# Outsmarting Delivery Barriers *In Vivo*: Base Editing via Next-Generation Virus-Like Particles

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***David Liu's team reports a big step forward in the delivery of gene editing cargo including base editors using engineered virus-like particles.***

CRISPR-based genome editing holds immense promise for enabling genetic therapies for countless diseases, but delivery technology—rife with limitations—remains a major bottleneck. A recent report in *Cell* by Banskota et al. from David Liu's laboratory at the Broad Institute[1] and elsewhere describes potent upgrades to a virus-like platform that embodies some of the best characteristics of both viral and nonviral technologies. Furthermore, this platform enables *in vivo* delivery of CRISPR base editors, which excel at correcting pathogenic single-nucleotide polymorphisms (SNPs) but can be difficult to deliver.

Decades of gene therapy research has identified useful viral delivery vectors such as lentivirus and adeno-associated virus (AAV), but these platforms are poorly suited for use with CRISPR enzymes. Gene therapies strive to introduce a gene that will be expressed indefinitely; doing so with CRISPR enzyme cargo could result in increased off-target editing[2] as well as immunogenicity in response to the microbe-derived protein.[3]

To avoid these issues, the most successful therapeutic applications so far have relied on delivery approaches that result in a transient dose of the genome editor. Last year, Intellia Therapeutics reported the use of lipid nanoparticles (LNPs) to deliver a guide RNA along with Cas9-encoding mRNA to the liver *in vivo* to address transthyretin amyloidosis.[4] This followed clinical success reported by CRISPR Therapeutics, which electroporated hematopoietic stem/progenitor cells *ex vivo* to introduce preformed Cas9 ribonucleoprotein (RNP) to address hemoglobinopathies.[5] Encouragingly, both approaches have favorably impacted biomarkers and ameliorated patient symptoms in initial clinical trials.

These clinical trials employed a transient dose of Cas9 nuclease, an enzyme with only limited capacity for correcting pathogenic SNPs because postmitotic cells tend not to perform efficient homology-directed repair (HDR), thus thwarting attempts to perform therapeutic knock-in. Base editing offers an appealing path to SNP correction, facilitating precise single-nucleotide changes without a requirement for double-strand breaks.

Although base editing simplifies corrective strategies by eliminating the need for a co-delivered HDR template, the large base editor complexes present their own challenges. Base editor genes are above the packaging capacity of an AAV, their mRNAs are extremely long and may complicate LNP-mediated delivery, and recombinant expression and purification of base editor proteins remain challenging to perform in high yield. In short, base editing presents an exciting therapeutic opportunity that poses its own distinct delivery challenges.

To slip past delivery barriers impeding therapeutic use of base editors *in vivo*, Banskota et al. devised a series of upgrades to the retrovirus-derived virus-like particle (VLP) platform, maturing it into an effective Trojan horse for sneaking preformed enzymes—but not their genetic code—into destination cells of interest. Sometimes branded as "nanoblades," VLPs have been used to deliver preformed CRISPR enzymes into cell types of therapeutic interest, including hematopoietic stem cells[6] and primary T cells.[7]

The beauty of the VLP approach lies in its ability to embody the best of two worlds: virus-like potency for sneaking into a cell's interior, coupled with the transient editor dosing characteristic of nonviral delivery. The VLP platform also holds the potential to be retargeted to specific cell types of interest[7] through an approach known as pseudotyping. But key foundational reports stopped short of robust *in vivo* genome editing, potentially because the VLPs lacked sufficient potency. Indeed, any macromolecular carrier can suffer if cargo loading and/or release is inefficient.

This is the challenge that Banskota et al. tackled, perhaps drawing inspiration from the Greeks of lore, who finally found a creative solution to penetrate the walls of Troy after a frustrating 10-year siege.

## Trojan Horses

Imagine the VLP as the Trojan horse, and adenine base editor (ABE) RNPs as the

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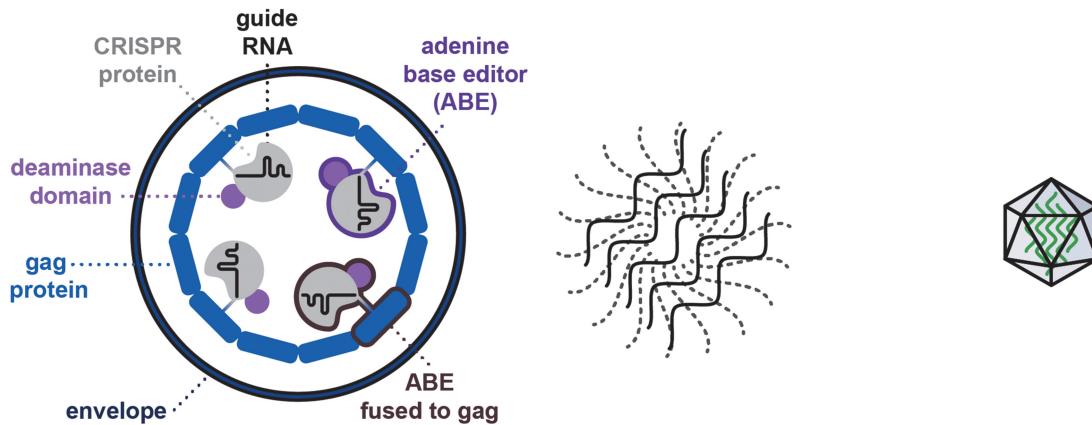
| Technology | VLP: RNP cargo | LNP: mRNA cargo | AAV: DNA cargo |
|---|---|---|---|
| Components | Retrovirus-derived virus-like particle (VLP) loaded with CRISPR protein & gRNA (RNP) | CRISPR-encoding mRNA carried by cationic lipid nanoparticles (LNP) | CRISPR-encoding DNA carried by adeno-associated virus (AAV) |
| Size | 100–150 nm | 50–100 nm | 25 nm |
| Manufacturing | Challenging to scale | Straightforward to scale | Challenging to scale |
| Editor lifetime | Transient (~1 day) | Transient (1–3 days) | Persisting / indefinite |
| Packaging limit | No fixed limit on cargo; DNA co-delivery is feasible | No fixed limit on cargo; DNA co-delivery is feasible | <5 kb; base editor cannot be packaged in single AAV |

**FIG. 1.   Comparison of delivery platforms and properties used for genome editing of the liver *in vivo*.** The depicted VLP is loaded with ABE, but several other cargo formats are also supported. ABE, adenine base editor; VLP, virus-like particle.

soldiers who must build, enter, and depart the carrier once they reach their destination. Considerable coordination will ensure that the mission runs smoothly, and that is precisely what Banskota et al. imparted through iterative design and testing. The VLP horse must be loaded with the right number of RNP soldiers; too many or too few results in either an overcrowded carrier or an underwhelming strike force. Liu's team thus determined the right balance of gag protein (a structural component of the VLP) to the ABE-gag fusion RNP that is loaded into the VLP (Fig. 1). Cleavage of the linker joining gag to the ABE RNP is necessary to make sure the soldiers swiftly disembark the horse upon reaching their destination.

Banskota et al. tuned this through comparison of several linker sequences, finding one with optimal cleavage kinetics. Finally, the soldiers must efficiently load themselves into the horse and then move with purpose once they have entered Troy undetected. This was emulated by tagging the ABE-gag fusion RNP containing both nuclear exclusion signals (NES) and nuclear localization signals (NLS). The gag-proximal NES initially predominates, ensuring that efficient VLP loading takes place in the cytosol of producer cells. The NES is removed from the cargo through linker cleavage during or after transit, allowing NLS still fused to the ABE RNP to direct the soldiers into the nucleus of the destination cell. In the spirit of sending only the best soldiers, the VLPs are loaded with an ABE construct featuring an evolved deaminase that confers extraordinary base editing activity.[8]

The engineered VLPs (eVLPs) resulting from these complementary improvements far surpassed the team's starting VLP platform. After intravenous delivery to the mouse liver, base editing rates using eVLP were 26-fold higher than rates attained using the initial VLPs. Administration of $7 \times 10^{11}$ eVLPs resulted in base editing efficiencies >60% in *PCSK9*, a gene target of interest for the treatment of familial hypercholesterolemia and cardiovascular disease,[9] and lowered serum levels of PCSK9 by 78%. When compared with a dual-AAV strategy for murine liver editing, eVLPs did not produce detectable off-target editing, whereas the viral delivery strategy caused low levels of off-target editing at three sites.

When eVLPs were injected subretinally to address a mouse model of genetic blindness, the platform mediated base editing with efficiency of >10% and resulted in improved visual function more than a month after treatment. After cerebroventricular injection into neonatal mice, a modest ~5% base editing efficiency was observed in a somewhat limited region of the brain. This result may be explained by the inherent difficulty that any retrovirus-based particle will face when introduced into the brain. Because retroviral particles (and VLPs) are about three times the diameter of the pores of the brain's extracellular space,[10] diffusion will be limited: even

the finest Trojan horse will struggle to travel through a dense forest. It remains encouraging that eVLPs showed promise in three very different tissue contexts *in vivo*.

The eVLPs developed by Liu's team represent a highly effective platform that can mediate efficient base editing in multiple clinically important tissues after *in vivo* administration. The eVLP platform is especially well suited to base editors, which are ideally delivered in a transient format, but can be challenging to express and purify recombinantly. Using eVLPs instead casts producer cells as the ABE protein factory, generating an all-in-one cell-invading carrier that is finely tuned to deploy its transient cargo for potent therapeutic base editing. Future optimization will likely focus on eVLP tropism, allowing this Trojan horse to breach any cellular stronghold of clinical interest.

## Author Disclosure Statement
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## Funding Information

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