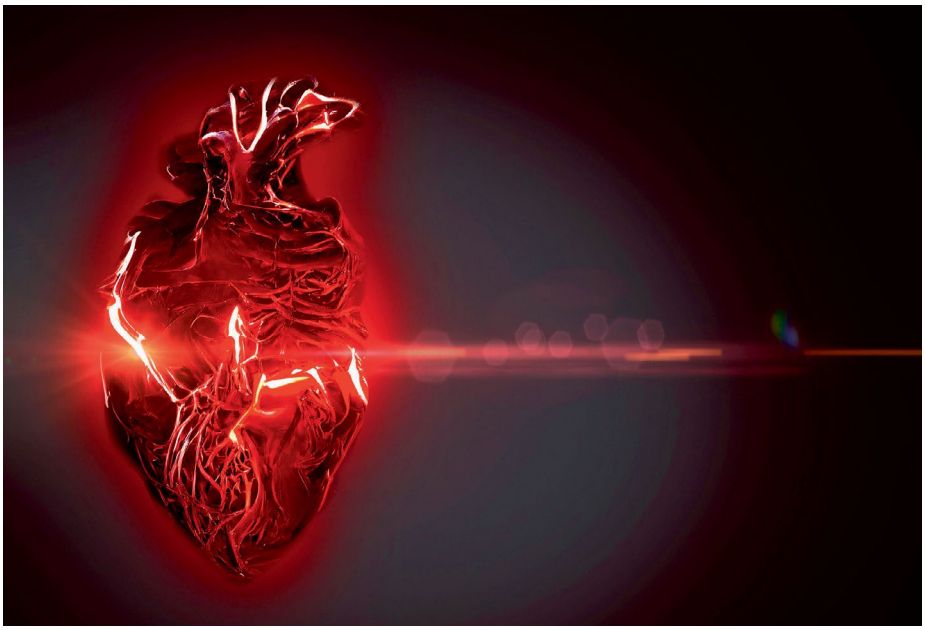
Genetic medicines aim straight for the heart

Promising gene therapy, gene editing and antisense oligonucleotide approaches herald a new era of cardiac medicine for patients with certain so-far intractable heart conditions.



Gene therapies could provide solutions for treating heart disease where traditional therapies have failed.

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By Cormac Sheridan

The first gene therapies for tackling heart failure are making progress in the clinic. Rocket Pharmaceuticals aims shortly to start a pivotal phase 2 study of a gene therapy for patients with Danon disease, an X-linked dominant disorder that causes progressive heart failure and death in early adulthood. If successful, the trial will encourage a slew of developers of genetic medicines (Table 1) to believe that arresting or even reversing progressive heart failure is feasible in different disease settings.

In addition, a trio of agenda-setting papers from the labs of Eric Olson at the University of Texas Southwestern Medical Center and a complementary study from Christine Seidman's lab at Harvard Medical School have shown that CRISPR–Cas9 editing, base editing and prime editing can all be harnessed to correct genetic models of cardiac disease in mice. Technical proof of concept has now been established for tackling cardiomyopathies caused by mutations in the *MYH7* and the *RBM20* genes, as well as for disrupting a pathological signaling mechanism caused by chronic overactivation of calcium/calmodulin-dependent protein kinase IIδ that is present in many patients with heart failure.

An important milestone in the field was the FDA's approval last year of Bristol Myers Squibb's oral cardiac myosin inhibitor Camzyos (mavacamten) for treating obstructive hypertrophic cardiomyopathy (HCM). Camzyos is the first therapy to target the underlying pathology in what is the most common genetic heart disorder. Originally developed by MyoKardia, a firm founded by Christine Seidman and her husband Jonathan Seidman (also of Harvard Medical School) and later acquired by Bristol Myers Squibb, it works by decreasing the elevated contractility that arises from excessive cross-bridge formation between actin and myosin, the proteins responsible for generating the force that gives rise to heart contractions. Cytokinetics is in phase 3 in obstructive HCM with another oral myosin inhibitor, aficamten. Neither agent is suitable for patients whose pathologies have a different genetic trigger, but a broad pipeline of therapies for tackling other genetically driven cardiomyopathies and cardiac diseases are starting to mature.

Danon disease, for example, is caused by mutations in the *LAMP2* gene, which encodes a protein involved in lysosome trafficking of proteins for degradation. When the protein is absent, autophagy is impaired, leading to the accumulation of autophagic vacuoles in cardiac and skeletal muscle cells. Rocket's therapy, RP-A501, comprises an adeno-associated virus serotype 9 (AAV9) vector encoding the B isoform of lysosome-associated membrane protein 2 (LAMP-2). It has already delivered promising, albeit preliminary data from a phase 1 trial in six young adult and pediatric patients. The transduction efficiency varied in different patients, but even a low level of gene expression was beneficial. "That's accompanied by a very marked improvement in cardiac histology," says Rocket's chief medical officer, Jonathan Schwartz. Patients' plasma levels of the heart stress biomarkers troponin and brain natriuretic peptide dropped, and reductions in autophagic vacuoles and in left ventricular wall thickness were visible. These were accompanied by functional and quality-of-life improvements. The upcoming, open-label study will recruit fewer than 50 patients, who will be compared with matched historical controls. The primary endpoint will be based on several biomarkers; it will also include functional and quality-of-life assessments. "The FDA has made it very clear, regardless of any striking benefits that are seen, that they want to make sure we are showing evidence of improvement in biomarkers that are likely to predict long-term clinical benefit," Schwartz says.

Tenaya Therapeutics is several years behind Rocket Pharma but is about to move its lead gene therapy program, TN-201, into a phase 1b trial in patients with HCM due to mutations in the *MYBPC3* gene, which encodes myosin-binding protein C3. The protein is involved in the organization and function of sarcomeres, the basic contractile units of cardiac muscle. Adults with heterozygous mutations suffer from haploinsufficiency – they cannot make enough protein from their remaining healthy allele – and develop heart failure. They are at risk of sudden death. In this population, the goal is to boost C3 expression

Table 1 | Selected development programs targeting cardiac disease

Developer	Therapy	Description	Indication	Status
Cardior Pharmaceuticals	CDR132L	Locked nucleic acid-based antisense oligonucleotide inhibitor of miR-132	Heart failure after myocardial infarction	Phase 2
Moderna	AZD8061	mRNA encoding VEGF-A injected directly into the myocardium	Patients undergoing elective coronary artery bypass and grafting surgery	Phase 2a
XyloCor Therapeutics	XC001 (encoberminogene rezmadenovec)	Epicardially delivered replication-incompetent adenovirus vector encoding VEGF to promote angiogenesis in the heart	Refractory angina due to obstructive coronary artery disease	Phase 1/2
Rocket Pharmaceuticals	RP-A501	AAV9 vector encoding LAMP-2 isoform B	Danon disease	Phase 1
Tenaya Therapeutics	TN-201	AAV vector encoding MYBPC3	Hypertrophic cardiomyopathy due to *MYBPC3* mutations	Phase 1b trial to start dosing patients in Q3 2023
BioMarin, Dinaqor (Pfäffikon, Switzerland)	BMN293 (DiNA-001)	AAV9 vector encoding *MYBPC3*	Hypertrophic cardiomyopathy due to *MYBPC3* mutations	Preclinical (IND filing planned for second half of 2023)
Haya Therapeutics	HTX-001	Antisense oligonucleotide targeting Wisper long noncoding RNA, which regulates cardiac fibrosis after injury	Heart failure due to non-obstructive hypertrophic cardiomyopathy	Preclinical
Phlox Therapeutics (Naarden, the Netherlands)	PHL-001	Short hairpin RNAs designed to achieve allele-specific gene silencing of mutated *LMNA* gene	Autosomal dominant cardiac laminopathies	Preclinical
Solid Biosciences	AVB-401	AAVrh74 vector encoding *BAG3*	BAG3-mediated dilated cardiomyopathy	Preclinical

VEGF, vascular endothelial growth factor; LAMP-2, lysosome-associated membrane protein 2; MYBPC3, cardiac myosin binding protein C3; Q3, third quarter; IND, investigational new drug.
Sources: ClinicalTrials.gov, company websites.

to healthy levels — and preclinical data in mouse and non-human primates suggest this is possible. "We are able to achieve expression levels of the protein at the wild-type level and even above the wild-type level," says CEO Faraz Ali. Tenaya is following Rocket's lead in employing an AAV9 vector because of its tropism for cardiac tissue. The company optimized the vector's promoter and regulatory region to enable it to package the full-length *MYBPC3* gene.

Tenaya aims initially to establish safety and efficacy in a small number of adult patients and will then pivot to newborns homozygous for the mutation, who rarely survive beyond infancy without a heart transplant. Also this year, Tenaya plans to submit an investigational new drug filing for its second gene therapy program, TN-401, for patients with genetic arrhythmogenic right ventricular cardiomyopathy arising from mutations in the *PKP2* gene. This encodes plakophilin 2, a protein found in the desmosomes — intercellular junctions — that connect neighboring cardiomyocytes. Its absence leads to the cells detaching from each other, followed by cell death and fibrosis.

Olson is a co-founder of Tenaya, but rights to his more recent gene editing work in cardiovascular disease have yet to be assigned. Olson's group developed both adenine base editing and prime editing approaches to correct pathogenic mutations in the *RBM20* gene, which encodes RNA binding motif 20 protein. They showed that the adenine base editing system could rescue cardiac function and extend lifespan in a mouse model of RBM20-mutated dilated cardiomyopathy. His group also developed an RNA-guided adenine base editor to correct a dominant-negative mutation in the *MYH7* gene, which encodes the β-myosin heavy chain. The mutation causes severe HCM, early onset of cardiac dysfunction, and sudden cardiac death. The editing components exceeded the packaging limit of a single AAV9 vector, so Olson and colleagues devised a dual AAV9 vector system, using trans-splicing inteins to reconstitute the full-length base editor after transduction. The therapy prevented onset of HCM in a humanized mouse model. Christine Seidman and colleagues reported similar findings in a non-humanized mouse model of the same condition.

Olson's third gene editing therapy could be more broadly applicable. To tackle the problem of overactivation of calcium/calmodulin-dependent protein kinase IIδ — an important regulator of cardiac signaling and function — his team used CRISPR–Cas9 adenine base editing to replace two oxidation-sensitive methionine residues with two valines, a strategy shown to be safe in earlier experiments in transgenic mice. They delivered the editing components by local injection, using an AAV9 vector, and expression of the constructs was controlled by a cardiac-specific promoter to minimize the risk of unwanted edits in other tissues. In mice subjected to induced ischemia and reperfusion injury, the edits promoted functional recovery, whereas control mice exhibited impaired cardiac function. Before testing in the clinic, however, further evaluation in a pig model will need to take place. "There are very large numbers of patients that could benefit from that therapy, independent of their genetics," says Olson.

Several other programs that are less precisely targeted but that may also be broadly applicable are also underway. XyloCor Therapeutics, for example, is in clinical development with an adenovirus-based gene therapy vector, XC001 (encoberminogene rezmadenovec), which encodes vascular endothelial growth factor (VEGF). The therapy is aimed at patients with refractory angina and severe

coronary artery disease who are ineligible for either a coronary artery bypass graft or a percutaneous coronary intervention, such as angioplasty and stenting. Instead of opening up blocked arteries, the treatment aims to encourage the growth of new blood vessels that will restore blood flow to the injured area. It employs an adenovirus vector to ensure the effect is transient. "We do not want chronic expression of VEGF in the heart," says XyloCor CEO Albert Gianchetti. The vector is delivered by direct injection into the epicardium, the epithelial layer covering the surface of the heart. "We are specifically delivering it to the heart so we can deliver a low dose," says XyloCor's chief medical officer Howard Dittrich. Preliminary data from a phase 1/2 trial were encouraging. "We actually unexpectedly saw a dose–response benefit in terms of exercise duration, PET [positron emission tomography] findings and CCS [Canadian Cardiovascular Society] class," he says.

XyloCor's program has a long clinical history: more than two decades ago, the company's founders, Ronald Crystal, of Weill Cornell Medical College, and Todd Rosengart, of Baylor College of Medicine, conducted an initial trial. Several other groups have pursued similar approaches, with mixed results. Delivering insufficient amounts of vector payload has been a problem for some. Moderna has tried to elicit similar effects by directly injecting mRNA into the epicardium of patients undergoing coronary artery bypass graft surgery, although its erstwhile partner, Cambridge, UK-based AstraZeneca, has dropped the program.

Several companies are using antisense oligonucleotides to target noncoding RNA species. If successful, these approaches to tackling cardiac disease would offer considerable cost advantages over gene therapy and gene editing approaches. Hannover, Germany-based Cardior Pharmaceuticals is in phase 2 with CDR132L, an antisense oligonucleotide directed against microRNA-132 (miR-132), in patients who have developed reduced ejection fraction after myocardial infarction. "This is roughly 10–15% of all patients with myocardial infarction," says Thomas Thum, Cardior founder and CSO. More than a decade ago, Thum and colleagues identified miR-132, along with a related species, miR-212, as being "necessary and sufficient" for driving HCM in mice, and they showed that this process could be prevented by antagonizing miR-132. A large-scale study in a pig model of heart failure following myocardial infarction extended those observations, and an initial study in patients with chronic ischemic heart failure indicated that CDR132L is safe and efficiently reduces plasma levels of miR-132 and several relevant biomarkers. That study was not powered to demonstrate clinical efficacy, but topline data from an ongoing phase 2 trial in 280 patients are expected in late 2024.

Haya Therapeutics, of Lausanne, Switzerland, is using an antisense approach to target a long noncoding RNA species, Wisper (Wisp2 super-enhancer-associated RNA), in an effort to arrest or reverse cardiac fibrosis following myocardial infarction or other forms of cardiac damage. Targeting fibrosis at the protein level has safety and tolerability problems because of on-target effects in off-target tissues, but Wisper is selectively expressed in cardiac fibroblasts, in which it regulates gene expression programs "critical for cell identity, extracellular matrix deposition, proliferation and survival," as Haya's CEO and co-founder Samir Ounzain and colleagues have previously reported. It also influences the differentiation of fibroblasts to proinflammatory myofibroblasts through its association with TIAR, an RNA-binding protein that is a key regulator of that process. Haya is 12 to 18 months from an investigational new drug filing but has already selected non-obstructive HCM as its lead indication. It will administer its drug, HTX-001, systemically, which results in most but not all of it being taken up by the liver. "The exposure in the heart is more than sufficient to achieve on-target effects," Ounzain says.

Although much work still needs to be done to realize the promise of most of these programs, there is a sense of optimism now in a field that had little of that commodity after the failure during the last decade of Celladon's AAV1-based gene therapy Mydicar in a phase 2b trial in heart failure. "Because of some of the hype around that program and the subsequent negative experience, many people though it was going to be difficult to transduce the heart," says Schwartz. That issue has been definitively addressed — and a new era of cardiac medicine beckons for many patients who currently have no viable options other than a heart transplant.

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