To VERV Or Not-To-VERV: Off-Tissue Base Editing Issues May Disarm Genetic Compliance

Summary: Delt4 Engine predicted scientific and regulatory outcomes for VERVE 101: 75% Probability Of VERVE-101 Approval In 2025 (PT $50); Black Swan scenario 15% probability of VERVE-101 rejection due to safety issues with off-tissue editing of PCSK9 (PT $10); Blue Swan scenario 10% probability of VERVE-101, -201 and "-X01" approvals by 2027, acquisition or multi-deal Horizon (PT $90).

- **DataChanneling suggests VERVE-101 IND hold is due to 1) Off-tissue editing of PCSK9 having deleterious effects**; 2) FDA attempting to delay trial until Phase 1 safety data from UK and NZ arrive in 2H23; 3) FDA backlogging due to limited internal bandwidth.
- **Hyperforecasting sets 80% probability VERV-101 FDA approval by 2025**; 75% probability IND hold comments will tank stock to $15 (Dec. 5th) with >300% upside in 2H23 (>$50 after positive Phase 1 data).
- **Safety: While the percentage of VERVE-101 off-tissue editing of PCSK9 varies (~1-10%) and increases with dosage and total number of doses, the Delt4 engine estimates ~ low single digits (<5%) per dose**. Low single-dose off-tissue editing is unlikely to trigger whole tissue failure (e.g., pancreas, heart) and disease (e.g., diabetes, heart failure).
- **Efficacy: In our view, enforcing genetic compliance by inactivating PCSK9 permanently is the major benefit from VERVE-101 compared to competitors** (lack of compliance in many cases). Maintaining lifelong stable low levels of LDL outweighs the risks associated with off-tissue on-target editing, in-tissue off-target editing, and LNP toxicity.
- **Numbers to keep an eye on:** *Safety and Efficacy*; total % of off-tissue on-target editing per treatment; % of lung, pancreas, and heart cells that lose function or die after off tissue on-target editing; % of liver cells that remain edited after 1y, 2y, and 5y. *Timeline*; at which age are patients being dosed (later the better), time to IND resubmission, time to Phase 1 safety and PD data release.
- **We are long-term bullish on VERV and see IND hold comments expected Monday Dec. 5th triggering weakness in PT as a good buying opportunity**. Note: Last Friday, BEAM 201, a base-editing cell therapy, saw its IND hold lifted by the FDA in what we believe is a positive readthrough for VERVE-101 (VERV up 5%). All data harvested by the Delt4 engine is publicly available. Please reach out at miguel@delt4.org for more information.

Blurb: In this note, we used DataChanneling and Hyperforecasting (a combination of data science methods using proprietary machine learning and natural language processing native to the Delt4 engine) to analyze all the available scientific literature, databases, and negative data in an unbiased way pertaining to VERVE-101. An advantage (and limitation) is that the focus of the analysis is arbitrarily defined by 1) quality and quantity of data harvested, and 2) the algorithm parameters inside the engine. For VERVE-101, Delt4 flagged the off-tissue editing of PCSK9 as a critical knowledge cluster, which pointed us in that direction. Conclusion: VERVE-101 is a viable drug pending keeping off-tissue editing below a safe threshold estimated by Delt4 at ~5%.

What is Delt4, How is Delt4, and When is Delt4? In lay terms, Delt4 uses the current available data to estimate the likelihood of future outcomes. The Delt4 engine is an ongoing machine learning and natural language processing effort to generate knowledge from data. Delt4 harvests data based on queries and deploys knowledge derived from a "free number" of "n" independent data-data interactions. The engine is still in early-stage development, and we are training and optimizing Delt4 to be fully mature in 6-12 months.

Similar to Janus, the two-faced Greek God, one staring at the past and another at the future, Delt4 has two inter-dependent components: DataChanneling and Hyperforecasting. DataChanneling harvests past knowledge from publicly available databases and generates "n" knowledge clusters (based on the strength of data-data interaction), normally deploying 3 central clusters: 1) knowledge supporting the query, 2) knowledge refuting the query as counterevidence, and 3) neutral knowledge. Hyperforecasting projects the future by weighing the relative impact of knowledge clusters, finding knowledge gaps, and generating probability estimates comprising binary outcomes (YES/NO) and a timeline estimate.

This methodology was used to answer questions 1-4 displayed below, which are the core of the analysis reported here. For sanity check, we compared Delt4 with elicit.org and galactica.org (before it was taken offline by MetaAI), and human-assisted pubmed.gov queries.

Delt4 Engine: Is VERVE-101 A Life-Long Genetic Compliance Drug for Heart Failure? Summarized answer integrating our 4 queries to the AI engine is "Yes, if off-tissue targeting is under a deleterious threshold, which we estimate at 5%."

Input Data Summary: VERVE-101 is a base-editor + standard lipid nano-particle (LNP) which inactivates PCSK9 in the liver, lowering circulating LDL levels, cholesterol levels, and the risk of heart failure. VERVE-101 is currently in Phase 1 dose-escalation trials in UK and NZ, with an independent safety committee recently supportive of up-dosing. The full Phase 1 safety + PD data arrives 2H23. Pre-clinical data in NHPs ($n \leq 6$ animals) has shown robust and durable inactivation of PCSK9, with 80% of liver hepatocytes being edited, resulting in 83% decrease in PCSK9 protein levels and 70% decrease of LDL-C levels compared to baseline (up to ~16 months). We believe this should represent a lifelong alleviation of cholesterol induced heart

failure. Liver biopsy in NHPs shows low off-target base-editing in liver and low on-target base editing across tissues, lowering the risk of CRISPR cancer or transgenerational edits in the germline.

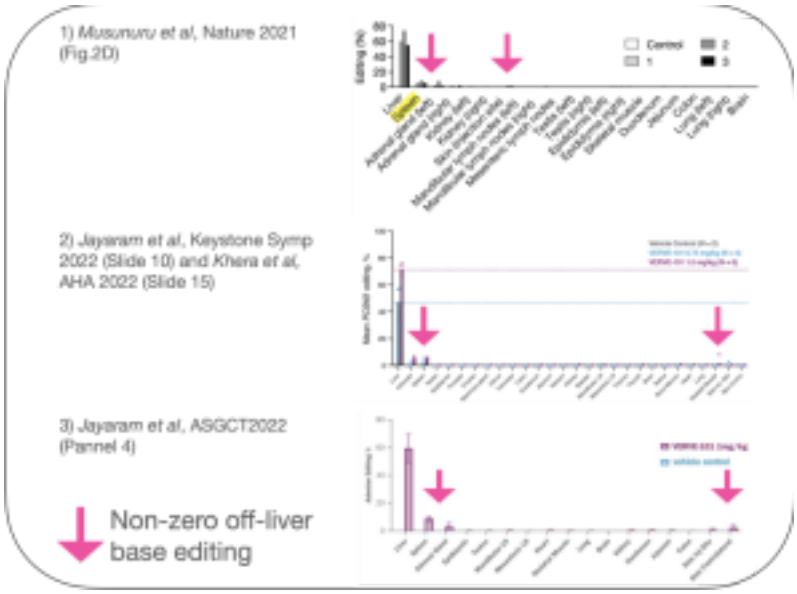
Q1: Will the FDA comments surrounding the IND hold on VERVE-101 be addressable in 2-6 months? Answer: YES

Delt4 thinks the FDA strategy is two-fold: 1) delay IND given that VERVE-101 is de-risked in Phase 1 elsewhere (meaning UK and NZ) hopefully until 2H23 full release, and 2) request additional data regarding safety (off-tissue editing, which was largely missed by 500X coverage, and additional LNP toxicity data). Verve has extensively de-risked off-target editing using 500X sequencing to show there is little to none, which suggests a very low risk of CRISPR cancer since randomly hitting a tumor suppressor gene or activating an oncogene is highly improbable. However, off-tissue on target editing was prevalent in the spleen and adrenal gland, and to a lesser extent in the skin, skeletal muscle, and importantly the heart. (This last sentence prompted question 2 below).

Q2: Is Off-tissue PCSK9 editing is likely to occur in humans? Answer: YES

While base-editing PCSK9 in liver cells to induce loss-of-function shows a negative correlation with LDL-C levels in the plasma, base-editing PCSK9 to disrupt its function in other cell types is deleterious. Off-tissue targeting of PCSK9 will always occurs to some extent, hence determining the % of non-toxic off-tissue PCSK9 editing is essential. The key parameters are 1) total % of edited cells, 2) % of edited cells that lose an essential function, and 3) are LNPs targeting stem cells, post-mitotic cells, and mitotic cells differently? Figure 1 shows off-tissue PCSK9 editing across tissues resulting from VERVE-101 treatment in NHPS (cynomolgus monkeys, i.e. macaca fascicularis, i.e. crab-eating monkeys). Delt4 extrapolated the data from the graphs, up-estimated based on highest individual datapoint or average + error (based on error bar when available) and concluded that the average off-tissue editing was between 1-10%, depending on the tissue type. This observation educated question 3. (Note: If you would like to consult the individual values extrapolated from Figure 1, data is available upon request at miguel@delt4.org).

Figure 1: Higher VERVE-101 Doses Lead to Increased Off-Liver Base-Editing of PCSK9.



Biochemical evidence supported rationale: Standard LNPs are not freely diffusing in circulation, being by default largely bound to APO-E lipoprotein and ending up internalized in liver (HERE). Decorating the LNP or changing its lipid composition can increase specificity to a tissue, as shown by Verve with GalNAc-LNP, which increases hepatocyte delivery (HERE). Verve therapeutics used a GalNAc-LNP for VERVE-201, which may result in lower off-target tissue editing, but also in lower liver-editing (40% GalNAc-LNP-ANGPTL3 vs ~65% with standard LNP-PCSK9 at 1.5mg/kg dose in NHPs). The correct experiment is missing, since Verve presented an apples-to-oranges comparison (changed two parameters at once, making it hard to distinguish whether the lower off-tissue editing is due to LNP composition or choice of target gene). This makes it hard to distinguish if the new GalNAc-LNP is superior, since the correct experiment would be to compare off-target between LNPs editing while keeping the same target gene (e.g., GalNAc-LNP-PCSK9 vs. Standard-LNP-PCSK9).

Biological conceptual rationale: So far, no one has commented on the issue of targeting different cell states. The long-term consequences of base-editing a post-mitotic long-lived cell are likely different than the ones resulting from editing a mitotic stem-cell. While a long-lived cell will carry the edit for the lifespan of the patient, mitotic cells compete with neighboring non-edited cells to survive, meaning that a small decrease (or increase) in cellular fitness due to removal of PCSK9 function can dictate whether the tissue will lose (or keep) the edit over time. Another issue pertains to tissue architecture: if the targeted tissue is structured (e.g., brain, heart, and eye) and largely post-mitotic, changes are likely to be permanent and there is the chance that they sub concentrate is a cluster of cells that are functionally relevant (e.g., amygdala, ventricular node,
or retina). This means that a 1% off-tissue editing rate might be locally amplified due to most edits hitting the same sub-tissue structure (and affecting e.g., the mitochondria resulting in cell death). There is no data to support or refute these hypotheses, so we will keep them as such.

Q3: Is off-tissue on-target PCSK9 editing a major safety issue? Answer: Undetermined

Delt4 harvested 3 sets of data: 1) *Nature* paper in Sep. 2021, (n=3 monkeys, and NO DATA for pancreas or heart tissue was shared (strange oversight since 2 of the most dangerous phenotypes described by independent research groups are PCSK9 deletion in pancreas HERE, HERE, and HERE, leading to decreased glucose tolerance and potentially diabetes; and heart HERE, HERE, HERE, and HERE leading to mitochondrial defects, changes in cardiac metabolism and cell death, which result in heart failure, the very same disease VERVE-101 aims to treat!). Original paper Figure 2d (HERE). 2) Keystone symposia presentation Apr. 2022 (HERE) and AHA presentation showing same dataset (HERE) that includes heart and pancreas data. 3) ASGCT 2022 poster May 22 (HERE) shows heart but pancreas data was removed (n=6 monkeys for both studies). Figure 1 shows a collage of the original data for illustrative purpose. **Take home message:** Even at a 500X sequencing depth (capable of detecting ~1 in 500 cells at best), there is non-zero signal editing in heart and pancreas, which means at least 1 in 500 cells received an off-tissue PCSK9 edit (captured by error bars). Of note, skeletal muscle went up to ~8% after a single dose (n=1 monkey).

In addition Delt4 detected anecdotal evidence in cluster 3 (neutral cluster) for other deleterious effects of removing PCSK9 outside the liver, such as sepsis (HERE) and potential effects in stem cell proliferation (HERE) and apoptosis in carcinoma cells (HERE). Delt4 ignored these observations, due to low numbers for counterevidence.

Note: VERVE-201 carrying novel GalNAc-LNP also shows non-zero off-tissue editing (slide 34 HERE).

Q4: Does human genetics support safety of PCSK9 knock-out approach? Answer: NO

Question 4 was formulated to present counterevidence to question 3. The major issue with using human genetic studies is that we cannot do the required experiments due to ethical reasons: removing the PCSK9 gene from a human embryo or germline to test its effect during embryonic development, gestation, and early infancy. Therefore, we are lacking "negative data" to assess if PCSK9 mutations affect human developmental biology. We know some humans live without PCSK9 and seem healthy, but other compensatory mutations or environmental factors could be conditioning this effect. Deleting PCSK9 is adults and comparing it to population studies is NOT an apples-to-apples comparison, since knocking out a gene from an adult organ which has not adapted to the lack of it during development (or lower function) could be problematic. Therefore, we do not think human population studies based on low numbers of individuals, with heterozygous and homozygous and heterogeneous mutations (fully inactivating or partially inactivating PCSK9), fully de-risk the PCSK9 base-editing approach. Phase 1 data available in 2H23 from VERVE-101 will be able to answer this question, at least for mid-term treatment (~up to 12 months).

Take home: To understand the likelihood of success of Verve Therapeutics as a company and VERV as a stock, given the first-of-its-kind nature of the lead drug VERVE-101, it is essential to establish safety and efficacy. In Delt4's opinion, Verve therapeutics publicly shared the most comprehensive safety data set for a genetic-medicines company, with extensive off-target base editing mapping (up to 500x coverage), off-tissue on target editing, and lipid-nanoparticle toxicity (including GalNAc LNPs), as suggested by the FDA's March 22 draft guidance (HERE). This dataset largely quenches FDA's concerns of toxicity and transgenerational editing (permanently editing the germline and changing the genome of future humans) and is in line with the independent monitoring committee decision to support VERVE-101 Phase 1 up-dosing in NZ. **However, the issue of off-tissue on-target editing is still looming and needs further scrutiny, as the data shared by Verve in multiple forms (paper, presentations, posters) was harvested by the Delt4 engine and flagged as a critical gating factor**. Nonetheless, we predict that a single-dose of VERVE-101 will create the "genetic compliance" needed to stabilize cholesterol levels and decrease heart failure in HeHF patients.

Disclosures: Delt4 engine is at early development stage, without any quantification of forecast accuracy yet available. The engine is still in beta-testing, and the results should not be considered 100% sure, nor supportive of investment decisions. Delt4 and its founder, Miguel Coelho, PhD, decline any responsibility for investment decisions based upon this report.

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Appendix I: Rationales and Methodology Explanation

Motivation and Inception: The predictions generated are the proof-of-concept deployment of Delt4, a product still in beta-testing phase and likely not yet optimized. VERVE-101 was chosen as a subject due to the opportunity to test 1) forecasting the outcome for a new technology in a highly controversial field (VERV has a high short-interest) 2) different timelines at play – short term IND hold comments on Dec. 5th), medium-term (Phase 1 data on 2nd half of 2023 at an undisclosed medical conference) catalysts and long-term development of VERVE-201 and "X01s" possibility to generate profitable deals and acquisition and 3) calibrating the probabilities of

likely scenarios, versus optimally negative (black swan) or positive (blue swan) outcomes.

We continued the analysis by focusing on 1) PCSK9 function and inactivation, 2) Off-tissue base editing of PCSK9, and 3) Long-term benefits and risks of genetic compliance (e.g., having permanently low levels of LDL-C). The objective is to inform academics, biotech, and investors on the most likely future outcomes of VERVE-101, via knowledge maps and attempting to predict future outcomes to guide decisions. For simplicity, we aimed the engine at VERVE-101 and let it build knowledge maps unbiasedly. This largely ignored competitor drugs, KOL opinions, pricing and market factors focusing instead on a single basic science question: will the issue of off-target editing compromise safety and efficacy of VERVE-101? *Of note, BEAM-201, another base-editing therapy, just received OK from the FDA (IND hold lifted on Dec 2nd).*

Appendix II: Supporting Rationales with Evidence and Counterevidence using Pubmed.gov

Opening the Black Box: Will VERVE-101 Become the First Life-Long Genetic Compliance Drug for Heart Failure?

Verve Therapeutics is a genetic medicines company, leveraging CRISPR technology, base-editing, and lipid nanoparticles (LNPs) to efficiently change the cellular genome and create single-shot lifelong therapies. The company was founded by the highly regarded cardiologist and savvy

entrepreneur, Dr. Sekar Kathiresan, who was the first to deploy base-editing, a form of genome editing similar to CRISPR-Cas9, into patients (HERE). Verve therapeutics licensed the base-editing technology from Beam therapeutics, which originated Dr. David Liu's lab.

Verve's first disease target is Heterozygous familial hypercholesterolemia (HeFH). HeFH occurs in 1/250 individuals and is a genetic disorder caused by elevated levels of low-density lipoprotein cholesterol (LDL-C) which leads to heart disease. The high LDL-C levels result in high cholesterol, which accumulates in the blood, arteries and ultimately drives heart failure. PCSK9 stands for proprotein convertase subtilisin/kexin type 9 (HERE), a protein widely expressed in tissues other than the liver, and is a well-defined drug target (small molecules, siRNA, antibodies HERE and HERE). Besides controlling the amount of low-density lipoprotein (LDL) receptors on the cell surface and regulating circulating LDL-C levels, PCSK9 is involved in mitochondrial function, and apoptosis, which can lead to cell death and inflammation (HERE, HERE and HERE).

Is Deleting PCSK9 Good or Bad? Harvesting Counterevidence. Gain-of-function mutations in PCSK9 cause hypercholesterolemia due to reduced number of lipoprotein receptors as the gain in PCSK9 activity results in faster membrane turnover (HERE). Loss-of-function mutations have the opposite effect, with higher presence of receptors in the cell membrane resulting in more transport of fat into cells, removing it from circulation and indirectly preventing accumulation in heart and therefore heart failure. While HeHF is caused by many genetic mutations in genes other than PCSK9 inactivating, inactivating PCSK9 counteracts the negative effect of those mutations via lowering LDL-C. Ablating PCSK9 creates a life-long genetic buffer, which we here term "genetic compliance", to counteract the effect of deleterious mutations causing HeHF, in a scenario where two wrongs (mutations + PCSK9 inactivation) make a right (decreasing heart failure). In support that PCSK9 genetic inactivation can be beneficial, or at least safe, in humans is the observation of multiple types of inactivating mutations in human adult populations with no reported health consequence (HERE and HERE). However, being born with an inactivated copy of PCSK9 and having PCSK9 inactivated as an adult is not an apples-to-apples comparison! Other background genetic mutations in multiple other genes might provide a beneficial interaction with PCSK9 inactivation which allow humans born without fully functional PCSK9 survive embryonic development, gestation, and infancy. How the adult body adapts to a "sudden lack" of PCSK9 is yet to be determined. The PCSK9 gene seems to be under evolutionary positive selection, which argues that its function might be required in tissues other than the liver (HERE). In fact, recent reports suggest that inactivating PCSK9 can lead to diabetes (HERE). The different phenotypes reported are likely localized by organ or tissue, therefore it is important to establish if PCSK9 is being edited outside the liver.

Appendix III: Mathematics and Biology behind Off-Tissue Targeting Issue and The Blue and Black Swan Scenarios (Bull vs. Bear analysis).

When can off-tissue targeting be a concern? We present below a back-of-the-envelope, Occam's razor type minimal calculation. Assuming the optimal situation for a patient's health, off-tissue editing rate for VERVE-101 is ~2% across tissues (4-fold lower than skeletal muscle outlier), the

LNP carrying the VERVE-101 base-editor will enter 2% of total cells within a tissue. For the sake of simplicity, we also assume that: 1) the off-target tissue is post-mitotic (meaning the division rate of cells in the tissue is close to zero), 2) the tissue is not structured (like the brain or heart), and 3) all cells have the same probability of being "targeted" by VERVE-101.

In scenario 1, the blue swan scenario, the best possible positive outcome arises: 1 dose of VERVE-101 affects 2% of cells, 2 doses affect 4%, 3 doses affect 6%, and 4 doses 8% of cells. Here, the total number of PCSK9 deleted cells in tissues other than liver increases slowly, before plateauing with some "unknown" kinetics. If 1 dose is sufficient to reach the desired low levels of LDL-C and has a protective effect against heart failure, risk is minimal. If we lose 2% of cardiomyocytes due to mitochondrial failure post PCSK9 editing (this is an overestimation since not all PCSK9 edited will die or completely lose function), maybe the benefit of lowering LDL-C through "genetic compliance" is overall positive and patients can live the rest of their lives without complications.

In scenario 2, the black swan scenario of ultimate failure, we change the assumptions: 1) tissue is mitotic, similar to liver and skin, 2) tissue is structured (brain and heart), with 3) cells in certain vital sub-structures having a higher chance of being targeted by VERVE-101. Also, we assume 4) a synergistic deleterious effect from knocking-out PCSK9 across tissues (meaning that editing multiple off-tissues is worse than the sum of each individually). In this case, VERVE-101 will not maintain durable efficacy and is likely to lead to serious long-term complications as explained: if the tissue is mitotic, and we know that knocking-out PCSK9 can decrease cellular fitness, the non edited cells will divide at a faster speed and overtake edited cells over time, which will require multiple dosing. This further increases the side impact on non-mitotic organs (brain and heart). Additionally, even if the total off-tissue base editing is only 2%, due to differences in cellular membrane composition, and receptor intake of LNPs, a specific subpopulation of cells in that tissue might have a higher likelihood of receiving VERVE-101 edits compared to their neighbors. In this situation, 2% general tissue editing could result in e.g. 70% editing of a specific cell type in the tissue and permanently disrupt the function of those cells (to be determined via comparative single-cell RNA seq and sub-cell population studies across tissues).

Appendix IV: Hyperforecasting Signals Verve Therapeutics to Dive Deep into Antagonistic Pleiotropy and Focus on Base-Editing for Anti-Aging Therapies.

The Delt4 engine predicted 3 possible outcomes, as follows. The text below is based on the

output metrics of the model and human calibration, for probability-of-success and time to event.

A. Occam's razor scenario (75% probability): VERVE-101 IND hold is an FDA play to de-risk Phase 1 in UK and NZ (trials already running) clinical and regulatory hurdles (ALT levels, LNP toxicity, BE immunogenicity, etc.) will be overcome. Verve convinces the FDA that permanently lowering LDL C via "genetic compliance" outweighs safety risks, approval ensues in 2025 and VERVE-201 follows in 2026, with a collection of VERVE-X01s in line for approval (also collaboration with Vertex, a likely acquirer of Verve). Company hits $2-5B market cap by 2027. Doctors and Patients are happy, insurance companies pay the bill.

B. Blue swan scenario (15% probability): VERVE-101 IND hold clears after benign FDA comments and enters clinic in US 2-6 months from now. Positive Phase 1 data arrives and path to approval by 2025 is clear. In 10 years, VERVE has become the leader in base-editing, with a focus on anti aging therapies ($10B market cap by 2027, too big to be bought). Delt4 picked up on a 4th knowledge cluster: antagonistic pleiotropy genes (HERE) which are essential during human embryonic development, early life, and reproduction; but later contribute to accelerate aging and health failure (HERE). Deletion of these genes in adulthood, post-reproductive age, would be safer and improve individual organ function and health, indirectly increasing the average lifespan of populations and filling a huge unmet need and improving the health of the elderly. Verve therapeutics picks up on this, creates a new FDA regulatory precedent and generates the first base-edited anti-aging drug. Inactivating PCSK9 in the liver could in fact be considered anti-aging, since by definition, lowering the rate of death by heart failure will result in an increase in average lifespan in patients (and beyond). Humans live a healthier, more productive life. Everyone benefits, including insurance companies that now have profited from early investments in the field.

C. Black swan scenario (10% probability): The issue of off-tissue on-target editing takes precedent. VERVE-101 requires repeat-dosing and this increases PCSK9 inactivation in heart cells >10%, promoting heart-failure, the very own condition it was designed to treat. Patients die, field gets discredited. Company goes bankrupt, investors become skeptical, Doctors shy away from new clinical trials. Insurance companies are happy. The FDA grows antibodies against base editing.