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Review Article

Barriers in Cardiac Gene Therapy: Mapping the Targeted Delivery Cascade

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Abstract

Cardiovascular disease remains the leading cause of death worldwide and a principal contributor to global disability, placing an immense burden on healthcare systems. Conventional pharmacological and device-based therapies have markedly improved symptoms and slowed disease progression, yet they do not fully address or reverse the underlying molecular causes. By directly targeting the genetic etiologies of cardiac disease, gene therapy offers a potentially curative paradigm, with the promise of reversing pathology and restoring cardiac function. Despite regulatory approvals across indications ranging from bleeding disorders to malignancy, no gene therapy has yet been approved for a myocardial indication. The translation of preclinical advances into the clinic has historically been hindered by the lack of safe, efficient, and cardiac-specific delivery systems. Progress has recently been made in optimizing viral capsid tropism, employing tissue-specific promoters, and refining non-viral lipid nanoparticle formulations; yet off-target hepatic sequestration, rapid microvascular clearance, and host immunity remain substantial obstacles. The distinctive anatomical and physiological environment of the cardiovascular system poses a further critical bottleneck. This review examines the biophysical and physiological barriers that constrain in vivo cardiac gene therapy, taking each tier of the delivery cascade in turn to specify where the administered dose is lost.

Keywords: *gene therapy; cardiovascular disease; drug delivery systems; adeno-associated virus; lipid nanoparticles*

1. Introduction

Cardiovascular disease (CVD) remains the leading cause of mortality globally, affecting millions of individuals and continuing to impose an enormous and growing burden on health systems [1,2]. Much of contemporary cardiology operates within a secondary prevention framework, in which treatments are directed to manage disease symptoms and optimize quality of life while the upstream genetic and cellular causes remain unaltered [3–7]. The recognition that a proportion of cardiovascular disease has a genetic basis, together with the rapid fall in the cost of genome sequencing and its wider availability, has fueled interest in gene-based therapeutic strategies capable of addressing the root molecular causes of disease [8].

The past decade has seen substantial progress in gene therapy, evidenced by accelerating regulatory approvals across indications ranging from bleeding disorders to malignancy [9,10]. As of October 2026, no gene therapy has been approved for a myocardial indication [8,9,11]. Several factors have delayed progress, including the technical difficulty of delivering therapeutic genetic material to the heart, the pathophysiological complexity of cardiovascular disease, and the stringent safety requirements of any intervention directed at a life-sustaining organ [3,8,12].

One of the clinical objectives of cardiac gene therapy is a delivery profile that is durable, cardiac-specific, titratable, immunologically silent, and effective [13]. Achieving therapeutic transgene expression confined to the working myocardium requires the administered drug product to cross a series of anatomical and physiological barriers [14,15]. These barriers are not an inventory of independent obstacles. A vector that fails at any tier is lost to every tier downstream, so the fractions transmitted at each step multiply, and the surviving dose is the product of the whole sequence. The productive strategies are those that raise delivery efficiency per unit dose and increase target transgene production without exceeding safety limits.

In this review, we first examine what the landmark trials of the past decades revealed about where delivery fails, then take each tier of the cascade in turn to delineate the attenuation at each step. Throughout, our purpose is to specify where the administered dose is lost, on the premise that this is the prerequisite for deciding where engineering effort is best directed.

2. A Historical Perspective: Lessons from the Early Studies

Cardiovascular gene therapy originated in the 1990s with trials of therapeutic angiogenesis, targeting ischemic myocardium through pro-angiogenic growth factors [16]. In a 1998 study reported by Losordo et al., naked plasmid DNA encoding vascular endothelial growth factor (phVEGF165) was delivered directly into the ischemic myocardium of five patients by intramyocardial injection and was associated with a reduction in anginal symptoms [16]. In the Angiogenic GENe Therapy (AGENT) trial, an adenoviral vector encoding fibroblast growth factor-4 (Ad5FGF-4) was administered by intracoronary infusion to 79 patients with chronic stable angina, establishing its tolerability [17]. A subsequent randomized, placebo-controlled study of the same vector in 52 patients (AGENT-2) found an improvement in myocardial perfusion that did not reach statistical significance[18].

Despite these early signals, larger trials failed to meet their clinical endpoints [19,20]. The Euroinject One trial randomized 80 patients with severe, refractory angina to percutaneous intramyocardial plasmid gene transfer of vascular endothelial growth factor (phVEGF-A165) or placebo. No significant difference in myocardial stress perfusion defects was found between groups, although local wall motion improved in the treatment group [20]. The AGENT-3 and AGENT-4 programs enrolled 532 patients and were halted at interim analysis when the primary exercise-treadmill endpoint was not met, with benefit apparent only in the pre-specified subgroup of women[19]. These trials nonetheless established the feasibility and safety of cardiac gene transfer and made clear that more capable delivery technology would be required.

The next major approach centered on calcium homeostasis in the failing heart, specifically targeting sarco/endoplasmic reticulum Ca^{2+}-ATPase 2a (SERCA2a) [21]. In the CUPID trial, a phase 1/2 study, 39 patients with advanced heart failure were randomized to one of three doses of AAV1-delivered SERCA2a (AAV1/SERCA2a) by intracoronary infusion or to placebo. Benefit across symptomatic, functional, and biomarker domains, with fewer cardiovascular events at 12 months, was confined to the high-dose group [21]. The subsequent pivotal CUPID 2 phase 2b trial randomly assigned 250 patients, of whom 243 comprised the modified intention-to-treat population, to a single intracoronary infusion of 1×10^{13} DNase-resistant particles of AAV1/SERCA2a or to placebo [22]. No significant improvement in time to recurrent heart failure events was observed (hazard ratio 0.93, 95% CI 0.53–1.65; p = 0.81), with no safety signal attributable to the vector [23]. After post-hoc analyses, the investigators identified insufficient myocardial delivery by the wild-type AAV1 vector, together with high pre-existing neutralizing antibody titers, as the primary cause of study failure. The endothelial barrier and myocardial scarring further restricted vector diffusion. The setback in CUPID 2 therefore redirected efforts toward engineering highly cardiotropic AAV capsids to optimize vector transduction.

In 2012, the development of the clustered regularly interspaced short palindromic repeats and associated Cas9 endonuclease (CRISPR-Cas9) system introduced the ability to make permanent and precise changes to the human genome [24]. NTLA-2001 is a lipid nanoparticle (LNP) carrying Cas9 messenger RNA and a guide RNA directed against transthyretin (TTR). This gene-editing product produced dose-dependent reductions in serum transthyretin of up to 87% after a single infusion, marking the first successful in vivo human genome editing for a cardiovascular disease [25]. Preclinical work has since sought to improve the precision of TTR knockdown. Structure-guided engineering of SpCas9 produced an ultrahigh-fidelity variant that markedly reduced off-target editing and chromosomal translocations at this locus without substantially impairing on-target activity, and that proved compatible with adenine base editing (ABE) [26].

Over the same period, preclinical work on VERVE-101, an ABE encapsulated in LNPs that permanently inactivates proprotein convertase subtilisin/kexin type 9 (PCSK9), raised the prospect of lifelong low-density lipoprotein (LDL) cholesterol reduction from a single treatment: a single infusion in cynomolgus monkeys lowered blood PCSK9 by 83% and LDL cholesterol by 69%, with effects durable to 476 days [27]. Following adverse effects attributed to the VERVE-101 delivery vehicle, development was redirected to a candidate carrying the same base editor and guide RNA in a proprietary N-acetylgalactosamine (GalNAc)-LNP designed for safer and more efficient hepatocyte targeting [28]. The base editing strategy for PCSK9 accordingly progressed to a phase 1, open-label, single-ascending-dose study of VERVE-102 in 35 participants, in which mean reductions in circulating PCSK9 ranged from 51% at the lowest dose to 88% at the highest dose of 1.0 mg/kg, with corresponding reductions in LDL cholesterol from 9% to 62%; these reductions appeared durable through at least one year of follow-up in 15 participants [29]. In a phase 1a trial of CRISPR-Cas9 editing of angiopoietin-like protein 3 (ANGPTL3) with CTX310 in 15 participants, the mean reduction in circulating ANGPTL3 at day 30 was 73.2% at the highest dose of 0.8 mg/kg and 79.7% at 0.7 mg/kg, with mean reductions at day 60 of 48.9% in LDL cholesterol and 55.2% in triglycerides at the highest dose; one participant died suddenly 179 days after receiving the lowest dose of 0.1 mg/kg, a death the investigators adjudicated as unrelated to the study drug [30].

The common feature of these successful therapies is that the targeted protein is synthesized in the liver [25,29,30]. The contrasting case, delivery of a transgene to the cardiomyocyte itself, is especially instructive. In the phase 1 study of RP-A501, an AAV9 vector encoding LAMP2B, seven male patients with Danon disease received a single infusion under a transient immunomodulatory regimen of prednisone, either tacrolimus or sirolimus, and rituximab. Cardiac LAMP2 protein expression was demonstrated, and among the six patients with normal baseline ejection fraction the left ventricular mass index fell or stabilized while cardiac troponin I and N-terminal pro-B-type natriuretic peptide (NT-proBNP) declined over 24 to 54 months of follow-up. One patient developed grade 4 complement-mediated thrombotic microangiopathy with thrombocytopenia and acute kidney injury; three had glucocorticoid-related exacerbation of skeletal myopathy; and the single patient with left ventricular systolic dysfunction at baseline progressed to transplantation five months after infusion [31].

The historical trajectory of cardiac gene therapy reveals a cell-specific dichotomy. When the hepatocyte is the target, delivery exploits a biological advantage: the liver's endogenous lipoprotein uptake pathways carry the vector to its target cell [32]. Direct myocardial targeting has no equivalent and remains constrained by biophysical and immunological barriers. Because cardiac delivery efficiency is low, therapeutic transgene expression in cardiomyocytes requires high systemic vector doses, and that vector load in turn provokes the dose-dependent host immune responses that have complicated every attempt at clinical translation [8,31]. The failures catalogued above provide context for evaluating barriers to modern cardiac gene therapy. The sections that follow take that cascade apart tier by tier.

3. Barriers to Success for Cardiac Gene Therapy

The obstacles to cardiac gene delivery have been catalogued as the anatomical challenge of access, the mechanical force of blood flow, the endothelial barrier, the cellular barrier, and the host immune response [15]. To reach its target, a vector must survive systemic distribution, cross the coronary endothelium within the transit time of a single pass, traverse an interstitium that is often fibrotic, enter a post-mitotic cell through a receptor-restricted pathway, escape the endosome, and reach the nucleus, all while evading humoral, innate, and adaptive immunity [14,33]. The fraction of the administered dose that completes this sequence, effectively the only therapeutically active portion, is therefore correspondingly small [34]. The sections that follow take the cascade in order, from systemic distribution to the nucleus, before turning to the constraints that act across the whole sequence and to the preclinical models on which these estimates depend. The cascade and its constituent barriers are summarized in Figure 1.

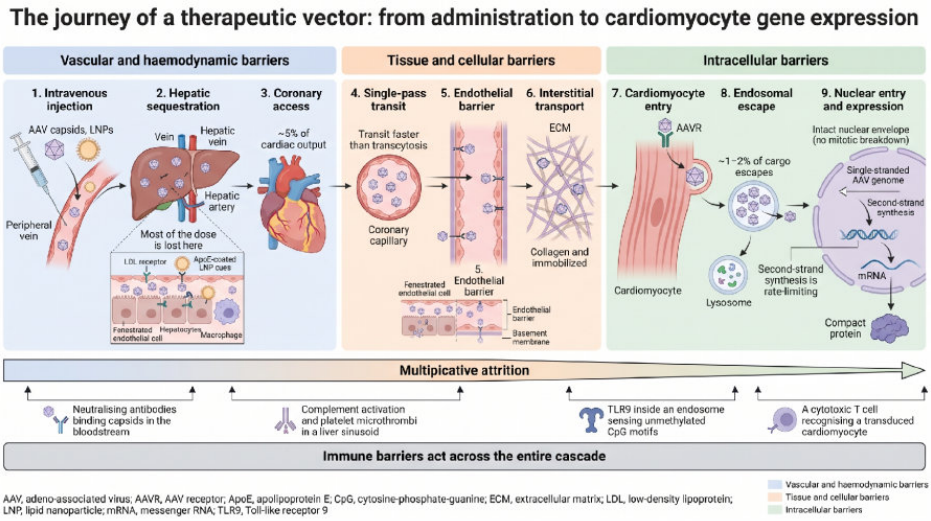


Figure 1. The journey of a therapeutic vector: from administration to cardiomyocyte gene expression.

3.1 Systemic Distribution

The first barrier to cardiac delivery is the off-target distribution of the vector [35]. Systemically administered particles are partially sequestered by the liver [11,15,35,36]. For LNPs, the mechanism is now defined at a molecular level. Ionizable LNPs adsorb apolipoprotein E (ApoE) from plasma, which functions as an endogenous targeting ligand delivering the particle to hepatocytes through the low-density lipoprotein (LDL) receptor [32,37]. This uptake pathway is part of what makes hepatically expressed cardiovascular targets, including TTR, PCSK9, and ANGPTL3, so tractable [25,29,30], and it has no counterpart in the heart.

The anatomical constraint arises because myocardial perfusion is low in absolute terms. Quantitative positron emission tomography places median resting myocardial blood flow between approximately 0.75 and 1.0 mL per minute per gram in adults without flow-limiting coronary disease [38,39], and the coronary circulation as a whole receives only about 5% of cardiac output at rest [40]. This restricted volumetric distribution limits the residence time available for either viral capsids or synthetic nanoparticles to undergo efficient endothelial binding and subsequent myocardial transudation. This creates a physical bottleneck at the point of entry to the coronary circulation. Capsid engineering alone is unlikely to resolve it, which gives the route of administration disproportionate leverage at this tier [41].

3.2 The Endothelial Barrier

The hepatic sinusoid is discontinuous and fenestrated, its endothelium perforated by transcellular pores averaging roughly 107 nm in diameter in healthy human liver and lacking an underlying basal lamina, so that a particle within the sinusoidal lumen has near-direct access to the hepatocyte surface [42,43]. An AAV capsid, at approximately 25 nm, passes this aperture without difficulty. The myocardial capillary is lined by an endothelium built to the opposite specification: a continuous layer joined by tight junctions and supported by an uninterrupted basement membrane [44]. A vector circulating in the coronary microvasculature is therefore not in contact with the cardiomyocyte at all, but is separated from it by an intact cellular layer [11,45]. Crossing that layer requires either a paracellular route, which is effectively closed to particles of this size under physiological conditions, or receptor-mediated transcytosis, a mechanism that remains largely unexploited for cardiac delivery [46,47]. The fraction of circulating vector that crosses the intact coronary endothelium in a single pass has not been directly measured in human myocardium.

3.3 Hemodynamics and Vector Residence Time

The dynamics of the coronary circulation add a further obstacle to cardiac delivery. Receptor-mediated transcytosis across the capillary wall proceeds more slowly than blood traverses the coronary bed, so a vector spends less time in contact with the endothelium than the transit time of blood in the coronary arteries [35,48]. A bolus delivered into a coronary artery is therefore largely washed through the microcirculation, collected by the coronary sinus, and returned to the systemic circulation, where it becomes subject to the hepatic sequestration described above. The transduction efficiency of a simple antegrade intracoronary bolus is correspondingly low [48].

Residence time is nonetheless the one variable in this cascade that can be manipulated without altering the vector itself. Across ex situ perfusion studies, longer exposure of the coronary endothelium to vector consistently increases delivery [49], which indicates that contact duration, rather than any intrinsic property of the vector, is rate-limiting at this tier. Contact time can be extended by procedural technique alone, and balloon occlusion, flow arrest, and mechanical circulatory support have all been tested to that end [41]. The optimal modality appears to be context-dependent and requires more in-depth analysis with human data.

3.4 Interstitial Transport and Fibrosis

Fibrotic remodeling is the common histological endpoint of ischemic, hypertensive, and most genetic cardiomyopathies, and the resulting deposition of extracellular matrix proteins impedes diffusion [50]. A delivery vehicle optimized in healthy, native myocardial architecture may not perform the same way in fibrotic tissue, which presents a distinct biophysical and physiological microenvironment [51].

The relationship between disease state and delivery is not uniform, and in some contexts, injury appears to improve rather than impair transduction. In one preclinical model, myocardial infarction substantially increased the transcriptional activity of AAV genomes [52]. Injured myocardium is also more permissive to LNP uptake, and which cells take up the particles depends on when the dose is given. Lineage tracing after ischemia-reperfusion injury identified myeloid cells as the primary recipients of mRNA-LNPs at three days, with uptake shared about equally between myeloid cells and fibroblasts by 14 to 28 days; delaying administration to three days after injury shifted uptake away from immune cells toward fibroblasts, pericytes, and endothelial cells [53].Because the diseased state of the heart can alter vector distribution, a platform characterized only in healthy myocardium may behave unpredictably in the patients who would receive it, whose hearts are, by definition, diseased. Evaluating delivery platforms in diseased rather than healthy models is therefore a prerequisite rather than a refinement, and it remains poorly characterized in the preclinical literature.

3.5 Cardiomyocyte Entry

Entry into the cardiomyocyte is receptor restricted. An unbiased genetic screen identified KIAA0319L, designated the AAV receptor (AAVR), as an essential host entry factor for all serotypes tested, and genetic ablation of AAVR renders a wide range of cell types, and mice, highly resistant to AAV transduction [54]. The dependence is near-universal rather than absolute: capsids of the AAV4-related Clade G lineage, including AAVrh32.33, enter cells by an AAVR-independent route through the sialomucin CD164, and AAVR-knockout mice remain fully permissive to their transduction [55,56]. For the cardiotropic serotypes in clinical use, however, AAVR remains an obligate chokepoint, and that imposes a design constraint. Capsid-engineering strategies aimed at optimizing endothelial transit must preserve productive engagement of the entry receptor, since crossing the vascular barrier is futile if the vector thereby loses the capacity to enter the target cardiomyocyte [57].

The permanently post-mitotic status of the adult cardiomyocyte cuts in both directions [58]. It is a liability at the point of nuclear access, because the absence of mitotic nuclear envelope breakdown removes the route by which vector genomes most readily reach the nucleus in dividing cells, and because the non-dividing state forecloses homology-directed repair as a gene-correction strategy [59,60]. A genome that reaches the nucleus still faces a further step: conventional single-stranded AAV genomes are transcriptionally inert until a complementary strand is synthesized, and this conversion is a principal rate-limiting step in transduction. Self-complementary vectors package a pre-annealed double-stranded genome and bypass it, achieving faster and more widespread myocardial transduction than single-stranded vectors of the same serotype, but they do so at the cost of roughly halving packaging capacity, which excludes most cardiomyopathy transgenes of clinical interest [61–63]. Conversely, the absence of cell division confers a therapeutic advantage: because episomal AAV genomes are not subject to mitotic dilution, a successful delivery event in a cardiomyocyte is expected to be durable [64].

3.6 Endosomal Escape

Even a vector that has entered the cardiomyocyte is not yet inside the cell in any useful sense, because its cargo remains sequestered within the endosomal compartment. Quantitative imaging of lipid nanoparticle-delivered small interfering RNA established that only approximately 1–2% of internalized cargo escapes the endosome into the cytosol, with the remainder recycled to the cell surface or trafficked for degradation [65]. That measurement was made in cultured cells and mouse hepatocytes rather than in myocardium, and the corresponding fraction for cardiomyocytes has not been established.

AAV escapes by a different route, in which endosomal acidification triggers a capsid conformational change that externalizes the VP1 unique region and its phospholipase A2 domain, permitting translocation across the vesicular membrane; this step additionally requires the Golgi-resident entry factor GPR108, which is dispensable for transduction by AAV5 but required across the other clades tested [66,67]. The efficiency of this route in post-mitotic cardiomyocytes likewise remains unquantified. Endosomal escape is nonetheless the step for which the most direct measurement of attenuation exists anywhere in the cascade, and on the available evidence it is among the least efficient, which makes it the point at which the largest proportional gains remain available [14].

3.7 The Dose Ceiling

Escalating the vector dose is the intuitive response to poor delivery efficiency, and it is the response the cascade forecloses. Because the losses at successive tiers compound, the dose required to offset them rises faster than the dose that can be given safely, and the toxicities that define that limit are severe and dose-dependent. High-dose systemic AAV administration provokes a marked immune response manifesting as hepatotoxicity, complement activation, and thrombotic microangiopathy [68,69]. In non-human primates and piglets given intravenous AAV at 2×10^{14} vector genomes per kilogram, approximately twice the dose used clinically in spinal muscular atrophy, toxicities included marked transaminase elevation, dorsal root ganglion degeneration, acute liver failure, and death [70]. These adverse events appear to be a property of high-dose systemic AAV exposure itself, arising independently of the specific capsid serotype or transgene design and independently of T-cell responses to capsid or transgene product.[71] Systematic toxicological profiling in macaques localized the injury to the hepatic sinusoidal endothelium, accompanied by sinusoidal platelet microthrombi, with transaminitis, thrombocytopenia, and alternative complement pathway activation peaking three days after administration [71]. The same dose dependence constrains cardiac applications. In a mouse model of hypertrophic cardiomyopathy, AAV9-delivered Cas9 nuclease inactivated the pathogenic myosin allele but impaired contractile function at higher doses, leaving only a narrow window in which the intervention was both effective and tolerated [72]. A ceiling on systemic vector exposure therefore exists, and it is set by the host rather than by the vector: the dose required to overcome poor cardiac tropism by brute force lies above the dose compatible with patient safety. Escalation cannot close a multiplicative gap, and the remaining tiers of the cascade must therefore be addressed by raising the efficiency of delivery per unit dose.

3.8 Immunity: A Cross-Cutting Barrier

The host immune system does not occupy a single position in the cascade. It acts at every stage, from neutralization of the vector in the circulation before it reaches the myocardium, through innate sensing of the vector genome after cell entry, to adaptive responses against capsid and transgene product that curtail expression once it is established [73,74]. Immunity is therefore better treated as a constraint acting across the whole sequence than as one tier within it, and on that basis it has repeatedly proved decisive in clinical translation [33].

Neutralizing antibodies (NAbs) against AAV are common in the adult population. In a survey of 502 adults across ten countries using a transduction inhibition assay, seropositivity at a 1:1 serum dilution ranged from 57.8% for AAV9, the most cardiotropic of the serotypes tested, to 74.9% for AAV1; prevalence varied considerably between countries, increased with age, and was higher in women, and AAV5 was consistently the least prevalent at dilutions of 1:2 and above [75]. These antibodies neutralize vectors in the circulation before they can reach the myocardium, with two clinical consequences. First, a substantial proportion of otherwise eligible patients is excluded from treatment at screening. Second, re-administration is effectively impossible, because even a single exposure induces durable neutralizing titers [76,77]. Strategies to circumvent pre-existing immunity have been reviewed elsewhere [77].

To overcome the barrier of pre-existing NAbs, several evasion strategies are being explored to safeguard vector delivery and permit re-administration [78]. Extrinsic approaches include IgG-degrading endopeptidases such as imlifidase and KJ103 to clear functional immunoglobulins transiently, tolerogenic nanoparticles to induce antigen-specific tolerance at the antigen-presenting cell level, and plasmapheresis or empty capsid decoys to deplete or saturate circulating antibodies [79]. Intrinsic modifications instead use rational capsid engineering and directed evolution to mutate solvent-exposed surface residues, yielding vectors that evade NAb recognition while preserving cardiotropic targeting [80].

Beyond antibody-mediated neutralization, the vector genome is itself immunostimulatory. Unmethylated CpG motifs within the AAV genome are recognized by Toll-like receptor 9 (TLR9), and this sensing drives the downstream cellular response: in Tlr9-deficient mice, interferon-γ T-cell responses against both capsid and transgene product were largely absent. Depleting CpG sequences from the vector genome reproduces this effect in immunocompetent animals, allowing the vector to evade detection and establish persistent transgene expression with minimal cellular infiltration [81]. That work used an AAVrh32.33 capsid in skeletal muscle, and the extent to which CpG depletion confers the same benefit in myocardium with cardiotropic serotypes has not been established. Systemic administration can additionally trigger acute activation of the complement cascade, producing the endothelial injury and thrombotic microangiopathy that set the dose ceiling described above [69,71,82,83].

3.9 The Translational Gap

The estimates in the preceding sections rest mostly on animal data, and the models differ from the human heart. Toxicity profiles diverge between humans and non-human primates, so findings in either cannot be assumed to transfer to the other [84]. High-dose systemic AAV administration produces hepatotoxicity and thrombotic microangiopathy in primates, and the threshold at which these occur must be established separately for each species rather than extrapolated [70,84,85]. The mouse, which supplies most of the efficacy literature, departs from the human heart on axes that bear directly on transduction: it beats roughly an order of magnitude faster and expresses a different predominant ventricular myosin heavy chain isoform, Myh6 rather than MYH7 [86]. Coronary transit time, interstitial architecture, and cardiomyocyte dimensions differ correspondingly, so a delivery efficiency measured in a mouse does not straightforwardly predict the equivalent figure in a patient. Mice nonetheless accounted for 86% of the animal models in a recent scoping review of cardiovascular gene editing studies [87]. Disease-specific preclinical models, adequately powered studies with meaningful endpoints, and precision diagnostics are therefore prerequisites for clinical translation. Strategies to overcome the barriers described in this section are summarized in Figure 2.

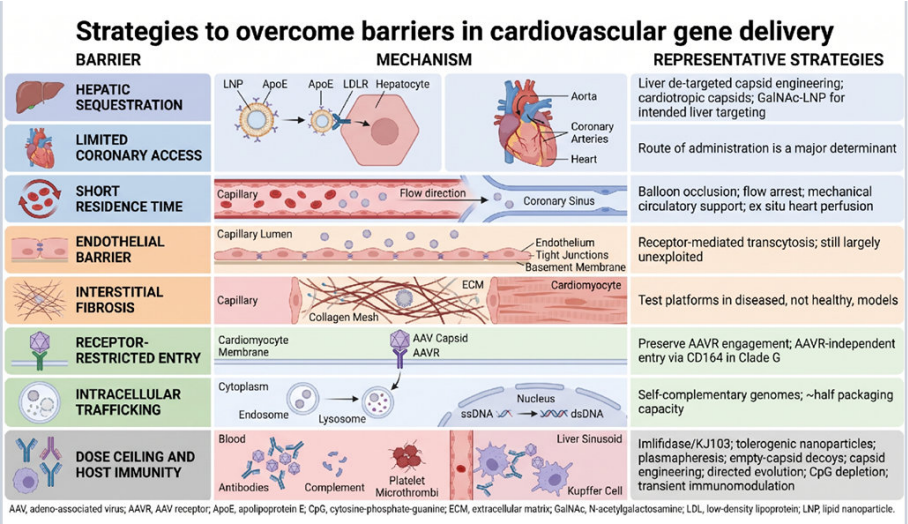


Figure 2. Strategies to overcome barriers in cardiovascular gene delivery.

4. Conclusions

No targeted gene therapy is yet approved for a myocardial indication, and the case for pursuing one rests on the shape of the burden ahead. Global Burden of Disease projections forecast that crude cardiovascular mortality will rise by 73.4% between 2025 and 2050, from 20.5 million to 35.6 million annual deaths.[88] Over the same interval, age-standardized cardiovascular mortality is projected to fall by 30.5% and age-standardized prevalence to remain essentially constant, so the growth in absolute deaths reflects an expanding and ageing population rather than any failure of contemporary prevention or treatment.[89] The argument for genetic medicine is therefore not that conventional care has failed, but that a durable, single-administration correction of the underlying molecular defect would address a category of disease that lifelong pharmacological management can contain but cannot resolve, in a population within which the absolute number of affected patients is set to grow substantially.

Realizing that potential depends on the delivery problem, and this review has argued that the delivery problem is best understood as a cascade rather than a list. The barriers are sequential and they act on the same drug product, so the surviving dose is the product of the fractions transmitted at each tier rather than the sum of independent losses. Two consequences follow. The first is that dose escalation cannot close the gap, because the host-imposed ceiling on systemic vector exposure lies below the dose that brute force would require; progress must instead come from raising delivery efficiency per unit dose. The second is that the field cannot presently say where most of the dose is lost, because the transmission fraction at most tiers has never been measured in human myocardium. Quantifying those fractions, in disease-relevant models and ultimately in patients, is the prerequisite for deciding which tier repays engineering effort. Until the cascade is measured rather than described, capsid design, route selection, and immunomodulation will continue to be optimized against a target that has not been located.

Ethics Approval and Consent to Participate

Not applicable.

Author Contributions

E.A.G. contributed to conceptualization, data curation, figure preparation, literature search, and writing of the original draft. A.A. contributed to the literature search, writing, review, and editing. B.B.G. contributed to writing, review, editing, and supervision. All authors have read and approved the final manuscript and agree to be accountable for its content.

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Conflict of Interest

The authors declare no conflict of interest.

Declaration of AI and AI-assisted Technologies

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