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AHA SCIENCE ADVISORY

Gene Therapy in Cardiovascular Disease: Recent Advances and Future Directions in Science: A Science Advisory From the American Heart Association

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ABSTRACT: Cardiovascular disease remains the foremost cause of morbidity and mortality globally, affecting millions of individuals. Recent discoveries illuminate the substantial role of genetics in cardiovascular disease pathogenesis, encompassing both monogenic and polygenic mechanisms and identifying tangible targets for gene therapies. Innovative strategies have emerged to rectify pathogenic variants that cause monogenic disorders such as hypertrophic, dilated, and arrhythmogenic cardiomyopathies and hypercholesterolemia. These include delivery of exogenous genes to supplement insufficient protein levels caused by pathogenic variants or genome editing to correct, delete, or modify mutant sequences to restore protein function. However, effective delivery of gene therapy to specified cells presents formidable challenges. Viral vectors, notably adeno-associated viruses and nonviral vectors such as lipid and engineered nanoparticles, offer distinct advantages and limitations. Additional risks and obstacles remain, including treatment durability, tissue-specific targeting, vector-associated adverse events, and off-target effects. Addressing these challenges is an ongoing imperative; several clinical gene therapy trials are underway, and many more first-in-human studies are anticipated. This science advisory reviews core concepts of gene therapy, key obstacles, patient risks, and ongoing research endeavors to enable clinicians to understand the complex landscape of this emerging therapy and its remarkable therapeutic potential to benefit cardiovascular disease.

Key Words: AHA Scientific Statements ■ cardiovascular diseases ■ gene editing ■ gene silencing ■ gene transfer techniques ■ genetic therapy

Cardiovascular disease (CVD) affects 28.6 million Americans >20 years of age[1] with enormous annual US health care expenditures and a profound impact on patients and families. Existing treatments and interventions are costly, typically require lifelong administration, and mitigate but rarely cure disease. The identification of genetic causes for many CVD enables an alternative approach: gene therapy to precisely target the molecular driver of disease pathogenesis.

Monogenic disorders arise from rare pathogenic variants (PVs) that alter the structure, function, or normal expression level of the gene-encoded protein. Monogenic disorders usually cause prominent clinical manifestations and increased morbidity and mortality. Dominant monogenic CVD results from PVs in 1 of 2 gene copies, such as dilated, hypertrophic, and arrhythmogenic cardiomyopathy, channelopathies, and aortopathies.[2–5] Recessive monogenic CVD arises from PVs in both gene copies such as homozygous familial hypercholesterolemia. PVs that cause monogenic disorders are identified in ≈1.7% of patients undergoing cardiac catheterization; they contribute substantially to the overall burden of CVD.[6] Clinical genetic testing is readily available that assesses for the presence of causal monogenic variants and enables early accurate diagnosis and interventions.[7]

Polygenic disorders arise from multiple genetic variants that occur in healthy populations and individually convey small effects but collectively increase disease susceptibility. Most polygenic variants alter nonprotein coding, regulatory sequences that influence gene expression, and are associated with prevalent CVD,

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including hypertension, diabetes, hypercholesterolemia, and atherosclerosis.[8–11] Genome analyses have identified specific polygenic variants that enabled the development of polygenic risk scores for some CVDs. However, evidence to support the use of polygenic risk scores in guiding clinical management remains limited.[12,13]

Studies of monogenic CVD have propelled insights into pathways, mechanisms, and therapeutic targets. For example, a gain-of-function dominant monogenic variant in the gene encoding proprotein convertase subtilisin/kexin type 9 (PCSK9) was first identified in a family with early-onset, rapidly progressive atherosclerosis.[14] Experiments revealed that PCSK9 degrades the low-density lipoprotein (LDL) receptor, which transports cholesterol-carrying lipoprotein particles into cells. Therefore, gain-of-function variants in *PCSK9* decrease the amount of LDL receptors and subsequently raise circulating LDL levels, similar to effects caused by loss-of-function variants in gene encoding LDL receptor, a cause of familial hypercholesterolemia. Conversely, other human *PCSK9* variants that cause loss of PCSK9 function reduce LDL levels and lifelong risk of CVD events without deleterious effects.[15,16] Together, these genetic findings propelled the development of PCSK9 inhibitors such as the monoclonal antibodies alirocumab[17] and evolocumab[18] and the small interfering RNA inclisiran[19] that target the PCSK9 protein and transcript, respectively. These novel therapies lower LDL cholesterol levels and reduce CVD events. Although effective, treatments by these PCSK9 inhibitors are not durable because of their half-life, requiring regular dosing. However, modeled on the salutary effects of human loss-of-function variants, efforts are ongoing to study a single treatment to permanently silence the *PCSK9* gene in patients with high-risk atherosclerotic disease.[20] Gene therapies harness fundamental knowledge of genes responsible for pathogenesis of CVD and aim to edit, replace, or modulate the activity of the causal genes to alter the disease course by preventing or curing the disease.

MECHANISMS OF GENE THERAPY

Gene therapies differ according to the mechanisms and functional consequences of causal genetic variants (Figure 1). PVs that inactivate 1 allele, resulting in insufficient protein production for normal function, cause haploinsufficiency. For these disorders, gene therapies aim to increase protein levels by delivering exogenous protein-encoding sequences, augmenting the expression of endogenous DNA sequences, or correcting or modifying mutated DNA sequences to produce normal or near-normal protein. PVs that produce sufficient amounts of protein but have deleterious function cause dominant negative effects. Gene therapies for dominant negative effects aim to correct the PV, inactivate the mutated DNA allele, or silence the abnormal transcripts using small interfering RNA or antisense oligonucleotides. Gene therapies that modify DNA sequences have permanent effects, whereas the durability of RNA-based strategies and exogenous protein-encoding sequence delivery is variable. However, gene therapies need to modify sufficient proportions of targeted cells to have salutary effects on tissue and reduce the likelihood for cell division by untreated cells to maintain or re-establish disease.

Tools for modifying DNA sequences continue to rapidly evolve. Current therapeutic strategies use a clustered regularly interspaced short palindromic repeats (CRISPR)/Cas9 nuclease system, base editors, and prime editors (Figure 2). Although the components of these genome editing tools differ, each includes a guide (RNA) molecule that targets specific sequences to be modified and molecules that cut DNA and substitute, insert, or delete ≥1 nucleotides. Genome editing tools that contain a CRISPR/Cas9 nuclease system generate double-strand breaks of DNA. These are repaired by nonhomologous end joining, during which random DNA sequences can be deleted or inserted, and frequently result in a stop codon that prematurely terminates gene transcription.[21] Accordingly, with appropriate target specificity, the CRISPR/Cas9 approach can inactivate the abnormal gene copy. Because multiple parameters influence target specificity, inactivation of both gene copies can occur with unacceptable consequences. Base editors are an alternative therapeutic complex that converts one nucleotide into another, specifically cytosine to thymine or adenine to guanine. Prime editors contain fusion proteins comprising Cas9 nickase and modified reverse transcriptase that can introduce, delete, or substitute multiple nucleotides, thereby offering a wider array of therapeutic options.[22] Base editors and prime editors do not introduce double-strand breaks and result in more predictable and precise changes than CRISPR/Cas9 approaches. Therefore, these gene editors can be used to introduce a stop codon to degrade the abnormal copy harboring the dominant negative variant, precisely restore normal DNA sequence, or potentially introduce activating sequences to increase gene expression.

DELIVERY OF GENE THERAPY

Effective in vivo delivery of gene therapy that specifically targets the affected cells within organs remains a challenge for gene therapies. Currently, 2 types of delivery vehicles, viral and nonviral vectors, are used with distinctive features (Figure 3). Viral vectors are composed of defective and nonpathogenic viral envelopes with packaging machinery and capsids that prevent immune-mediated degradation during systemic delivery. Adeno-associated virus (AAV) is a commonly used vector because it has multiple serotypes with tissue-specific tropism[23] and preferential delivery into a specific tissue and because it rarely integrates into the genome of the cell. AAVs can persist in organs with nondividing cardiomyocytes or

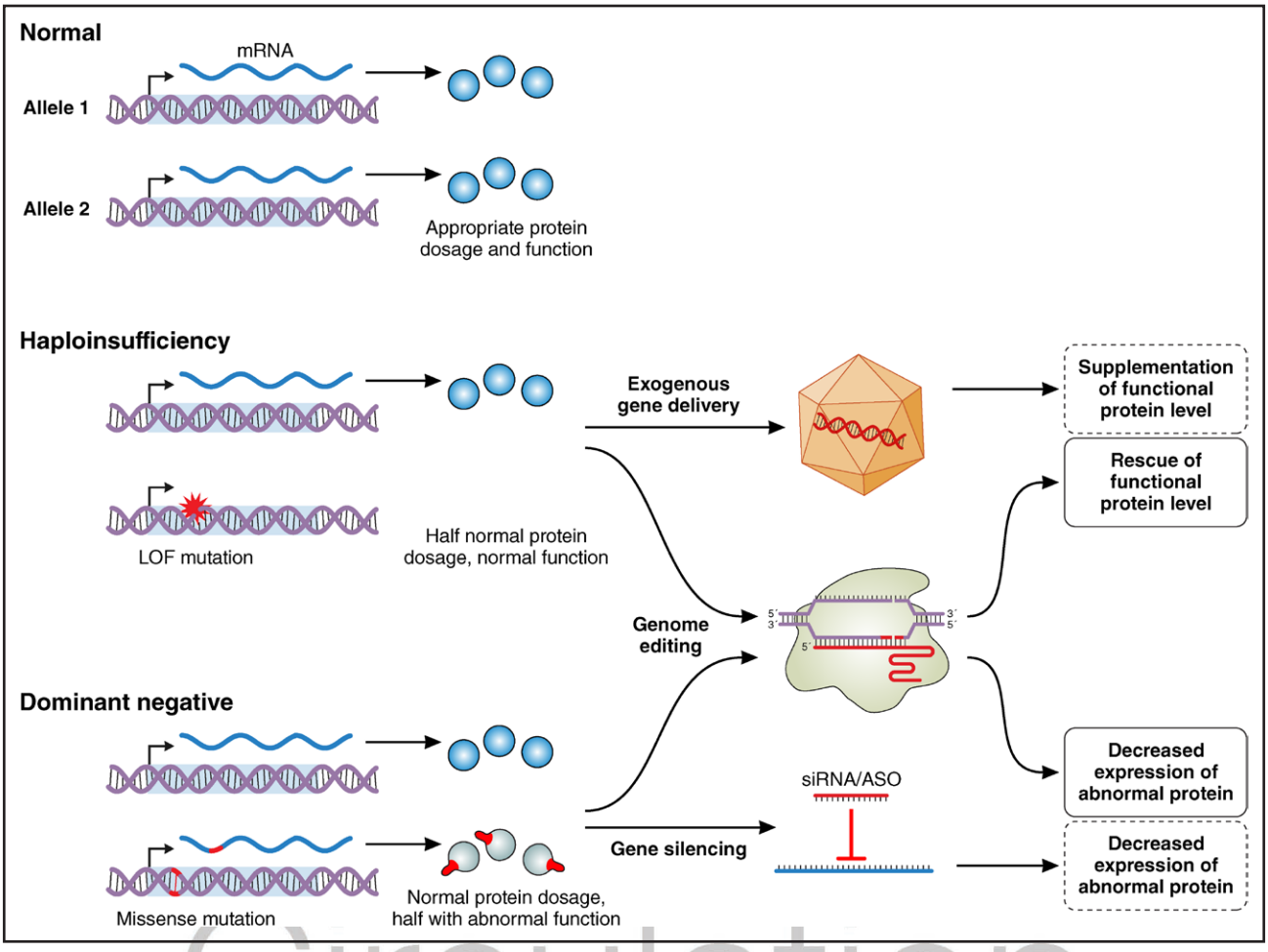

Figure 1. Mechanisms and strategies of gene therapy.
Pathogenic variants (PVs) in cardiovascular disease often lead to either haploinsufficiency, in which the amount of essential protein is insufficient, or dominant negative effects of genes critical in cellular function, in which protein with abnormal function is produced. Strategies to correct PVs differ according to the mechanisms of disease pathogenesis. Haploinsufficiency can be overcome through supplementation with exogenous proteins or direct editing of the causal variants in the genome. Targeted editing of the pathogenic variants or degradation of the mutant alleles to prevent production of harmful proteins can be used to treat disease caused by dominant negative effects. ASO indicates antisense oligonucleotide; LOF, loss-of-function; and siRNA, small interfering RNA. Solid and dashed boxes indicate permanent and temporary effects by gene therapy, respectively.

slowly dividing liver cells for prolonged periods without adverse effects. These advantages are offset by a limited cargo size of AAV vectors and existing or newly developed neutralizing antibodies against AAV.

Nonviral delivery systems such as lipid nanoparticles (LNPs) were developed to overcome limitations associated with viral vectors. LNPs generally consist of ionizable lipids, amphipathic phospholipids, cholesterol, and polyethylene glycol lipids. These lipid particles encapsulate nucleic acids encoding genome editors or a supplementary gene and facilitate their delivery into the cells.[24] LNPs have the advantages of ease of manufacturing and reduced immune responses. They also have demonstrated success as delivery vehicles for genetic materials for therapeutic applications such as small interfering RNA against transthyretin for treatment of cardiac amyloidosis[25] and COVID-19 mRNA vaccines.[26,27] However, LNPs and related vectors have low tissue specificity and accumulate primarily in liver because of inherent liver tropism, thereby limiting effective delivery to cardiomyocytes.

RECENT ADVANCES IN GENE THERAPY FOR CVD

Notable progress in the development and clinical use of gene therapy for CVD includes small interfering RNAs to treat hyperlipidemia[19] and to reduce levels of damaging proteins in cardiac amyloidosis.[25] Alternative therapeutic approaches to permanently inactivate the mutant gene allele through the CRISPR/Cas9 nuclease system while preserving the other allele with normal DNA sequences are being investigated in both animal models[28] and human patients[29] with CVD. Gene therapies for CVD caused by haploinsufficiency are also under active

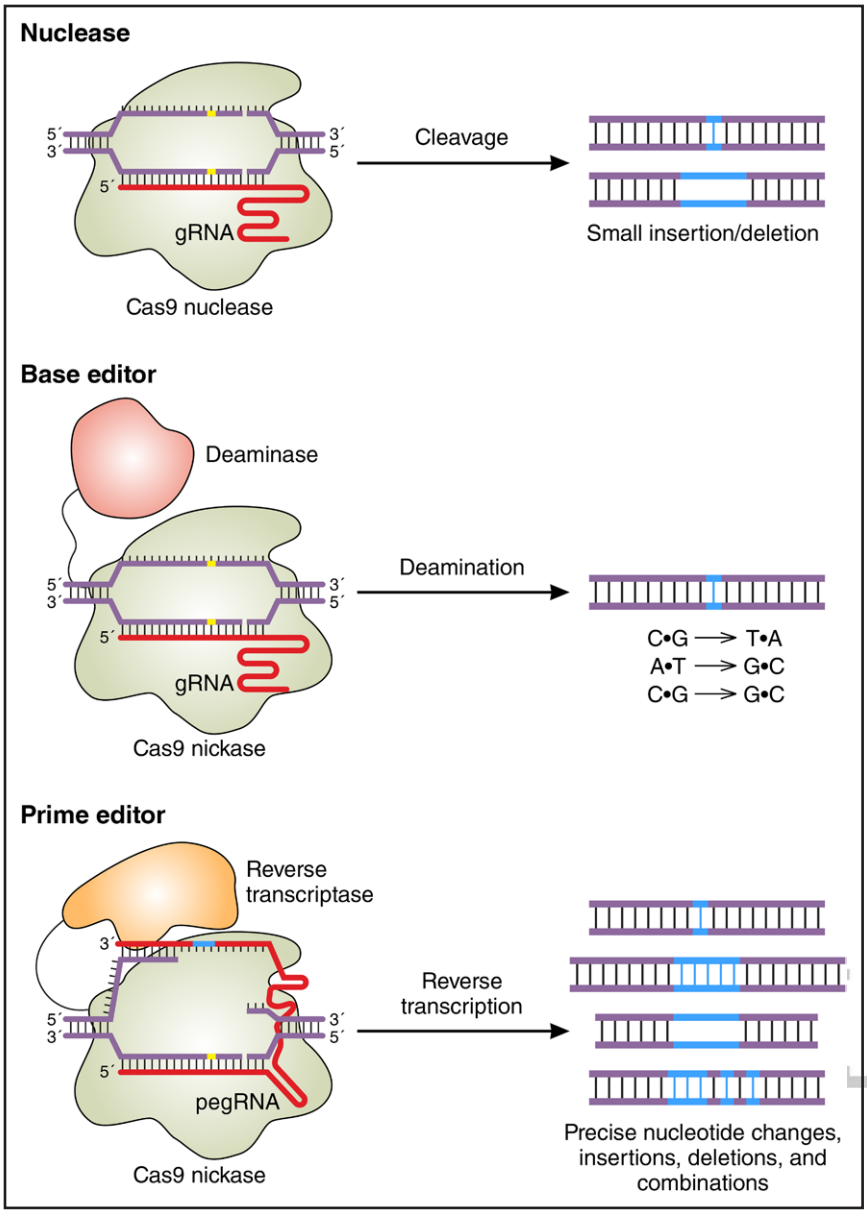

Figure 2. Genome editing tools.
Three main types of genome editors are currently available. Guide RNA (gRNA) encodes target DNA sequences and directs clustered regularly interspaced short palindromic repeats/Cas9 nuclease to specific location within the genome with the pathogenic variant (PV). Nuclease cuts both strands of DNA, which is repaired by an intrinsic process available in cells within the body. Small insertions and deletions are often introduced during the repair process, which create a nonfunctioning DNA allele and ultimately premature truncation of proteins. Base editor, also recruited to a desired location within the genome through gRNA, nicks only 1 strand of DNA and substitutes the mutated nucleotide to another nucleotide such as cytosine to thymine and adenine to guanine. Prime editor is the newest genome editing tool and enables more versatile editing of nucleotides. Desired genome edit sequences can be included in prime editing gRNA (pegRNA), which can facilitate replacement, deletion, and insertion of multiple nucleotides. Target DNA sequences are marked with yellow; edited sequence changes are depicted in blue.

investigation. For example, the US Food and Drug Administration has recently approved AAV delivery of sequences encoding a microdystrophin protein to address dystrophin haploinsufficiency, a cause of Duchenne muscular dystrophy. Full results from a randomized, double-blind, placebo-controlled phase 3 clinical trial evaluating the safety and efficacy of microdystrophin have not been published yet.[30] In parallel, both preclinical and clinical investigations are ongoing to develop therapeutic methods to correct the underlying PV by genome editors. Studies demonstrate the potential to permanently correct PVs by AAV9 delivery of base editors in animal models of hypertrophic cardiomyopathy and dilated cardiomyopathy.[31] Notably, these preclinical studies prevent the emergence of disease, but to date, no study has reversed established disease. LNP delivery of base editors that inactivate the *PCSK9* gene has been effective in lowering LDL with durable effects in nonhuman primates[32] and human patients with heterozygous familial hypercholesterolemia or uncontrolled hypercholesterolemia.[33] An open-label phase 1b clinical trial was recently halted because of transaminitis and thrombocytopenia,[34] and a new phase 1b clinical trial with an alternative LNP delivery system is currently underway.[20] Multiple early-phase clinical trials are ongoing to examine the safety and efficacy of gene replacement and genome editing through viral and nonviral delivery as a novel treatment option for various types of CVD (Table).

CHALLENGES AND LIMITATIONS

Successful gene therapy for CVD promises exciting opportunities for patients. Administration of a single treatment that indefinitely prevents disease emergence or

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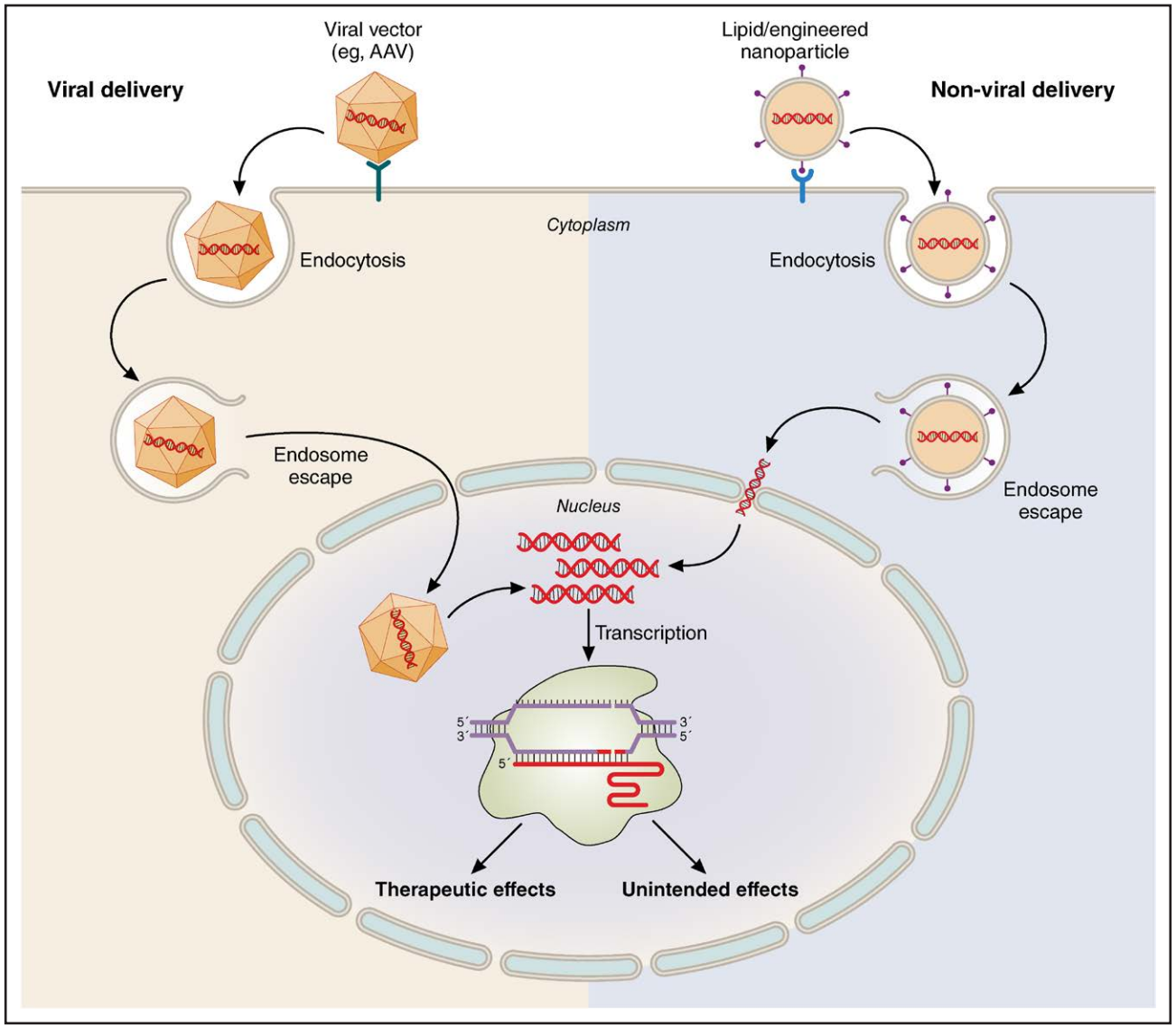

Figure 3. Delivery methods for gene therapy.
Gene therapy vector serve as an envelope to deliver genetic materials for gene therapy (eg, exogenous gene for supplementation or genome editing tools). Viral or nonviral vehicles carrying gene therapy tools bind to receptors on the surface of cells within the diseased organ (eg, heart) and enter the cells through endocytosis. After endosomal escape in which the gene therapy vectors are released and localized to the nucleus, the DNA-encoded gene therapy tool is transcribed to produce supplementary proteins or genome editors. AAV indicates adeno-associated virus.

progression could eliminate the need for lifelong medication use and associated side effects; prevent serious disease sequelae such as arrhythmias and heart failure; and reduce the overall burden of disease for patients, health care systems, and society. Achieving these benefits will require addressing ethical issues, regulatory matters, long-term monitoring, and extremely high cost with input from multiple stakeholders, including patients, the public, pharmaceutical companies, communities, and policymakers. Although they are beyond the scope of this science advisory, we highlight major scientific challenges.

Gene therapy is predicated on the precise knowledge of an individual's PV. Although clinical genetic testing is currently available, multiple barriers, including lack of access, mistrust of health systems, especially in historically underrepresented communities, lack of insurance coverage, and insufficient awareness by both patients and clinicians, have limited its uptake in the United States and many other developed countries. Education to increase the understanding of genetic concepts and genotype interpretation is needed for clinicians, patients, and society to ensure the appropriate use of gene therapies.

Achieving target specificity of gene therapy for tissue, cell type, and DNA sequence is paramount for therapeutic success. The inclusion of cell-specific promoters can limit the expression of CRISPR/Cas9, base editors, and prime editors to selected cells, but unintended DNA changes, both nearby and distant from the targeted DNA sequence, still occur with variable frequency. Preclinical studies indicated the highest target specificity

Table. Recent and Ongoing Clinical Trials in Gene Therapy of CVD

Trial ID*	Disease	Target	Inheritance	Approach	Therapy	Delivery	Current trial phase
Genetic cardiomyopathy							
NCT05885412	ACM	*PKP2*	AD	Gene replacement	RP-A601	AAV	1
NCT06109181	ACM	*PKP2*	AD	Gene replacement	LX2020	AAV	1, 2
NCT06228924	ACM	*PKP2*	AD	Gene replacement	TN-401	AAV	1
NCT05836259	HCM	*MYBPC3*	AD	Gene replacement	TN-201	AAV	1b
Heart failure							
NCT01966887	Nonischemic cardiomyopathy and heart failure	*SERCA2a*	N/A	Gene replacement	MYDICAR	AAV	2 (terminated)
NCT05598333	Ischemic cardiomyopathy and heart failure	*PP1*	N/A	Protein inhibition	AB-1002	AAV	2
Hyperlipidemia							
NCT02651675	FH	*LDLR*	AR	Gene replacement	AAV-LDLR	AAV	1, 2 (terminated)
NCT06125847	FH	*LDLR*	AR	Gene replacement	NGGT006	AAV	1
NCT00891306	FH	*LDLR*	AR	Gene replacement	LPLS447X	AAV	2, 3
NCT06293729	FH	*LDLR*	AR	Gene replacement	NGGT006	AAV	1
NCT06112327	FH	*PCSK9*	AR	Gene editing	VERVE-101	LNP	1
NCT05860569	Hypertriglyceridemia	*LPL*	AR	Gene replacement	GC304	AAV	1
Muscular dystrophy							
NCT03333590	Duchenne muscular dystrophy	*DMD*	XR	Alternative gene replacement	AAV-GALGT2	AAV	1, 2a
NCT02376816	Duchenne muscular dystrophy	*DMD*	XR	Gene replacement	AAV-microdystrophin	AAV	1
NCT05693142	Duchenne muscular dystrophy	*DMD*	XR	Gene replacement	RGX-202	AAV	1, 2
NCT05689164	Duchenne muscular dystrophy	*DMD*	XR	Gene replacement	AAV9-minidystrophin	AAV	3
NCT03368742	Duchenne muscular dystrophy	*DMD*	XR	Gene replacement	SGT-001	AAV	1, 2
NCT06138639	Duchenne muscular dystrophy	*DMD*	XR	Gene replacement	SGT-003	AAV	1, 2
NCT02354781	Duchenne muscular dystrophy	*DMD*	XR	Myostatin inhibitor	AAV-follistatin	AAV	1, 2
NCT06114056	Duchenne muscular dystrophy	*DMD*	XR	Gene replacement	JWK007	AAV	1
NCT05096221	Duchenne muscular dystrophy	*DMD*	XR	Gene replacement	Delandistrogene moxeparvovec (SRP-9001)	AAV	3
NCT06392724	Duchenne muscular dystrophy	*DMD*	XR	Base editing/exon skipping	GEN6050X	AAV	1
NCT05429372	Duchenne muscular dystrophy	*DMD*	XR	Gene replacement	Fordadistrogene movaparvovec (PF-06939926)	AAV	2
Genetic syndrome							
NCT04601051	Transthyretin amyloidosis	*TTR*	AD	Gene editing	NTLA-2001	LNP	1
NCT05445323	Friedrich ataxia	*FXN*	AR	Gene replacement	LX2006	AAV	1, 2
NCT05302271	Friedrich ataxia	*FXN*	AR	Gene replacement	AAVrh.10hFXN	AAV	1a
NCT04174105	Pompe disease	*GAA*	AR	Gene replacement	AT845	AAV	1
NCT03533673	Pompe disease	*GAA*	AR	Gene replacement	ACTUS-101	AAV	1, 2
NCT04093349	Pompe disease	*GAA*	AR	Gene replacement	SPK-3006	AAV	1, 2
NCT00976352	Pompe disease	*GAA*	AR	Gene replacement	AAV-GAA	AAV	1, 2
NCT02240407	Pompe disease	*GAA*	AR	Gene replacement	AAV-GAA	AAV	1
NCT03454893	Fabry disease	*GLA*	XR	Gene replacement	AVR-RD-01	Lentivirus	1, 2 (terminated)
NCT04046224	Fabry disease	*GLA*	XR	Gene replacement	ST-920	AAV	1, 2
NCT04519749	Fabry disease	*GLA*	XR	Gene replacement	4D-310	AAV	1, 2
NCT06092034	Danon disease	*LAMP2*	XR	Gene replacement	RP-A501	AAV	2

AAV indicates adeno-associated virus; ACM, arrhythmogenic cardiomyopathy; AD, autosomal dominant; AR, autosomal recessive; CVD, cardiovascular disease; FH, familial hypercholesterolemia; HCM, hypertrophic cardiomyopathy; LDLR, low-density lipoprotein receptor; LNP, lipid nanoparticle; and XR, X-linked recessive.

*Clinicaltrials.gov (NCT) or EudraCT identifier.

and lowest off-target event with prime editors,[35] but their effective delivery remains below clinical utility. Studies are needed to examine unintended nearby and distant mutations,[36,37] and predicting and assessing their potential, particularly late-onset adverse effects such as malignancy, remain difficult. Unlike in other organ systems,[38,39] the potential for cardiac gene therapy to inadvertently activate malignant transformation is limited by the low oncogenic potential of cardiomyocytes.

Tissue tropism is highest with AAVs, but multiple organs take up these vectors, reducing transduction efficiency in targeted cells.[40] Intravenously administered LNPs accumulate in the liver, providing an effective vector for hepatic indications,[41] but the potential for liver toxicity remains. Ongoing strategies that detarget the liver,[42] incorporate cell type–specific ligands, conjugate molecules for receptor-mediated uptake,[43] and refine engineered viral-like particles[44] with capacity for large sized cargo remain under study.

Vectors and nonhuman sequences of gene therapies can elicit immune responses. Preexisting neutralizing AAV antibodies occur in ≈25% of individuals that limit the effectiveness of initial treatments and prevent repeated administrations.[45] These issues are particularly pertinent for nondurable therapies and early treatment strategies that may limit future options.

Minimizing acute adverse reactions while maximizing therapeutic efficacy is a paramount concern in gene therapy for CVD. More than 30% of clinical trials that principally used AAV vectors reported serious adverse events, including hepatotoxicity, thrombotic microangiopathy (systemic delivery), neurotoxicity (central nervous system delivery), acute respiratory distress syndrome, and death.[46,47] Adverse effects appear greatest among patients receiving high-dose AAV.[46] Nonviral vectors also carry potential side effects. Intravenous LNPs can cause hepatotoxicity and infusion-related hypersensitivity reactions.[48]

Last, although preclinical studies and early-phase clinical trials demonstrate the potential of gene therapy for treating CVD, robust evidence of long-term efficacy and safety in larger patient populations is still lacking. Identification of patient populations with greatest risk for adverse outcomes, combined with evidence for the effectiveness of gene therapy to stabilize or reverse disease, is critically needed; this will be essential for determining optimal timing for gene therapy administration in selected patients. Biomarkers, functional assessments, and patient-reported parameters, including quality of life measures, are needed to assess effectiveness, compare with established medicines and interventions, and position gene therapy among other CVD treatments.

CONCLUSION AND FUTURE DIRECTIONS

Genetic understanding and therapeutic innovations are propelling a dramatic change in the landscape of CVD treatments. Foundational genetic insights, creative molecular engineering strategies, and emerging successes are testaments to the potential for genetic interventions that mitigate disease burden and improve patient outcomes. Gene therapy holds immense promise as a paradigm-shifting approach in the management of CVD, offering the prospect of disease prevention, long-lasting cures, and alleviation of lifelong pharmacotherapy. Translating these promises into clinical reality necessitates surmounting significant challenges. Precise targeting of therapeutic genes to specific cardiovascular tissues, minimizing immune responses to delivery vectors, mitigating off-target effects of genome editing tools, identifying the patient population with the greatest therapeutic potential, and establishing long-term efficacy and safety profiles are among the key hurdles that must be overcome. With collaborative efforts across multidisciplinary domains, gene therapy holds enormous potential to revolutionize personalized medicine tailored to individual genetic and clinical profiles.

ARTICLE INFORMATION

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*Modest.

†Significant.

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Reviewer	Employment	Research grant	Other research support	Speakers' bureau/ honoraria	Expert witness	Ownership interest	Consultant/ advisory board	Other
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Joshua M. Hare	University of Miami Miller School of Medicine	AskBio (I am an investigator in a gene therapy trial sponsored by AskBio)*	None	None	None	Longeveron, Inc†	None	None
Anjali T. Owens	University of Pennsylvania Perelman School of Medicine	BMS (site PI for research study)*; NIH (site PI for research study)*; Array Biopharma (site PI for research study)*		None	None	None	BMS†; Cytokinetics†; BioMarin†; Tenaya*; Alexion†; Pfizer*; Lexeo†	None
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