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CRISPR DELIVERY IN LIVER-TARGETED GENE THERAPY: VECTORS, DISEASE APPLICATIONS, AND EMERGING STRATEGIES

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Abstract:

CRISPR-Cas genome editing has rapidly evolved from a molecular biology tool into a clinically validated therapeutic modality, with the liver serving as the primary proving ground for in vivo human applications. Hepatocytes are uniquely suited for systemic genetic interventions due to high hepatic blood flow, natural receptor accessibility for delivery vehicles, and a comparatively tolerant immune microenvironment that supports sustained expression. These physiological advantages have facilitated the clinical translation of both viral vectors, such as adeno-associated virus (AAV), and non-viral platforms, particularly lipid nanoparticles (LNPs), to target severe monogenic and metabolic disorders. Conditions amenable to hepatic DNA-level intervention include hemophilia B, hereditary transthyretin amyloidosis, familial hypercholesterolemia, alpha-1 antitrypsin deficiency, Wilson disease, and chronic hepatitis B reservoirs. Despite achieving regulatory-grade proof-of-concept across multiple indications, widespread clinical adoption faces critical hurdles, notably vector immunogenicity, potential off-target activity, re-dosing constraints, and poor delivery efficiency to non-parenchymal cells. Ongoing innovations—such as base, prime, and epigenetic editing, alongside capsid and lipid engineering—aim to refine targeting and safety. Ultimately, overcoming these delivery barriers, standardizing scalable manufacturing, and ensuring equitable global access will determine how successfully liver-directed CRISPR therapies transition into standard medical practice.

Keywords: *CRISPR-Cas9, Liver-Targeted Gene Therapy, Lipid Nanoparticles, Adeno-Associated Virus, Hepatocyte Genome Editing, Inherited Metabolic Liver Disease.*

1. Introduction

The discovery that the bacterial CRISPR-Cas9 system could be reprogrammed as a sequence-specific genome-editing tool transformed the trajectory of gene therapy research [1]. Where earlier approaches were largely restricted to adding a functional copy of a gene, CRISPR-based nucleases, base editors and prime editors allow disease-causing mutations to be corrected, disrupted or precisely rewritten at their native genomic location [2,3].

Translating this precision into a clinical benefit, however, depends entirely on delivery: the editing machinery must reach the correct cell type, in sufficient quantity, without provoking a damaging immune response or unacceptable off-target activity [3,4].

Among candidate target organs, the liver occupies a privileged position. It receives roughly a quarter of resting cardiac output and is perfused through fenestrated sinusoidal endothelium that allows macromolecular and nanoparticulate cargoes to reach hepatocytes with comparatively little anatomical obstruction [5,6]. Hepatocytes also express receptors, such as the asialoglycoprotein receptor and low-density lipoprotein receptor, that many synthetic and viral delivery vehicles exploit for productive uptake, and the hepatic microenvironment is intrinsically tolerogenic, which favours durable transgene expression relative to more immunologically active tissues [7,8]. These properties, combined with the sheer number of monogenic disorders of hepatic origin, have made the liver the organ in which in vivo CRISPR therapeutics have advanced furthest toward the clinic [5,6,9].

This review has three aims. First, we outline why the liver is both an accessible delivery target and a disease-relevant one, drawing together the vascular, cellular and immunological features that favour hepatic gene editing with an overview of the inherited and acquired liver disorders most amenable to correction. Second, we compare the principal delivery platforms currently used for liver-directed CRISPR therapeutics, namely AAV vectors, lipid nanoparticles and adjunct physical or hybrid methods, and we critically discuss the recent preclinical and clinical literature for each. Third, we consider the challenges that continue to limit broader application, including immunogenicity, durability, off-target risk and access to non-hepatocyte liver populations, before outlining emerging strategies, such as base and prime editing and next-generation nanoparticle engineering, that are being developed to address them.

2. The liver as a target for gene editing: anatomical basis and disease burden

The suitability of the liver for systemic gene therapy is not incidental. Hepatic sinusoids lack a continuous basement membrane and are lined by fenestrated endothelial cells, which permits an unusually direct exchange between the bloodstream and the space of Disse that surrounds hepatocytes [6,7]. This anatomical arrangement, together with the liver's role as the principal site of plasma protein synthesis and xenobiotic clearance, means that a therapeutic transgene or edited gene expressed in hepatocytes can correct disorders that manifest far beyond the liver itself, such as bleeding disorders or systemic amyloid deposition, simply by restoring normal circulating protein levels [5,9].

Several hundred monogenic disorders originate wholly or partly in the liver, and together they constitute a substantial share of the rare-disease burden addressed by gene therapy research [5,6]. Disorders of coagulation, such as hemophilia A and B, reflect deficient synthesis of clotting factors VIII and IX by hepatocytes and were among the first indications for liver-directed AAV gene addition [7,10]. Hereditary transthyretin amyloidosis arises when hepatocytes secrete a misfolding-prone variant of transthyretin that deposits as amyloid in the heart and peripheral nerves; because the protein is produced almost exclusively by the liver, hepatic gene silencing or knockout is sufficient to halt disease progression [11]. Disorders of lipid metabolism, including homozygous familial hypercholesterolemia and other severe dyslipidaemias, result from excessive activity of PCSK9 or ANGPTL3, both of which are chiefly expressed in hepatocytes and regulate clearance of low-density lipoprotein cholesterol [12,13]. Inherited metabolic disorders such as hereditary tyrosinemia type 1, in which a defective fumarylacetoacetate hydrolase enzyme allows toxic tyrosine metabolites to accumulate, likewise localise

pathology to the hepatocyte and have served as influential preclinical models for genome-editing correction [2]. Alpha-1 antitrypsin deficiency and Wilson disease represent a further category in which a hepatocyte-autonomous mutation causes both loss of a circulating protein's function and toxic intracellular accumulation of the mutant protein or a metal substrate, driving progressive liver fibrosis in addition to extrahepatic disease [14,15]. Finally, chronic hepatitis B infection persists because of a stable, non-integrated viral DNA reservoir, the covalently closed circular DNA, that resides within infected hepatocytes and is not eliminated by conventional antiviral therapy, making it an attractive target for nuclease-based disruption [2,16].

This convergence of anatomical accessibility and disease relevance explains why the liver has functioned as a proving ground for in vivo CRISPR therapeutics: successful hepatic delivery platforms developed for one indication are frequently repurposed across this broader spectrum of hepatic and hepatically-secreted disorders [5,7].

3. Delivery platforms for liver-directed CRISPR-Cas9

Delivery remains the central bottleneck determining whether a CRISPR-based therapeutic can be translated from a cell-culture demonstration into an effective in vivo treatment [3,4]. Two platforms dominate current liver-directed clinical development: recombinant AAV vectors and lipid nanoparticles, each with a distinct risk-benefit profile that has shaped which indications they have been applied to.

3.1 Adeno-associated viral vectors

Recombinant AAV vectors were the first delivery platform to achieve durable, clinically meaningful hepatic gene transfer and remain central to liver-directed gene addition [7,10]. Their principal strength lies in the capacity of the vector genome to persist episomally in post-mitotic hepatocytes, supporting transgene expression that has been documented for a decade or more after a single infusion in some hemophilia cohorts [10,17]. Engineered capsids, such as the synthetic AAVS3 serotype used in the FLT180a hemophilia B programme, have been developed specifically to improve hepatocyte transduction efficiency relative to naturally occurring serotypes, allowing therapeutic factor IX activity to be achieved at lower vector doses [10,17].

Despite these strengths, AAV-based delivery faces well-documented constraints when applied to CRISPR-Cas9 rather than simple gene addition. The packaging capacity of AAV capsids, approximately 4.7 kilobases, is insufficient to accommodate a single-vector cassette encoding a full-length Cas9 nuclease together with its guide RNA and any homology-directed repair template, which has driven the use of smaller Cas9 orthologues, such as Staphylococcus aureus Cas9, or split dual-AAV strategies that reconstitute the nuclease only after co-transduction [2,9]. A further limitation is pre-existing humoral immunity: a substantial proportion of the general population carries neutralising antibodies against common AAV serotypes as a consequence of natural infection, which can exclude patients from treatment eligibility and precludes vector re-administration once an immune response has been mounted [18,8]. High systemic AAV doses have also been associated with dose-dependent hepatotoxicity and, in rare cases, complement-mediated thrombotic microangiopathy, prompting closer scrutiny of dosing strategies and prophylactic immunosuppression in ongoing trials [18,8]. Because AAV genomes persist as largely non-integrating episomes, the risk of insertional mutagenesis is low relative to earlier integrating vectors, though it is not entirely absent and continues to be monitored in long-term follow-up studies [7,10].

3.2 Lipid nanoparticles

Lipid nanoparticles have become the dominant non-viral platform for delivering CRISPR-Cas9 mRNA and guide RNA to the liver, building directly on the formulation science developed for small interfering RNA and mRNA vaccine products [19,12]. Following intravenous administration, ionisable lipid nanoparticles accumulate preferentially in hepatocytes through apolipoprotein E-mediated uptake, making the liver the default organ of LNP tropism even before any active targeting ligand is incorporated [19,13]. Because the Cas9 component is delivered as transient messenger RNA rather than as a persistently expressed transgene, the editing machinery is cleared from the cell within days, which is thought to reduce the cumulative risk of off-target modification compared with platforms that support prolonged nuclease expression [19,12].

The clinical significance of this platform was established when a lipid nanoparticle formulation encoding Cas9 mRNA and a guide RNA targeting the transthyretin gene, NTLA-2001, produced dose-dependent and durable reductions in serum transthyretin in patients with hereditary transthyretin amyloidosis, representing the first published clinical demonstration of systemically delivered in vivo CRISPR gene editing in humans [11]. Related LNP-based work has extended this approach to cardiovascular risk genes: adenine base editors targeting PCSK9 delivered by LNP achieved durable reductions in circulating PCSK9 and low-density lipoprotein cholesterol in non-human primates, and a comparable mRNA/guide RNA LNP platform achieved liver-specific disruption of Angptl3 [12,13]. These studies collectively demonstrate that LNP-mediated hepatic editing can be both efficient and reproducible across independent laboratories and target genes.

LNP delivery is not without constraints. Its efficiency is closely tied to formulation chemistry, and small changes in ionisable lipid structure, helper lipid ratio or particle size can substantially alter hepatocyte uptake, endosomal escape and, consequently, editing efficiency [19]. The same apolipoprotein E-dependent tropism that favours hepatic delivery also limits the platform's applicability to extrahepatic tissues without additional targeting modifications, and repeated dosing can provoke anti-Cas9 protein immune responses that attenuate the effect of subsequent infusions [12,13].

3.3 Physical and adjunct delivery methods

Physical and early viral methods, including hydrodynamic tail-vein injection of naked plasmid DNA and adenoviral vectors, played an important historical role in establishing proof-of-concept for CRISPR-mediated correction of hemophilia B and hereditary tyrosinemia in mouse models [2,3]. Hydrodynamic delivery, which relies on rapid, high-volume intravenous injection to transiently permeabilise hepatocyte membranes, achieves efficient murine hepatic gene transfer but has no viable analogue in human-scale intravenous administration and is therefore restricted to preclinical research [3]. Adenoviral vectors achieved higher correction efficiency than early plasmid-based methods in some hemophilia B models but were also associated with pronounced hepatotoxicity attributable to their high intrinsic immunogenicity, which has limited their further clinical development for liver-directed CRISPR delivery [3].

Table 1 summarises the comparative properties of the principal delivery platforms discussed above. This comparative table evaluates viral and non-viral delivery platforms for liver-directed gene editing. It contrasts the natural hepatotropism and durability of AAV vectors against the scalable, repeatable delivery of lipid nanoparticles, highlighting key trade-offs regarding packaging limits, immunogenicity, toxicity, and translational feasibility across clinical and preclinical hepatic applications.

Table 1: Comparative analysis of viral and non-viral delivery platforms for liver-directed gene and genome editing

Delivery platform	Principal advantages	Principal limitations	Representative liver application
Adeno-associated virus (AAV)	Strong natural hepatotropism; long-term episomal transgene persistence; low pathogenicity; multiple engineered serotypes/capsids available	Pre-existing neutralising antibodies exclude many patients; limited packaging capacity (~4.7 kb) constrains large Cas9 payloads; dose-dependent hepatotoxicity and complement-mediated thrombotic microangiopathy at high doses; largely unsuitable for re-dosing	Hemophilia B (FLT180a/verbrinacogene setparvovec)
Lipid nanoparticles (LNP)	Transient mRNA-based expression limits genotoxic risk; intrinsic accumulation in hepatocytes after intravenous delivery; scalable, cGMP-compatible manufacturing; repeat dosing feasible	Predominant liver tropism restricts extrahepatic targeting; formulation-dependent innate immune activation; large Cas9 mRNA cargo can reduce encapsulation efficiency	Hereditary transthyretin amyloidosis (NTLA-2001); PCSK9/ANGPTL3 base editing
Adenoviral / hydrodynamic and other physical methods	High transient transduction efficiency; simple plasmid-based delivery for preclinical proof-of-concept	Marked immunogenicity and hepatotoxicity with adenovirus; hydrodynamic injection is not translatable to human-scale intravenous delivery	Early proof-of-concept correction of hemophilia B and hereditary tyrosinemia in mice

4. Disease-specific applications

The delivery platforms described above have been applied across a widening spectrum of liver and liver-secreted disorders, several of which have already reached human clinical trials while others remain at the preclinical stage. Table 2 summarises representative examples discussed in the current literature. This table outlines major genetic and viral liver disorders amenable to genome modification, detailing their underlying molecular defects and therapeutic strategies. It charts interventions ranging from AAV- and LNP-mediated gene addition, knockout, and base editing to viral DNA disruption, summarizing developmental progression from murine and non-human primate models to clinical applications.

Table 2: Targeted liver diseases, molecular pathologies, therapeutic editing strategies, and current translational development stages

Liver disease	Underlying defect	Editing strategy / delivery	Stage of development
Hemophilia B	Loss-of-function mutation in F9 (factor IX)	AAV-delivered gene addition/repair; nuclease-based knock-in of a gain-of-function factor IX variant	Clinical (approved AAV gene-addition products; editing-based approaches preclinical/early clinical)
Hereditary transthyretin (ATTR) amyloidosis	Toxic misfolded transthyretin protein produced by hepatocytes	LNP-delivered CRISPR-Cas9 mRNA/sgRNA knockout of TTR	Clinical (NTLA-2001)
Homozygous familial hypercholesterolemia / atherosclerotic risk	Elevated PCSK9 or ANGPTL3 activity reducing LDL-receptor recycling	LNP-delivered adenine base editing of PCSK9; mRNA/sgRNA knockout of Angptl3	Preclinical (non-human primate)
Hereditary tyrosinemia type 1	Deficient fumarylacetoacetate hydrolase (FAH) causing toxic metabolite accumulation	AAV/LNP-assisted homology-directed repair or prime editing of Fah; Hpd disruption to reroute metabolism	Preclinical (murine models)
Alpha-1 antitrypsin deficiency	Misfolded Z-variant SERPINA1 protein accumulates in hepatocytes, causing toxic gain-of-function liver injury alongside loss-of-function lung disease	Dual-AAV base editing/epigenetic silencing of mutant SERPINA1	Preclinical
Wilson disease	Loss-of-function mutation in ATP7B causing hepatic copper accumulation	AAV-delivered mini-ATP7B gene addition; nuclease-free targeted integration under investigation	Early clinical (gene addition); editing-based strategies preclinical
Chronic hepatitis B infection	Persistent covalently closed circular DNA (cccDNA) reservoir in hepatocytes	CRISPR-Cas9/Cas13-mediated cleavage of viral DNA/RNA	Preclinical

Hemophilia B remains the indication with the longest clinical track record for liver-directed gene therapy, and although the majority of approved products rely on conventional AAV-mediated gene addition rather than nuclease-based editing, long-term follow-up of trials such as the AAVS3-based FLT180a programme has been instructive in defining the durability and immunogenicity profile that any editing-based successor must match or improve upon [10,17]. Hereditary transthyretin amyloidosis illustrates the opposite trajectory: NTLA-2001 was developed as a genome-editing therapy from the outset, and its clinical success has served as a template for subsequent LNP-based editing programmes targeting other hepatically expressed genes [11]. Early proof-of-concept for hepatic PCSK9 disruption was established using adenovirus-delivered CRISPR-Cas9 in mice, which achieved substantial on-target editing and reduced plasma cholesterol, laying groundwork for the non-viral and base-editing strategies subsequently tested in non-human primates [20]. The PCSK9 and ANGPTL3 base-editing studies in non-human primates extend this logic to a common, rather than rare, disease indication, raising the possibility that a single-administration "install-and-forget" approach to cardiovascular risk reduction could eventually complement or substitute for lifelong pharmacological LDL-lowering therapy [12,13]. Preclinical work in hereditary tyrosinemia type 1 has been particularly informative regarding the biology of edited-cell selection, since correction of even a small proportion of hepatocytes can confer a survival and proliferative advantage that allows corrected cells to progressively repopulate the liver [2]. Alpha-1 antitrypsin deficiency and Wilson disease both illustrate a more complex editing challenge, in which the therapeutic goal is not simply to restore a missing protein but to simultaneously silence or correct a toxic mutant allele, an objective that has motivated interest in base editing and epigenetic silencing approaches that avoid double-strand breaks [14,15]. Finally, nuclease-based disruption of the hepatitis B viral genome represents a qualitatively different application, in which the CRISPR target is exogenous viral DNA rather than a host gene, and progress here has been constrained less by delivery than by the difficulty of accessing the full cccDNA reservoir within infected hepatocytes [2,16].

5. Critical discussion of recent literature

Read together, the studies summarised above indicate that the field has moved decisively from establishing whether liver-directed CRISPR editing is feasible to determining how it can be made safe, durable and broadly accessible. The clinical validation provided by NTLA-2001 is arguably the single most important development in this literature, because it demonstrated for the first time that systemically administered, non-viral CRISPR editing could achieve a dose-dependent, apparently durable biochemical effect in humans without the packaging and immunogenicity constraints associated with AAV [11]. This result has lent credibility to the broader LNP-based editing programmes targeting PCSK9 and ANGPTL3, whose data remain confined to non-human primates but whose editing efficiencies and lipid-lowering effects are comparable in magnitude to those achieved by long-established pharmacological agents [12,13].

At the same time, the AAV literature illustrates that platform maturity does not eliminate risk. Long-term hemophilia trial data show that AAV-mediated hepatic gene transfer can remain transcriptionally active for a decade, yet the same body of evidence documents transient transaminase elevations, the persistence of high neutralising antibody titres that would preclude re-dosing, and occasional serious adverse events unrelated to editing itself, underscoring that immunogenicity rather than editing efficiency is now the principal constraint on AAV-based liver editing platforms [18,10,8]. A recurring theme across both viral and non-viral studies is that editing efficiency reported in murine liver, which is often characterised by more permissive homology-directed

repair and more accessible hepatocyte turnover, does not translate directly to non-human primates or humans, where editing rates are consistently lower; this gap has been attributed variously to differences in nuclease or base-editor delivery kinetics, hepatocyte proliferation rates, and species-specific immune recognition of the editing machinery [2,12,13]. The comparative literature also suggests that base editing and other nick-based strategies are gradually displacing double-strand-break-inducing nucleases for liver applications where the therapeutic goal is a discrete point correction, reflecting a broader shift toward editing modalities perceived to carry lower genotoxic risk [12,13].

6. Challenges

6.1 Immunogenicity and limited re-dosing

Both AAV capsid proteins and bacterially derived Cas9 protein are immunogenic in humans, and pre-existing or treatment-induced antibodies against either component can compromise efficacy or preclude retreatment [18,8,21]. This is a particular concern for AAV, where population seroprevalence against common serotypes can exclude a substantial fraction of otherwise eligible patients, but LNP-delivered Cas9 mRNA is not immune to this problem, since repeated exposure to the translated Cas9 protein can elicit anti-Cas9 humoral and cellular responses that blunt subsequent doses [18,12].

6.2 Off-target editing and genotoxicity

Unintended editing at sequences homologous to the intended target, together with the possibility of large structural rearrangements at on-target sites following double-strand breaks, remains a central safety consideration for any nuclease-based hepatic therapy [3,4]. Base and prime editing strategies are designed to mitigate this risk by avoiding double-strand breaks altogether, but they introduce their own class of off-target effects, including bystander editing of nearby bases and, for cytidine deaminase-based editors, occasional RNA-level off-target activity, both of which require dedicated characterisation for each new liver-directed construct [12,13].

6.3 Restricted access to non-parenchymal liver cells

Most current delivery platforms are optimised for hepatocyte uptake and are comparatively inefficient at reaching non-parenchymal liver populations, such as Kupffer cells, hepatic stellate cells and sinusoidal endothelial cells, which nonetheless contribute meaningfully to diseases such as liver fibrosis and viral persistence [7]. Extending editing efficiency beyond hepatocytes will likely require targeting ligands or capsid modifications distinct from those already optimised for parenchymal delivery [7,19].

6.4 Manufacturing, cost and equitable access

The clinical-grade production of either AAV vectors or lipid nanoparticle formulations carrying nucleic acid cargo is technically demanding and costly, and this manufacturing complexity is reflected in the high projected list prices of approved liver-directed gene therapies, raising sustainability and equitable-access questions that are increasingly discussed alongside the underlying science [6,9].

7. Future prospects

Several converging developments are likely to shape the next phase of liver-targeted CRISPR therapeutics. First, the continued refinement of base and prime editors, which correct point mutations without generating double-strand breaks, is expected to further narrow the gap between editing precision demonstrated in animal models and the safety margins required for widespread clinical use, particularly for indications such as alpha-1 antitrypsin

deficiency and Wilson disease in which the pathogenic allele must be corrected rather than merely disrupted [12,13,14]. Second, rational engineering of both AAV capsids and lipid nanoparticle surface chemistry, including the incorporation of hepatocyte-selective ligands such as N-acetylgalactosamine, is being pursued to improve on-target potency at lower administered doses, which should in turn reduce the immunogenic and hepatotoxic burden associated with current dosing regimens [18,19]. Third, epigenetic editing approaches that modulate gene expression without altering the underlying DNA sequence are gaining attention as a potentially reversible alternative to permanent genome editing, an attractive property for indications where long-term safety data are still being accumulated [14]. Finally, as the number of approved and late-stage liver-directed gene and editing therapies grows, parallel investment in manufacturing scale-up, biosimilar-style cost reduction, and international regulatory harmonisation will be necessary if these therapies are to move from isolated clinical trials to broadly accessible standard-of-care treatments [6,9].

8. Conclusion

The liver's unique combination of vascular accessibility, receptor-mediated uptake and disproportionate contribution to monogenic and hepatically secreted disease has positioned it as the leading edge of in vivo CRISPR-Cas9 therapeutics. Adeno-associated viral vectors and lipid nanoparticles have each demonstrated clinically meaningful hepatic gene delivery, and their complementary strengths and weaknesses, durable expression versus transient dosing flexibility, established manufacturing versus non-integrating safety profile, are likely to keep both platforms in active clinical use for different indications rather than resolving into a single dominant technology. The clinical translation of transthyretin-targeted and PCSK9-targeted editing programmes demonstrates that the fundamental feasibility question has been answered; the central task for the coming years is to consolidate these early successes into safer, more precise and more equitably accessible therapies across the full spectrum of liver disease.

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