

## Review article

## Gene therapy for heart failure and cardiomyopathies

Alessia Argirò,<sup>a,\*</sup> Jeffrey Ding,<sup>b</sup> and Eric Adler<sup>b</sup><sup>a</sup> Cardiomyopathy Unit, Careggi University Hospital, Florence, Italy<sup>b</sup> Division of Cardiovascular Medicine, Department of Medicine, University of California, San Diego, San Diego, CA, United States

## Article history:

Received 31 March 2023

Accepted 28 June 2023

Available online 26 July 2023

## Keywords:

Gene therapy

Cardiomyopathies

Genetic diseases

## ABSTRACT

Gene therapy strategies encompass a range of approaches, including gene replacement and gene editing. Gene replacement involves providing a functional copy of a modified gene, while gene editing allows for the correction of existing genetic mutations. Gene therapy has already received approval for treating genetic disorders like Leber's congenital amaurosis and spinal muscular atrophy. Currently, research is being conducted to explore its potential use in cardiology. This review aims to summarize the mechanisms behind different gene therapy strategies, the available delivery systems, the primary risks associated with gene therapy, ongoing clinical trials, and future targets, with a particular emphasis on cardiomyopathies.

© 2023 Sociedad Española de Cardiología. Published by Elsevier España, S.L.U. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

## Terapia génica para la insuficiencia cardiaca y las miocardiopatías

## RESUMEN

Las estrategias de terapia génica incluyen diversos enfoques, como la sustitución y la edición de genes. La sustitución proporciona una copia funcional de un gen alterado y la edición permite corregir una mutación genética preexistente. La terapia génica ya está aprobada para trastornos genéticos como la amaurosis congénita de Leber y la atrofia muscular espinal, y actualmente se estudia su uso en cardiología. En esta revisión se resume el mecanismo de las distintas estrategias de terapia génica, los sistemas de administración disponibles, los principales riesgos relacionados con la terapia génica, los ensayos clínicos en curso y los objetivos futuros, con especial atención a las miocardiopatías.

© 2023 Sociedad Española de Cardiología. Publicado por Elsevier España, S.L.U. Este es un artículo Open Access bajo la licencia CC BY-NC-ND (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

## Palabras clave:

Terapia génica

Miocardiopatías

Enfermedades genéticas

## Abbreviations

AAV: adeno-associated virus

DD: Danon disease

DMD: Duchenne muscular dystrophy

FD: Fabry disease

HCM: hypertrophic cardiomyopathy

LNP: lipid nanoparticle

Nab: neutralizing antibodies

## INTRODUCTION

Human gene therapy exerts its impact through the transcription and/or translation of transferred genetic material, and/or by integrating into the host genome. These therapies are administered

in the form of nucleic acids, viruses, or genetically engineered microorganisms. The objective of this review is to examine the present status of gene therapies, their application in the treatment of cardiovascular diseases, and future challenges, with a specific emphasis on cardiomyopathies.

## HISTORY OF GENE THERAPIES

Human gene therapy has undergone rapid development since Theodore Friedmann and Richard Roblin published the first accounts on its potential and challenges in 1972.<sup>1</sup> Since then, scientists have strived to develop a safe, long-lasting, and effective method for modifying and manipulating human cells to achieve therapeutic effects for various heritable diseases. In 1989, the first approved human gene therapy trial took place, using a retroviral vector to insert a gene encoding neomycin resistance into human tumor-infiltrating lymphocytes for the treatment of metastatic melanoma.<sup>2</sup> Just under a year later, another group of researchers conducted the first approved gene therapy trial for heritable genetic diseases. In that study, a retroviral vector delivered a functional copy of the adenosine deaminase gene to explanted T lymphocytes obtained from patients with adenosine deaminase-

\* Corresponding author.

E-mail address: [argiro.alessia@gmail.com](mailto:argiro.alessia@gmail.com) (A. Argirò).

✉ @AlessiaArgiro

deficient severe combined immune deficiency. Over a 2-year period, the 2 patients received multiple autologous infusions of corrected T cells.<sup>3</sup> These clinical trials laid the groundwork for the potential of gene therapy by demonstrating the feasibility, durability, and potential safety of using viral vectors to deliver genetic material as a therapeutic approach.

Despite these landmark studies, the death of 18-year-old Jesse Gelsinger in 1999 highlighted the dangers of gene therapy at the time. Mr. Gelsinger had partial ornithine transcarbamylase deficiency and participated in a trial aiming to use adenoviral vectors to deliver functional ornithine transcarbamylase genes. More than  $3.8 \times 10^{13}$  adenoviral particles were injected into his liver, triggering a cytokine storm and acute respiratory distress leading to his death.<sup>4</sup>

Clinical trials conducted throughout the 1990s and early 2000s encountered various challenges primarily related to unforeseen long-term toxicities and immune responses. A subset of patients enrolled in these trials developed leukemic malignancies as the retroviral vector used had the potential to activate proto-oncogenes.<sup>5,6</sup> Other patients mounted large immune responses against the viral vectors as well as to the newly transduced cells expressing the protein products of the gene therapy.<sup>7</sup>

In 2012, a bacterial defense mechanism called CRISPR/Cas9 that could be used to easily manipulate genomes, was discovered by the Nobel laureates Jennifer Doudna and Emmanuelle Charpentier laying the foundations for gene editing.<sup>8,9</sup> A month later, the first gene therapy was approved by the European Medicines Agency (an adeno-associated virus [AAV] vector encoding lipoprotein lipase, for the treatment of lipoprotein lipase deficiency).<sup>10</sup> In 2017, the first gene therapy was approved in the United States by the Food and Drug Administration (FDA) for the treatment of Leber congenital amaurosis.<sup>11</sup>

In 2020, the FDA released 6 guidelines, standardizing and streamlining the drug approval process for new gene therapies.<sup>12</sup> In response to the SARS-CoV-2 pandemic, gene therapy achieved a major breakthrough with the approval and adoption of 2 messenger RNA (mRNA)-based vaccines. These vaccines used novel lipoprotein nanoparticles to safely deliver the genetic payload into the cell cytoplasm.<sup>13,14</sup> Currently, 27 approved gene therapies are available in the United States with dozens of clinical trials underway.<sup>15</sup>

In the field of cardiology, some pioneering studies of gene therapy were first performed in 1990, in which a murine retrovirus was used to deliver a recombinant beta-galactosidase gene into the wall of an arterial segment.<sup>16</sup> In 1996, Dr Jeffrey Isner used a plasmid containing vascular endothelial growth factors, which was suspended in a hydrogel polymer and coated onto an angioplasty balloon that was applied to the distal popliteal artery to induce angiogenesis.<sup>17</sup> This approach laid the foundation for a series of clinical trials aimed at injecting naked plasmid DNA encoding for angiogenic factors like vascular endothelial growth factor into or at the walls of coronary and peripheral arteries, as well as into the myocardium itself.<sup>18–20</sup> However, none of these trials had a significant impact on clinical outcomes.

In 2007, the first gene therapy clinical trial for patients with heart failure aimed to determine the effects of delivering a gene expressing SERCA2a through an AAV to improve calcium handling within cardiomyocytes where the  $\text{Ca}^{2+}$  ATP-ase was down-regulated.<sup>21</sup> Unfortunately, the treatment failed to improve clinical outcomes.<sup>21,22</sup>

## TYPES OF GENE THERAPIES

In genetic diseases, loss-of-function mutations, usually non-sense, splice, frameshift or missense, may lead to a lack of protein

production or to the release of a nonfunctional protein. Gain-of-function mutations, typically from missense variants, determine the production of a protein with increased or different activity compared with the wild-type form.

Appropriate gene therapy approaches may target these alterations (figure 1). It is helpful to break down the different gene therapies into 2 categories based on their general treatment goals. Gene silencing through RNA interference or antisense oligonucleotide are not strictly considered to be gene therapy and are therefore not discussed in this manuscript.

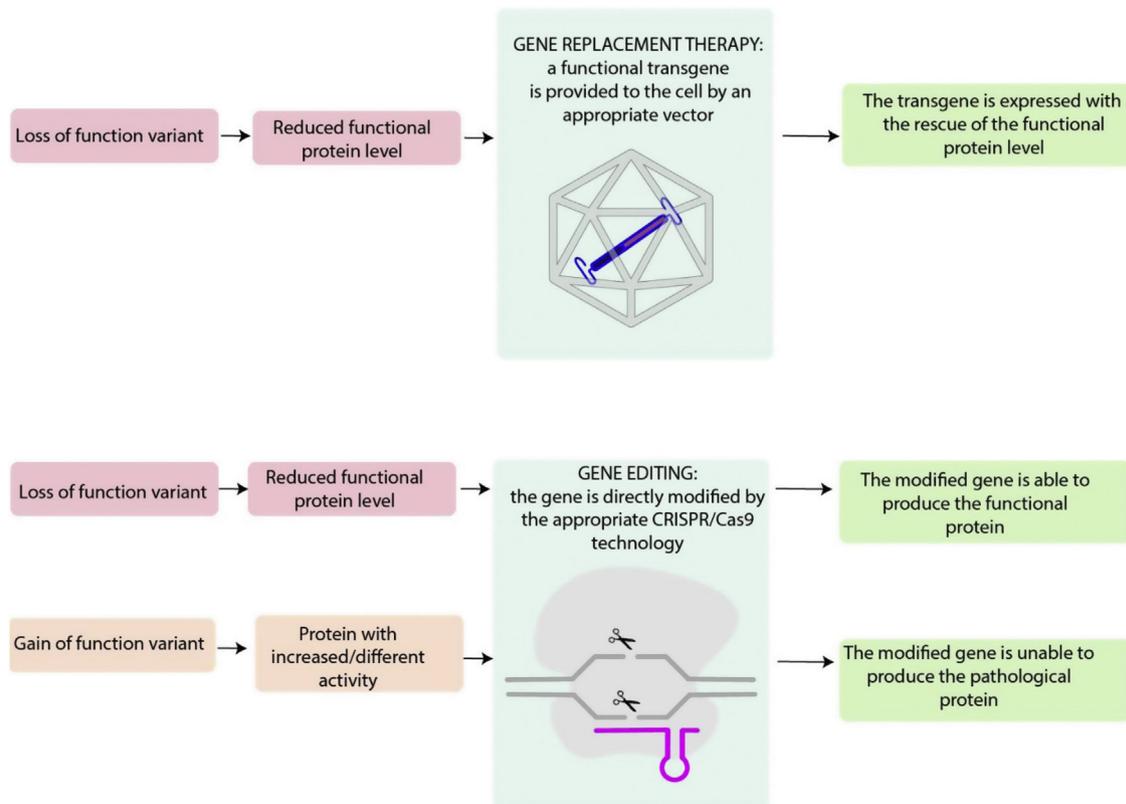
## GENE REPLACEMENT

Gene replacement aims to replace a defective gene with a functional copy to achieve a therapeutic effect mitigating the effects of a loss-of-function mutation (figure 2).<sup>23</sup> Gene replacement products can include plasmid DNA or genetic material enclosed in viral vectors, which allow the transduction of a foreign copy of the gene of interest into the target cell.<sup>24,25</sup> Plasmid-based gene therapy may potentially produce a durable effect while minimizing the genetic disruption of the cell's genome, resulting in a lower oncogenic potential compared with viral vector-based gene therapies.<sup>26</sup> However, plasmid delivery to the nucleus is difficult and protein expression may not be durable, especially in dividing cells.<sup>27,28</sup> Viral vector-based gene therapy is able to produce durable expression of gene products,<sup>29</sup> but this approach has toxicity risks that will be evaluated later in this article.

## GENOME EDITING

Unlike gene replacement therapy, in which a new gene is provided through an appropriate vector, genome editing consists of modifying the cell's endogenous genomic sequence and could be used for both loss and gain-of-function mutations. The type of edits desired may be obtained through CRISPR/Cas9 technology and include: conversion of DNA base pairs, deletion of DNA base pairs, insertion of DNA base pairs or a combination of the above (figure 3). CRISPR/Cas9 includes 2 fundamental components: a guide RNA to match a target gene (CRISPR, clustered regularly interspaced palindromic repeats), and Cas 9, an endonuclease that determines double-stranded DNA breaks (DSBs). DSB repair may be effected through nonhomologous end-joining, in which the broken DNA ends are religated, often with additional nucleotide deletions or insertions. Another mechanism is homology-directed repair (HDR), which involves using DNA templates to accurately repair the damaged DNA. HDR is only active in dividing cells.<sup>30</sup> CRISPR-Cas nucleases are usually used to disrupt target genes, since the indels after nonhomologous end-joining cause frameshift mutations that impede the production of a functional protein. Base editors contain a CRISPR-Cas nuclease unable to make DSBs (nickase) and a DNA deaminase enzyme and include cytosine base editors (CG- to TA) and adenine base editors (AT to GC). This technique does not require DSB or donor DNA templates, but cannot determine transversion point mutations.<sup>30</sup> Cas transposases may integrate large genomic sequences in target sites, but this technique has not been reported in mammalian cells.

Prime editing is a new technology that may determine all possible types of point mutations, small insertions and small deletions in a targeted way. Prime editors are composed of a Cas 9 domain fused to a reverse transcriptase and are guided to the target DNA by a prime editing guide RNA (pegRNA) that also encodes the desired edit. The reverse transcriptase copies the template from the pegRNA into the DNA that is incorporated into



**Figure 1.** Central illustration. Loss-of-function variants result in a diminished level of functional proteins, which can potentially be addressed through gene replacement therapy. This approach involves introducing a functional transgene into the affected cell, allowing for the restoration of the proper expression of functional protein. Genome editing includes several techniques that allow direct modification of the endogenous genome and, in the case of loss-of-function variants, may restore the functional protein, and, in the case of gain-of-function variant, may impede pathological protein expression.

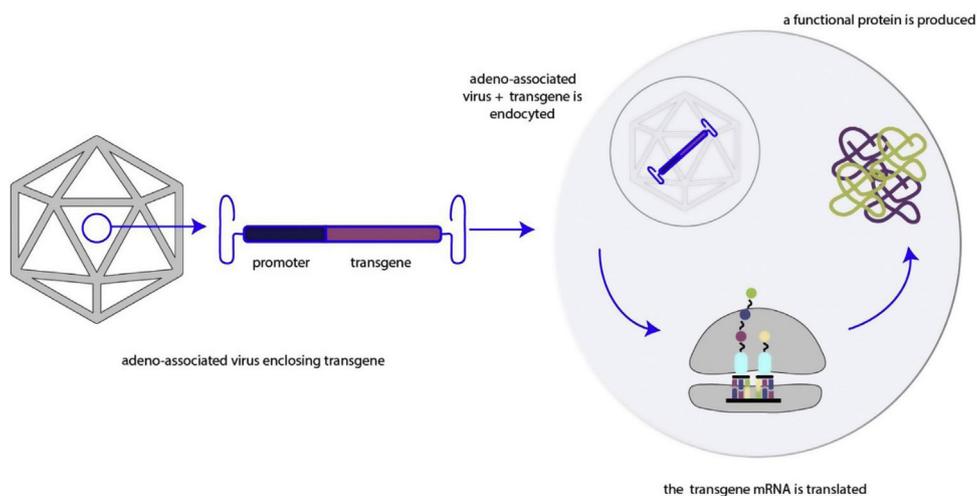
the DNA duplex.<sup>30</sup> Although highly promising, this approach is limited by the dimensions of the edited DNA and by the development of adequate delivery strategies.

## DELIVERY SYSTEMS

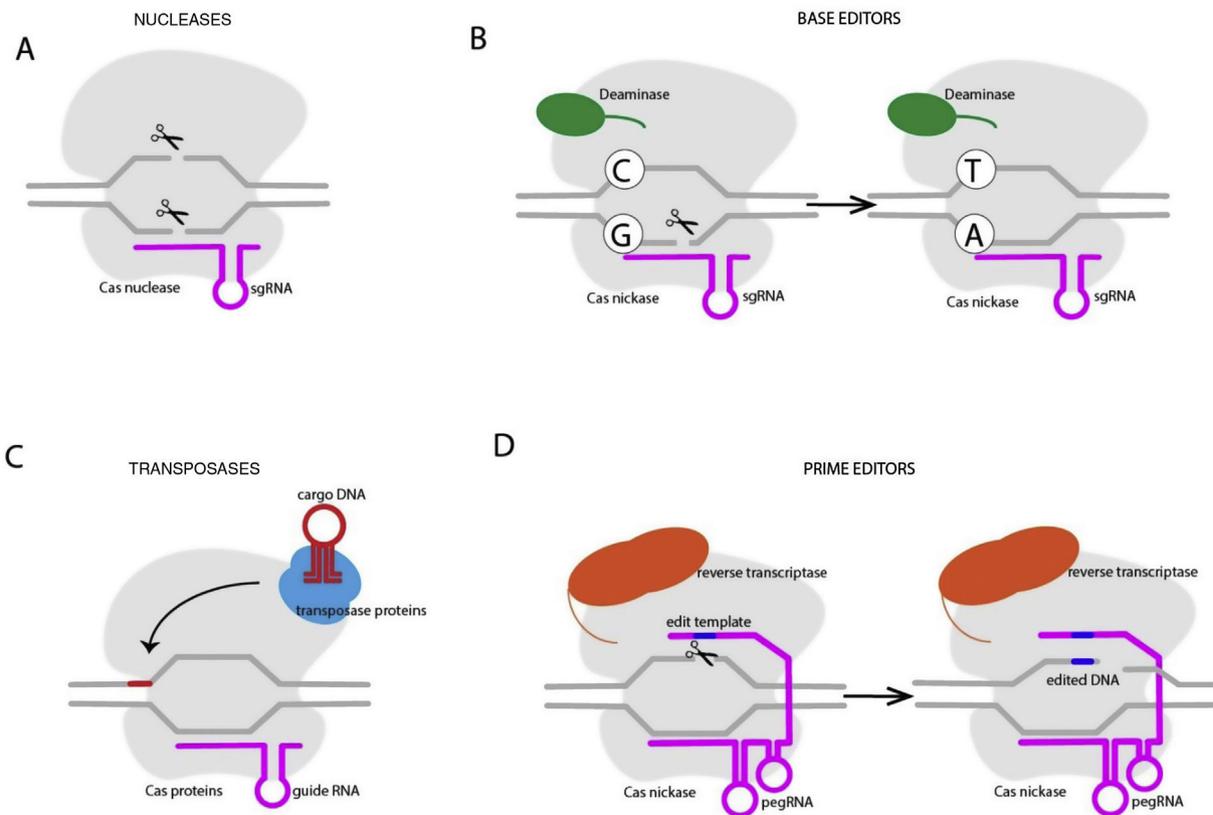
Several viral vectors are used to effectively deliver genetic material.

## Lentivirus

Lentiviruses are enveloped single-stranded RNA viruses that are able to infect both dividing and nondividing cells.<sup>31</sup> These viruses deliver their genetic payload by integrating it into the host cell's genome, creating a durable response within the infected cell and subsequent progeny cells.<sup>31</sup> However, this mechanism increases the potential for insertional oncogenesis, an adverse event that has been mitigated by the development of self-inactivating lentiviral vectors.<sup>32–34</sup>



**Figure 2.** An appropriate vector, in this case an adeno-associated virus, carries the transgene. The complex AAV + transgene is encycted by the cell and the transgene is transcribed and translated, allowing the production of a functional protein. AAV, adeno-associated virus; mRNA, messenger RNA.



**Figure 3.** Genome editing strategies. A: nucleases. B: base editors. C: transposases. D: prime editors. pegRNA, prime editing guide RNA; sgRNA, single guide RNA.

## Adenovirus

Adenoviruses (AVs) are nonenveloped icosahedral viruses with a double-stranded DNA genome that can hold large transgenes up to 54 kb.<sup>35</sup> The adenovirus has a high rate of gene transduction, including in cardiomyocytes, but the protein expression may be short-lived, lasting up to 4 weeks.<sup>36</sup> Adenovirus can also elicit a significant inflammatory response, and the presence of pre-existing antibodies resulting from prior exposure to wild-type adenovirus can diminish its transduction efficiency.<sup>37</sup>

## Adeno-associated virus

AAV is a nonenveloped virus with a single-stranded DNA genome that is replication-deficient.<sup>36</sup> Unlike wtAAV, recombinant AAVs (rAAV) do not integrate into the human chromosome and the viral genome remain extrachromosomal in a circular episomal form.<sup>38</sup> Some serotypes of AAV have a natural tropism for specific tissues, such as AAV9 for the liver and heart. Although AAVs are less immunogenic than AVs, they still have the potential for immune activation and increased clearance by pre-existing neutralizing antibodies.<sup>36,39</sup> The gene size capacity in AAV is limited to 4.7 kb. Therefore, an approach using dual AAV vectors expressing different genome sequences that merge before translation is under development.<sup>40</sup> rAAV are mostly sequestered in the liver after systemic injection and gene delivery to specific tissues, like muscular tissue including the heart, requires high doses of the virus. Hence, AAV capsid containing specific peptides have been developed to improve transfection in muscular cells known as MyoAAV.<sup>41</sup>

## Nonviral vectors

Nonviral vectors have been developed to overcome the limitations of the viral vectors and include polymers, lipids, inorganic particles and combined approaches. Most of these systems are cationic and may be combined with negatively charged DNA generating an overall positive vector-gene complex that may bind to the negatively charged molecules of the cellular membrane and be internalized.<sup>42</sup>

Lipid nanoparticles (LNPs) are an attractive solution as they can be conjugated with specific targeting ligands to reach specific tissues. These nanoparticles can be modified to reduce immunogenicity, and are affordable to produce at scale.<sup>43–46</sup> Much of the research around this novel vehicle centers around the delivery of RNA-based therapies. LNPs have been recently used in gene editing, but their effectiveness in transducing cardiomyocytes has been limited.<sup>47,48</sup>

## TOXICITY RISKS OF ADENO-ASSOCIATED VIRUS VECTORS

Immune response to AAV vectors may cause several adverse events, including liver toxicity and thrombotic microangiopathy. The immunological mechanisms and potential immunosuppressive treatments are reported in [table 1](#).

## Liver tototoxicity

Hepatotoxicity is the most common adverse event associated with systemic administration of AAV vectors. This adverse effect may present as elevated liver enzymes (AST, ALT), and liver failure.

**Table 1**  
Immune response to adeno-associated virus

Immune response to AAV	Trigger	Effect	Treatment/prevention
Pre-existing immunity	Originates from previous exposure to wtAAV Nab vs wtAAV prevalence up to 70% for AAV1 and AAV2, 45% for AAV6 and AAV9, and 38% for AAV8. <sup>49</sup> Nab seroprevalence is age-dependent, lowest value between 3 and 17 years <sup>50</sup>	Vector neutralization	Exclusion of patients with high titer Nab
Innate immunity	Nonspecific response and does not generate immunological memory triggered by capsid, genome, transgene product, dsDNA, dsRNA, unmethylated CpG motifs. <sup>51,52</sup>	Transduced cell damage Loss of transgene expression	Glucocorticoids
Adaptive immunity	Develops after innate immunity Activation and differentiations of T and B lymphocytes and creation of memory lymphocytes that may be reactivated	Block readministration Complement activation Neutralization of transgene product Transduced cell damage Loss of transgene expression	Glucocorticoids Rapamycin Tacrolimus Rituximab

dsDNA, double-stranded; dsRNA, double-stranded RNA; Nab, neutralizing antibodies; wtAAV, wild-type adeno-associated virus.

In clinical trials that evaluated the safety and efficacy of AAV carrying the FIX transgene to treat hemophilia B,<sup>53,54</sup> transgene expression gradually declined after 4 to 8 weeks and was associated with a transaminase increase and the detection of AAV-specific CD8 + T cells. The adverse event was limited, excluding patients with neutralizing antibodies (Nab) and improving liver expression of the transgene, and the transaminase elevation was controlled with a tapering course of steroids.<sup>54</sup>

### Thrombotic microangiopathy

Thrombotic microangiopathy is characterized by damage to arterioles and capillaries, leading to microvascular thrombosis with clinical manifestations including hemolytic anemia, thrombocytopenia, and acute kidney injury. Complement-mediated thrombotic microangiopathy (atypical hemolytic-uremic syndrome, aHUS) is a dose-related class effect of intravenous (iv) administration of AAV for cardiac and muscle tissues. The mechanism includes rapid IgM rise, capsid binding and classic and alternative complement pathway activation. aHUS has been described in patients in gene therapy clinical trials for spinal muscular atrophy, Duchenne muscular dystrophy (DMD), Danon disease and Fabry disease (FD). In some cases, eculizumab, a monoclonal IgG antibody that prevents the cleavage of C5 into C5a and AC5b and avoiding the formation of the membrane attack complex, was used to effectively treat the condition.<sup>55,56</sup> It is still unknown whether prophylactic use of eculizumab may be effective in preventing this adverse event.

### CLINICAL TRIALS EVALUATING GENE THERAPY IN CARDIOMYOPATHIES

Several clinical trials evaluating gene therapy in cardiomyopathies are ongoing (table 2). In this section, we describe the preliminary data from these trials, with a particular focus on gene replacement therapy using AAVs, currently the most frequently used technique.

#### Danon disease

Danon disease is a rare X-linked lysosomal disorder due to pathogenic variants in the *LAMP2* (lysosomal associated membrane protein 2) gene causing impaired autophagy and the accumulation of autophagic vacuoles in muscle tissue. Clinical

manifestations include rapidly progressive hypertrophic cardiomyopathy (HCM) with a median survival for males of 19 years without heart transplantation.<sup>57</sup> No disease-modifying pharmacological therapies are available for this condition. A single iv infusion of RP-A501, an AAV9 vector expressing *LAMP2B*, at a low dose ( $6.7 \times 10^{13}$  GC/kg) or high dose ( $1.1 \times 10^{14}$  GC/kg) in male patients aged 8 to 14 years and  $\geq 15$  years has been evaluated in an open-label phase 1 study.<sup>58</sup> The preliminary results of the study were presented at the 2022 Heart Failure Society of America annual scientific meeting. In 3 patients in cohort 1 ( $\geq 15$  years, low dose), RP-A501 increased *LAMP2B* expression by Western blot and immunochemistry in myocardial tissue. The 2 patients with observed immunosuppressive regime compliance showed high cardiac *LAMP2B* expression (67.8% and 92.4% vs normal control by immunochemistry) and improvement in New York Heart Association (NYHA) class and natriuretic peptides. All 3 patients showed improvement or stability of functional capacity measured by the 6-minute walk test, an increase in physical activity, and decreased autophagic vacuoles on endomyocardial biopsy. The most common adverse events included reversible platelet decrease, transaminase elevation, and steroid-induced myopathy. In the high-dose adult cohort, 1 patient developed complement-mediated thrombotic microangiopathy with thrombocytopenia and acute kidney injury requiring transient hemodialysis. Consequently, no further patients have been enrolled in the higher dose cohort; furthermore, the immunosuppressive regimen was adjusted, limiting the daily dose of corticosteroids, using sirolimus instead of tacrolimus to minimize the renal impact and continuing rituximab. In September 2022, an update of the phase 1 trial was presented.<sup>59</sup> In the adult cohort, a significant reduction in biomarkers, reduced left ventricular wall thickness, improvement or stability of NYHA class and Kansas city cardiomyopathy questionnaire score were sustained up to 36 months of follow-up. In the 2 patients in the pediatric cohort, with the improved immunomodulation protocol, there were no reports of thrombocytopenia, skeletal myopathy, late transaminitis or complement activation-related adverse events. Protein expression was improved at the latest follow-up at 3 and 6 months. Moreover, an improvement in biomarkers, reported symptoms and NYHA class was seen at 6 and 9 months. *LAMP2* expression was increased similar to the adult cohort at the same timeframe and the vacuolar area at the available endomyocardial biopsy was significantly reduced. On the basis of these positive results, the United States FDA has granted Regenerative Medicine Advanced Therapy designation to RP-A501, and phase 2 trial is scheduled to start in the second quarter of 2023.<sup>60</sup>

**Table 2**

Clinical trials on gene therapies in cardiomyopathies

Disease	Trial	Therapy	Actual enrolment	Main preliminary findings	Status
Danon disease X-linked LAMP2 deficiency Prevalence < 1/1*10 <sup>6</sup>	Gene Therapy for Male Patients With Danon disease Using RP-A501; AAV9.LAMP2B (NCT03882437)	RP-A501 rAAV9 capsid containing the human LAMP2B	5 adults and 2 pediatric patients	PEDIATRIC COHORT (6 & 9 months of FU): on enhanced immunomodulation (steroid + sirolimus) all AE were transient and reversible Absent or limited worsening of skeletal myopathy Increased LAMP2 expression, improvement in cardiac biomarkers, QOL, NYHA class  ADULT COHORT: Low dose well tolerated at 2-3-years Increased LAMP2 expression, improvement or stabilization NYHA class, QOL, LV hypertrophy, cardiac biomarkers	Not recruiting
Fabry disease X-linked Gal A deficiency Prevalence 1-5/10 000	Open Label, Study Of Efficacy and Safety Of AVR-RD-01 for Treatment-Naive Subjects With Classic Fabry Disease (NCT03454893)	AVR-RD-01 Autologous stem cell transplantation of CD34+ cells transduced with the lentivirus vector containing the human GLA gene	11 participants	Terminated due to disappointing engraftment data in 5 participants	Terminated
	Dose-Ranging Study of ST-920, an AAV2/6 Human Alpha-Galactosidase A Gene Therapy in Subjects With Fabry Disease (NCT04046224)	ST-920 recombinant AAV2/6 vector containing functional GLA gene	12 classic males and 1 female	Well tolerated at all doses No requirement for prophylactic immunosuppression Stable expression of Gal A for more than 2 years Patients on replacement therapy continue to be off it ↓40-65% lyso-Gb3 ↑QOL	Recruiting
	An Open label, Phase 1/2 Trial of Gene Therapy 4D-310 in Adults With Fabry Disease (NCT04519749)	4D-310 Engineered AAV vector C102 with high tropism for cardiomyocytes enclosing GLA transgene	6 patients with corticosteroids prophylaxis	At 12 months improvement in exercise capacity, QOL, GLS Adequate transgene delivery and expression 3 transient acute aHUS	Active, not recruiting
Duchenne muscular dystrophy X-linked DMD gene Prevalence 1-9/100 000	A Gene Transfer Therapy Study to Evaluate the Safety of SRP-9001 (Delandistrogene Moxeparvovec) in Participants With Duchenne Muscular Dystrophy (NCT03375164) Open label	SRP-9001 rAAVrh74 + codon-optimized human micro-dystrophin driven by a skeletal and cardiac muscle-specific promoter	4 pediatric patients Daily prednisolone 1 mg/kg, started 1 day before gene delivery	At 4 years increase of 9.4 points on North Star Ambulatory Assessment (NSAA) compared with a propensity-weighted external control	Active, not recruiting
	A Gene Transfer Therapy Study to Evaluate the Safety of and Expression From SRP-9001 (Delandistrogene Moxeparvovec) in Participants With Duchenne Muscular Dystrophy (DMD) (ENDEAVOR) (NCT04626674) Open label	SRP-9001	20 pediatric patients Expected enrolment 46 patients	3.2 points improvement in NSAA compared with propensity-weighted external control Two treatment related SAEs	Enrolling by invitation

**Table 2** (Continued)

Clinical trials on gene therapies in cardiomyopathies

Disease	Trial	Therapy	Actual enrolment	Main preliminary findings	Status
	Study of Fordadistrogene Movaparovec in Early Stage Duchenne Muscular Dystrophy (NCT05429372) Open label	Fordadistrogene movaparovec AAV9 vector expressing mini dystrophin	Estimated enrolment: 10 patients		Recruiting
	Study to Evaluate the Safety and Efficacy of PF-06939926 for the Treatment of Duchenne Muscular Dystrophy (NCT04281485) Phase 3	Fordadistrogene movaparovec	Estimated enrolment: 99 patients		Recruiting
	Microdystrophin Gene Transfer Study in Adolescents and Children With DMD (IGNITE DMD) (NCT03368742) Open label	SGT-001 AAV9 expressing microdystrophin	Estimated enrolment: 16 patients		Active, not recruiting
Limb-girdle muscular dystrophy	Gene Delivery Clinical Trial of SRP-9003 (Bidridistrogene Xeboparovec) for Participants With Limb-Girdle Muscular Dystrophy, Type 2E (LGMD2E) (Beta-Sarcoglycan Deficiency) (NCT03652259) Open label	SRP-9003	6 patients	Not presented yet	Active, not recruiting
Friedreich ataxia Autosomal recessive GAA hyper expansion in intron 1 of frataxin (FXN) gene	Gene Therapy for Cardiomyopathy Associated With Friedreich's Ataxia (NCT05445323) Open label	LX2006 AAVrh.10hFXN	Estimated enrolment: 10 patients	Not yet presented	Recruiting
Pompe disease Autosomal recessive Mutations in acid alpha-glucosidase gene (GAA)	Gene Transfer Study in Patients With Late-Onset Pompe Disease (FORTIS) (NCT04174105) Open label	AT845 AAV8 carrying functional copy of GAA gene	Estimated enrolment: 12 patients	3 of 4 patients withdrew from ERT at 17, 10 and 24weeks All patients showed stable force capacity Stable or improved PRO Most AEs were grade 1 and unrelated to drug 1 patient had peripheral sensory neuropathy and the trial was put on clinical hold until January 2023	Recruiting
	AAV2/8-LSPhGAA (ACTUS-101) in Late-Onset Pompe Disease (NCT03533673) Open label	ACTUS-101 AAV8-LSPhGAA	Estimated enrolment: 13 patients Prednisone as immunoprophylaxis	3 patients low dose (1.6 × 10 <sup>12</sup> vector genomes/kg), 52 weeks Sustained serum GAA activities from 101% to 235% of baseline Off ERT from week 26 No treatment relates SAEs No anti-capsid T cell responses	Recruiting
	A Gene Transfer Study for Late-Onset Pompe Disease (RESOLUTE) (NCT04093349) Open label	SPK-3006 rAAV with bioengineered Rh74-derived capsid and an expression cassette to drive the expression of secretable GAA protein	Estimated enrolment: 30 patients	Not yet presented	Active, not recruiting

**Table 2** (Continued)  
Clinical trials on gene therapies in cardiomyopathies

Disease	Trial	Therapy	Actual enrolment	Main preliminary findings	Status
TTR amyloidosis Autosomal dominant TTR gene	Safety Study of Recombinant Adeno-Associated Virus Acid Alpha-Glucosidase to Treat Pompe Disease (NCT00976352) Open label	rAAV1-CMV-hGAA Engineered AAV that carried a normal copy of the GAA gene Intradiaphragmatic administration	9 patients with early onset Pompe disease	No drug-related adverse events but the surgical procedure led to pain and pneumothorax In 5 ventilator-dependent patients, larger unassisted tidal volume	Completed but no further trials announced
	Readministration of Intramuscular AAV9 in Patients With Late-Onset Pompe Disease (AAV9-GAA_IM) (NCT02240407)	rAAV9-DES-hGAA intramuscular administration after immunomodulation strategy (rituximab+sirolimus) and readministration	Current enrollment: 2 patients	Not yet released	Completed
	Study to Evaluate Safety, Tolerability, Pharmacokinetics, and Pharmacodynamics of NTLA-2001 in Patients With Hereditary Transthyretin Amyloidosis With Polyneuropathy (ATTRv-PN) and Patients With Transthyretin Amyloidosis-Related Cardiomyopathy (ATTR-CM) (NCT04601051)	NTLA-2001 Lipid nanoparticle enclosing a messenger RNA for Cas 9 protein and a single guide RNA targeting the human gene encoding TTR	12 patients	1 grade 3 transient infusion-related reaction Rapid and sustained TTR reduction greater than 90% sustained up to 6 months of FU	Recruiting

AAV, adeno-associated virus; AE, adverse events; AHUS, atypical hemolytic uremic syndrome; DMD, Duchenne muscular dystrophy; ERT, enzyme replacement therapy; FU, follow-up; GAA, acid alpha-glucosidase gene; Gal A, alpha-galactosidase A; GLA, galactosidase alpha gene; GLS, global longitudinal strain; LAMP, lysosomal associated membrane protein-2; LAMP2B, lysosome-associated membrane protein 2 isoform B; Lyso-Gb3, globotriaosylsphingosine; LV, left ventricle/ventricular; NYHA, New York Heart Association; NSAA, North Star Ambulatory Assessment; PRO, patient reported outcome; QOL, quality of life; rAAV, recombinant adeno-associated; TTR, transthyretin.

## Fabry disease

FD is an X-linked lysosomal storage disorder due to pathogenic variants in the *GLA* (galactosidase alpha) gene. This alteration leads to various degrees of alpha-galactosidase (Gal A) deficiency and accumulation of glycosphingolipids, mostly globotriaosylceramide (Gb3) and its deacylated derivative globotriaosylsphingosine (lyso-Gb3), in target organs such as the heart, kidney and blood vessels. The current standard of care includes iv enzyme replacement therapy or oral chaperone therapy, which, although effective in ameliorating quality of life, neuropathic pain, gastrointestinal manifestation and renal function, has limited efficacy in cardiomyopathy.<sup>61</sup>

FD is a suitable candidate for gene therapy as it is a monogenic disease and clinical effects can be achieved with 10% activity of normal Gal A levels. Several studies are currently evaluating the safety and efficacy of gene therapy in FD.

### AVR-RD-01

In an open-label study assessing AVR-RD-01, autologous stem cell transplantation of CD34+ cells transduced with a lentivirus vector containing the human *GLA* gene was used. However, recruitment for the trial has been halted due to disappointing engraftment data in 5 participants.<sup>62</sup>

### ST-920

By means of ST-920 (isaralgagene civaparvovec), functional *GLA* gene is delivered to the liver via one-time infusion of a recombinant AAV2/6 vector without the need for immunosuppressive therapy. This approach is currently under study in the phase 1/2 STAAR trial (NCT04046224). In the dose escalation phase of the trial, 9 male patients with classic FD with mild/moderate disease severity were enrolled. In the expansion phase, 5 patients received a dose of  $5 \times 10^{13}$  vg/kg. As of the October 2022 data cutoff date, no treatment-related adverse events greater than grade 2 have been reported in the 13 patients studied.

All 9 patients treated in the dose escalation phase across the 4 dose cohorts ( $0.5 \times 10^{13}$  vg/kg,  $1 \times 10^{13}$  vg/kg,  $3 \times 10^{13}$  vg/kg, and  $5 \times 10^{13}$  vg/kg) showed dose-dependent, supraphysiological and sustained Gal A activity ranging from nearly 4-fold to 68-fold of the mean normal levels for more than 2 years for the patient with the longest follow-up. A stable and dose-dependent reduction in Lysogb3 concentration was observed at high baseline levels ( $> 80$  ng/mL) and, at the highest dose, also at low plasma levels ( $< 25$  ng/mL) up to 25 months of follow-up. The therapy was also associated with a potential improvement in Fabry nephropathy. Indeed, a reduction in Gb3 inclusions and podocyte loss has been reported in patient 9.

### C102

C102, an engineered AAV vector with high tropism for cardiomyocytes, was found to transduce nonhuman primate heart tissue in vivo along with human cardiomyocytes and endothelial cells in vitro better than AAV1, AAV8 or AAV9-serotyped vectors, allowing the use of low dose iv delivery.<sup>63</sup>

4D-310 (vector C102 enclosing *GLA* transgene) promotes high and stable blood Gal A levels that can be taken up by cells in the proximity, but also permits transduction directly to target tissue cells such as cardiomyocytes, kidney (glomeruli including podocytes), and blood vessels.<sup>64</sup>

The phase 1/2 open-label clinical trial INGLAXA-1 and -2 is currently assessing the safety and tolerability of 4D-310 in patients with classic or late-onset FD with cardiac involvement on or off enzyme replacement therapy with corticosteroid prophylactic immunosuppression. In cohort 1 (1<sup>^</sup>13 vg/kg with corticosteroid immune regimen), 6 patients have been enrolled, 4 had enzyme replacement therapy experience and 1 was on chaperone therapy. Global longitudinal strain and peak VO<sub>2</sub> improved from baseline to week 52 in 2 of 3 patients. One patient underwent cardiac biopsy in week 6, and the tissue proved healthy with no signs of inflammation. In situ hybridization showed uptake in cardiomyocytes only, with around 50% of cardiomyocytes positive for transgene expression. On Immunohistochemistry, AGA protein was expressed in all samples. Genome delivery was adequate, being 4.4 vector copies per cardiomyocyte and RNA expression was 16.2 transcript copies per cardiomyocyte.

Three patients experienced transient acute aHUS, with onset at day 3 to 7; 1 patient required no intervention, 1 was treated with eculizumab, and 1 was treated with eculizumab and temporary dialysis. After the aHUS dose limiting toxicity, the FDA put the program on clinical hold.

The patients' immune response was therefore thoroughly assessed and the patients developed aHUS showed predosing complement activation. The next steps for this trial include alignment with the FDA to remove the clinical hold and resume enrolment following protocol amendment including implementation of a rituximab/sirolimus immunosuppressive regimen, and adding prescreening for complement and excluding patients with predosing complement activation.

### Duchenne muscular dystrophy

The large size of the *DMD* gene poses challenges for the potential application of gene transfer therapies. However, a smaller gene construct may be able to alleviate the disease phenotype and several recombinant genes encoding mini/micro-dystrophin have been created.<sup>65</sup>

A phase 3 clinical trial on fordadistrogene movaparvovec (SRP-9001), an AAV9 vector expressing mini-dystrophin, is ongoing after an initial clinical hold from the FDA due to the death of a participant (NCT04281485).<sup>66</sup>

SRP-9001 is formed by rAAV rh74 (rAAVrh74) and a codon-optimized human micro-dystrophin driven by a skeletal and cardiac muscle-specific promoter with enhanced cardiac expression.<sup>67</sup> Preliminary results of the phase 1/2a nonrandomized controlled trial have shown an improvement in functional motor abilities in 4 pediatric patients after 4 years and a phase 3 trial is ongoing.<sup>68</sup>

The exon skipping technique allows silencing of specific exons determining the restoration of the reading frame of a mutated gene by means of silencing RNA, antisense oligonucleotides or gene editing. Exon skipping is a mutation-specific technique and, in *DMD* it leads to the production of shortened but functional dystrophin protein. Four antisense oligonucleotide therapies have been approved by the FDA for *DMD* treatment and an AAV vector delivering small nuclear RNA that contains antisense sequences targeting the duplicated *DMD* exon 2 is under study.<sup>65</sup>

### Friedreich ataxia

A phase 1A/1B study is currently evaluating the safety and efficacy of a serotype rh.10 AAV delivering a functional frataxin (*FXN*) gene for the treatment of Friedreich ataxia cardiomyopathy (NCT05445323). In a murine model, this approach reversed cardiac

mass, improved cardiac function, corrected *FXN*-related biochemical defects and reversed stress-induced cardiac dysfunction.<sup>69,70</sup>

Ribonucleoprotein complex for the excision of the acid alpha-glucosidase (GAA) repeat sequences in *FXN* has shown promising results in a murine model and a clinical trial is in the planning phase.<sup>71</sup>

### Pompe disease

Several gene therapies designed to provide a functional copy of the *GAA* gene are at various stages of development.

In 2015, the first-in-human trial of diaphragmatic gene therapy (AAV1-CMV-GAA) to treat respiratory and neural dysfunction in early-onset Pompe disease was conducted. No adverse events related to the drug have been reported, but intradiaphragmatic injection led to pneumothorax and lung contusion, and ventilatory performance was only modestly improved.

The initial analysis of 3 patients with late-onset Pompe disease in the trial of ACTUS-101, and AAV8 vector expressing human *GAA* under the transcriptional control of a liver-specific promoter (AAV2/8-LSPH-GAA), showed a sustained increase in *GAA* levels and no treatment-related SAEs.<sup>72</sup>

AT845 is an AAV8 designed to express human *GAA* specifically in skeletal muscle and heart and is currently under study in patients with late-onset Pompe disease with promising preliminary results.<sup>73</sup>

### TRANSTHYRETIN AMYLOIDOSIS

The CRISPR-Cas9 system is under evaluation for the treatment of transthyretin (TTR) amyloidosis. TTR amyloidosis is characterized by progressive deposition of misfolded senescent or mutated transthyretin in various tissues including the heart and peripheral nervous system. NTLA-2001 is included in an LNP opsonized by apolipoprotein E that acts as a carrier system. The active components are a messenger RNA for a Cas 9 protein and a single guide RNA (sgRNA) targeting the human gene encoding TTR. NTLA-2001 LNP is administered iv and reaches the liver where is taken up by low-density lipoprotein receptor expressed by hepatocytes. The Cas9 RNA molecule is translated in the cytoplasm, producing the Cas9 endonuclease enzyme. Along with the Cas9 endonuclease, the TTR-specific sgRNA forms a CRISPR-Cas9 ribonucleoprotein complex. The latter enters the nucleus, the sgRNA binds the target DNA, and the CRISPR-Cas9 complex may unwind the helix and reach the target gene. The *TTR* gene is eventually cleaved and endogenous DNA repair through nonhomologous end-joining introduces insertions or deletions of bases (indels) that result in the reduction in the levels of functional mRNA and transthyretin. In human hepatocytes, NTLA-2001 produced saturating levels of *TTR* editing ( $\geq 93.7\%$ ), resulting in a reduction of at least 91% of *TTR* mRNA expression and TTR protein. Next-generation sequencing showed that NTLA-2001 induced TTR knock-out. Preclinical studies in transgenic mice and cynomolgus monkeys showed that *TTR* editing led to a sustained reduction in TTR levels over a 12-month period. An open-label, single dose, proof-of-concept study has enrolled 6 patients, aged 46 to 64 years, with hereditary TTR amyloidosis polyneuropathy. All patients completed the infusion and 1 patient had a grade 1 infusion-related reaction. Coagulation values, platelet count and transaminase levels remained within the reference ranges. A rapid and sustained dose-dependent reduction in TTR concentration was observed, with a reduction at day 28 between 47% and 56% in the lower dose group (0.1 mg/kg) and between 80% and 96% in the higher dose group (0.3 mg/kg).<sup>47</sup>

Data from the 12 patients with hereditary or wild-type cardiomyopathy in the dose escalation phase (9 patients on 0.7 mg/kg, 3 patients on 1.0 mg/kg) have been presented. NTLA-2001 was well tolerated and there was a single grade 3 infusion-related reaction, which resolved without clinical sequelae. All patients achieved a TTR concentration reduction greater than 90% by day 28 that was sustained throughout the 6 months of follow-up.<sup>74</sup>

## FUTURE TARGETS

### MYH7

Preclinical models for CRISPR-Cas9 gene editing are also under study for HCM. The latter is defined by a left ventricular thickness greater than 15 mm and the disease may be caused by pathogenic variants in genes encoding sarcomeric proteins. The main clinical manifestations include reduced exercise capacity, heart failure, and arrhythmias. Several approaches have been proposed. Chai et al.<sup>75</sup> used a dual AAV9 delivery system enclosing an adenine base editing (ABEmax-VRQR, which mediates the conversion of A•T to G•C without DNA cleavage) and a guide RNA (h403\_sgRNA) to target the pathogenic missense variant in the *MYH7* gene p.Arg403Gln. The treatment corrected the target pathogenic adenine at 98% efficiency in induced pluripotent stem cells derived from patients with HCM with minimal bystander editing of nearby adenines and low DNA off-target editing. The differentiated cardiomyocytes had a normal contractile force and cellular energetics compared with uncorrected cardiomyocytes. In a humanized mouse model of HCM, intrathoracic injection of a single dose of dual AAV9 encoding ABEmax-VRQR and the h403\_sgRNA resulted in correction of the pathogenic allele, a reduction in pathogenic transcripts, and a reduction in cardiac hypertrophy and histopathologic remodeling.

Reichart et al.<sup>40</sup> used a similar dual AAV-based strategy to deliver an ABE and gRNA to correct the same pathogenic variant in a nonhumanized mice model, obtaining a correction of the pathogenic variant in  $\geq 70\%$  of ventricular cardiomyocytes and preventing the development of the pathological phenotype. As an alternative approach, AAV9 delivery of RNA-guided Cas9 nuclease was tested. The technique effectively inactivated the pathogenic allele, but there was also reduced contractile function with higher doses, demonstrating accidental editing of the wild-type allele.

### MYBPC3

Loss-of-function mutations in myosin-binding protein C3 (*MYBPC3*) together with *MYH7* are the most common genetic causes of HCM. Reduced *MYBPC3* in the sarcomere eventually leads to an increase in myosin heads available for actin interaction and hypercontractility.

A single administration of AAV9-*MYBPC3* in homozygous *MYBPC3*-targeted knock-in mice mimicking a neonatal form of hypertrophic cardiomyopathy prevented the development of cardiac hypertrophy through 34 weeks of observation, and, in a dose-dependent manner, increased *MYBPC3* mRNA and protein. Furthermore, the therapy unexpectedly reduced the pathological mRNA species concentration. Probably, since the transgene *MYBPC3* protein would be more stably integrated in the sarcomere, a feedback mechanism may downregulate the transcription of the mutant protein.<sup>76</sup> In a further study, since the *MYBPC3* N-terminal domain is involved in the interactions with actin and myosin, the transfer of the sole N-terminal domain of *MYBPC3* through AAV9 compared with full-length has been evaluated in a cMyBPC-null

murine model (cMyBPC<sup>-/-</sup>). The transfer of the N-terminal domain was sufficient to prevent the macroscopic (left ventricular hypertrophy) and microscopic (myocardial disarray and fibrosis) HCM phenotype. However, it is still unknown whether AAV therapy may rescue the HCM phenotype and induce reverse remodeling in overt HCM.<sup>77</sup> A phase 1 clinical trial to evaluate the safety, tolerability and clinical efficacy of a single iv infusion of an AAV gene therapy for the treatment of *MYBPC3* mutation-related HCM is scheduled to begin in the third quarter of 2023.<sup>78</sup>

### BAG3

The *BAG3* (BCL2-associated athanogene 3) gene is highly expressed in the heart, skeletal muscle, and central nervous system. *BAG3* in cardiomyocytes enhances contractility by linking the beta-adrenergic receptor and L-type Ca<sup>2+</sup> channel, facilitates autophagy interacting with heat shock proteins, provides support for the sarcomere by linking actin with the Z-disc, and has antiapoptotic properties through BCL2 binding.<sup>79</sup> Truncating or deletion variants in the *BAG3* gene causing reduced levels of *BAG3* protein have been associated with dilated cardiomyopathy (DCM).<sup>80</sup> Human DCM cardiomyocytes have reduced sarcomere contractile function and impaired myofilament turnover with misfolded/dysfunctional proteins that remain integrated in the sarcomere. Moreover, myofilament expression of *BAG3* decreases in DCM and is correlated with reduced contractile function. In mice receiving a myocardial infarction, the administration of recombinant AAV9 vector expressing mouse *BAG3* gene restored cardiac function and myofilament turnover.<sup>81</sup> Furthermore, iv delivery of adeno-associated virus 9 (AAV9)-*BAG3* in *Bag3* haplo-insufficient mice prevented the onset of reduced ejection fraction.<sup>82</sup>

In a recent report, catheter-based retrograde coronary sinus infusion was safely used to deliver low doses of AAV9-*BAG3* in healthy mini pigs, resulting in diffuse transduction in the myocardium.<sup>83</sup> A phase 1/2 clinical trial to evaluate the safety and efficacy of a single administration via retrograde coronary sinus infusion in patients with reduced ejection fraction and *BAG3* haploinsufficiency is the planning phase.<sup>60</sup>

### PKP2

Arrhythmogenic cardiomyopathy is a myocardial disease characterized by fibrofatty replacement of the right or left ventricle, or both. The structural modifications create a proarrhythmic milieu and determine progressive chamber dilation and systolic dysfunction. Arrhythmogenic cardiomyopathy in 40% to 50% of cases is associated with mutations in desmosomal genes, with plakophilin-2 (*PKP2*) being the most frequent. An AAVrh10 vector expressing the *PKP2* gene (LX2020) has been evaluated in *PKP2* mutant mice. LX2020 showed a dose-dependent improvement in survival of the mice, a reduction in ectopic beats, and amelioration of left and right ejection fraction.<sup>84</sup> Preclinical data on *PKP2* knock-out mice have also been reported for TN-401 (AAV9 vector expressing *PKP2*), showing a reduction in ventricular arrhythmias, prevention of right and left ventricular dysfunction, and extension of the life span. Furthermore, the therapy proved effective in restoring desmosomes and gap junctions and in preventing fibrosis.<sup>85</sup> Clinical trials evaluating these strategies are in the design phase.

### PRKAG2

*PRKAG2* mutations lead to a glycogen storage cardiomyopathy characterized by hypertrophic phenotype, supraventricular

arrhythmias and atrioventricular blocks. A CRISPR-Cas9 system combined with AAV9 has been used to disrupt the mutant *PRKAG2* allele encoding the H503 mutation in knock-in mice. A single systemic injection of the product at day 4 or day 42 restored the morphology and function in the mice model, reducing left ventricular thickness and myocardial glycogen content.<sup>86</sup>

## LMNA

Hutchinson-Gilford progeria syndrome is characterized by premature aging and death due to cardiovascular causes and is provoked by mutations in the *LMNA* gene that is translated into progerin, exerting a dominant-negative effect. CRISPR-Cas9 technology has been used to generate mice that ubiquitously express progerin and lamin C and lack lamin A and allows progerin suppression and lamin A restoration in a time- and cell-type-specific manner on Cre recombinase activation. Progerin suppression and lamin A restoration restricted to vascular smooth cells and cardiomyocytes prevented vascular damage and normalized the lifespan in this murine model.<sup>87</sup>

## CURRENT CHALLENGES

Gene therapy could be a real game changer for different diseases; however, several issues need to be resolved. The greatest challenges with AAV gene therapy include improving transduction efficacy, finding the best strategy to target specific tissues, reducing the administered doses to minimize possible adverse events, identifying the most effective immunosuppressive strategies to blunt the immune response and eventually allow a readministration of the drug.

Gene editing systems may have off-target activity, namely altering the genome in regions other than the target site. High fidelity Cas variants can reduce off-target editing but are also associated with lower on-target activity.<sup>30</sup>

Although several options have been developed, a tissue-specific, nonimmunogenic adequate delivery system remains an unmet need.

Patient selection remains unsolved; indeed, it is still unknown whether the treatment could rescue an already overt phenotype or if it should be administered in the early phase of the disease.

Long-term durability and long-term effects are still unknown.

Further problems include affordability, regulation, and access to the therapy. All these approaches have high manufacturing costs and access to therapy may be limited.

## CONCLUSION

Gene therapy is an evolving and promising field that already allows targeted treatment for severe diseases like Leber congenital amaurosis and spinal muscular atrophy. Safety and durability issues remain to be resolved; however, several ongoing clinical trials will hopefully provide answers and expand the technique to other conditions.

## FUNDING

The authors received no specific funding for this work.

## AUTHORS' CONTRIBUTIONS

E. Adler contributed in the work through the activities of conceptualization, supervision, review, and editing. A. Argirò and

J. Ding wrote the original draft and made edits under E. Adler's supervision.

## CONFLICTS OF INTEREST

E. Adler participates in the Scientific Advisory Board for Medtronic, Fuji, and SanaChief. E. Adler works as scientific officer for Lexeo Therapeutics, and as a consultant for Abiomed, AstraZeneca, Ionis, Medtronic, Abbott, and Novartis. E. Adler is part of the advisory board and shareholder for Rocket Pharmaceuticals. E. Adler is a founder of the scientific board and shareholder of ResQue Therapeutics. A. Argirò and J. Ding have no conflicts of interest to declare.

## REFERENCES

- Friedmann T, Roblin R. Gene Therapy for Human Genetic Disease?: Proposals for genetic manipulation in humans raise difficult scientific and ethical problems. *Science*. 1972;175:949–955.
- Rosenberg SA, Aebbersold P, Cornetta K, et al. Gene Transfer into Humans – Immunotherapy of Patients with Advanced Melanoma Using Tumor-Infiltrating Lymphocytes Modified by Retroviral Gene Transduction. *N Engl J Med*. 1990;323:570–578.
- Blaese RM, Culver KW, Miller AD, et al. T Lymphocyte-Directed Gene Therapy for ADA-SCID: Initial Trial Results After 4 Years. *Science*. 1995;270:475–480.
- Verma IM. A Tumultuous Year for Gene Therapy. *Mol Ther*. 2000;2:415–416.
- Hacein-Bey-Abina S, Garrigue A, Wang GP, et al. Insertional oncogenesis in 4 patients after retrovirus-mediated gene therapy of SCID-X1. *J Clin Invest*. 2008;118:3132–3142.
- Nienhuis AW, Dunbar CE, Sorrentino BP. Genotoxicity of Retroviral Integration In Hematopoietic Cells. *Mol Ther*. 2006;13:1031–1049.
- Mingozzi F, High AK. Immune Responses to AAV in Clinical Trials. *Curr Gene Ther*. 2011;11:321–330.
- Jinek M, Chylinski K, Fonfara I, Hauer M, Doudna JA, Charpentier E. A Programmable Dual-RNA-Guided DNA Endonuclease in Adaptive Bacterial Immunity. *Science*. 2012;337:816–821.
- Doudna JA, Charpentier E. The new frontier of genome engineering with CRISPR-Cas9. *Science*. 2014;346:1258096.
- Flemming A. Pioneering gene therapy on brink of approval. *Nat Rev Drug Discov*. 2012;11:664–664.
- Morrison C. Landmark gene therapy poised for US approval. *Nat Rev Drug Discov*. 2017;16:739–741.
- U.S. Food and Drug Administration. Cellular & Gene Therapy Guidelines. 2 June 2023. Available at: <https://www.fda.gov/vaccines-blood-biologics/biologics-guidances/cellular-gene-therapy-guidances>. Accessed 6 Mar 2023.
- Walsh EE, Frenck RW, Falsey AR, et al. Safety and Immunogenicity of Two RNA-Based Covid-19 Vaccine Candidates. *N Engl J Med*. 2020;383:2439–2450.
- Abu Abed OS. Gene therapy avenues and COVID-19 vaccines. *Genes Immun*. 2021;22:120–124.
- Mullard A. Gene-editing pipeline takes off. *Nat Rev Drug Discov*. 2020;19:367–372.
- Nabel EG, Plautz G, Nabel GJ. Site-Specific Gene Expression in Vivo by Direct Gene Transfer into the Arterial Wall. *Science*. 1990;249:1285–1288.
- Isner JM, Pieczek A, Schainfeld R, et al. Clinical evidence of angiogenesis after arterial gene transfer of phVEGF165 in patient with ischaemic limb. *Lancet*. 1996;348:370–374.
- Kastrup J, Jørgensen E, Rück A, et al. Direct intramyocardial plasmid vascular endothelial growth factor-A165 gene therapy in patients with stable severe angina pectoris. *J Am Coll Cardiol*. 2005;45:982–988.
- Stewart DJ, Kutryk MJ, Fitchett D, et al. VEGF Gene Therapy Fails to Improve Perfusion of Ischemic Myocardium in Patients With Advanced Coronary Disease: Results of the NORTHERN Trial. *Mol Ther*. 2009;17:1109–1115.
- Hedman M, Hartikainen J, Syväne M, et al. Safety and Feasibility of Catheter-Based Local Intracoronary Vascular Endothelial Growth Factor Gene Transfer in the Prevention of Postangioplasty and In-Stent Restenosis and in the Treatment of Chronic Myocardial Ischemia: Phase II Results of the Kuopio Angiogenesis Trial (KAT). *Circulation*. 2003;107:2677–2683.
- Greenberg B, Butler J, Felker GM, et al. Calcium upregulation by percutaneous administration of gene therapy in patients with cardiac disease (CUPID 2): a randomised, multinational, double-blind, placebo-controlled, phase 2b trial. *Lancet*. 2016;387:1178–1186.
- Cannatà A, Ali H, Sinagra G, Giacca M. Gene Therapy for the Heart Lessons Learned and Future Perspectives. *Circ Res*. 2020;126:1394–1414.
- Pipe SW, Gonen-Yaacovi G, Segurado OG. Hemophilia A gene therapy: current and next-generation approaches. *Expert Opin Biol Ther*. 2022;22:1099–1115.
- Gill DR, Pringle IA, Hyde SC. Progress and Prospects: The design and production of plasmid vectors. *Gene Ther*. 2009;16:165–171.
- Deyle DR, Russell DW. Adeno-associated virus vector integration. *Curr Opin Mol Ther*. 2009;11:442–447.

26. Lufino MM, Edser PA, Wade-Martins R. Advances in High-capacity Extrachromosomal Vector Technology: Episomal Maintenance. *Vector Delivery and Transgene Expression Mol Ther.* 2008;16:1525–1538.
27. Wooddell CI, Reppen T, Wolff JA, Herweijer H. Sustained liver-specific transgene expression from the albumin promoter in mice following hydrodynamic plasmid DNA delivery. *J Gene Med.* 2008;10:551–563.
28. Herweijer H, Zhang G, Subbotin VM, Budker V, Williams P, Wolff JA. Time course of gene expression after plasmid DNA gene transfer to the liver. *J Gene Med.* 2001;3:280–291.
29. Mendell JR, Al-Zaidy SA, Rodino-Klapac LR, et al. Current Clinical Applications of In Vivo Gene Therapy with AAVs. *Mol Ther.* 2021;29:464–488.
30. Anzalone AV, Koblan LW, Liu DR. Genome editing with CRISPR-Cas nucleases, base transposases and prime. *Nat Biotechnol.* 2020;38:824–844.
31. Fleury S, Simeoni E, Zuppinger C, et al. Multiply Attenuated, Self-Inactivating Lentiviral Vectors Efficiently Deliver and Express Genes for Extended Periods of Time in Adult Rat Cardiomyocytes In Vivo. *Circulation.* 2003;107:2375–2382.
32. Naldini L, Blömer U, Gallay P, et al. In Vivo Gene Delivery and Stable Transduction of Nondividing Cells by a Lentiviral Vector. *Science.* 1996;272:263–267.
33. Montini E, Cesana D, Schmidt M, et al. The genotoxic potential of retroviral vectors is strongly modulated by vector design and integration site selection in a mouse model of HSC gene therapy. *J Clin Invest.* 2009;119:964–975.
34. Papayannakos C, Daniel R. Understanding lentiviral vector chromatin targeting: working to reduce insertional mutagenic potential for gene therapy. *Gene Ther.* 2013;20:581–588.
35. Davison AJ, Benkó M, Harrach B. Genetic content and evolution of adenoviruses. *J Gen Virol.* 2003;84:2895–2908.
36. Vassalli G, Büeler H, Dudler J, von Segesser LK, Kappenberger L. Adeno-associated virus (AAV) vectors achieve prolonged transgene expression in mouse myocardium and arteries in vivo: a comparative study with adenovirus vectors. *Int J Cardiol.* 2003;90:229–238.
37. Rincon MY, VandenDriessche T, Chuah MK. Gene therapy for cardiovascular disease: advances in vector development, targeting, and delivery for clinical translation. *Cardiovasc Res.* 2015;108:4–20.
38. McCarty DM, Young SM, Samulski RJ. Integration of Adeno-Associated Virus (AAV) and Recombinant AAV Vectors. *Annu Rev Genet.* 2004;38:819–845.
39. Wang D, Tai PWL, Gao G. Adeno-associated virus vector as a platform for gene therapy delivery. *Nat Rev Drug Discov.* 2019;18:358–378.
40. Reichart D, Newby GA, Wakimoto H, et al. Efficient in vivo genome editing prevents hypertrophic cardiomyopathy in mice. *Nat Med.* 2023;29:412–421.
41. El Andari J, Renaud-Gabardos E, Tulalamba W, et al. Semirational bioengineering of AAV vectors with increased potency and specificity for systemic gene therapy of muscle disorders. *Sci Adv.* 2022;8:eabn4704.
42. Butt M, Zaman M, Ahmad A, et al. Appraisal for the Potential of Viral and Nonviral Vectors in Gene Therapy: A Review. *Genes.* 2022;13:1370.
43. Szóke D, Kovács G, Kemecei É, et al. Nucleoside-modified VEGFC mRNA induces organ-specific lymphatic growth and reverses experimental lymphedema. *Nat Commun.* 2021;12:3460.
44. Pardi N, Hogan MJ, Porter FW, Weissman D. mRNA vaccines — a new era in vaccinology. *Nat Rev Drug Discov.* 2018;17:261–279.
45. Rizvi F, Everton E, Smith AR, et al. Murine liver repair via transient activation of regenerative pathways in hepatocytes using lipid nanoparticle-complexed nucleoside-modified mRNA. *Nat Commun.* 2021;12:613.
46. Krienke C, Kolb L, Diken E, et al. A noninflammatory mRNA vaccine for treatment of experimental autoimmune encephalomyelitis. *Science.* 2021;371:145–153.
47. Gillmore JD, Gane E, Taubel J, et al. CRISPR-Cas9 In Vivo Gene Editing for Transthyretin Amyloidosis. *N Engl J Med.* 2021;385:493–502.
48. Rurik JG, Tombácz I, Yadegari A, et al. CAR T cells produced in vivo to treat cardiac injury. *Science.* 2022;375:91–96.
49. Boutin S, Monteilhet V, Veron P, et al. Prevalence of Serum IgG and Neutralizing Factors Against Adeno-Associated Virus (AAV) Types 1, 2, 5, 6, 8, and 9 in the Healthy Population: Implications for Gene Therapy Using AAV Vectors. *Hum Gene Ther.* 2010;21:704–712.
50. Perocheau DP, Cunningham SC, Lee J, et al. Age-Related Seroprevalence of Antibodies Against AAV-LK03 in a UK Population Cohort. *Hum Gene Ther.* 2019;30:79–87.
51. Martino AT, Suzuki M, Markusic DM, et al. The genome of self-complementary adeno-associated viral vectors increases Toll-like receptor 9-dependent innate immune responses in the liver. *Blood.* 2011;117:6459–6468.
52. Shao W, Earley LF, Chai Z, et al. Double-stranded RNA innate immune response activation from long-term adeno-associated virus vector transduction. *JCI Insight.* 2018;3:e120474.
53. Manno CS, Pierce GF, Arruda VR, et al. Successful transduction of liver in hemophilia by AAV-Factor IX and limitations imposed by the host immune response. *Nat Med.* 2006;12:342–347.
54. Nathwani AC, Tuddenham EGD, Rangarajan S, et al. Adenovirus-Associated Virus Vector-Mediated Gene Transfer in Hemophilia B. *N Engl J Med.* 2011;365:2357–2365.
55. Chand DH, Zaidman C, Arya K, et al. Thrombotic Microangiopathy Following Onasemnogene Apeparvec for Spinal Muscular Atrophy: A Case Series. *J Pediatr.* 2021;231:265–268.
56. Ertl HCJ. Mitigating Serious Adverse Events in Gene Therapy with AAV Vectors: Vector Dose and Immunosuppression. *Drugs.* 2023;83:287–298.
57. Boucek D, Jirikowic J, Taylor M. Natural history of Danon disease. *Genet Med.* 2011;13:563–568.
58. Greenberg B, Eshraghian E, Battiprolu P, et al. Results from First-in-Human Clinical Trial of RP-A501 (AAV9:LAMP2B) Gene Therapy Treatment for Danon Disease [Abstract]. *Circulation.* 2021;144(Suppl\_1):A10727.
59. Rocket Pharmaceuticals Announces Positive Updates from Phase 1 Clinical Trial for RP-A501 in Danon Disease at the Heart Failure Society of America (HFSA) Annual Scientific Meeting 2022. Businesswire. 30 Sept 2022. Available at: <https://www.businesswire.com/news/home/20220930005139/en/%C2%A0Rocket-Pharmaceuticals-Announces-Positive-Updates-from-Phase-1-Clinical-Trial-for-RP-A501-in-Danon-Disease-at-the-Heart-Failure-Society-of-America-HFSA-Annual-Scientific-Meeting-2022>. Accessed 15 May 2023.
60. Rocket Pharmaceuticals to Acquire Renovacor, Extending Leadership in AAV-based Cardiac Gene Therapy. *Rocket Pharmaceuticals.* Available at: <https://ir.rocketpharma.com/news-releases/news-release-details/rocket-pharmaceuticals-acquire-renovacor-extending-leadership/>. Accessed 6 March 2023.
61. Pieroni M, Ciabatti M, Graziani F, et al. The Heart in Fabry Disease: Mechanisms Beyond Storage and Forthcoming Therapies. *Rev Cardiovasc Med.* 2022;23:196.
62. AVROBIO. AVROBIO Reprioritizes Pipeline Programs. Available at: <https://investors.avrobio.com/news-releases/news-release-details/avrobio-reprioritizes-pipeline-programs/>. Accessed 23 Jun 2023.
63. Felis A, Whitlow M, Kraus A, Warnock DG, Wallace E. Current and Investigational Therapeutics for Fabry Disease. *Kidney Int Rep.* 2020;5:407–413.
64. 4D Molecular Therapeutics Presents Interim Data from 4D-310 INGLAXA Phase 1/2 Clinical Trials & Development Plans for Fabry Disease Cardiomyopathy at WORLD-SymposiumTM. 4D Molecular Therapeutics. 22 Feb 2023. Available at: <https://ir.4dmolecularterapeutics.com/news-releases/news-release-details/4d-molecular-therapeutics-presents-interim-data-4d-310-inglaxa/>. Accessed 6 March 2023.
65. Elangkovan N, Dickson G. Gene Therapy for Duchenne Muscular Dystrophy. Jaiswal J, Nagaraju K, eds. *J Neuromuscul Dis.* 2021;8(Suppl 2):S303–S316.
66. Deng J, Zhang J, Shi K, Liu Z. Drug development progress in duchenne muscular dystrophy. *Front Pharmacol.* 2022;13:950651.
67. Mendell JR, Sahenk Z, Lehman K, et al. Assessment of Systemic Delivery of rAAVrh74.MHCK7.micro-dystrophin in Children With Duchenne Muscular Dystrophy: A Nonrandomized Controlled Trial. *JAMA Neurol.* 2020;77:1122.
68. Sarepta Therapeutics. Sarepta Therapeutics Presents New Data from its Gene Therapy and RNA Platforms at World Muscle Society 2022. Available at: <https://investorrelations.sarepta.com/news-releases/news-release-details/sarepta-therapeutics-presents-new-data-its-gene-therapy-and-rna>. Accessed 6 May 2023.
69. Perdomini M, Belbella B, Monassier L, et al. Prevention and reversal of severe mitochondrial cardiomyopathy by gene therapy in a mouse model of Friedreich's ataxia. *Nat Med.* 2014;20:542–547.
70. Salami CO, Jackson K, Jose C, et al. Stress-Induced Mouse Model of the Cardiac Manifestations of Friedreich's Ataxia Corrected by AAV-mediated Gene Therapy. *Hum Gene Ther.* 2020;31:819–827.
71. Sivakumar A, Cherqui S. Advantages and Limitations of Gene Therapy and Gene Editing for Friedreich's Ataxia. *Front Genome Ed.* 2022;4:903139.
72. Smith EC, Hopkins S, Case LE, et al. Phase I study of liver depot gene therapy in late-onset Pompe disease. *Mol Ther.* 2023. <http://dx.doi.org/10.1016/j.jymthe.2023.02.014>.
73. Eggers M, Vannoy CH, Huang J, et al. Muscle-directed gene therapy corrects Pompe disease and uncovers species-specific GAA immunogenicity. *EMBO Mol Med.* 2022;14:e13968.
74. Gillmore J. AHA 22: Results of the CRISPR-Cas9 First-in-Human Trial. Available at: <https://www.uscjournal.com/video-index/aha-22-crispr-crispr-cas9-vivo-gene-editing-patients-transthyretin-amyloidosis>. Accessed March 6 2023.
75. Chai AC, Cui M, Chemello F, et al. Base editing correction of hypertrophic cardiomyopathy in human cardiomyocytes and humanized mice. *Nat Med.* 2023;29:401–411.
76. Mearini G, Stimpel D, Geertz B, et al. Mybpc3 gene therapy for neonatal cardiomyopathy enables long-term disease prevention in mice. *Nat Commun.* 2014;5:5515.
77. Li J, Mamidi R, Doh CY, et al. AAV9 gene transfer of cMyBPC N-terminal domains ameliorates cardiomyopathy in cMyBPC-deficient mice. *JCI Insight.* 2020;5:e130182.
78. Tenaya Therapeutics Announces TN-201 IND Clearance and Anticipated 2023 Milestones. *GlobeNewswire News Room.* 1 Sep 2023. Available at: <https://www.globenewswire.com/en/news-release/2023/01/09/2585026/0/en/Tenaya-Therapeutics-Announces-TN-201-IND-Clearance-and-Anticipated-2023-Milestones.html>. Accessed 15 May 2023.
79. Knezevic T, Myers VD, Su F, et al. Adeno-Associated Virus Serotype 9–Driven Expression of BAG3 Improves Left Ventricular Function in Murine Hearts With Left Ventricular Dysfunction Secondary to a Myocardial Infarction. *JACC Basic Transl Sci.* 2016;1:647–656.
80. Domínguez F, Cuenca S, Bilińska Z, et al. Dilated Cardiomyopathy Due to BLC2-Associated Athanogene 3 (BAG3) Mutations. *J Am Coll Cardiol.* 2018;72:2471–2481.
81. Martin TG, Myers VD, Dubey P, et al. Cardiomyocyte contractile impairment in heart failure results from reduced BAG3-mediated sarcomeric protein turnover. *Nat Commun.* 2021;12:2942.
82. Myers VD, Gerhard GS, McNamara DM, et al. Association of Variants in BAG3 With Cardiomyopathy Outcomes in African American Individuals. *JAMA Cardiol.* 2018;3:929.
83. Myers VD, Landesberg GP, Bologna ML, Semigran MJ, Feldman AM. Cardiac Transduction in Mini-Pigs After Low-Dose Retrograde Coronary Sinus Infusion of AAV9-BAG3. *JACC Basic Transl Sci.* 2022;7:951–953.

84. Sheikh F, Zhang J, Wang J, et al. Abstract 13599: LX2020, an Adeno Associated Viral-Based Plakophilin 2 Gene Therapy Stabilizes Cardiac Disease Phenotype in a Severe Mouse Model of Arrhythmogenic Right Ventricular Cardiomyopathy. *Circulation*. 2022;146(Suppl\_1):A13599–A13599.
85. Tenaya Therapeutics, Inc. Tenaya Therapeutics Receives Orphan Drug Designation from the U.S. Food and Drug Administration for its Gene Therapy for Genetic Arrhythmogenic Right Ventricular Cardiomyopathy. Available at: <https://investors.tenayatherapeutics.com/news-releases/news-release-details/tenaya-therapeutics-receives-orphan-drug-designation-us-food-and/>. Accessed 31 March 2023.
86. Xie C, Zhang YP, Song L, et al. Genome editing with CRISPR/Cas9 in postnatal mice corrects PRKAG2 cardiac syndrome. *Cell Res*. 2016;26:1099–1111.
87. Sánchez-López A, Espinós-Estévez C, González-Gómez C, et al. Cardiovascular Progerin Suppression. Lamin A. Restoration Rescue Hutchinson-Gilford Progeria Syndrome. *Circulation*. 2021;144:1777–1794.