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Perspective of Viral Genetherapy in Cardiovascular Diseases

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Abstract

Cardiovascular diseases (CVDs) remain the leading cause of death worldwide and are often inadequately managed by standard pharmacologic or surgical treatments. As a result, viral gene therapy has emerged as a promising strategy to address the molecular roots of CVD by enabling gene delivery, correction, or modulation directly within cardiac and vascular tissues. In this approach, therapeutic genes are introduced using viral vectors such as adeno-associated virus (AAV), adenovirus, and lentivirus. These vectors can be engineered to target specific cell types—including cardiomyocytes, endothelial cells, and smooth muscle cells—with high precision. Such targeting allows for potential treatment of conditions like heart failure, myocardial infarction, arrhythmias, and atherosclerosis. Preclinical and clinical studies have demonstrated that viral gene therapy can enhance cardiac contractility, promote vascular regeneration, and reverse maladaptive structural remodeling of the heart and vessels. Several clinical trials have attracted global attention, particularly those aiming to restore SERCA2a function in chronic heart failure and to use VEGF for ischemic angiogenesis. These trials showed acceptable safety profiles and modest therapeutic benefits. However, issues such as vector immunogenicity, suboptimal delivery efficiency, limited tissue specificity, restricted packaging capacity, and challenges in sustaining long-term transgene expression have so far prevented widespread clinical adoption. This article offers an in-depth review of the mechanisms underlying viral gene therapy, the characteristics of different viral vectors, key outcomes from clinical trials, strategies for targeted delivery, and emerging innovations in the field. As gene editing technologies and vector engineering continue to advance, viral gene therapy is poised to become a cornerstone of precision cardiology and regenerative cardiovascular medicine.

Keywords: Viral gene therapy; Myocardial infarction; Gene delivery; CRISPR; Vascular targeting

1. Introduction

Cardiovascular diseases (CVDs) cause roughly 17.9 million deaths annually, representing 32% of all mortalities worldwide (World Health Organization, 2023). This group covers a broad spectrum of disorders, including ischemic heart disease, cardiomyopathy, heart failure, arrhythmias, and congenital malformations. Most of these disorders are chronic, evolving over time, and interplay of many factors, including genetic predisposition, environmental triggers, and maladaptive tissue remodeling. Although recent interventions like drugs, percutaneous coronary interventions, and implantable devices have increased survival for CVD patients, they mostly focus on symptom relief rather than cure, and in cases of advanced disease, they can be accompanied by long-term complications or reduced effectiveness (Benjamin et al., 2022).

The emergence of gene therapy as a precision medicine approach has since offered revolutionary ways to treat CVD. Gene therapy, in contrast to treating symptoms or trying to arrest the progression of the disease, modifies the molecular and cellular dysfunctions that constitute pathological manifestations of the targeted disease. One of the approaches

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explored, viral gene therapy-with the use of engineered viral vectors to deliver therapeutic genes to the cardiac or vascular tissues--has witnessed considerable attention due to the exceedingly high transduction efficiency, stable expression profiles, and evolving reports of safety profiles (Kobayashi et al., 2019).

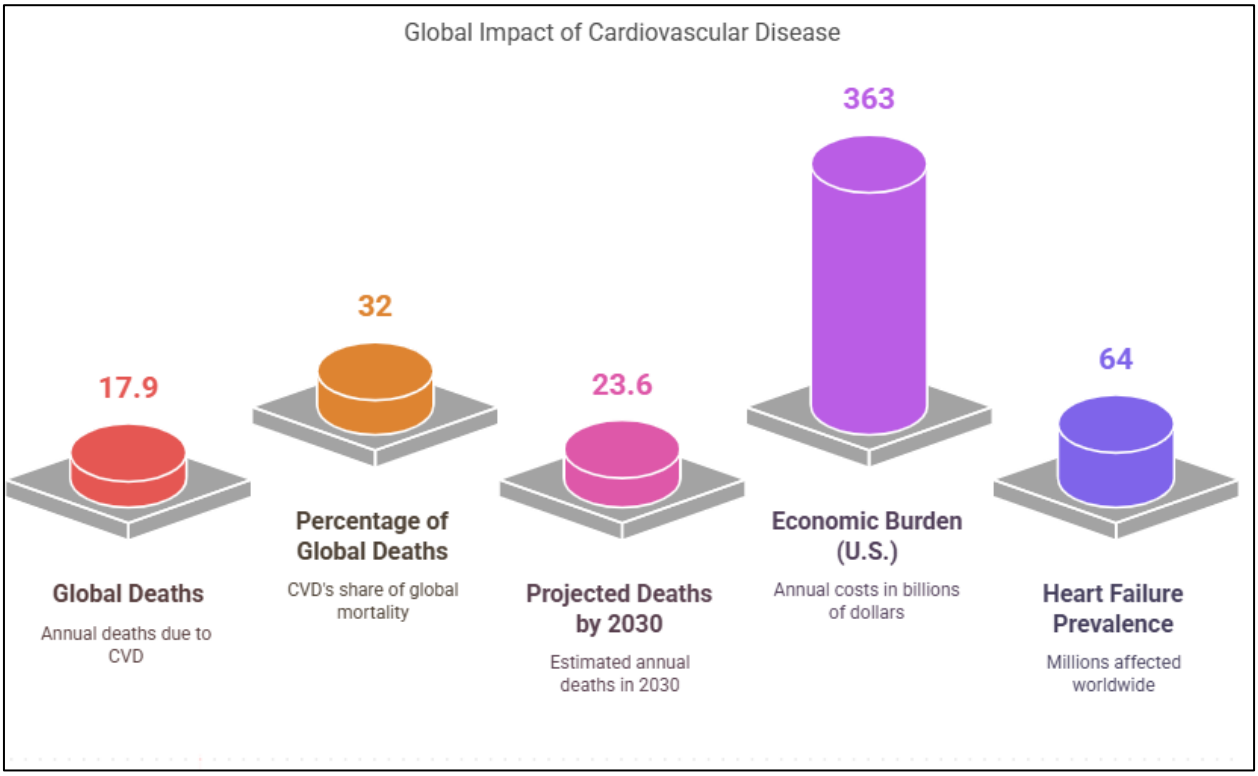

Figure 1 Global impact of cardiovascular disease. This figure illustrates the worldwide prevalence, mortality rates, and economic burden of cardiovascular disease (CVD). It highlights key statistics such as the number of affected individuals, leading causes of death, and regional variations, emphasizing the urgent need for improved prevention and treatment strategies

After their very early exploration in monogenic diseases, gene therapy has extended its reach to more polygenic and complex diseases like CVD by identifying key candidates involved in calcium handling (e.g., SERCA2a), nitric oxide signaling (eNOS), apoptosis, fibrosis, and angiogenesis (Jaski et al., 2009). Initial approaches using plasmid vectors were hampered by the poor uptake of the introduced gene and its transient expression. Thereafter, vector systems comprising viral vectors, such as AAV, lentivirus, and adenovirus, were introduced, which are far more competent in transferring genes into post-mitotic cells like cardiomyocytes and vascular endothelium and their precursors (Zacchigna et al., 2014).

Additionally, vector engineering and improvements of its components, capsids, and promoters, conferred tissue-specificity, reduced immunogenicity, and enhanced gene expression longevity (Kawase et al., 2011). The 1980s can be considered an important period in gene therapy development in the sense that the CUPID trial, in which AAV1 was used to deliver SERCA2a for heart failure, initially showed signs of efficacy and laid the basis for further clinical investigations (Greenberg et al., 2016).

Table 1 Evolution of Gene Therapy Tools in Cardiovascular Medicine

Era/Phase	Technological Milestone	Impact on Cardiac Applications
1990s	Non-viral plasmid vectors	Poor efficiency in cardiac cells
Early 2000s	First-generation adenoviruses	High transduction, but immunogenic
Mid-2000s	AAV vector optimization (AAV1, AAV9)	Targeted cardiomyocyte delivery, prolonged expression

2010s	Clinical trials (CUPID, SDF-1, VEGF plasmid)	Showed feasibility, mixed long-term results
2020s–present	CRISPR, base editors, capsid engineering, dual-vector systems	Enabling precision editing and broader therapeutic scope

Table1- Evolution of gene therapy tools in cardiovascular medicine. This table outlines the progression of gene therapy technologies used in cardiovascular treatments, from early viral vectors to advanced non-viral delivery systems and genome editing tools. It highlights improvements in targeting specificity, efficiency, safety, and therapeutic potential over time.

As the field advances, gene therapy is considered for many cardiovascular applications, from revascularization for ischemic disease to anti-fibrotic modulation for heart failure and repolarization correction in arrhythmogenic syndromes (Kupatt et al., 2010; Jessup et al, 2011). With CRISPR-based editing, non-integrating lentiviral systems, and AI-predicted target selection, options for safer, more efficacious, and patient-specific interventions are laid out.

Henceforth, this paper attempts to provide a thorough review of the mechanisms, viral platforms, clinical trial status, and future perspectives for viral gene therapy for cardiovascular diseases. This involves presenting both the opportunities and challenges in the current approaches while leading up to novel technologies that may solve existing barriers.

2. Mechanisms of Viral Gene Therapy

Gene transfer is manipulation of host cells so as to introduce functional genetic material to either correct, replace, or modulate the expression of genes that are dysfunctional or missing and that participate in the manifestation of the given disease. Hence, in cardiovascular gene therapy, the viral gene therapy attempts to undo the maladaptive cardiac remodeling, restore ionic homeostasis, angiogenesis, and fibrosis, or regulate apoptotic and inflammatory pathways (Kobayashi et al., 2019).

On the other hand, traditional small molecules are designed to elicit transient biological responses at the receptor or enzyme levels, whereas gene therapy seeks to produce sustained molecular level biological changes in one or a few administrations. Viral vector gene delivery permits expression for a long time in postmitotic cells, such as cardiomyocytes, vascular smooth muscle cells, and endothelial cells (Zacchigna et al., 2014).

2.1. Overview of Mechanism: From Vector to Therapeutic Effect

Gene therapy begins with vector generation, in which a therapeutic gene is inserted into the viral genome often under the control of a tissue-specific promoter. Antigens featuring in the packaging step include those from adeno-associated virus (AAV), lentivirus, or adenovirus. The virus is then injected systemically, locally, or with a combination of both (Kawase et al., 2011).

Once the virus reaches its target site, it binds to receptors on the cell membrane surface, enters via endocytosis, and releases its genetic material into the nucleus. AAVs and lentiviruses usually remain either as episomal forms or co-transcribe therapeutic proteins at designated loci within the genome, such as SERCA2a, VEGF, eNOS, or anti-fibrotic factors (Greenberg et al., 2016; Jaski et al., 2009).

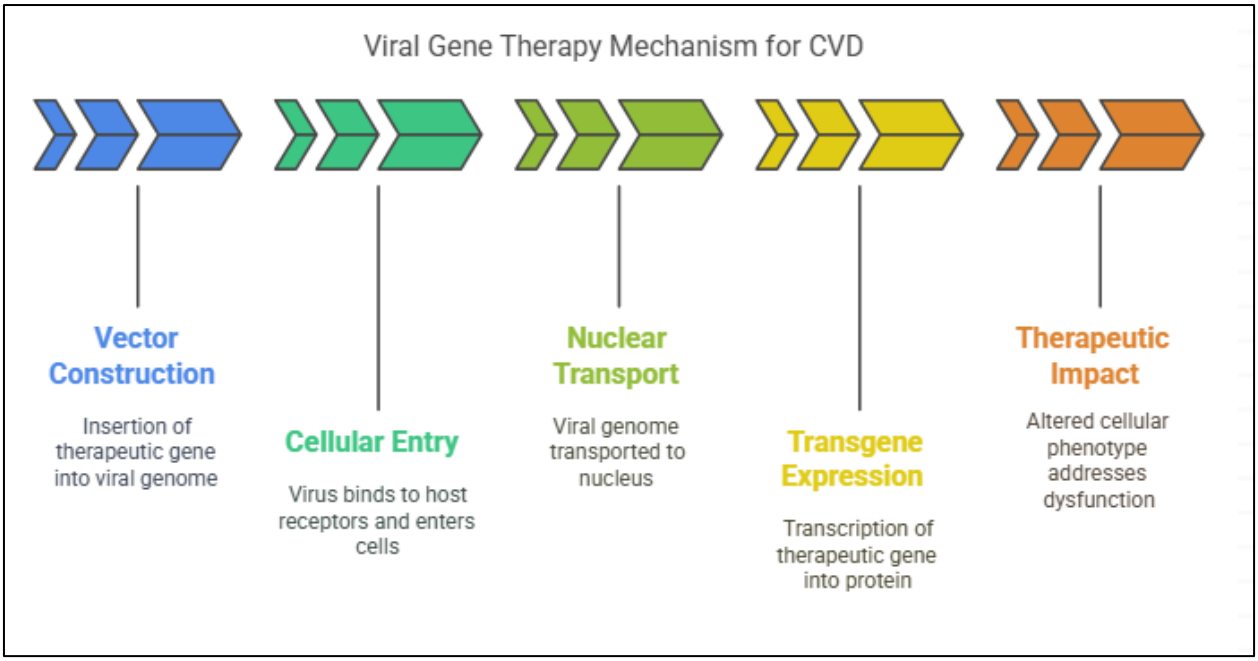

Figure 2 Viral gene therapy mechanism for cardiovascular disease (CVD). This figure illustrates how viral vectors deliver therapeutic genes into cardiac cells, enabling the correction or modulation of disease-related pathways. It highlights key steps including vector entry, gene expression, and the resulting therapeutic effects on cardiac function

2.2. Approaches: Gene Augmentation, Silencing, and Editing

The three major molecular strategies in viral gene therapy are:

2.2.1. Gene Augmentation Therapy (GAT)

Used to supplement defective or downregulated genes. Another example concerns SERCA2a, a sarcoplasmic reticulum calcium pump, which, being downregulated in heart failure, was delivered by AAV vectors for improving calcium handling and contractility (Jessup et al., 2011).

2.2.2. Gene Silencing

Target overactive or maladaptive genes using shRNA or miRNA cassettes delivered by viral vectors. This method is useful in repressing pro-fibrotic or pro-apoptotic pathways involved in heart failure and hypertrophy (Kupatt et al., 2010).

2.2.3. Genome Editing

Newer generation systems like CRISPR-Cas9, TALENs, and ZFNs can be packed in lentiviral or AAV vectors for removing mutations in genes responsible for inherited cardiomyopathies or arrhythmias such as LMNA, RYR2, and SCN5A (Musunuru et al., 2019).

2.3. Therapeutic Targets Investigated in Cardiovascular Gene Therapy

Over the years, some molecular targets have indeed been investigated from a therapeutic standpoint in order to modify the course of the disease. These include one set of proteins involved in calcium cycling and another with interest in vasodilation, angiogenesis, ECM remodeling, and anti-inflammatory signaling.

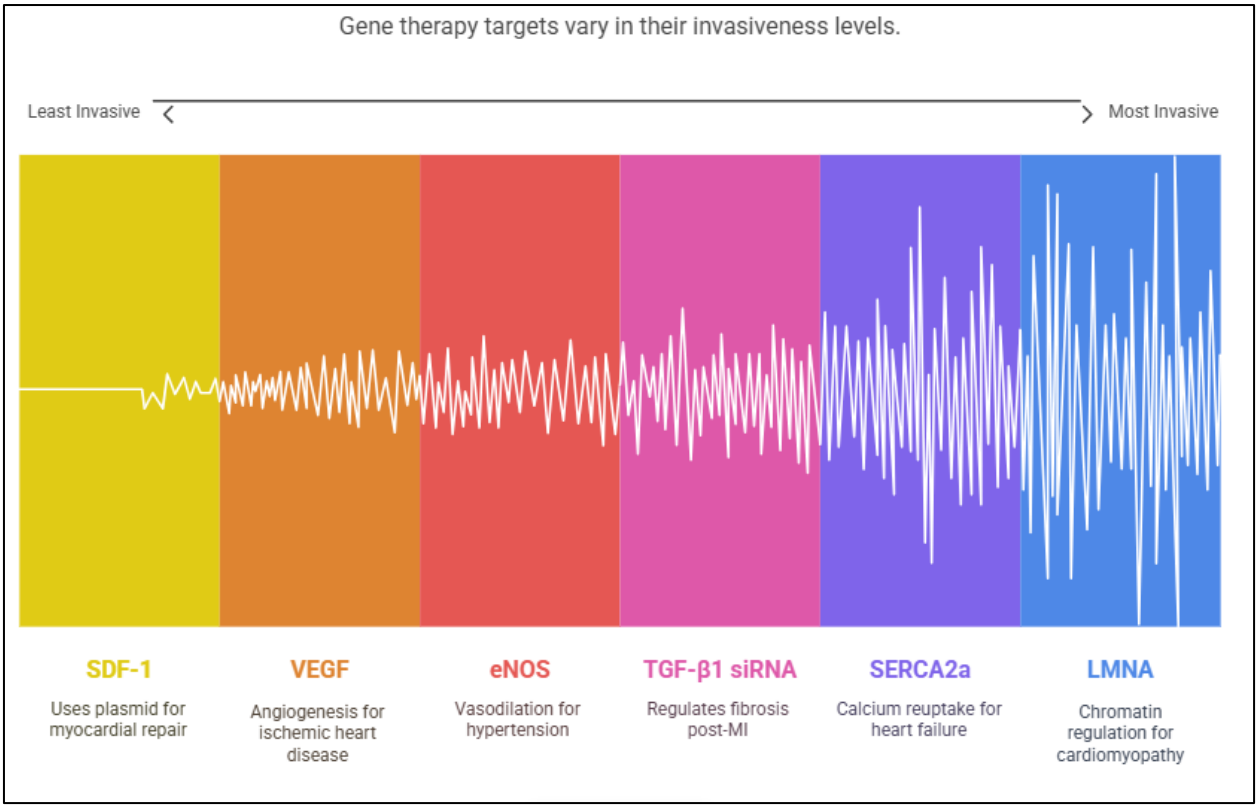

Figure 3 Gene therapy targets vary in their invasiveness levels. This figure categorizes different cardiovascular gene therapy targets based on the invasiveness of their delivery methods. It compares minimally invasive approaches, such as systemic intravenous delivery, with more invasive strategies like direct myocardial injection or catheter-based administration, highlighting the trade-offs between precision, efficacy, and patient safety.

2.4. Expression Kinetics and Duration

The expression pattern and duration rely heavily on the type of vector used:

- AAVs provide long-term, non-integrating expression (6 months to several years)-strictly for stable gene augmentation in post-mitotic cardiomyocytes.
- Lentiviruses provide for stable integration into the genome and are preferred for those cases requiring a permanent correction, for example, channelopathies inherited.
- Adenoviruses, being efficient but inducing transient expression and strong immune responses, are generally limited to short-term goals (Kobayashi et al., 2019).

2.5. Functional Outcomes in Preclinical Models

Animal models receiving viral gene therapy exhibit:

Improved ejection fraction and stroke volume, particularly post-delivery of SERCA2a and VEGF. Reduction in infarct size and fibrosis in MI models placed with anti-TGF-β and pro-angiogenic genes. Respects arrhythmogenic phenotypes after CRISPR corrections of genes in mouse models for Brugada and LQTS (Musunuru et al., 2019).

3. Cardiovascular Therapeutic Viral Vector Systems

Viral vectors form the actual essence of gene therapy, working essentially as delivery vehicles for the transfer of therapeutic DNA/RNA into the target cell. In cardiovascular diseases, since target cells such as cardiomyocytes, endothelial cells, and VSMCs are largely non-dividing, there needs to be a high vector efficiency, long-term expression, tissue specificity, and low immunogenicity. Hence, the three mostly used platforms are AAV, lentivirus, and adenovirus (Zacchigna et al., 2014; Kobayashi et al., 2019, Behetizadeh et al.,2025).

Each of the systems has individual characteristics, which thus set the clinical suitability for a specific cardiovascular target and disease.

3.1. AAV

According to the literature, AAV vectors are the most frequently used platform in cardiac gene therapy given they do not cause disease and possess only low levels of immunogenicity in addition to being able to transduce non-dividing cells. AAVs exist in more than a hundred natural and engineered serotypes, with each of the serotypes displaying different tissue tropisms. Significantly:

AAV1 and AAV9 present a strong tropism toward cardiac and skeletal muscle, AAV6 and AAV2 are the usual suspects when considering the cardiac vascular endothelium and smooth muscle (Kawase et al., 2011). Due to their episomal nature, AAV vectors circumvent genome integration and could decrease cancer risk; in dividing cells, however, episomal nature of AAV may counter durability.

3.2. Lentivirus

Integration-competent vectors derived from HIV-1, lentiviruses are able to deliver genes into dividing and non-dividing cells equally. For stable, long-term expression lentiviral vectors are most suitable and may find applications in inherited cardiomyopathies, where lifelong correction is required (Musunuru et al., 2019).

Nonetheless, they induce worries on their integration capacity onto the host's genome for insertional mutagenesis, requiring further design of vectors and regulatory measures. Ex vivo gene-modified stem cell therapies are increasingly adopting lentiviral integration, in which modified stem cells are expanded and validated before transplantation.

3.3. Adenovirus

The original first-generation adenoviruses are highly effective but also very immunogenic, inducing massive innate and adaptive immune responses. Though they allow very high levels of gene expression, their application has generally been limited to short-term expression such as in acute myocardial infarction models or for the induction of angiogenesis (e.g., VEGF delivery).

With enhanced safety profile and large genetic cargo capacity (~36 kb), these newer 'gutless' or helper-dependent adenoviral systems might be ideally suited for gene cassettes encumbered with several regulatory elements (Ginn et al., 2013).

Table 2 Comparative Features of Major Viral Vectors in Cardiovascular Gene Therapy

Feature/Vector	AAV	Lentivirus	Adenovirus
Genome Type	ssDNA	ssRNA (reverse-transcribed)	dsDNA
Transduction Target	Non-dividing cells	Dividing and non-dividing cells	Broad (high efficiency)
Integration	No (episomal)	Yes (random integration)	No (episomal)
Expression Duration	Long-term (months to years)	Permanent	Short-term (days to weeks)
Packaging Capacity	~4.7 kb	~8 kb	~36 kb
Immunogenicity	Low to moderate	Moderate	High
Clinical Examples	CUPID (SERCA2a), SDF-1 trials	LMNA correction, stem cell engineering	VEGF, eNOS angiogenic therapies

Table 2- Comparative features of major viral vectors in cardiovascular gene therapy. This table summarizes key characteristics of commonly used viral vectors— including adenoviruses, adeno-associated viruses (AAV), and lentiviruses—focusing on factors such as packaging capacity, tropism, immunogenicity, duration of gene expression, and safety profiles relevant to cardiovascular applications.

3.4. Vector Choice Based on Serotype

The AAV serotype or the pseudotype used for the lentivirus can greatly affect the efficiency of the vector and even its biodistribution and immunogenicity. For example:

AAV9 can cross the endothelial barrier and transduce the heart upon intravenous injection, making it an ideal vector for systemic gene therapy for heart failure.

AAV1 and AAV6, on the other hand, require intramyocardial or intracoronary delivery, which is more invasive, but may arguably enhance local uptake.

Lentiviral vectors can be pseudotyped with VSV-G for a broad tropism or with an envelope specific for endothelium restricted expression.

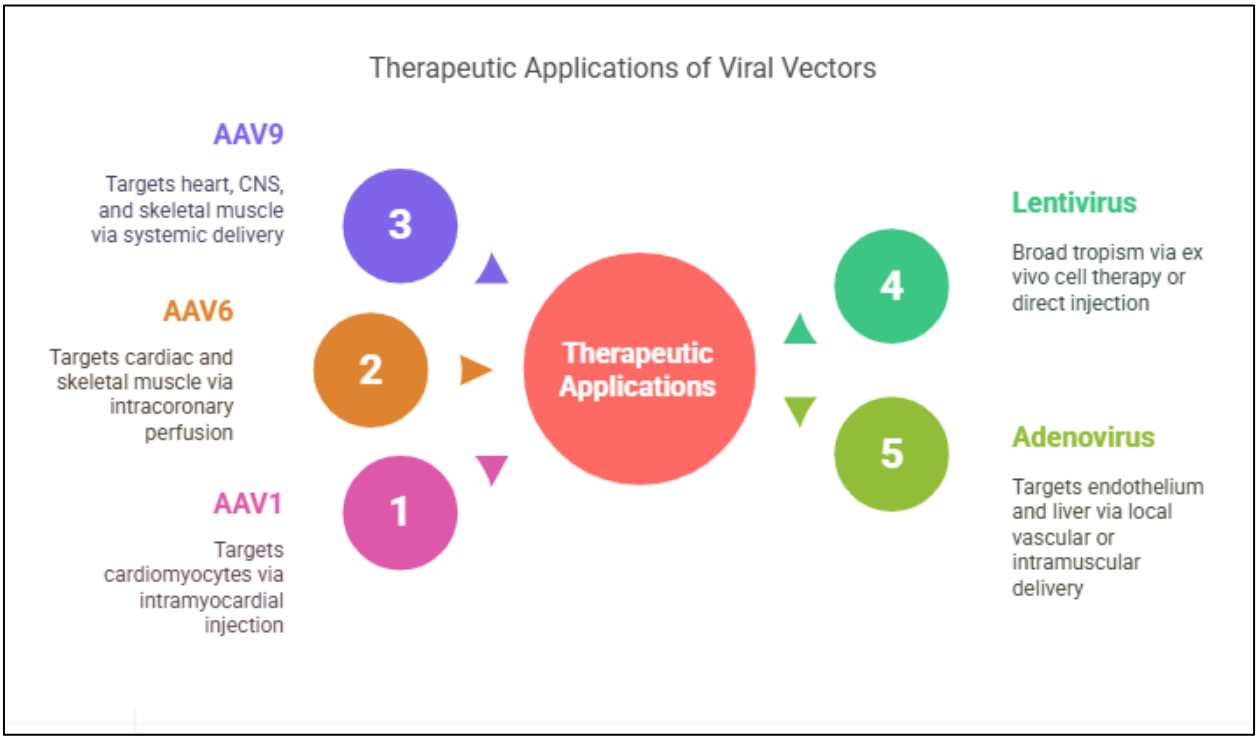

Figure 4 Therapeutic applications of viral vectors in cardiovascular medicine. This figure highlights various uses of viral vectors for gene delivery, including promoting angiogenesis, reducing fibrosis, enhancing cardiomyocyte survival, and correcting genetic mutations. It emphasizes how these applications contribute to repairing damaged heart tissue and improving cardiac function.

3.5. Choosing the Right Vector for Disease Context

Depending on the variables under consideration, the choice for an optimum viral vector would be:

Duration of disease: acute, chronic;

- Type of target cell: myocytes, fibroblasts, endothelial cells;
- Expression: transient, lifelong;
- Delivery: intravenous, surgical injection;
- Cargo size: gene + promoter + regulatory RNA, etc.

Hence, the proper clinical development would be in line with the approach, which integrates vector biology and disease pathophysiology.

4. Preclinical and Clinical Applications

Viral therapy for genes entered practical medicine with sound proof coming from preclinical animal experimentation and human clinical trials. The last 20 years have witnessed a body of literature grow enormous, emphasizing the feasibility and demonstrating the safety and efficacy of gene transfer to the heart and blood vessels by viral vectors. The most intensely studied cardiovascular applications are heart failure, myocardial infarction (MI), arrhythmias, and ischemic vascular disease.

4.1. Heart Failure: SERCA2a and the CUPID Trials

In cardiac gene therapy, one of the more landmark efforts had to do with calcium handling in the failing heart: the delivery of the SERCA2a gene using AAV1 vectors. SERCA2a is involved in the uptake of calcium by the sarcoplasmic reticulum, and its downregulation is responsible for an impaired contractile response in heart failure.

The CUPID (Calcium Upregulation by Percutaneous Administration of Gene Therapy in Cardiac Disease) program was a series of randomized controlled trials designed to evaluate the AAV1-SERCA2a delivery in patients with advanced systolic heart failure (Greenberg et al., 2016).

CUPID I (Phase 1/2a) demonstrated safety and dose-dependent improvement of cardiac function, fewer hospitalizations, and increased exercise tolerance.

CUPID II (Phase 2b), while safe, failed to meet the primary efficacy endpoint, provoking another look at delivery and stratification of patients.

In spite of their mixed results, these trials did manage to demonstrate proof of principle along with providing useful information about vector delivery, dosing, and the choice of endpoints.

4.2. Myocardial Infarction: Angiogenesis and Tissue Repair

Gene therapies after myocardial infarction (MI) mainly target: Improving perfusion by angiogenesis, Modulating inflammatory and fibrotic responses, and Ensuring cardiomyocyte survival and regeneration.

Probably the most intensely studied target is Vascular Endothelial Growth Factor (VEGF). Adenoviral or AAV-mediated gene transfer of VEGF in animal models and early human trials enhanced neovascularization and decreased infarct size (Kastrup et al., 2005).

Another interesting target is Stromal Cell-Derived Factor-1 (SDF-1), which may promote tissue repair by recruiting endogenous stem cells to ischemic myocardium (Penn et al., 2012).

Table 3 Selected Clinical Trials of Viral Gene Therapy for Cardiovascular Diseases

Trial Name	Vector Used	Target Gene	Condition Treated	Phase	Outcome Summary
CUPID I	AAV1	SERCA2a	Systolic heart failure	I/IIa	Improved EF, reduced symptoms
CUPID II	AAV1	SERCA2a	Systolic heart failure	IIb	Safe but did not meet primary endpoints
STOP-HF	Adenovirus	SDF-1	Ischemic cardiomyopathy	II	Increased perfusion and reduced scar size
REVASC	Adenovirus	VEGF121	Peripheral ischemia	I/II	Increased vascular density, improved ABI
AGENT-3/4	Adenovirus	FGF-4	Angina	III	Mixed results; improved symptoms in subgroups

Table 3- Selected clinical trials of viral gene therapy for cardiovascular diseases. This table summarizes key clinical studies investigating the safety and efficacy of viral vector-based gene therapies in cardiovascular patients. It includes

trial phases, target genes, vector types, delivery methods, and reported outcomes, highlighting progress and challenges in translating gene therapy to clinical practice

4.3. Arrhythmias: Ion Channels and Gap Junctions as Therapeutic Targets

Germline arrhythmias are actually ideal conditions for gene therapy in that many clinical phenotypes such as LQTS or Brugada syndrome are due to mutations in single genes affecting ion channel functioning. CRISPR-Cas9 or lentiviral correction of mutations in SCN5A, KCNQ1, or RYR2 have restored normal electrophysiological functioning in both animal models and iPSC-derived cardiomyocytes (Musunuru et al., 2019).

In addition, connexin 43, the primary gap junction protein, has been delivered using AAV vectors in post-myocardial infarction models to restore electrical coupling and prevent arrhythmogenesis (Gutstein et al., 2001).

4.4. Vascular Disease and Atherosclerosis

From the perspective of myocardial gene therapy, the vascular treatment paradigm aims at modulating vascular smooth muscle cell proliferation, plaque stability, and endothelial function. For example:

In hypertensive models, eNOS delivery via AAV was found to improve endothelial function and reduce blood pressure (Channon & Guzik, 2002).

An adenovirus encoding TIMP-1 (tissue inhibitor of metalloproteinases) made atherosclerotic mice less susceptible to plaque rupture (Bendall et al., 2002).

Hereagain, we see the wide-ranging utility of viral vectors in targeting both myocardial and vascular pathologies.

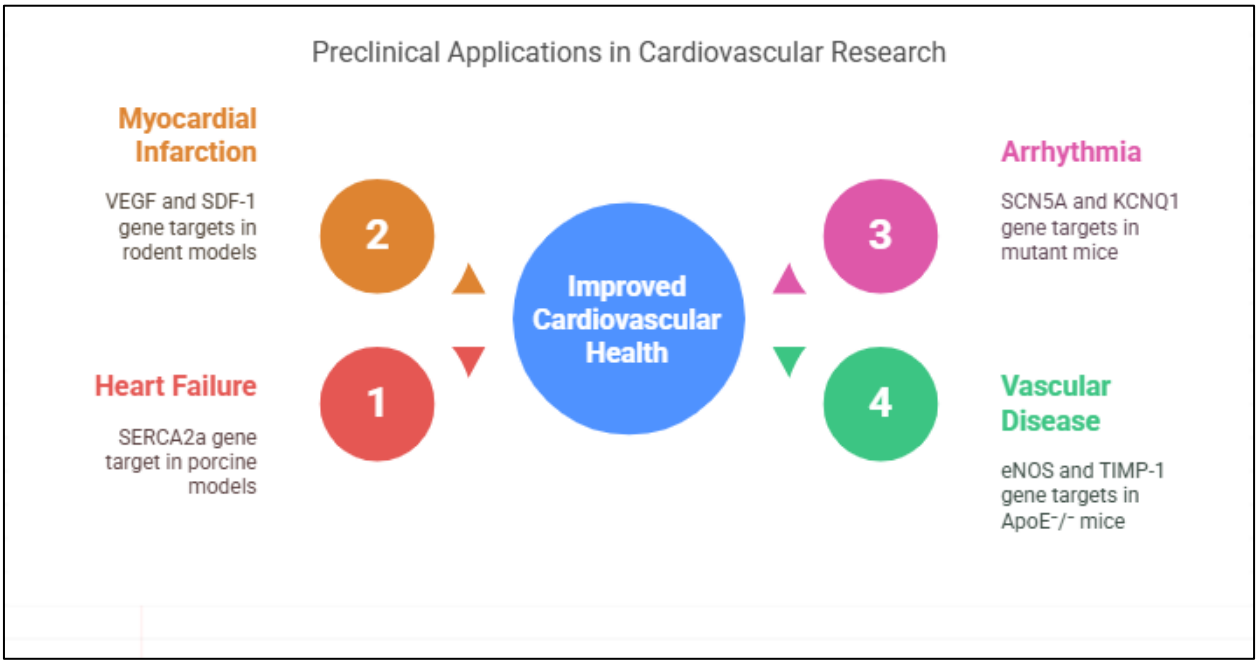

Figure 5 Preclinical applications of gene therapy in cardiovascular research. This figure illustrates how gene therapy is utilized in animal models and laboratory studies to explore cardiac repair, angiogenesis, arrhythmia control, and prevention of heart failure. It highlights the role of preclinical research in optimizing delivery methods, evaluating therapeutic targets, and assessing safety before clinical translation

4.5. Limitations to Clinical Translation

Notwithstanding promising results, translation into clinical therapies has been slow for some reasons. Namely:

- Variable results in different trials (e.g., CUPID II), Immunogenicity issues of adenoviral vectors,

- Lack of data on long-term efficacy, Issues regarding delivery of the vector to patients (e.g., via intracoronary infusion), Restrictions from regulatory and ethical bodies concerning gene editing.

Nonetheless, the lessons learned from these trials will continue to drive the next generation of vector engineering, patient stratification on the basis of answers to their own biological questions, and even some regulatory pathways.

4.6. Gene Therapy in Congenital and Pediatric Cardiomyopathies

Gene therapy may offer new hope for the congenital and inherited cardiomyopathies occurring in pediatric populations beyond late-onset heart failure and ischemic heart diseases. Conditions such as hypertrophic cardiomyopathy (HCM), now caused by mutations in the genes MYH7 or MYBPC3, and dilated cardiomyopathy (DCM), linked to LMNA or truncating mutations in TTN, have long been without available therapies affecting the course of the disease (Yotti et al., 2019). By administering diverse AAV9 vectors into neonatal mouse hearts at birth or 3 days of age, investigators have been able to salvage a murine model of neonatal cardiomyopathy that suffers from sarcomeric protein insufficiency, thereby restoring contractility and preventing early death (Prondzynski et al., 2019).

Gene therapy is also being tried in rare syndromes, including Danon disease (LAMP2 deficiency), where lentiviral and AAV systems are being used for restoring lysosomal function in cardiomyocytes (Sugie et al., 2020). This context embodies a frontier for pediatric precision cardiology, in which offensive therapeutic strategies can be implemented well before the onset of irreversible myocardial damage.

4.7. Immunomodulatory Gene Therapy

A growing number of works focus on immunomodulation as chronic inflammation and immune dysregulation are often elements of heart failure and atherosclerosis. For example, interleukin-10 (IL-10) gene transfer via a lentiviral vector reduced macrophage infiltrates, suppressed TNF-α expression, and inhibited ventricular remodeling in rodent models of MI (Grisanti et al., 2014).

Another interesting clinical avenue proposes to deliver checkpoint regulators such as PD-L1 or CTLA-4 to inflamed vascular tissue to mitigate autoimmune-like processes involved in vasculitis and coronary artery inflammation. This could show the promise that viral gene therapy has, not just for correcting structural and electrical abnormalities but also for curing pathological immune signaling of CVD.

4.8. Combined Gene and Cell Therapy Approaches

More established strategies combine viral gene therapy with cell-based delivery platforms to improve therapeutic efficacy and circumvent delivery hurdles. One such strategy involves ex vivo transduction of mesenchymal stem cells (MSCs) or cardiac progenitor cells with therapeutic genes (such as VEGF, IGF-1, or SERCA2a) before administrating the cells into the damaged myocardium (Silvestre et al., 2011).

The approach synergizes the homing and paracrine capacity of stem cells with the stable expression of therapeutic genes conferred by viral vectors. These interventions prove particularly potently beneficial in fibrotic or infarcted regions where viral uptake is impaired by facilitating "biological targeting" of gene delivery along the migratory path of stem cells.

4.9. Lessons from Failed and Ambiguous Trials

Several clinical trials that have failed to meet primary efficacy endpoints have nevertheless illuminated barriers that must be addressed. For example:

While CUPID II was deemed safe, results suggested that patient selection should be improved, perhaps selecting for patients able to preserve vector uptake and with lower antibody titers in further studies (Greenberg et al., 2016).

The AGENT-3/4 trials assessing FGF-4 in patients with stable angina revealed only modest improvements in symptoms, and none in exercise tolerance, indicating that outcome is strongly influenced by timing and mode of delivery as well as the stage of the disease (Grines et al., 2003).

These experiences highlight that vector efficacy needs to be aligned with the execution strategy, patient phenotype, and when the disease presents itself in order to render tangible clinical changes.

4.10. Trials Now Underway and Future Enrollments

Several new and ongoing trials of viral vectors for cardiovascular disorders currently exist: REVEAL trial (NCT04347271): A study of AAV9 microdystrophin delivery for the treatment of cardiomyopathy of Duchenne muscular dystrophy. PRECISE trial (NCT04852552): Gene-correcting LMNA cardiomyopathy with a lentiviral vector. ENHANCE trial (NCT04752598): Gene therapy combined with stem cell–modulated cardiac fibroblasts. These later-generation trials increasingly incorporate genotyping, imaging biomarkers, and electrophysiological monitoring for better stratification of responders and therapy personalization.

5. Targeting Strategies and Delivery Approaches

More than any other factor, successful viral gene therapy relies heavily on effective delivery, which needs to be not only efficient but also specific to the target. Although viral vectors essentially induce high-level transduction, it is equally important to tactfully decide on the route of administration, promoter design, and tropism modification depending on the tissue that is being targeted, type of disease, and timing of treatment (Zacchigna et al., 2014; Kobayashi et al., 2019). Hence, in cardiovascular settings, where it is often necessary to target endothelium, myocardium, or vascular smooth muscle in an environment that is fibrotic, ischemic, or inflamed, an all-encompassing strategy must be conceived.

5.1. Cardiovascular Gene Therapy Delivery Routes

Three major delivery routes are commonly practiced in cardiac and vascular gene therapy: intracoronary, intramyocardial, and systemic intravenous administration.

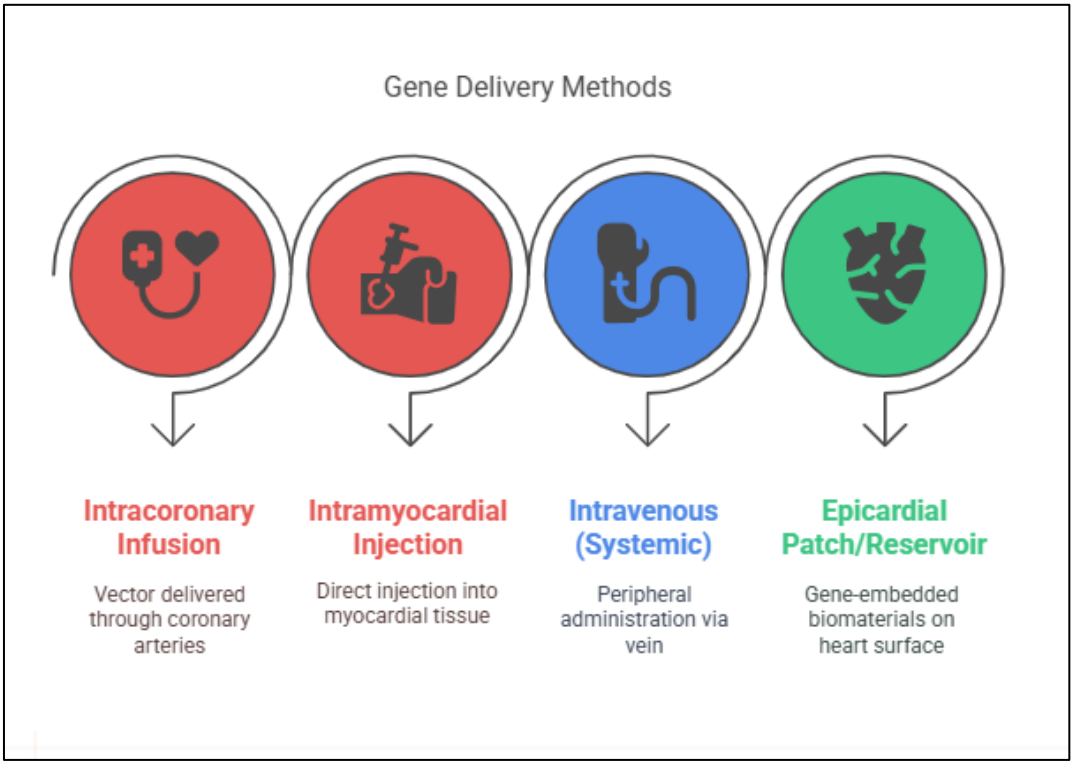

Figure 6 Gene delivery methods in cardiovascular therapy. This figure depicts various techniques for delivering therapeutic genes to the heart, including viral vector injection, catheter-based delivery, systemic administration, and emerging non-viral approaches. It highlights the advantages and limitations of each method in terms of efficiency, targeting accuracy, invasiveness, and safety

Intracoronary infusion has conventionally been implemented in trials such as CUPID (AAV1–SERCA2a) and STOP-HF (Ad–SDF1) (Greenberg et al., 2016; Penn et al., 2012). It allows wide dispersal within the perfused myocardium; however, its activity could be attenuated in hypoperfused or infarcted areas.

Intramyocardial injection is usually accompanied by electromechanical mapping or imaging modalities to ensure localized gene delivery and thus sets the gene delivery idealistic to border zones of infarcts, fibrotic scars, or pacing

nodes (Kupatt et al., 2010). There is, however, a risk for myocardial trauma, and this mode of gene delivery also generally warrants an interventional or surgical setting.

Systemic delivery is becoming increasingly popular through methods using AAV9 vectors, which can traverse the vascular endothelium to transduce cardiomyocytes after peripheral injection (Wang et al., 2023). This means that very high dosages must be given, which brings about concerns regarding vector immunogenicity and liver toxicity and, of course, non-cardiac transduction.

5.2. Use of Cardiac-Specific Promoters

Another main consideration in targeting is promoter selection. Many early gene therapy trials used ubiquitous viral promoters, such as CMV or SV40; however, these promoters are often implicated in off-target expression and immune response. More advanced ways utilize tissue-specific promoters for a higher degree of safety and efficacy (Kawase et al., 2011).

Table 4 Common Promoters in Cardiovascular Gene Therapy

Promoter Type	Specificity	Common Use in Therapy	Vector Compatibility
CMV (cytomegalovirus)	High expression in all cells	Early trials; transient expression	AAV, Adenovirus
α-MHC (α-myosin heavy chain)	Specific to cardiomyocytes	SERCA2a, connexin gene therapy	AAV1, AAV9
cTnT (cardiac troponin T)	Cardiac muscle–specific	Heart failure models; pediatric therapy	AAV9, Lentivirus
SM22α	Vascular smooth muscle	Atherosclerosis, hypertension models	Lentivirus, Adenovirus
VE-cadherin	Endothelial-specific	Angiogenesis studies	AAV2, AAV6

Table 4 Common promoters used in cardiovascular gene therapy. This table lists frequently used genetic promoters that drive gene expression in cardiovascular gene therapy, highlighting their specificity, strength, and suitability for targeting cardiac cells or vascular tissues to achieve optimal therapeutic outcomes

α-MHC and cTnT promoters have been in use in the past for cardiomyocyte-specific expression, reducing the chances of their transgene activity in other muscle types. SM22α and VE-cadherin promoters carry out vascular-specific gene therapy for pulmonary hypertension, atherosclerosis, and endothelial dysfunction (Musunuru et al., 2019).

Newer systems include synthetic hybrid promoters and microRNA-responsive systems that improve targeting based on gene regulatory elements present only in diseased cardiac cells, such as miR-1 or -208a, which are upregulated in heart failure (Callis et al., 2009).

5.3. Enhancing Vector Tropism and Retention

These targeting strategies have been further enhanced by adding the following:

Inserting peptide ligands within the AAV capsids to promote cardiotropism, Magnetic-nanoparticle-conjugated vectors to direct delivery, Electroporation and ultrasonic-facilitated transduction to raise uptake in fibrotic or calcified tissues (Reddy et al., 2021).

Gene-delivery reservoirs such as biodegradable patches loaded with AAV vectors can also act to permit localized sustained release during open-heart surgery or device implantation.

5.4. Timing and Disease Stage Considerations

Overtly, the timing of delivery is the deciding factor in the success of the therapy:

The intervention should be early in acute MI so that necrosis and adverse remodeling could be prevented. In chronic heart failure, gene expression, wherein possible, may need to continue for several months in order to have a meaningful

clinical benefit. Therapy for genetic arrhythmias ideally should take place before symptom onset for preventive correction. Advanced imaging (MRI, PET) and circulating biomarkers (NT-proBNP, troponin, miRNA signatures) could guide patient stratification for best timing of delivery and vector choice (Musunuru et al., 2019).

6. Challenges and Limitations

Gene therapy by viral vectors has transformative potential in the treatment of cardiovascular diseases, but this potential is tempered by some serious limitations that hinder its passage into routine clinical applications. Some of these limitations could be biological, such as immune response, off-target effects, etc.; others could be technical constraints, such as limited transgene capacity, delivery efficiency, etc. Furthermore, ethical, regulatory, and manufacturing concerns need to be addressed to guarantee the safety, equity, and scalability of the new therapies (Ginn et al., 2013; Zacchigna et al., 2014).

6.1. Immune Response toward Viral Vectors

Host immune reactions against viral vectors and the gene products they express represent some of the most pressing impediments to vector utilization. Pre-existing immunity is sometimes present against viral capsids, especially in the case of AAV and adenovirus serotypes, thus limiting the transduction efficiency and contributing to risks of systemic inflammation (Mingozzi & High, 2011). Moreover, repeated administration would become compromised by the formation of neutralizing antibodies (NAbs), which prevent not only therapeutic efficacy but might also evoke hypersensitivity responses, elevations of liver enzymes, or complement activation (Li et al., 2013).

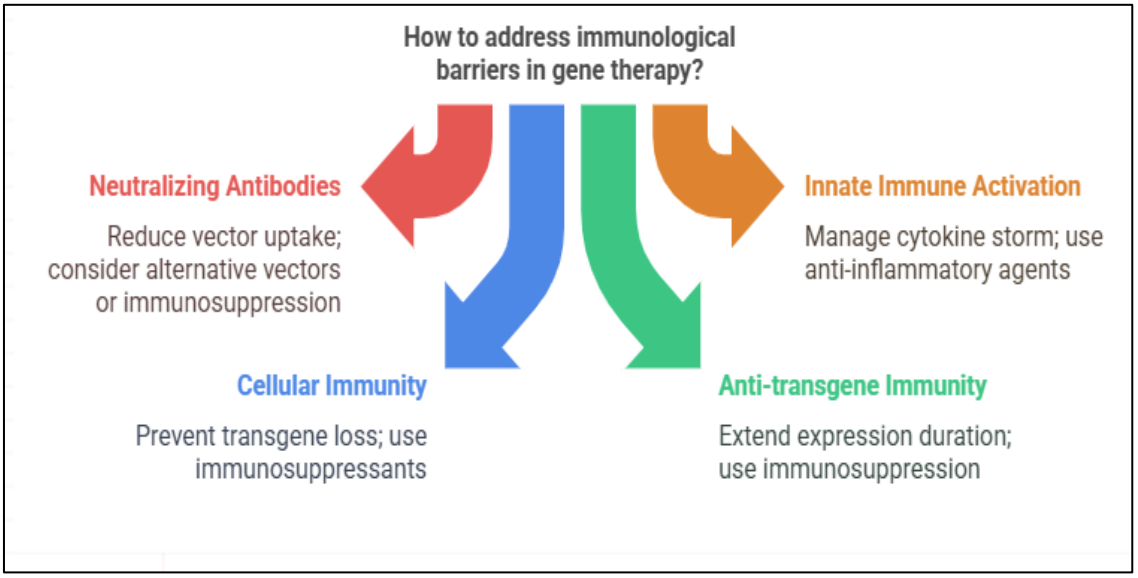

Figure 7 Strategies to address immunological barriers in gene therapy. This figure outlines approaches to overcome immune responses against viral vectors and transgene products, including vector engineering, immunosuppressive treatments, immune evasion techniques, and repeated dosing protocols, aimed at enhancing the safety and efficacy of gene therapy in cardiovascular applications

The biologic reaction commonly stems from the AAV capsid and can be somewhat mitigated by:

- Using rare AAV serotypes (e.g., AAVrh10),
- Transient immunosuppression upon vector administration,
- Developing immune escape mechanisms, e.g., decoy capsids and stealth vectors,
- Ex vivo gene editing of autologous cells, then transplantation.

6.2. Vector Capacity and Gene-Size-Constraints

Each viral vector has a limited packaging capacity, which can constrain application in systems requiring larger or multiple genes. For example:

- AAV vector can accommodate only ~4.7kb and is NOT enough for large cDNAs such as dystrophin or multigene constructs (Chamberlain et al. 2016).
- Lentiviruses may accommodate up to ~8 kb but present a risk for insertional mutagenesis and thus raise safety concerns.

Strategies under study include:

- Dual-vector system where two separate AAVs reconstitute a full gene in vivo,
- Mini-genes or truncated versions retaining the relevant therapeutic domains of larger proteins,
- Non-viral delivery of oversized genes by way of nanoparticles or mRNA-based methods.

6.3. Off-Target Effects and Tissue Leakage

Another limitation is in the execution of non-cardiac transduction, more so when systemic routes or non-specific promoters are used. For instance, AAV9, while excellent at traversing endothelial barriers, also transduces skeletal muscle and liver, thereby causing off-target protein expression, immunotoxicity, or metabolic disturbances (Wang et al., 2023).

Even within the cardiac tissues, heterogeneous uptake can lead to areas of varying gene expression, which in turn reduces overall efficacy or contributes to electrical instability in arrhythmia applications.

- Areas under research include the following:
- Synthetic promoters active only under disease conditions,
- microRNA-regulated expression systems,
- Spatial targeting, either via catheter-based or patch-based gene release (Zacchigna et al., 2014).

6.4. Genomic Integration and Insertional Mutagenesis

Lentiviral vectors, while good at long-term expression, integrate randomly into the host genome. This confers a risk of activation of proto-oncogenes or disruption of tumor-suppressor genes, leading to malignancy (Hacein-Bey-Abina et al., 2003).

While this is less so in adult somatic cells as opposed to hematopoietic stem cells, it nevertheless requires strict surveillance, particularly where pediatric or preventive interventions are concerned. Alternative methods employing site-specific genome editors, such as CRISPR/Cas9, TALENs, and non-integrating lentiviral systems, are currently being developed (Musunuru et al., 2019).

6.5. Regulatory and Manufacturing Barriers

There are a number of regulatory hurdles to consider in translation from lab-to-clinic, including gaining:

- Approval of Good Manufacturing Practices (GMP) facility,
- Batch testing for purity, potency, and vector copy number,
- Long-term studies on biosafety and oncogenicity.

Still, there remains the issue of scalability and cost. The production of AAV of clinical grade and at clinical volume is exorbitantly priced, a circumstance which may, in turn, pose barriers to gene therapies in lower-income regions.

Table 5 Translational and Regulatory Challenges in Cardiovascular Gene Therapy

Challenge Area	Description	Implication for Clinical Adoption
Manufacturing scalability	High cost and low yield of GMP-grade vectors	Limits availability and commercial viability
Regulatory approval	Complex, multi-stage FDA and EMA pathways	Slows trial initiation and market entry
Ethical concerns	Germline modification, pediatric application risks	Requires case-by-case ethics board review

Long-term follow-up	Need for 5–15 year safety tracking in trials	Increases trial cost and timeline

Table 5- Translational and regulatory challenges in cardiovascular gene therapy. This table summarizes key obstacles in moving gene therapies from research to clinical practice, including issues related to manufacturing consistency, safety evaluation, regulatory approval processes, ethical considerations, and long-term monitoring of treatment effects

Besides these challenges, much has been done to integrate production, automate quality control, and develop international regulatory harmonization. Additionally, the establishment of public-private partnerships that include the Accelerating Medicines Partnership (AMP) has helped pre-empt any erosion of that innovation-access divide.

7. Conclusion

Viral gene therapy has arisen and hence burgeoned into a desirable alternative for combating the molecular bases of cardiovascular disease, which is certainly one of the biggest health pandemics of this generation. Design of viral vectors with high levels of precision, durability, and function has enabled clinicians and researchers to deliver therapeutic genes in cardiac and vascular tissues. With preclinical and clinical studies completed on genes such as SERCA2a, VEGF, SDF-1, and eNOS, improvement in cardiac function, vascular regeneration, and myocardial remodeling has been made concrete.

Nevertheless, huge challenges confront the field, such as immune response, restriction of capacity for packaging, off-target effect, and regulation. The continuous innovation to improve the safety and specificity of gene therapy include capsid engineering, microRNA-based control elements, and non-integrating vectors. Moreover, further expansion of the therapeutic horizon will be facilitated by interfacing gene therapy with CRISPR-based genome editing, 3D bioprinting, AI-driven diagnostics, and biomaterial-assisted delivery technologies.

Going forward, concerted efforts on the side of the biological community, technologists, and the clinical fraternity shall be critical to attaining successes in the realm of viral gene therapy for cardiovascular applications. Thus, landscape changes call for multidisciplinary collaborative inter-relationships between molecular biologists, cardiologists, regulatory specialists, and bioengineers. Given prudent stewardship, the gene therapy shall very soon be out of its experimental phase into an operative treatment regimen, heralding a radical change in the way we treat and potentially cure cardiovascular disease right at the genetic bases.

Compliance with ethical standards

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Disclosure of conflict of interest

Authors has no conflict of interest.

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