Comprehensive evaluation for off-target editing of *in vivo* base editing medicines targeting the PCSK9 gene

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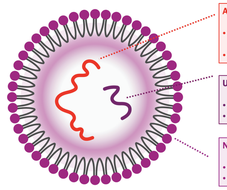
What is the risk of off-target editing posed by VERVE-101, an intravenously administered *in vivo* base editing medicine targeting PCSK9, at pharmacologically relevant doses?

1 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

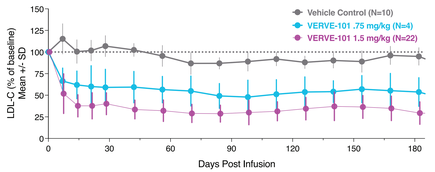
Non-viral LNP delivery
- Delivery predominantly to liver
- Transient exposure < 7 days

Comprehensive approach to off-target assessment aligned with March '22 draft U.S. regulatory guidance

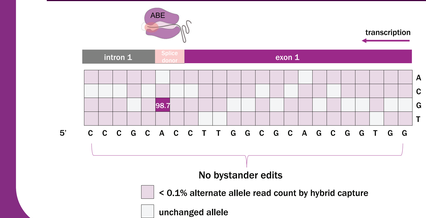
FDA Draft Guidance	VERVE-101 Approach
"…to limit the degree of potential off-target editing, the duration of GE component persistence should be minimized…"	• Non-viral lipid nanoparticle delivery strategy eliminates possibility of DNA integration and results in transient exposure of < 7 days
"We recommend biodistribution studies be conducted to characterize the distribution, persistence, and clearance of the GE product, as well as any expressed GE components in vivo."	• Detailed assessment of VERVE-101 biodistribution in mouse and nonhuman primate models, as assessed by both adenine base editor mRNA quantification and on-target base editing
"The use of multiple orthogonal methods (e.g., in silico, biochemical, cellular-based assays) that include an unbiased genome-wide analysis is recommended for identification of potential off-target sites."	• Nomination of >3,000 candidate sites using an unbiased genome-wide analysis (ABE-digenome-seq), a homology-based barcoding library approach (ONE-Seq), and an in silico analysis of DNA sites with the greatest sequence homology
"When possible, the analysis should be performed using the target human cell type(s) from multiple donors."	• Assessment for potential off-target editing in primary cells from multiple human donors
"Verification of bona fide off-target sites should be conducted using methods with adequate sensitivity to detect low frequency events."	• Assessment of nominated sites using highly sensitive hybrid capture and targeted amplicon sequencing • High coverage 500x whole genome sequencing to detect non-gRNA dependent global adenine editing
"The use of in vitro models should be considered for evaluating the activity of a GE product in the target cell type(s) for genomic modification."	• Analysis of a range of primary human cell types based on empirical assessment of biodistribution – including primary human hepatocytes, adrenal cells, splenic cells – and primary human hematopoietic stem cells to reflect an alternate cellular context
"Assessment of genomic integrity, including chromosomal rearrangements, large insertions or deletions, integration of exogenous DNA, and potential oncogenicity or insertional mutagenesis."	• Adenine base editor minimizes potential for double-strand breaks in DNA • Lipid nanoparticle delivery eliminates potential for exogenous DNA integration • Assessment for structural variants performed using whole-genome optical mapping of VERVE-101 treated primary human hepatocytes
"Evaluation of the potential for inadvertent germline modification."	• Potential for germline editing in males evaluated in study of sexually-mature NHPs to assess for any editing in sperm samples
"Each GE product lot evaluated in the preclinical studies should be characterized according to appropriate specifications, consistent with the stage of product."	• Analysis of batch-to-batch variability in on- and off-target editing across product development, engineering, and GMP batches of VERVE-101

2 VERVE-101: one-time infusion in non-human primates targeting PCSK9, durable lowering of blood LDL-C by >60%

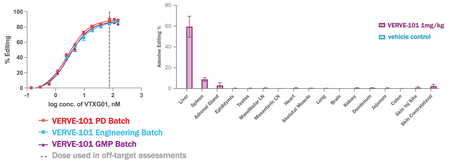
Phase 1 study initiation for VERVE-101 planned



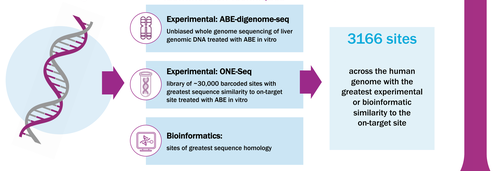
3 Base editing of the PCSK9 on-target site allows for a precise single base pair change without bystander edits



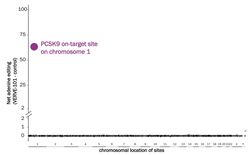
4 For an *in vivo* gene editing medicine, pharmacologically relevant context means: dose and biodistribution



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



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



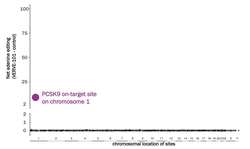
- Manhattan style plot of ~3000 candidate sites
- No candidate sites show statistically significant net editing
- Extension of this result to primary cells from a second donor, also noting no statistically significant net editing

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

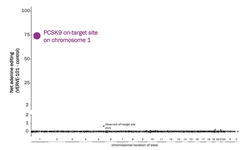
Sites of somatic variation seen in the untreated primary cells have been removed from the plot for clarity

No observed off-target editing 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

7 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

8 Off-target risk assessment of VERVE-101

Comprehensive analysis of >3000 sites in multiple cellular settings

two potential sites of A-> G changes identified

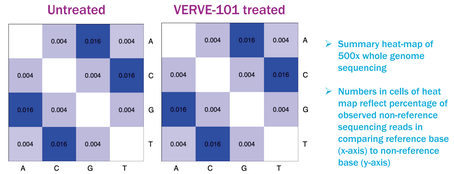
Risk Assessment Criteria for Potential off-target sites

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

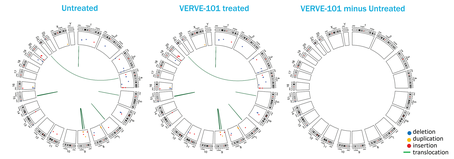
low risk of off-target genomic modifications that could be expected to have an associated clinical adverse effect

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



- 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)

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

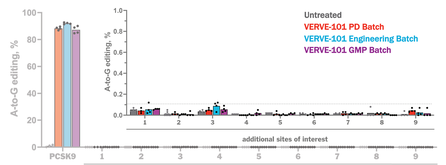


Structural variants are observed in control untreated PHH donor cells

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

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

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



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

Drs. Jayaram, Kathiresan, and Bellinger are employees of Verve Therapeutics.