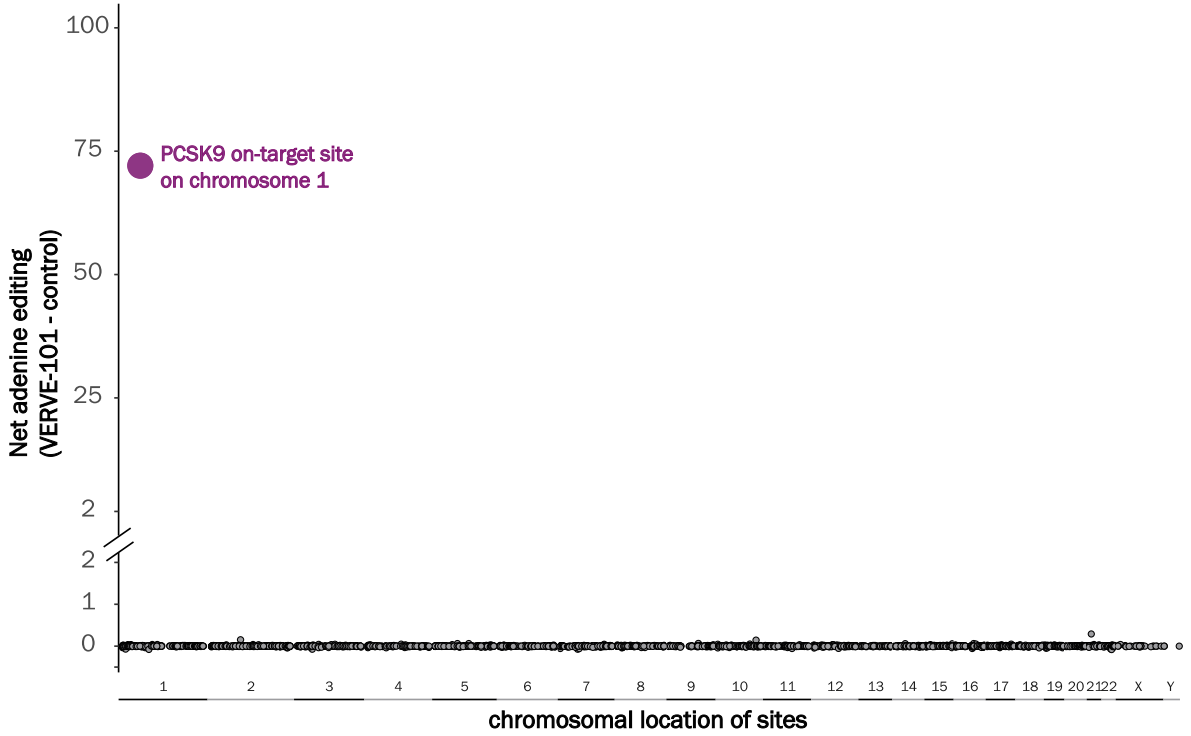
- Y axis indicates net editing (alternate allele frequency in treated primary human hepatocytes - matched untreated controls)
- Logistic regression statistical test is performed at each candidate site comparing alternate read counts in treated cells versus untreated cells
- Sites of somatic variation seen in the untreated primary cells have been removed from the plot for clarity

No observed off-target editing at ~3000 candidate sites in multiple lots of primary human liver cells

Donor lot 1

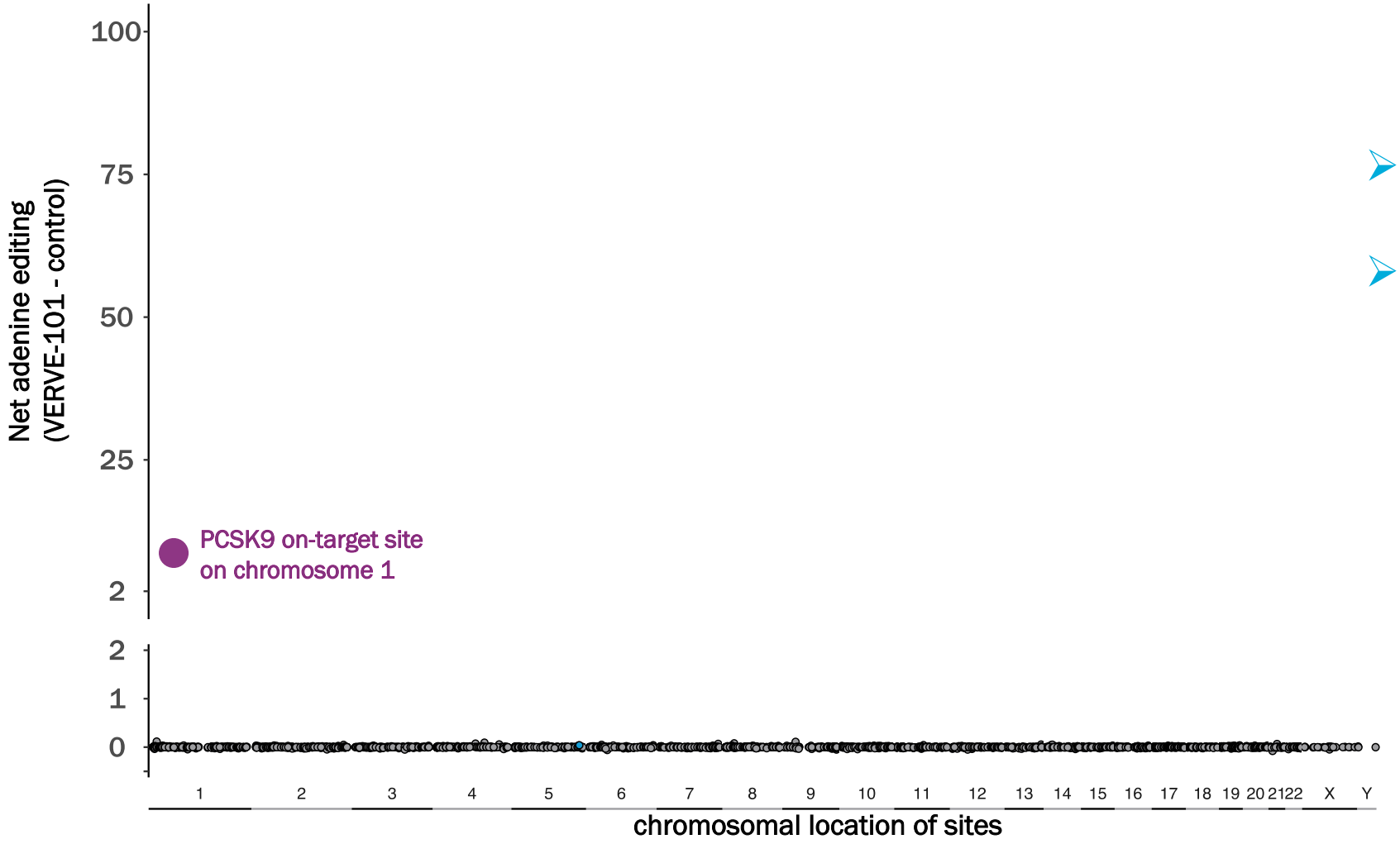


Donor lot 2



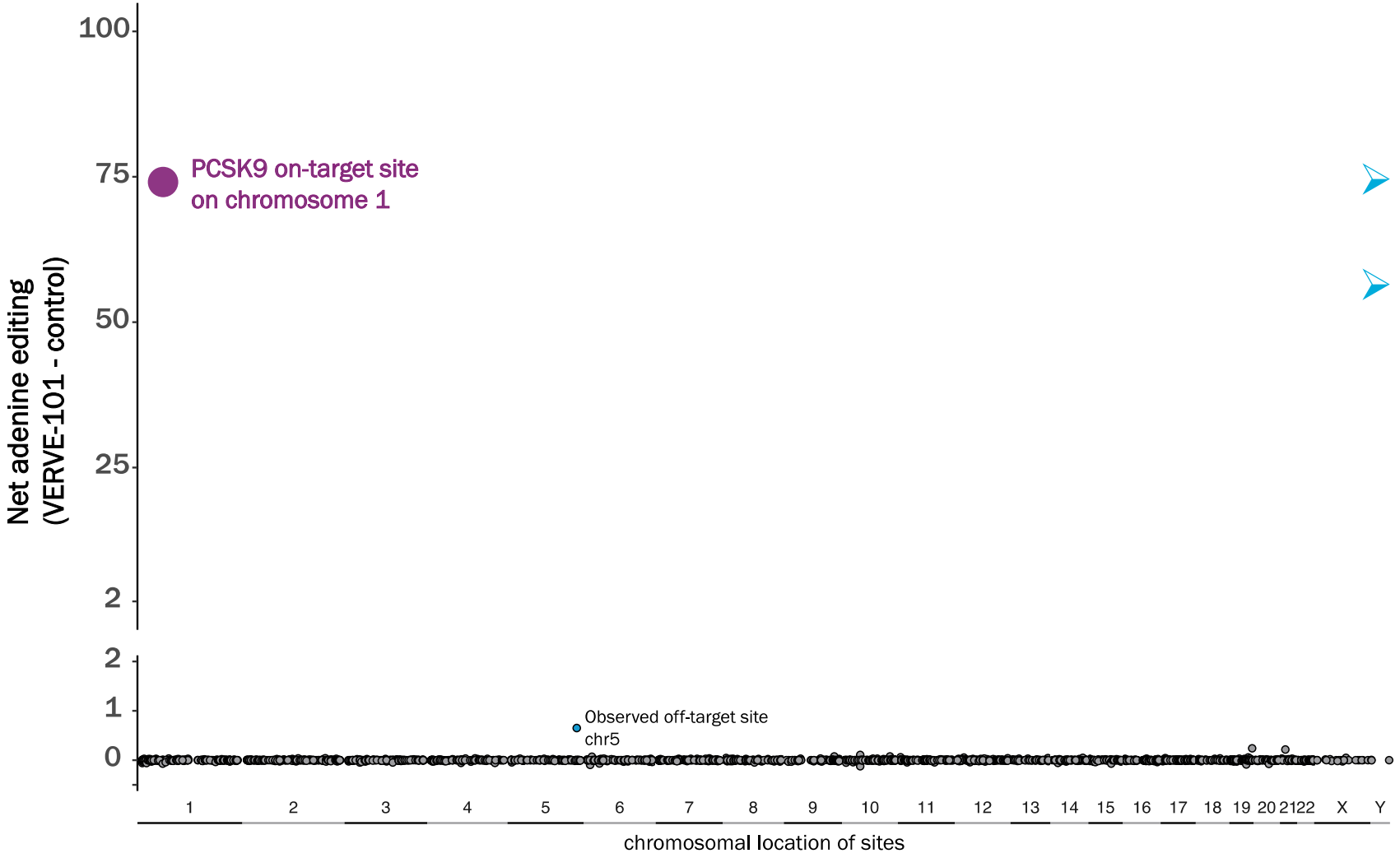
➢ No candidate sites show statistically significant net editing

No observed off-target editing in primary human adrenal cells



- Cells dosed *in vitro* at liver saturating dose of VERVE-101
- No candidate sites show statistically significant net editing

In primary human splenic cells, observed one site where editing in treated cells exceeded untreated



- Cells dosed *in vitro* at liver saturating dose of VERVE-101
- One site on chromosome 5 showed statistically significant editing compared to untreated

Extensive evaluation of VERVE-101 in multiple other cellular contexts

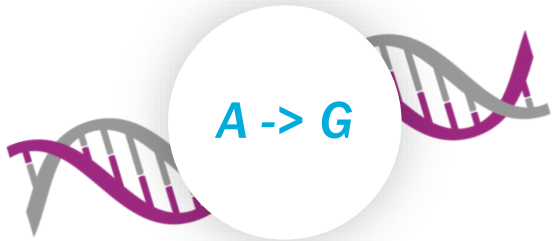
- Human liver cell lines (eg. huh-7, hepG2)
- Primary hematopoietic stem cells
- Pediatric donor primary human hepatocytes

One additional site identified where off-target editing might occur

Off-target risk assessment of VERVE-101

Comprehensive analysis of >3000 sites in multiple cellular settings

two potential sites of A -> G changes identified



Risk Assessment Criteria for Potential off-target sites

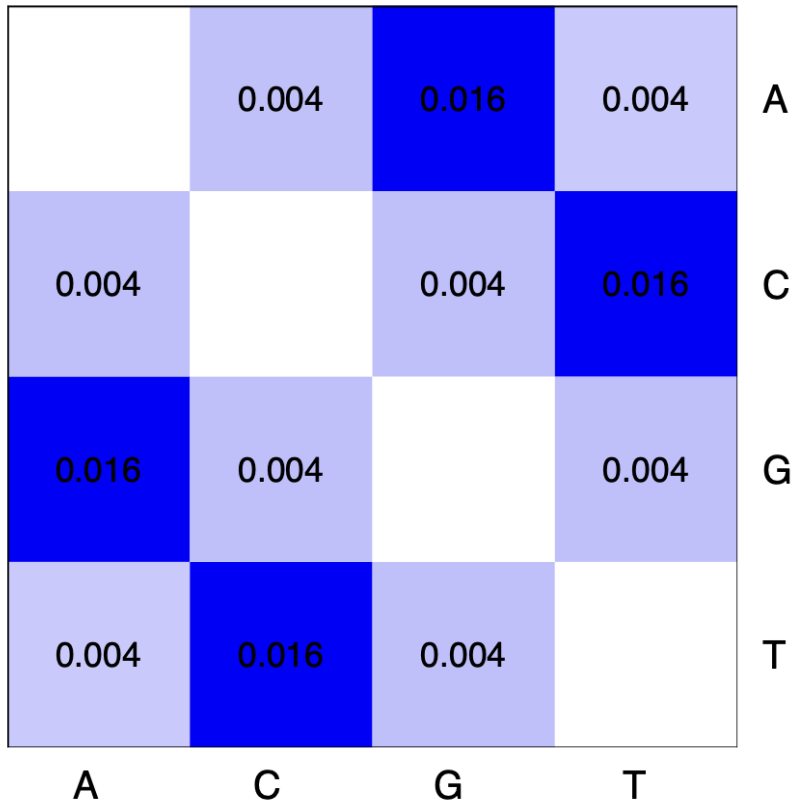
1. In protein-coding region of the genome?
2. In or near a gene associated with cancer?
3. Likely to impact nearby gene expression in liver or spleen?
4. Structural variants or translocations noted with VERVE-101?
5. Editing likely to occur at pharmacological doses in vivo?

Clinical Relevance Conclusions

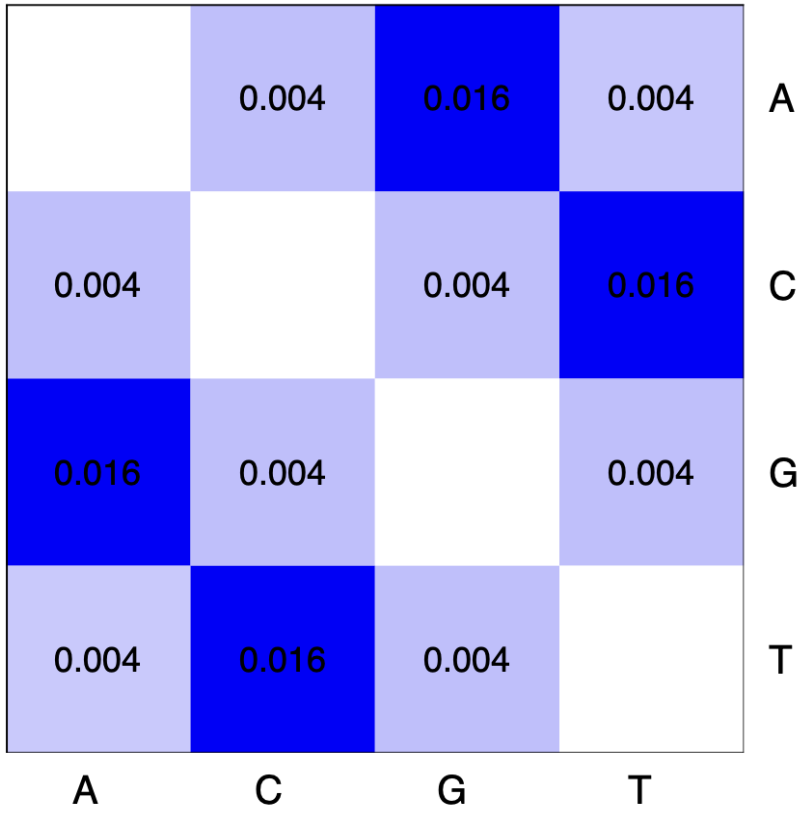
low risk of off-target genomic modifications that could be expected to have an associated clinical adverse effect

Whole Genome Sequencing of VERVE-101 treated huh-7 liver cells shows no increase in global adenine editing compared to untreated controls

Untreated



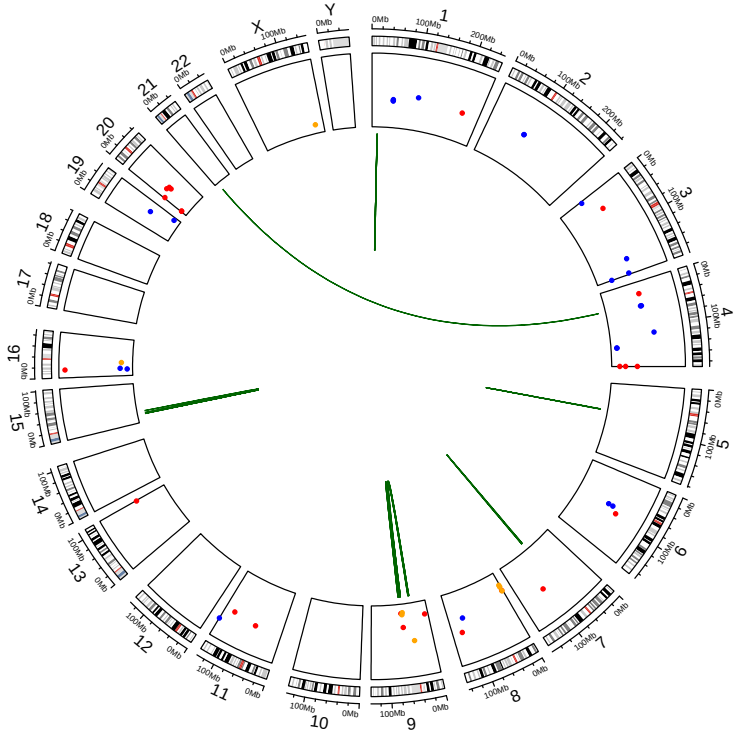
VERVE-101 treated



- Summary heat-map of 500x whole genome sequencing
- Numbers in cells of heat map reflect percentage of observed non-reference sequencing reads in comparing reference base (x-axis) to non-reference base (y-axis)

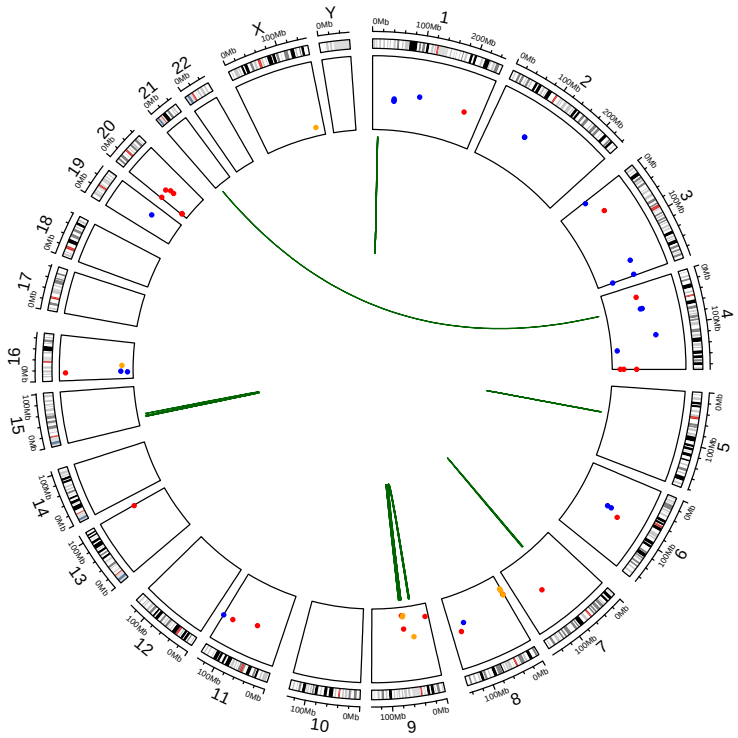
No structural variants observed from VERVE-101 treatment in primary human hepatocytes: whole genome optical mapping

Untreated



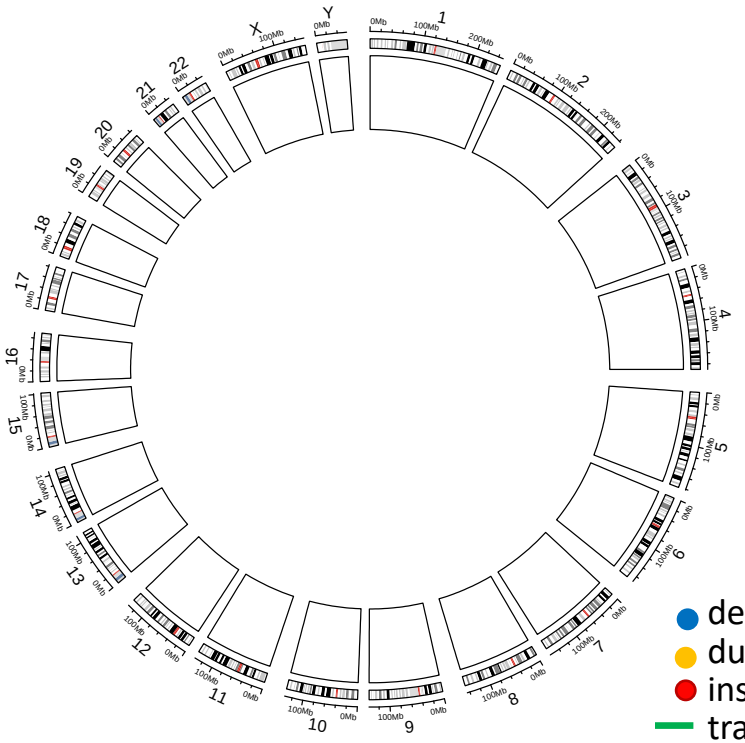
Structural variants are observed in control untreated PHH donor cells

VERVE-101 treated



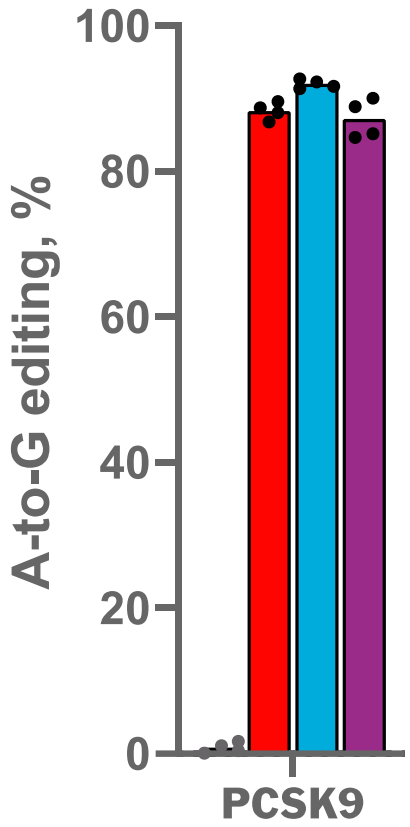
Identical structural variants are observed in the VERVE-101 treated PHH donor cells

VERVE-101 minus Untreated



No treatment-related structural variants are observed in VERVE-101 treated PHH donor cells

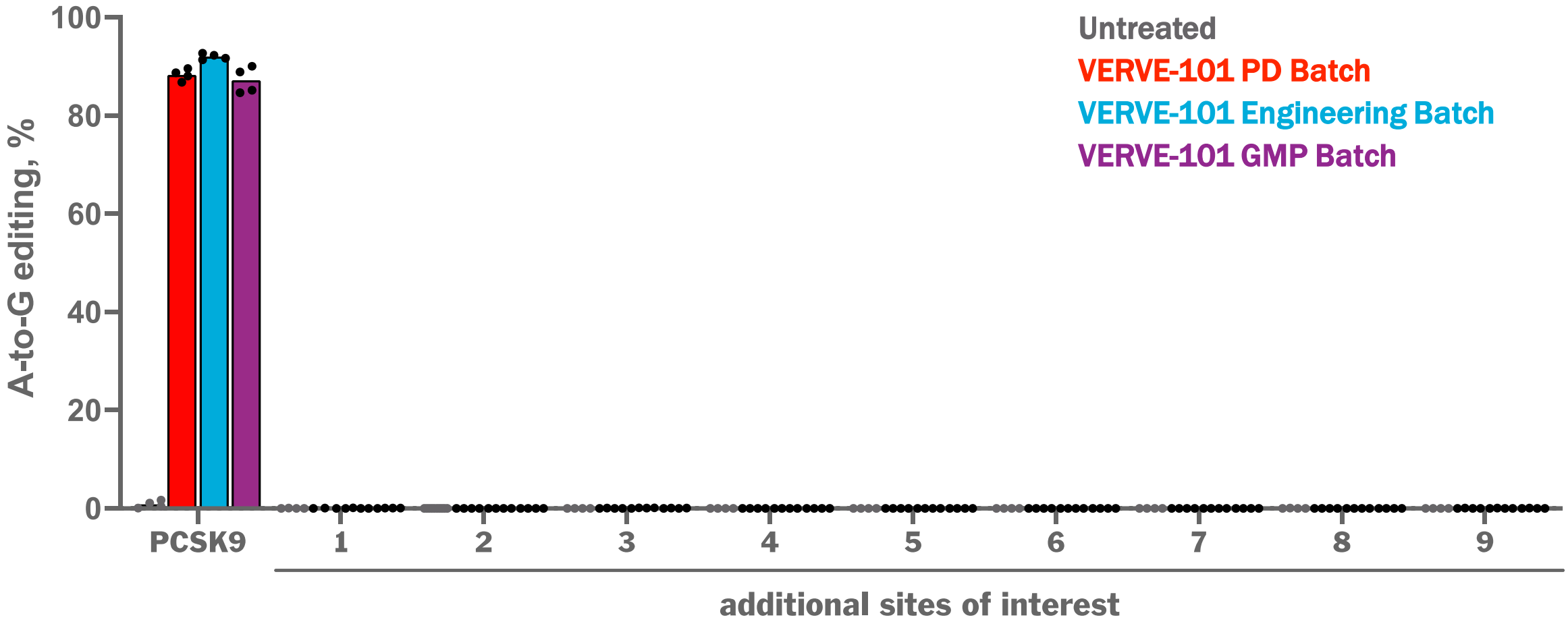
Editing outcomes at *PCSK9* and additional sites of interest:
No evidence of batch-to-batch variability



Untreated
VERVE-101 PD Batch
VERVE-101 Engineering Batch
VERVE-101 GMP Batch

Primary Human Hepatocytes treated with a saturating dose

Editing outcomes at *PCSK9* and additional sites of interest: No evidence of batch-to-batch variability



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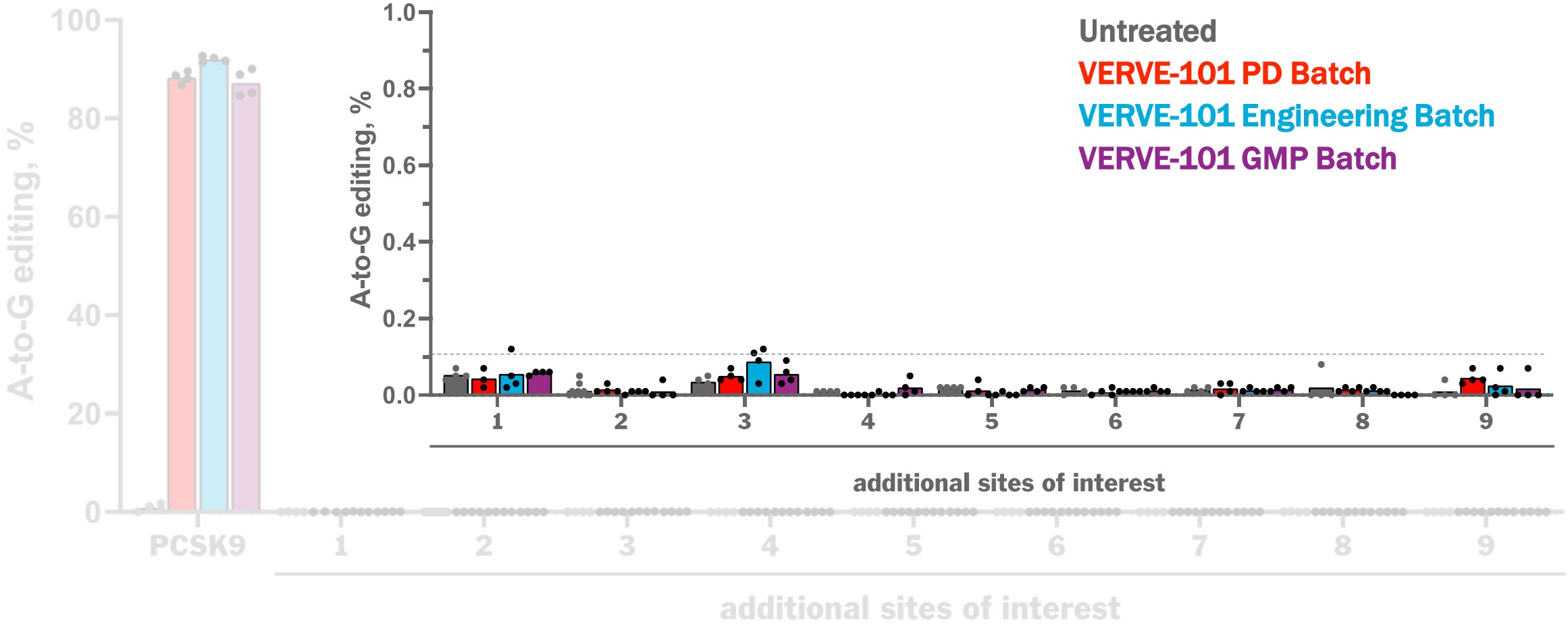

Primary Human Hepatocytes treated with a saturating dose

Conclusions

Assessed ~3000 candidate off-target sites in primary human liver, spleen and adrenal cells

At doses in primary human cells greater than the EC90 for on-target editing:

- **Two low-level potential off-target A → G edits observed which we assess as low risk**
- **No variability in off-target editing by batch**

These data support initiation of the first human trial of VERVE-101 (anticipated in the second half of 2022)