



Advances in Engineered Virus-Like Particles for Genome Editing and Therapy

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Abstract

Engineered virus-like particles (eVLPs) have emerged as a promising class of delivery systems for genome editing agents. By combining the efficient cellular entry of viral vectors with the safety advantages of nonviral platforms, eVLPs enable transient delivery of ribonucleoproteins such as Cas9, base editors, and prime editors. Successive design strategies, from rational engineering to directed optimization, have progressively improved particle assembly, cargo stability, and editing efficiency. In parallel, pseudotyping approaches have expanded the versatility of eVLPs for cell-type-specific targeting. Recent preclinical studies highlight their potential across diverse applications, ranging from monogenic disease models to complex disorders, and support their advancement toward clinical translation. This review summarizes the structural and production principles of eVLPs, surveys key advances in their development, and discusses therapeutic opportunities and future challenges for their application in genome editing.

Key Points

Engineered virus-like particles (eVLPs) have emerged as innovative delivery vehicles for genome editing tools, combining viral entry efficiency with the safety of nonviral systems.

Distinct platforms such as Nanoblades, base editor-eVLPs, prime editor-eVLPs, and RIDE have advanced the field, yet a comprehensive review uniting these developments has been lacking.

By integrating structural principles, engineering strategies, and therapeutic applications, this review outlines the opportunities and challenges of eVLPs as next-generation genome editing platforms.

1 Introduction

Advances in gene editing technologies, most notably the clustered regularly interspaced short palindromic repeats (CRISPR)–CRISPR associated protein 9 (Cas9) system, have enabled precise and programmable modification of genomic DNA and driven the development of innovative gene therapies [1–10]. To translate these powerful tools into therapeutic applications across diverse tissues, the development of safe and efficient delivery systems remains a central challenge. Adeno-associated viruses (AAVs) are among the most widely used vectors in clinical gene therapy due to their well-characterized tissue tropism and long-term transgene expression; however, their limited DNA cargo capacity constrains the delivery of large genome editors such as Cas9 and more complex editing systems [11, 12]. Lipid nanoparticles (LNPs) represent a scalable, nonviral alternative and have enabled clinically advanced in vivo genome editing, particularly through liver-targeted delivery of mRNA or ribonucleoprotein (RNP) cargoes [13–15]. Direct delivery of RNP complexes provides highly transient editor activity and minimizes prolonged nuclease exposure, and its therapeutic potential has been most prominently demonstrated in liver-directed applications using LNPs [16, 17], including ongoing clinical studies [18, 19]. Nevertheless, despite these advances, RNP-based in vivo genome editing remains largely constrained to specific organs and delivery routes

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[20], and broader tissue targeting and delivery efficiency continue to pose significant challenges.

Physical delivery approaches such as electroporation are widely used in ex vivo cell engineering and have enabled several clinical successes, particularly for hematological disorders, while offering reduced cytotoxicity compared with nucleic acid-based electroporation [21, 22]. However, their reliance on transient membrane disruption limits applicability to in vivo settings and can compromise cell viability in sensitive cell types [23, 24]. In this landscape, virus-like particles (VLPs) have emerged as a complementary delivery platform that enables direct protein delivery ex vivo and in vivo without permanent transgene integration or sustained editor expression [25, 26]. By combining transient editor exposure with modular cargo packaging and targeting capabilities, engineered VLPs expand the genome-editing delivery toolbox by addressing specific use cases where protein-based, nonintegrating delivery is advantageous. In this review, we focus primarily on VLP platforms developed for protein-mediated delivery of genome-editing tools, particularly RNP-based editors, while acknowledging that VLP-based systems have also been explored for the delivery of other cargo modalities.

Classical VLPs were first established as noninfectious assemblies of viral structural proteins and rapidly gained prominence as vaccine platforms, where they could present antigens without encoding viral genomes [27–29]. Beyond vaccination, retroviral Group-specific antigen (Gag) polyproteins were shown to drive the assembly of particles incorporating not only viral but also cellular proteins, hinting at their utility for protein transfer [30]. In parallel, overexpression of vesicular stomatitis virus glycoprotein (VSV-G) was found to induce the release of fusogenic nanovesicles, termed “gesicles,” which could directly transfer functional proteins into recipient cells [31]. Although distinct from modern eVLPs, these early studies provided key conceptual foundations that inspired the development of eVLPs for genome editing applications.

eVLPs represent a next-generation platform that combines the efficient cellular entry of viruses with the favorable safety profile of non-viral systems [32, 33]. Unlike naturally secreted exosomes, which load cargo stochastically and have limited targeting capacity, eVLPs are rationally designed to control both payload and cell specificity. They also avoid the long-term risks of viral vectors, because they deliver genome editors as transient RNP complexes rather than genome-encoding cassettes.

By definition, eVLPs are viral genome-free and nonreplicating, yet they self-assemble via the viral Gag polyprotein, placing them conceptually between exosomes and viral vectors [34]. This narrow definition, however, overlooks their engineered potential. In practice, eVLPs function as modular platforms: scaffold, cargo-loading modules, and envelope

design can be flexibly engineered to produce tailored particles with defined tropism and efficient, transient activity.

A central challenge has been to redirect their natural role of packaging viral genomes toward the efficient encapsidation and delivery of large, heterologous RNP complexes [35, 36]. Successive iterations of rational engineering and directed optimization have progressively improved particle assembly, cargo stability, and tissue targeting, culminating in eVLP platforms capable of delivering Cas9, base editors (BEs), and prime editors (PEs) with demonstrated therapeutic relevance in preclinical models [32, 37].

This review will summarize the structural and production principles of eVLPs, survey key advances in their engineering, and discuss their emerging therapeutic applications and future challenges for clinical translation.

2 Structure and Production of eVLPs

eVLPs are nanoscale particles (100–200 nm) assembled from retroviral structural proteins, most notably the Gag polyprotein [38, 39]. They mimic the morphology of native viruses but lack a genome, making them noninfectious [32, 33]. Gag forms the inner capsid core, which is surrounded by a lipid envelope derived from the host cell membrane [40] (Fig. 1).

Production is typically carried out in HEK293T producer cells by transient cotransfection of multiple plasmids (Fig. 1) [35, 41]. A Gag plasmid provides the structural scaffold [39, 42], while a cargo plasmid encodes genome editors such as *Streptococcus pyogenes* Cas9 (SpCas9), BEs, or PEs. To ensure encapsidation, editors are often expressed as Gag-cargo fusions. In addition, recruitment tags or aptamer-binding protein (ABP) systems can be employed to further enhance cargo loading efficiency [43]. In parallel, a guide RNA plasmid supplies the single-guide RNA (sgRNA), which assembles with the cargo protein into a functional RNP [44]. Aptamer–ABP pairs such as MS2–MCP or COM–Com enhance sgRNA packaging and stability, ensuring efficient incorporation [37, 45–47]. Finally, an envelope plasmid encodes the surface glycoprotein, which dictates tropism. VSV-G is most common owing to its broad entry range [38, 48], whereas alternatives such as baboon retroviral envelope (BaEV) can improve editing of hematopoietic cells [49, 50]. This pseudotyping process provides a key source of modularity in eVLP design [51–53].

Once expressed within the producer cell, the components initiate a highly orchestrated assembly process. The newly synthesized Gag and Gag–cargo fusion polyproteins are trafficked to the inner leaflet of the producer cell’s plasma membrane, a process primarily mediated by the Matrix (MA) domain of Gag [39, 42] (Fig. 1). At the plasma membrane, thousands of Gag polyproteins multimerize, driven largely

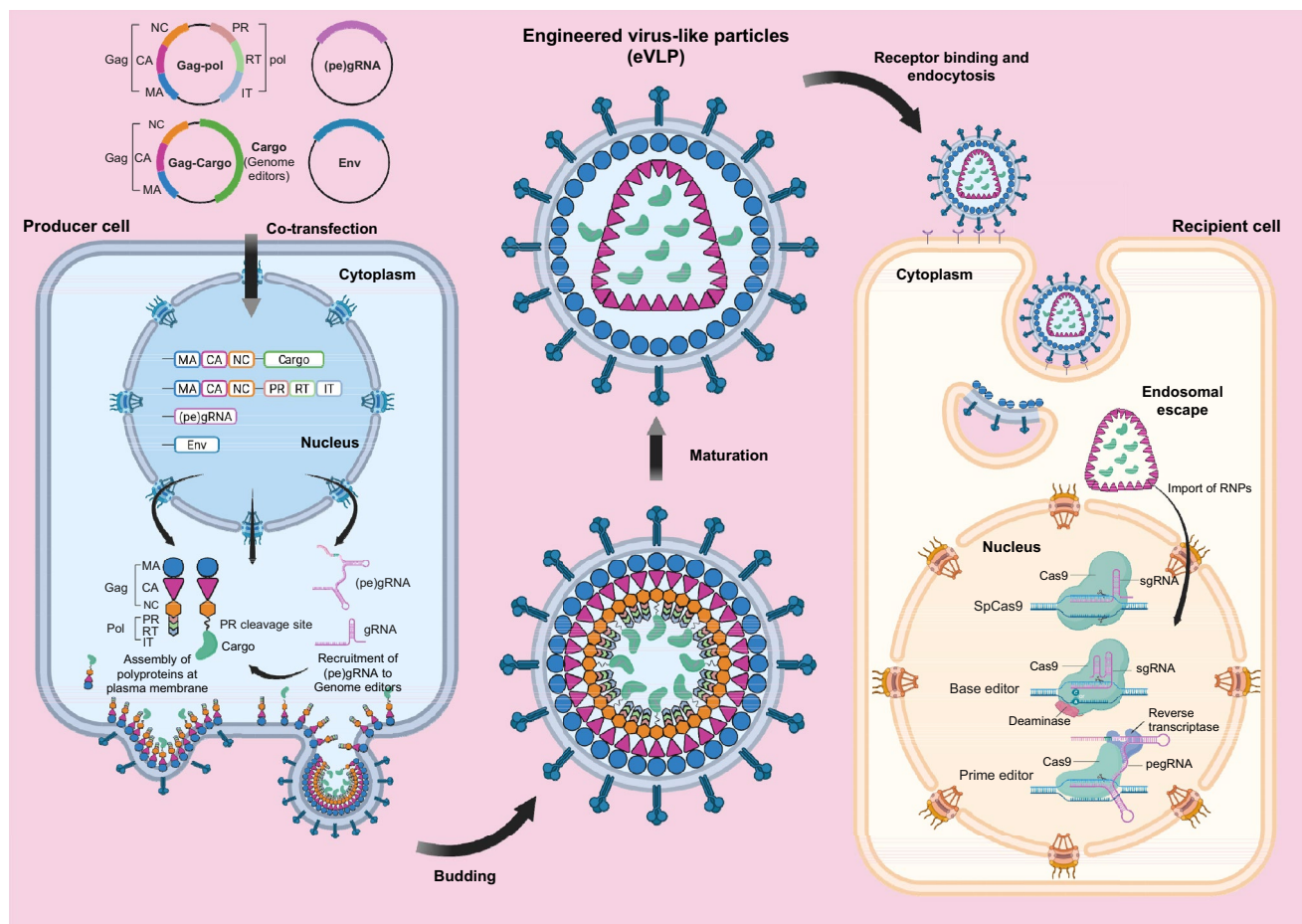


Fig. 1 Schematic illustration of eVLP production and delivery of genome editing agents. The figure shows principles of production and delivery of Cas9/sgRNA ribonucleotides. In producer cells, eVLPs are generated by transient cotransfection of plasmids encoding Gag-pol, Gag-cargo fusion (e.g., SpCas9/base editor/prime editor), an envelope glycoprotein (Env), and the corresponding guide RNA (sgRNA or pegRNA). The guide RNA recruits Cas9 to form RNP complexes. Gag and Gag-cargo polyproteins self-assemble at the plasma membrane of the producer cell, inducing membrane curvature. Continued assembly drives budding and eVLPs bud from cell in an immature state. After budding, the viral protease (PR) becomes active and cleaves Gag into its constituent domains, triggering core maturation. The PR also cleaves the Gag-cargo linker, releasing the

therapeutic cargo (e.g., Cas9, BE, and PE RNP) into the lumen of the particle. Mature, cargo-loaded eVLPs are harvested from the supernatant. Harvested eVLPs are then applied to recipient cells, where glycoproteins in eVLPs envelope bind to specific receptors on the target cell surface, initiating endocytosis. After membrane fusion and uncoating, the encapsidated RNPs escape into the cytoplasm. Guided by their intrinsic nuclear localization signals (NLSs), the RNP complexes engage the importin- α/β transport machinery to translocate into the nucleus, where they recognize target genomic DNA sequences and catalyze genome editing. MA matrix, CA capsid, NC nucleocapsid, PR protease, RT reverse transcriptase, IT integrase, gRNA guide RNA, pegRNA prime editing guide RNA, Env envelope glycoprotein, RNP ribonucleoprotein, sgRNA single-guide RNA

by interactions between their Capsid (CA) domains, to form a spherical, immature lattice [39]. This growing assembly induces outward membrane curvature, and the budding process is finalized when the particle pinches off from the cell, a step facilitated by the Gag Late (L) domain through recruitment of the host cell's Endosomal Sorting Complexes Required for Transport (ESCRT) machinery [54, 55].

After budding, the eVLP undergoes a crucial maturation step (Fig. 1). The copackaged viral protease becomes active and cleaves the Gag polyproteins into their constituent domains, triggering a dramatic structural rearrangement of the particle's core into its mature form [39, 56].

During this process, the protease also cleaves the linker between Gag and the cargo protein and releases the therapeutic cargo into the lumen of the eVLP [32, 35]. The resulting mature, cargo-loaded eVLPs are harvested from the supernatant and, when administered to a target cell, use their surface envelope glycoprotein to mediate entry [38, 48] (Fig. 1). Following membrane fusion and uncoating, the encapsidated RNPs are liberated into cytoplasm. Once released, the RNPs utilize their intrinsic nuclear localization signals (NLSs) to engage the host importin α/β transport machinery [57–59]. This ensures their nuclear import,

where they recognize target sequences and catalyze the intended genomic modifications [44, 57] (Fig. 1).

3 Advances in VLP Engineering for Enhanced Gene Editing and Delivery Efficiency

The primary goal in developing VLP-based delivery systems has been to continually improve their capacity to package and deliver gene-editing agents into target cells [38]. Early approaches largely adapted retroviral assembly principles to demonstrate proof-of-concept protein transfer, but these platforms were limited in packaging efficiency and cargo stability [30, 31]. Over the past decade, VLP engineering has advanced from simple structural scaffolds to systems specifically optimized for genome editors. Rational design strategies enabled stepwise improvements in packaging and release [32, 37], and iterative optimization has further refined particle assembly, cargo loading, and cell targeting [36].

Together, these advances have culminated in highly sophisticated eVLP platforms capable of packaging large and fragile ribonucleoproteins, demonstrating improved gene-editing efficiency while achieving minimal off-target effect and preserving cell viability [32, 35, 43, 60, 61]. The following section will trace their successive generations to illustrate how iterative engineering strategies achieved these gains.

3.1 The Foundational Approach: Direct Gag-Cargo Fusion

The concept of fusing heterologous proteins to retroviral Gag for particle incorporation dates back to early work by Jones et al. (1990) [62], who showed that Gag- β -galactosidase fusion proteins could assemble into retrovirus-like particles. These findings established that Gag could act as a versatile scaffold not only for viral proteins but also for engineered fusion partners. Later studies extended this principle to functional protein transfer, demonstrating that Gag-based particles could incorporate cellular proteins [30] and that vesicles induced by VSV-G overexpression (“gesicles”) were capable of transferring active proteins into recipient cells [38].

A major milestone toward therapeutic genome editing was achieved by Choi et al. [63], who showed that lentiviral particles prepackaged with Cas9 protein could mediate safer gene editing by avoiding long-term transgene expression. Building on this foundation, Mangeot et al. [35] introduced “Nanoblades,” the first dedicated eVLP platform for genome editing, using MMLV Gag–Cas9 fusions. This design, incorporating a protease-cleavable linker to release cargo during maturation, became the template for subsequent systems [32,

35]. While effective, it suffered from suboptimal packaging and incomplete release, highlighting the need for further engineering [32]. To date, eVLP-based platforms have been successfully developed for the delivery of multiple genome-editing tools [64–66], including nuclease-active Cas9, BEs, and PEs, underscoring the modularity of the Gag–cargo fusion strategy (Fig. 2).

Notably, while eVLP-based delivery of RNA-targeting CRISPR systems such as Cas13 has been reported in limited contexts [67], a dedicated and systematically optimized eVLP platform for Cas13 delivery has not yet been established. Given the predominantly cytoplasmic mode of action of Cas13 [68, 69], future development of eVLP–Cas13 platforms could further highlight the capacity of eVLPs to efficiently release functional cargos into the cytosol [70] and expand their applicability beyond DNA editing.

A conceptual overview of eVLP-mediated delivery of Cas9, BE, and PE cargos is provided in Fig. 2, and representative studies employing diverse genome editors are summarized in Table 1.

3.2 The Lineage of Gag-cargo Fusion eVLPs: Tailored Advances for Different Editors

The direct Gag-cargo fusion approach provided the initial framework [32, 35, 37]. However, the distinct molecular properties of different gene editors, such as BEs and the much larger PEs, required separate engineering strategies to enable efficient delivery. This section traces the divergent advancement paths of BE-eVLPs and PE-eVLPs, showing how the system was progressively adapted to overcome the unique challenges associated with each cargo.

3.2.1 The BE-eVLP Lineage: A Stepwise Journey v1–v5

The progression from v1–v4 eVLPs illustrates how rational design was applied to systematically overcome specific bottlenecks in packaging and delivery [32]. Key improvements included the optimization of the Gag-cargo linker, in which the protease cleavage site was engineered to balance stability during particle production with efficient release upon maturation. In addition, incorporation of a nuclear export signal (NES) into the Gag-cargo fusion enhanced cytoplasmic localization of the cargo in producer cells, thereby improving packaging efficiency. Further refinements were achieved through stoichiometric tuning, where the expression ratios of the individual plasmids (Gag, Gag-cargo fusion, and envelope) were optimized to maximize both particle yield and the potency of the final product. Specifically, adjusting the ratio of structural Gag to Gag-cargo fusion plasmids identified an optimal balance that simultaneously improved particle yield and the functional potency of the packaged RNPs [36].

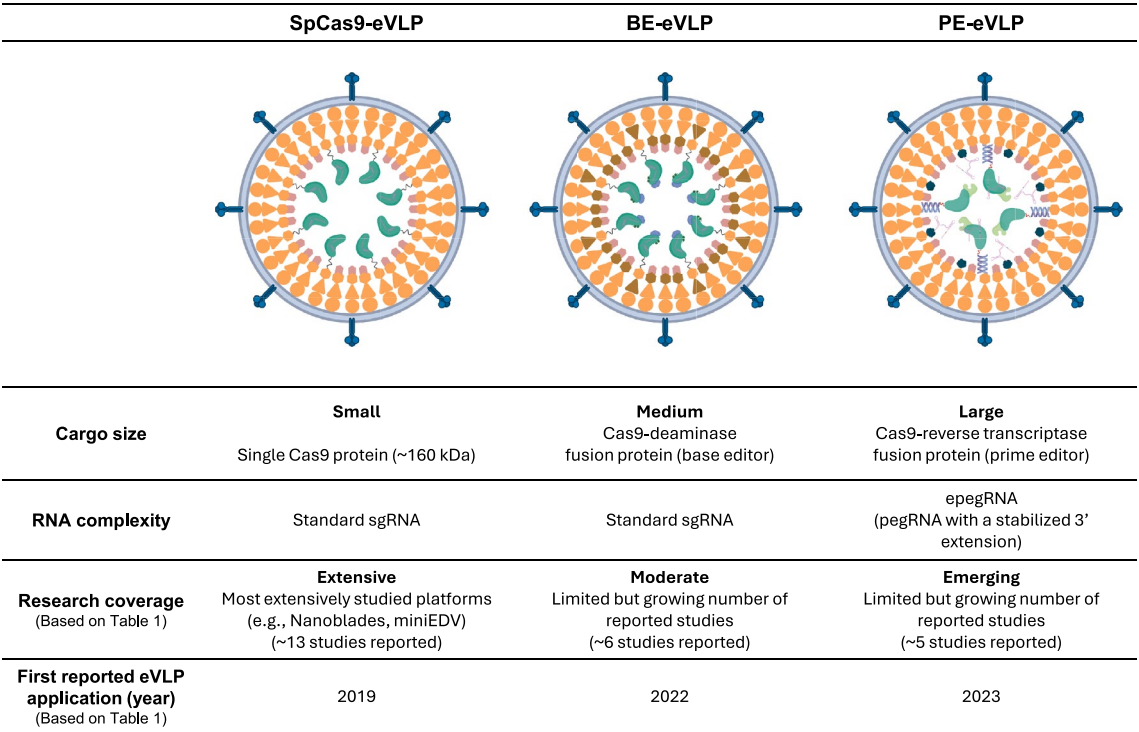


Fig. 2 Schematic comparison of eVLPs delivering Cas9, base editor, and prime editor. Schematic illustration of eVLPs packaging genome-editing cargos of increasing size and RNA complexity. Left, eVLPs encapsulating Cas9 RNP complexes composed of Cas9 protein and a standard single-guide RNA (sgRNA), representing the smallest and structurally simplest cargo configuration. Middle, eVLPs delivering base editor proteins, in which Cas9 is fused to a deaminase and guided by a standard sgRNA, resulting in increased protein cargo

size while maintaining low RNA complexity. Right, eVLPs packaging prime editor cargos consisting of a Cas9-reverse transcriptase fusion protein together with a prime editing guide RNA (pegRNA), which contains an extended 3' region and exhibits higher RNA structural complexity (epegRNA). Together, these schematics highlight how eVLP platforms accommodate progressively larger and more complex genome-editing cargos, with implications for packaging efficiency, RNA stability, and delivery performance

These refinements enabled v4 eVLPs to mediate robust *in vivo* editing across multiple tissues. In a mouse liver, a single intravenous injection achieved 63% editing of the *Proprotein convertase subtilisin/kexin type 9 (Pcsk9)* gene, resulting in a sustained 78% reduction in serum PCSK9 protein levels. In the central nervous system, local injection into the brain yielded up to 60% editing in transduced cells. Similarly, in a mouse model of inherited blindness *retinal degeneration 12 (rd12)*, a single subretinal injection corrected the pathogenic *Retinal pigment epithelium-specific 65 kDa protein (Rpe65)* mutation in retinal pigment epithelium cells with up to 30% efficiency—sufficient to restore visual function. Collectively, these studies demonstrated that eVLPs can support therapeutically relevant levels of *in vivo* genome editing across multiple tissues and disease models [32], although the reported editing efficiencies reflect outcomes obtained in distinct cell types and delivery contexts rather than direct comparisons of editor-intrinsic activity or delivery efficiency. However, when this v4 architecture was directly applied to prime editors, the oversized cargo and unstable prime editing guide RNA (pegRNA) led to

markedly reduced efficiency, underscoring the need for a separate, tailored strategy [37] (Fig. 3).

The transition to v5 eVLPs represented a shift from rational design to library-based selection [36]. Achieving potent editing at lower doses required further gains in packaging and transduction efficiency. In conventional viral capsid screening systems, most notably those developed for AAV, the genetic identity of each capsid variant is inherently preserved through the packaged viral genome, which enables a direct linkage of phenotype to genotype and facilitates the recovery of high-performing variants [79]. eVLPs, in contrast, lack a viral genome, rendering these standard methods inapplicable. To overcome this limitation, a barcoding-based strategy was implemented in which each Gag capsid mutant was tagged with a unique sequence embedded within the copackaged sgRNA, thereby enabling efficient tracking and selection of high-performing variants [36].

The library-based selection identified two critical mutations, Q226P and C507V, that cooperatively reprogrammed the capsid interior to improve compatibility with large RNP cargoes. The Q226P substitution is thought to alter

Table 1 Experimental settings, target cells, and genes edited by eVLP-based delivery systems

Genome editing agent	Experimental setting	Target cell/tissue	Target gene	Platform	References
SpCas9	in vitro	HEK293T cells, NIH-3T3 cells	<i>AAVS1, DDX3, EMX1, MYD88, 45S rDNA, TTN, Fto, Hpd, Tyr, LoxP</i>	Nanoblades	[35]
		HEK293T cells, K562 cells, Raji B-cell line	<i>WAS</i>	Nanoblades (VSV-G and BaEV co-pseudotyped)	[49]
		HEK293T cells	<i>EMX1</i>	SpCas9-v4 eVLP	[32]
		Jurkat cells, A549 cells	<i>B2M, TRAC</i>	Cas9-VLP	[52]
		HEK293T cells, Murine AML12 hepatocytes	<i>AFF1, Pcsk9, Vegfa, SERP-ING1, Fah</i>	LVNP	[61]
		Mouse prostate organoids	<i>AR</i>	Nanoblades (VSV-G and BaE-VRless co-pseudotyped)	[50]
		Mouse and human colon organoids	<i>CFTR</i>	Nanoblades (VSV-G and BaE-VRless co-pseudotyped)	[50]
		HEK293T cells	<i>BCL11A</i> enhancer, <i>EMX1</i>	eVLP (v4, v5 Cas9-eVLP)	[36]
		Jurkat cells	<i>B2M, PDCD1, TRAC</i>	EDV	[53]
		HEK293T cells	<i>B2M</i>	miniEDV	[71]
		iPSCs, iPSC-derived neurons	<i>B2M, NEFL, HSPB1</i>	VSVG pseudotyped VLP, VSVG/BRL co-pseudotyped VLP	[72]
		HEK293T cells, iPSCs	<i>B2M</i>	ENVLPE	[73]
		HEK293T, NIH3T3, Cos-7 cells, primary NHP fibroblast	<i>AAVS1, B2M, NECTIN1, Vegfa</i>	RIDE	[74]
	ex vivo	Primary human B cells, primary human T cells, CD34+ HSPCs	<i>WAS</i>	Nanoblades	[49]
		Activated primary human T cells	<i>B2M</i>	miniEDV	[71]
	in vivo	Mouse zygotes	<i>Tyr</i>	Nanoblades	[35]
		Mouse embryo	<i>LoxP</i>	Nanoblades	[35]
		Mouse liver	<i>Hpd</i>	Nanoblades	[35]
		Mouse retina (retinal pigment epithelium)	<i>Vegfa</i>	LVNP	[61]
		PBMC-humanized mice	<i>TRAC</i>	EDV	[53]
		Mouse retina (retinal pigment epithelium)	<i>Vegfa</i>	RIDE	[74]
		Mouse brain	<i>Htt, hmHTT</i>	RIDE	[74]
		NHP brain	<i>HTT</i>	RIDE	[74]
	in vitro	HEK293T, <i>Venus</i> reporter cells, CEM/CCR5, Jurkat cells	<i>CXCR4, CCR5, Venus</i>	NanoMEDIC	[75]

Table 1 (continued)

Genome editing agent	Experimental setting	Target cell/tissue	Target gene	Platform	References
Base editor	in vitro	HEK293T cells, NIH-3T3 cells, Neuro-2a cells	<i>BCL11A</i> enhancer, <i>Dnmt1</i> , <i>COL7A1</i> , <i>Idua</i> , <i>B2M</i> , <i>CIITA</i>	BE-eVLP	[32]
		HEK293T cells	Genomic site 2	LVNP	[61]
		HEK293T cells, Neuro-2a cells	<i>BCL11A</i> enhancer, <i>Angptl3</i> , <i>Rosa26</i> , <i>Dnmt1</i> , <i>Pcsk9</i>	BE-eVLP	[36]
		HEK293T cells, Jurkat cells	<i>PD1</i> , <i>B2M</i> , <i>CIITA</i> , <i>TIGIT</i>	HDVrz-psi-LVLP	[76]
		hiPSC-derived cortical neurons, Jurkat E6.1, primary bronchial epithelial cells, hiPSC	<i>B2M</i> , <i>TRBC1/2</i>	ENVLPE	[73]
	ex vivo	patient-derived intestinal organoids	<i>CFTR</i>	eVLP	[77]
		Primary human T lymphocytes	<i>B2M</i> , <i>TRBC1/2</i>	ENVLPE+	[73]
	in vivo	Mouse brain	<i>Dnmt1</i>	BE-eVLP	[32]
		Mouse Liver	<i>Pcsk9</i>	BE-eVLP	[32]
		Mouse retina (retinal pigment epithelium)	<i>Rpe65</i>	BE-eVLP	[32]
Prime editor	in vitro	HEK293T cells	<i>HEK3</i>	LVNP	[61]
		HEK293T cells, Neuro-2a cells	<i>HEK3</i> , <i>HEK4</i> , <i>FANCF</i> , <i>Col12a1</i> , <i>Dnmt1</i>	PE-eVLP	[37]
		HEK293T reporter cells (YFPs)	YFPs, <i>HEK3</i> , <i>RNF2</i>	NanoScribes	[78]
		HEK293T cells	<i>HEK3</i>	PE-eVLP	[36]
		HEK293T cells, Neuro-2a cells	<i>Dnmt1</i> , <i>RUNX1</i> , <i>HEK3</i> , <i>HEK4</i>	ENVLPE	[73]
	ex vivo	hiPSCs, hiPSC-derived HSCs, primary myoblasts	<i>HEK3</i>	NanoScribes	[78]
	in vivo	Mouse brain	<i>Dnmt1</i>	PE-eVLP	[37]
		Mouse retina (retinal pigment epithelium)	<i>Mfrp</i> , <i>Rpe65</i>	PE-eVLP	[37]
		Mouse retina (retinal pigment epithelium)	<i>Mfrp</i> , <i>Rpe65</i>	ENVLPE, ENVLPE+	[73]

AAVS1 adeno-associated virus integration site 1, *AFF1* AF4/FMR2 family member 1, *Angptl3* angiopoietin like 3, *AR* androgen receptor, *B2M* beta-2 microglobulin, *BCL11A* B cell lymphoma/leukemia 11A, *CCR5* chemokine receptor type 5, *CFTR* cystic fibrosis transmembrane conductance regulator, *CIITA* class II major histocompatibility complex transactivator, *Col12a1* collagen type XII alpha 1 chain, *Col7A1* collagen type VII alpha 1 chain, *CXCR4* C-X-C chemokine receptor type 4, *DDX3* dead-box RNA helicase 3, *Dnmt1* DNA methyltransferase 1, *EMX1* empty spiracles homeobox 1, *Fah* fumarylacetoacetate hydrolase, *FANCF* Fanconi anemia group F, *Fto* fat mass and obesity-associated gene, *Hpd* 4-hydroxyphenylpyruvate dioxygenase, *HSPB1* heat shock protein family B member 1, *HTT* huntingtin, *Idua* alpha-L-iduronidase, *LoxP* locus of X-over P1, *Mfrp* membrane frizzled-related protein, *MYD88* myeloid differentiation primary response 88, *NEFL* neurofilament light chain, *Pcsk9* proprotein convertase subtilisin/kexin type 9, *PD1* programmed cell death protein 1, *PDCD1* programmed cell death protein 1, *RNF2* ring finger protein 2, *Rpe65* retinal pigment epithelium-specific 65 kDa protein, *RUNX1* Runt-related transcription factor 1, *SERP-ING1* serpin family G member 1, *TIGIT* T cell immunoreceptor with immunoglobulin and ITIM domain, *TRAC* T cell receptor alpha constant, *TRBC1/2* T cell receptor beta constant 1/2, *TTN* titin, *Tyr* tyrosinase, *Vegfa* vascular endothelial growth factor A, *WAS* Wiskott–Aldrich syndrome, *YFPs* yellow fluorescent proteins, *45s rDNA* 45s ribosomal DNA

local capsid conformation, enhancing structural flexibility and facilitating RNP accommodation, whereas the C507V mutation disrupts a zinc-finger motif normally required for viral RNA packaging, thereby redirecting the capsid toward

efficient loading of protein–RNA complexes. Together, these modifications improved both packaging and cargo release, resulting in a two- to fourfold increase in editing potency compared with v4 eVLPs. Beyond generating a

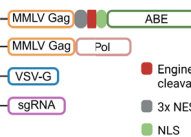
BE-eVLP version	Components	Bottleneck	Engineering strategy	Key improvement	References
v1 (baseline)		- Suboptimal protease-site cleavage incomplete cargo release - Nuclear bias of cargo reduces loading	Baseline comparator (no optimization yet)		
v2.4		Inefficient Gag–cargo linker cleavage limits release	- Switch retroviral scaffold from FMLV to MMLV - Optimized MLV protease-cleavable linker (screened sequences).	Higher base editing vs v1 (cell and in vivo assays).	[32]
v3.4		- Gag–cargo retained in nucleus (NLS bias) - Earlier variants required two protease cuts	- Add 3 × NES to shift producer-cell localization to cytoplasm - Simplify to a single protease cut.	1.1 to 2.1-fold higher editing efficiency (vs v2.4) - Improved cytoplasmic localization	
v4	Same as v3.4	Imbalanced Gag–cargo : Gag–Pol expression limits per-particle activity and titers	Tune Gag–BE proportion to ~25% while keeping v3.4 design.	5.6-fold (vs v1) and 2.2-fold (vs v2.4) higher editing efficiency per unit volume 8.5-fold higher editing efficiency per viral particle (vs v1) - Produced at higher titer	
v5		- Need to boost RNP packaging/release and transduction beyond v4 - Suboptimal capsid maturation kinetics	- Barcoded-sgRNA–linked directed evolution - Combine Q226P (near p12/CA cleavage) and C507V (NC zinc-finger) to favor RNP loading/release.	- Improved cargo release 1.8-fold protein and 4.3-fold more sgRNA per particle (vs v4) 2.6-fold potency in primary human HSPCs - Altered capsid maturation	[36]

Fig. 3 Summary of BE-eVLPs development step and schematic of their components. The figure shows stepwise development of BE-eVLPs from the baseline design (v1) to the direct-evolved design (v5). In the Components column, the plasmids used to produce each version of the eVLP are depicted schematically. The Bottleneck column summarizes the limitations identified in the preceding version. The Engineering strategy column describes the methods used to create the current version to address those limitations. The Key improvement column shows how much the current version improves relative

to earlier versions. *BE* base editor, *FMLV* friend murine leukemia virus, *Gag* Group-specific antigen, *ABE* adenine base editor, *Pol* polymerase, *VSV-G* vesicular stomatitis virus glycoprotein, *sgRNA* single guide RNA, *MMLV* Moloney murine leukemia virus, *MLV* murine leukemia virus, *NES* nuclear export signal, *NLS* nuclear localization signal, *POL* polymerase, *MA* matrix, *CA* capsid, *NC* nucleocapsid, *RNP* ribonucleoprotein, *HSPCs* human hematopoietic stem and progenitor cells

more effective v5 particle, this study also established a generalizable framework for improving VLPs, highlighting the feasibility of reprogramming the platform for functions not readily accessible through rational design alone [36] (Fig. 3).

3.2.2 PE-eVLP Lineage: Engineering Solutions for a Bulkier Cargo (v1–v3b)

The delivery of PEs posed unique challenges compared with BEs, owing to the large size of the PE–reverse transcriptase fusion protein and the inherent instability of the pegRNA, whose long, single-stranded 3' extension is vulnerable to exonucleolytic degradation, reducing editing efficiency [80]. As noted above, simply adapting the potent v4 BE-eVLP design was insufficient, necessitating a tailored engineering strategy specifically optimized for PEs [37] (Fig. 4).

The first-generation v1 and v2 PE-eVLPs revealed persistent inefficiency in packaging PE cargo, with only modest gains achieved through structural adjustments and plasmid ratio tuning, which remained inadequate for therapeutic applications. A major advance was realized with the v3 PE-eVLP, which introduced a multi-component scaffold to improve recruitment of the large PE RNP complex [37]. In parallel, an aptamer-based recruitment strategy was adopted. Aptamers are nucleic acid ligands that bind target proteins with high affinity and specificity and can be engineered as short RNA hairpin motifs into guide RNAs, where they are recognized by cognate ABPs fused to Gag. This principle was first established in CRISPR systems as a modular RNA scaffold for recruiting effector proteins to Cas9-targeted loci [81], and later extended to eVLP contexts, where aptamer–ABP interactions enabled efficient packaging and delivery of Cas9 RNPs using lentiviral capsid-based particles [43]. Building on these precedents, the

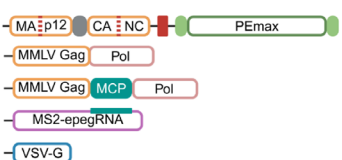
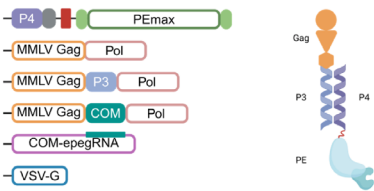
PE-eVLP version	Components	Bottleneck	Engineering strategy	Key improvement	Reference
v1.1		- Very low prime editing efficiency in cells (<1% at benchmark loci) - Poor RNP loading and pegRNA stability/affinity	Baseline comparator (no optimization yet)		
v2.3		- Endogenous PR cleavage in RT removed the C-terminal NLS - Incomplete Gag cleavage left NES attached, impairing nuclear import - Limited cargo release	- Eliminate RT protease site - Reposition NES upstream of cleavage - Add flexible GGS linkers to improve PR access	2.6-fold higher editing efficiency (vs v1.3) (<i>HEK3, Dnmt1</i>) at the highest tested dose	
v3		- Insufficient epegRNA loading was the dominant limiter - Need for coordinated loading of PE protein and epegRNA	- RNA-protein tethering via MCP-MS2 to recruit epegRNA - Stoichiometry re-tuning - Single-particle PE3 packaging	4-fold at <i>Dnmt1</i> (vs v2.3) and up to 170-fold at <i>HEK3</i> (vs v1.1) higher editing efficiency 2.2-fold more epegRNA and 1.3-fold more PE protein per particle	[37]
v3b		- Large Gag-PE fusion limited PE expression and packaging - Need further boost to RNA loading without perturbing maturation	- Replace Gag-PE with smaller P4-PE recruited by coiled-coil (P3/P4) - Replace MS2/MCP with compact COM/Com pair - Stoichiometry optimization	1.3-fold (vs v2.3) 1.2-fold at <i>Dnmt1</i> and 2.9-fold at <i>FANCF</i> (vs v3) higher editing efficiency - up to 150-fold at <i>HEK3</i> (vs v1.1)	

Fig. 4 Summary of PE-eVLPs development step and schematic of their components. The figure shows stepwise development of PE-eVLPs from the baseline design (v1) to the state-of-the-art design (v3b). In the Components column, the plasmids used to produce each version of the eVLP are depicted schematically. The Bottleneck column summarizes the limitations identified in the preceding version. The Engineering strategy column describes the methods used to create the current version to address those limitations. The Key improvement column shows how much the current version improves relative

to earlier versions. *PE* prime editor, *MMLV*: moloney murine leukemia virus, *Gag* Group-specific antigen, *Pol* polymerase, *VSV-G* vesicular stomatitis virus glycoprotein, *pegRNA* prime editing guide RNA, *NES* nuclear export signal, *NLS* nuclear localization signal, *RNP* ribonucleoprotein, *MA* matrix, *CA* capsid, *NC* nucleocapsid, *GGS* gly-glyser, *HEK3* human embryonic kidney 293T site 3, *Dnmt1* DNA methyltransferase 1, *MCP* MS2 coat protein, *epgRNA* engineered prime editing guide RNA, *FANCF* Fanconi anemia complementation group F

v3 PE-eVLP incorporated a Gag fusion with the MS2 coat protein (Gag-MCP), which specifically recognized MS2-tagged pegRNAs and thereby stabilized and enriched the full RNP complex during particle assembly [37] (Fig. 4).

The v3b PE-eVLP further improved pegRNA recruitment by replacing the MS2/MCP system with the higher-affinity COM/com pair, resulting in more efficient and stable pegRNA capture [37]. This architecture, which combined coiled-coil-mediated protein recruitment with enhanced pegRNA stabilization, enabled prime editing at therapeutically relevant levels (Fig. 4). In vivo, a single subretinal injection corrected ~ 15 % of the pathogenic *Membrane frizzled-related protein* (*Mfrp*) mutation in the *rd6* mouse model and ~ 7.2 % of the *Rpe65* substitution in the *rd12* model, restoring partial visual function. Taken together, the advancement from v1 to v3b illustrates the adaptability of the eVLP platform [37] and its capacity to be tailored for the distinct requirements of different gene editors [32, 35–37].

3.3 Recent Innovations in eVLP Engineering: NanoScribes, ENVLPE, and NanoMEDIC Systems

Beyond the BE and PE lineages, further innovations have substantially improved the efficiency of eVLP-mediated genome editing by addressing critical bottlenecks in RNP assembly and packaging. Extending engineering strategies in a lentiviral context, Haldrup et al. [61] applied protease-releasable Gag-cargo fusions, sgRNA scaffold and plasmid stoichiometry optimization, and envelope pseudotyping to build human immunodeficiency virus-1 (HIV-1) derived lentivirus-derived nanoparticles (LVNPs) that support transient and efficient delivery of Cas9 as well as base/prime editor RNPs. In a PE-focused eVLP lineage, Halegua et al. [78] introduced “NanoScribes,” an MLV-Gag-based PE-eVLP that couples iterative Gag-prime-editor design (removal of the RNase H domain, strategic placement of 3×NES and a single protease cleavage site) with pegRNA stabilization (3' structural motifs) and a Pol II/Csy4 intronic-pegRNA

(ipegrNA) strategy to boost RNA processing and packaging. Together with multi-envelope pseudotyping (e.g., VSV-G + BaEV + hSyncytin-1), these interventions delivered near-transfection-level PE efficiencies in reporter assays and enabled editing in fragile human cells (human induced pluripotent stem cells (hiPSCs), hiPSC-derived hematopoietic stem cells (HSCs), primary myoblasts) while maintaining high target fidelity in mismatch-tolerance tests [78].

Building on these PE-centric advances, Geilenkeuser et al. [73] developed the engineered nucleocytosolic vehicles for loading of programmable editors (ENVLPE) platform, which integrates aptamer-mediated RNP recruitment, nucleocytosolic shuttling, and pegRNA stabilization to selectively encapsidate complete RNPs. The optimized ENVLPE+ achieved near-stoichiometric Cas9:pegRNA ratios and demonstrated ~5.5-fold greater functional rescue in retinal models compared to v3 PE-eVLPs at ten-fold lower particle doses.

Borovikova et al. [75] optimized the NanoMEDIC system by incorporating AsCas12a with Pol II-driven CRISPR RNA (crRNA) transcription, enabling cytoplasmic crRNA processing, efficient RNP incorporation, and multiplex editing. This strategy yielded 2–3 fold higher editing efficiencies than SpCas9-eVLPs and enabled C-X-C chemokine receptor type 4 (*CXCR4*) knockout in T cells. Together, these advances exemplify a new generation of eVLP architectures that enhance per-particle activity and broaden therapeutic applicability.

Collectively, the engineering advances described above—optimized Gag-cargo architectures, improved RNP recruitment and release, and programmable pseudotyping—convert incremental particle-level gains into application-level benefits. Higher per-particle activity enables therapeutically relevant editing at lower doses; editor-specific scaffolds (e.g., for BEs and PEs) extend the usable cargo space to bulky RNPs; and envelope modularity opens cell-type-specific access. We next examine how these properties translate into disease-model efficacy, tissue targeting, and safety signals across preclinical systems.

4 Positioning eVLPs within the Genome-Editing Delivery Landscape

The rapid expansion of genome-editing technologies has been accompanied by the parallel development of diverse delivery platforms, each optimized for distinct therapeutic contexts. Among these, AAVs, LNPs, and eVLPs represent three conceptually and mechanistically distinct approaches to in vivo genome-editing delivery. Rather than functioning as mutually exclusive competitors, these platforms occupy complementary positions within the evolving delivery

landscape, shaped by trade-offs in cargo capacity, expression kinetics, tissue tropism, and translational maturity.

AAV vectors remain the most extensively validated viral delivery system for in vivo gene therapy, with a long history of clinical use and well-characterized tissue tropism profiles [82, 83]. In the context of genome editing, however, AAV-based delivery presents intrinsic challenges. The limited packaging capacity of AAV constrains the delivery of large or multi-component editing systems, such as BEs and PEs, often necessitating split or dual-vector strategies that increase complexity and variability [84–86]. Moreover, AAV-mediated delivery results in sustained transgene expression [87], which can be advantageous for gene replacement but raises concerns for genome editing applications that favor transient nuclease exposure. These issues are particularly salient in post-mitotic tissues such as the central nervous system, where prolonged expression of genome-editing enzymes may increase cumulative off-target activity, immunogenicity, and long-term safety risks [88]. In addition, preexisting and treatment-induced neutralizing antibodies against AAVs represent a well-recognized limitation for in vivo applications, particularly with respect to patient eligibility and redosing. As a result, AAV vectors are well-suited for durable gene augmentation but exhibit a fundamental tension with the transient exposure paradigm increasingly favored for therapeutic genome editing.

LNPs represent the most clinically advanced nonviral delivery platform for in vivo genome editing and have demonstrated robust efficacy in liver-targeted applications. Their modular composition, scalable manufacturing, and regulatory familiarity have enabled rapid clinical translation of mRNA- and RNP-based genome-editing therapeutics [89]. In particular, LNP-mediated delivery has established liver-directed in vivo editing as a clinical reality [18, 19, 90]. Nevertheless, despite ongoing advances in lipid chemistry and formulation, LNP biodistribution remains predominantly biased toward the liver, largely driven by endogenous lipid transport pathways [16]. In addition, inefficient endosomal escape continues to represent a major bottleneck for cytosolic delivery, limiting editing efficiency across tissues [91]. These features position LNPs as a powerful and clinically mature platform, while highlighting persistent challenges in achieving efficient, extrahepatic genome editing.

eVLPs occupy a distinct and emerging niche within this delivery ecosystem. Conceptually, eVLPs have been described as “Trojan horse-like” delivery vehicles that combine virus-derived entry efficiency with the safety advantages of genome-free, transient cargo delivery [34]. By packaging preassembled genome-editing proteins or ribonucleoprotein complexes, eVLPs enable direct cytoplasmic delivery without reliance on transcription or translation, thereby minimizing prolonged nuclease exposure. Unlike AAV vectors, eVLPs avoid sustained expression of editing

enzymes, and unlike LNPs, they leverage virus-inspired membrane fusion mechanisms that can facilitate efficient cytosolic release [32, 36]. Importantly, eVLP tropism can be modulated through envelope pseudotyping [53], offering a flexible strategy to tailor delivery toward specific cell types.

At the same time, eVLPs remain at an earlier stage of translational development compared with AAVs and LNPs, with challenges related to standardized manufacturing, large-scale production, and quality control yet to be fully addressed. Accordingly, eVLPs are not positioned to replace established delivery platforms, but rather to complement them by addressing specific use cases where transient, protein-based delivery is advantageous. In this context, AAVs, LNPs, and eVLPs can be viewed as complementary solutions within the genome-editing delivery landscape: AAVs provide robust *in vivo* delivery for durable gene expression, LNPs offer clinically validated and scalable nonviral delivery for liver-targeted editing, and eVLPs introduce a modular platform optimized for transient protein delivery with a reduced genomic footprint. Appreciating the context-dependent strengths and limitations of each platform will be critical for selecting appropriate delivery strategies and guiding the future development of genome-editing therapeutics (Table 2).

5 Therapeutic Applications and Clinical Outlook for eVLPs

eVLPs represent a promising next-generation platform for therapeutic genome editing, combining the efficiency of viral entry with the safety advantages of nonviral systems. Recent studies have demonstrated that eVLPs can overcome key limitations inherent to existing delivery strategies [32, 35–37, 49, 50, 53, 73]. Unlike integrating viral vectors such as lentiviruses, eVLPs do not integrate DNA into host genomes and instead deliver genome editors in their most transient ribonucleoprotein form, thereby reducing prolonged exposure and minimizing the risk of off-target activity [51].

Moreover, eVLPs can be pseudotyped with diverse glycoproteins, offering the potential for cell type-specific targeting through rational envelope engineering [49, 50, 53, 78]. This section will first summarize the key preclinical successes that validate the potential of eVLPs, then explore their future applications in precision medicine, and finally address the critical research directions needed to overcome hurdles for clinical translation.

Table 2 Comparative landscape of delivery platforms for *in vivo* genome editing

Feature	AAV	LNP	eVLP
Cargo type	DNA (expression cassette)	mRNA, sgRNA, RNP	Protein, RNP (optionally mRNA)
Cargo size compatibility	Limited (~4.7 kb); large editors require split/dual AAV	Flexible; supports large mRNA and RNP cargos	High; accommodates large protein and multi-component editors
Expression/activity duration	Long-term transgene expression	Transient (translation-dependent)	Highly transient (direct protein delivery)
Control of nuclease exposure	Limited; sustained expression	Moderate; depends on mRNA stability	High; rapid clearance after delivery
Primary delivery mechanism	Viral transduction and nuclear entry	Endocytosis followed by endosomal escape	Virus-inspired membrane fusion and cytoplasmic release
Endosomal escape requirement	Required	Required; major efficiency bottleneck	Minimal; fusion-mediated entry
Tissue tropism	Serotype/capsid-dependent	Predominantly liver-biased	Envelope/pseudotyping-dependent
<i>In vivo</i> delivery maturity	Clinically approved	Clinically advanced	Preclinical
Redosing potential	Limited by immunity	Potentially feasible	Not yet established
Immunogenicity considerations	Pre-existing immunity; adaptive responses	PEG-related and innate immune responses	Under investigation; envelope-dependent [74]
Manufacturing and scalability	Established GMP pipelines	Highly scalable; GMP-ready	Emerging; standardization needed
Representative clinical context	Gene replacement	Liver-targeted genome editing	Not yet established
Key limitations for genome editing	Cargo size, sustained expression	Liver bias, endosomal escape	Manufacturing, translational readiness

This table summarizes key features of three major delivery platforms, AAVs, LNPs, and eVLPs, used for *in vivo* genome-editing applications. The comparison highlights differences in cargo type and size compatibility, duration of editor activity, delivery mechanisms, tissue tropism, translational maturity, and key limitations relevant to genome-editing therapeutics

GMP, Good Manufacturing Practice; PEG, polyethylene glycol; RNP, ribonucleoprotein

5.1 Demonstrating Therapeutic Efficacy Across a Spectrum of Preclinical Models

A critical step in validating eVLPs as therapeutic tools has been their successful application in preclinical models, beginning with foundational studies in cells and organoids (Table 2). Early proof-of-concept work established the utility of eVLPs for modeling monogenic diseases through efficient genome editing in primary cells and organoid systems [35, 50]. Demonstrating translational potential, adenine BE-eVLPs successfully corrected the *Cystic Fibrosis Transmembrane Conductance Regulator* (*CFTR*) G542X mutation in patient-derived intestinal organoids, leading to the functional rescue of chloride channel activity [77]. Beyond simple editing efficiency, complementary work in iPSC-derived neurons revealed that Cas9-eVLPs can also shed light on DNA repair pathway preferences in specific cell lineages, informing strategies to improve precision [72]. Together, these studies underscore the versatility of eVLPs as tools for disease modeling and therapeutic development in diverse in vitro and ex vivo contexts.

Building on these cellular studies, eVLP-based delivery has achieved therapeutically relevant outcomes in various animal models (Table 2). A landmark example is the systemic delivery of eVLPs to edit disease genes such as *Pcsk9* in the liver, resulting in long-term modulation of serum protein levels [32]. Local administration has also proven highly effective, enabling efficient editing in challenging tissues such as the retina and brain, with functional rescue demonstrated in models of inherited blindness [32, 37]. The therapeutic applicability has been further broadened to ocular neovascular disease and Huntington's disease, where targeted in vivo delivery produced efficient editing and significant phenotypic improvement [74]. In addition to direct therapeutic applications, RNP-loaded eVLPs have been used to generate knockout mouse models with high efficiency, providing a rapid and nonintegrating alternative to traditional transgenesis methods [92].

5.2 Pseudotyping Strategies to Enhance eVLP Tropism for Precision Medicine

The next frontier for eVLPs lies in precision therapy, where rational envelope engineering enables highly tailored interventions. The principle of pseudotyping—equipping eVLPs with diverse viral glycoproteins to alter tropism—has been significantly advanced through rational design. A key early example is the use of a BaEV, which enabled the “Nano-blades” platform to efficiently edit clinically relevant human T, B, and CD34⁺ hematopoietic stem cells, demonstrating the power of targeted delivery in immune cell engineering [49]. Building on this, more sophisticated programmable enveloped delivery vehicles (EDVs) have shown the

feasibility of customizing glycoprotein composition to program cell tropism, and VSVGmut pseudotyping has enabled selective T-cell editing in vivo, highlighting the translational potential of modular engineering for applications such as adoptive immunotherapy [52, 53]. Nevertheless, earlier approaches on the basis of VSV-G variants often suffered from reduced titers, limiting their translational potential [93]. In this context, the Delivery to Intended REcipient Cells Through Envelope Design (DIRECTED) provides a major advance by decoupling the fusogenic and targeting functions of viral envelopes. By disabling the native receptor-binding activity of a potent fusogen including VSV-G while preserving its fusion machinery, the system creates a functionally “blind” but fusion-competent particle. Specificity is then conferred by tethering interchangeable antibodies or ligands to the envelope surface, allowing transduction to occur only in the presence of the chosen targeting moiety. This modular design enables highly selective delivery into defined cell populations, including human T and B cells, while maintaining compatibility with diverse fusogens and eVLP backbones [94] (Table 1).

The ultimate goal of these precision technologies is to facilitate more disease-tailored and preclinical frameworks for genome editing, transitioning from broad disease models to more disease-tailored approaches. The integration of engineered eVLPs with patient-derived organoids provides a powerful paradigm for this future: organoids can serve as a preclinical platform to test and validate the efficacy of a specifically targeted eVLP before its potential therapeutic application [95]. This approach moves beyond proof-of-concept and toward a progressive translational workflow where interventions are tailored to the individual. As emphasized in recent reviews, the unique combination of envelope modularity and transient RNP delivery positions eVLPs to effectively balance safety with precision in the clinic [33, 51].

5.3 The RIDE Platform: From Preclinical Validation to Translational Potential

A prominent example of the therapeutic advancement of eVLPs is the recently developed Ribonucleoprotein Delivery (RIDE) system [74]. RIDE is a customizable, tropism-programmable eVLP that, as in the v3b PE-eVLP, employs MS2–MCP-mediated recruitment to copackage preassembled CRISPR–Cas9 RNPs during particle assembly. Exploiting eVLP modularity, tropism can then be redirected by swapping only the plasmid encoding the envelope glycoprotein, with no changes to the RNP payload.

Instead of detailing individual disease models, the RIDE platform can be more concisely summarized as having demonstrated effective, tropism-adjustable RNP delivery across both ocular and neurological preclinical settings, with evidence of sustained therapeutic benefit and acceptable

long-term safety profiles in small-animal models, as well as initial tolerability in a large-animal context [74].

While these findings underscore the translational promise of RIDE, direct quantitative comparisons with AAV- or LNP-based systems remain limited and highly context dependent. Accordingly, RIDE is best viewed as a proof-of-concept demonstration of how eVLP modularity can support efficient and transient *in vivo* genome editing, rather than as a benchmarked alternative to clinically established platforms.

6 Future Research Directions for Advancing eVLP Therapeutics

Although eVLPs have demonstrated compelling preclinical efficacy across diverse genome-editing applications, several critical biological and translational challenges must be addressed to enable their progression toward clinical development. Addressing these gaps will require a shift from proof-of-concept demonstrations toward systematic evaluation of safety, biodistribution, immunogenicity, and manufacturability under translationally relevant conditions.

A first important direction concerns reduction of viral-derived components to mitigate immunogenicity while preserving delivery efficiency. Early-generation eVLPs retain substantial portions of retroviral structural proteins, raising concerns regarding adaptive immune responses and redosing feasibility. Recent mechanism-guided studies have shown that efficient genome editing does not strictly depend on an intact viral capsid core, but rather on effective cytosolic release and nuclear import of editor RNPs [71]. Guided by this principle, minimal particle architectures such as miniEDV and miniENVLPE have demonstrated that extensive removal of viral protein content—up to ~80–90% of native Gag sequences—can be achieved without major loss of editing potency [71, 73]. These approaches represent promising strategies to lower immunogenic burden and simplify particle composition, but further *in vivo* validation will be required to determine whether reduced viral content translates into improved tolerability, redosing potential, and regulatory acceptability.

eVLP-mediated mRNA delivery has also emerged as a conceptually distinct modality for genome editing [76]; however, its implications for therapeutic applications require careful evaluation. In contrast to RNP delivery, which enables highly transient editor exposure while achieving permanent genomic modification, mRNA-based delivery inherently prolongs editor expression and may increase cumulative off-target risk. Accordingly, most eVLP platforms developed for base and prime editing have prioritized RNP delivery to minimize prolonged nuclease exposure [32, 37]. Moreover, although eVLP-based mRNA encapsulation

offers additional modularity, current studies provide limited quantitative assessment of packaging efficiency and report lower editing efficiencies than optimized eVLP-RNP systems, underscoring the need for further optimization and context-specific evaluation in future studies.

Beyond particle composition, a major unresolved gap lies in systemic delivery and biodistribution profiling of eVLPs. To date, most *in vivo* studies have relied on localized administration routes, such as subretinal or intracerebral injection, which effectively demonstrate target engagement but provide limited insight into whole-body pharmacokinetics and off-target exposure [32, 37, 74]. Robust evaluation of intravenously or intramuscularly administered eVLPs remains sparse, leaving critical questions unanswered regarding circulation time, organ accumulation, clearance pathways, and dose-dependent toxicity. Comprehensive biodistribution studies, particularly in nonhuman primates, will be indispensable for defining therapeutic windows and for satisfying regulatory expectations prior to first-in-human studies.

Closely related to biodistribution is the need for systematic assessment of human-relevant immune responses. While transient RNP delivery inherently reduces prolonged nuclease exposure, the immunogenicity of viral envelopes and residual structural proteins in humans remains largely uncharacterized. Current datasets are insufficient to predict adaptive immune activation, neutralizing antibody formation, or T-cell responses following repeated administration. Future work should therefore prioritize standardized immunotoxicology studies, incorporating cytokine profiling, antibody titers, and cellular immune assays, ideally under GLP-compliant conditions to enable regulatory translation [96].

Another critical future direction involves comprehensive evaluation of long-term safety and genotoxicity. Although eVLPs avoid genomic integration and prolonged editor expression, transient delivery does not inherently eliminate the risk of rare off-target edits or chromosomal rearrangements, particularly at higher doses or with repeated exposure. Rigorous safety frameworks combining short- and long-read whole-genome sequencing, transcriptomic and epigenomic profiling, and long-term functional monitoring in relevant animal models will be essential to establish a robust safety profile [97, 98]. Such analyses will also be crucial for benchmarking eVLPs against clinically advancing LNP- and AAV-based genome-editing platforms.

Finally, scalable and GMP-compatible manufacturing remains a major translational bottleneck. Current eVLP production typically relies on transient transfection of HEK293T cells, a process that introduces batch-to-batch variability and is poorly suited for large-scale clinical manufacturing [41]. In addition, challenges related to particle stability, freeze–thaw sensitivity, purification efficiency, and empty/full particle heterogeneity remain unresolved. Addressing these issues will require the development of

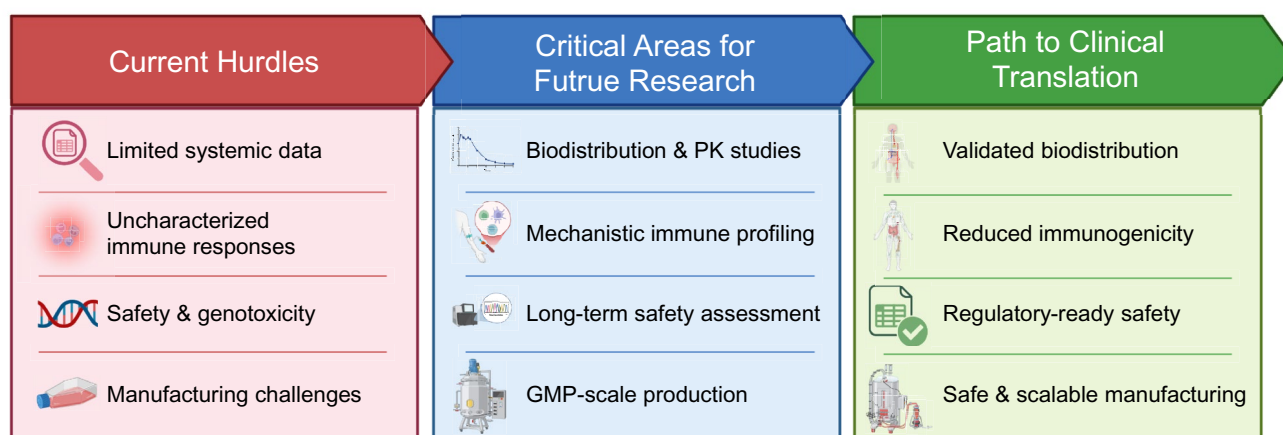


Fig. 5 Conceptual roadmap highlighting current hurdles, key research priorities, and the requirements for clinical translation of eVLP-based genome editing platforms. This schematic summarizes the major

stable producer cell lines, standardized purification workflows, and formulation strategies compatible with long-term storage and distribution, drawing on lessons learned from both viral vector and nanoparticle manufacturing pipelines [99].

Taken together, future progress in the eVLP field will depend less on incremental improvements in editing efficiency and more on systematic resolution of translational barriers, including immunogenicity, systemic biodistribution, safety validation, and manufacturing scalability. Addressing these challenges will be essential to move eVLPs beyond promising preclinical tools toward viable clinical delivery platforms, a trajectory that is schematically summarized in Fig. 5.

7 Conclusions

eVLPs stand at a pivotal juncture in the development of next-generation genome editing delivery systems. AAVs, LNPs, and eVLPs each represent important tools for in vivo delivery of genome-editing technologies, with distinct strengths and inherent limitations depending on the therapeutic context. LNPs have already advanced into clinical testing and demonstrated therapeutic efficacy in vivo, supported by extensive optimization of their physicochemical properties, tissue targeting strategies, and regulatory experience [100]. However, similar to all delivery platforms, LNP-based systems face context-dependent challenges, including dose-dependent toxicity [101, 102] and considerations surrounding biodistribution [17, 20], highlighting that no single modality is universally optimal. In contrast, eVLPs remain in the preclinical stage, yet they have shown remarkable versatility by enabling efficient ribonucleoprotein delivery across diverse tissues

challenges and future directions that must be addressed to enable the clinical translation of eVLP-based genome editors

and disease models. Platforms such as RIDE exemplify the potential of programmable, single-particle delivery with encouraging efficacy in ocular and neurological disease models, and early safety validation in nonhuman primates [74].

Nevertheless, critical hurdles must still be addressed before eVLPs can be realistically considered for clinical translation. These include the intrinsic immunogenicity of viral structural proteins, limited data on systemic administration and biodistribution, unresolved questions about long-term safety and genotoxicity, and challenges in achieving scalable, GMP-compliant manufacturing. In particular, the feasibility of repeated administration and the development of manufacturing strategies compatible with intravenous clinical delivery remain key determinants of translational success. Future work focusing on immune evasion, precise tropism engineering, robust safety evaluation, and bioprocess innovation will be essential to move the field forward. If these hurdles are systematically addressed, eVLPs could transition from preclinical promise to viable candidates for clinical translation.

Taken together, although eVLPs currently lag behind LNPs in clinical readiness, their position alongside AAV- and LNP-based systems underscores that eVLPs are best viewed as a complementary rather than competitive platform, and their unique ability to deliver large and complex genome-editing RNPs in a transient, nonintegrating manner makes them an especially intriguing addition to the genome-editing delivery toolkit. With continued iterative improvements, rigorous translational studies, and eventual regulatory validation, eVLPs may expand the therapeutic toolbox and open new avenues for safe and precise in vivo genome editing.

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Declarations

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Ethics approval Not applicable.

Consent to participate Consent to participate was obtained.

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