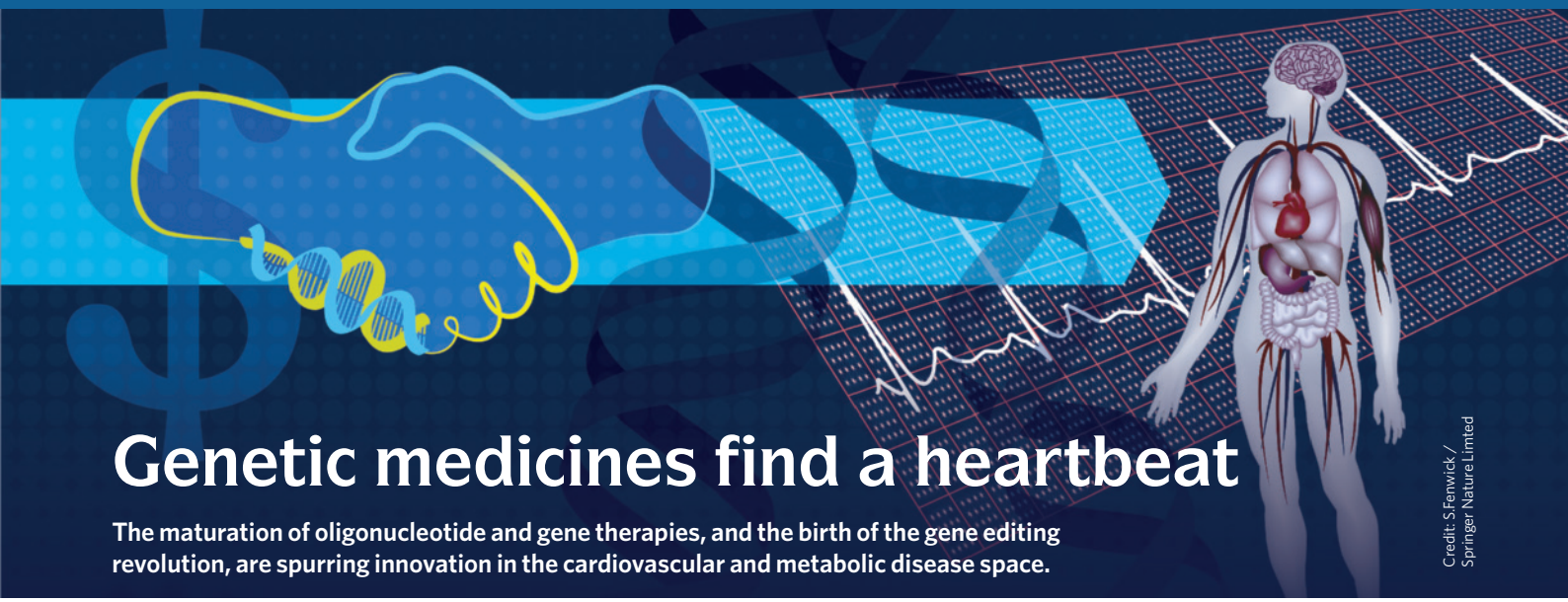

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# Genetic medicines find a heartbeat

**The maturation of oligonucleotide and gene therapies, and the birth of the gene editing revolution, are spurring innovation in the cardiovascular and metabolic disease space.**

Mark Zipkin

A vanguard of nucleic acid-based medicines advancing through phase 2 and 3 clinical trials is laying the groundwork for a paradigm shift in treating and preventing cardiovascular and metabolic diseases via the liver. As the key underlying platform technologies—antisense oligonucleotides (ASOs) and small interfering RNAs (siRNAs)—gain traction, pharma and investors are taking notice. Novartis, Pfizer, AstraZeneca, Novo Nordisk and Eli Lilly all have active collaborations or partnerships with biotech companies in the field, including six signed in the past 2 years (Table 1), and momentum for gene therapy with viral vectors and gene-editing technology such as CRISPR is building as well.

Nucleic acid-based therapies have so far been approved to treat rare diseases caused by mutations in a particular gene. But the recent deal flow suggests the industry is positioning itself to expand into broader patient populations in the cardiovascular and metabolic disease field through targets linked genetically to disease risk, such as PCSK9, lipoprotein(a) (Lp(a)) and ANGPTL3.

The biggest recent deal in the cardiovascular space was Novartis's $21 billion purchase of The Medicines Company in November 2019. Novartis gained inclisiran, an siRNA-targeting PCSK9 that originated from Alnylam, which was then in phase 3 testing for heterozygous familial hypercholesterolemia and atherosclerotic cardiovascular disease. Novartis also exercised the option to in-license a phase 2 ASO therapy that lowers Lp(a) levels from Ionis Pharmaceuticals's subsidiary, Akcea Therapeutics, for $150 million up front in February 2019. And Akcea licensed another phase 2 ASO against a third key cardiovascular disease target—ANGPTL3—to Pfizer in October 2019 for $250 million up front.

The two most recent pharma deals in the area are from AstraZeneca, which partnered with Silence Therapeutics in March 2020 to develop siRNA therapies in cardiovascular, metabolic, and other disease areas. The partners will focus on liver targets, but also explore targets in other tissues. And in January 2020, it announced a collaboration with MiNA Therapeutics to develop small activating RNA (saRNA) therapies for metabolic diseases. Financial terms were not disclosed (Table 1).

While the bulk of the pharma deals are with biotech companies focused on ASOs or siRNAs, investors are backing multiple approaches (Table 2). Cardiovascular gene editing company Verve Therapeutics has raised $123 million in two series A rounds since 2019—with the most recent series A2 financing in June 2020 led by GV (formerly Google Ventures). Omega Therapeutics, which is

**Table 1 | Selected metabolic and cardiovascular genetic medicine deals 2018–2020**

| Date | Partner 1 | Partner 2 | Disease indication/s | Summary |
|---|---|---|---|---|
| 03/05/2020 | BioMarin | DiNAQOR | Rare genetic cardiomyopathies | BioMarin signs preclinical deal with DiNAQOR to develop gene therapies. |
| 25/03/2020 | AstraZeneca | Silence Therapeutics | Cardiovascular, renal, metabolic and respiratory diseases | AstraZeneca partners with Silence Therapeutics to discover, develop and commercialize siRNA therapeutics. |
| 07/01/2020 | AstraZeneca | MiNA Therapeutics | Metabolic diseases | RNA activation therapies pioneer, MiNA Therapeutics, signs research collaboration with AstraZeneca to evaluate saRNA molecules. |
| 24/11/2019 | Novartis | The Medicines Company | Cardiovascular diseases | Novartis acquires The Medicines Company for $9.7 billion, including inclisiran, an siRNA drug in late-stage development for lowering cholesterol. |
| 18/11/2019 | Novo Nordisk | Dicerna Pharmaceuticals | Chronic liver disease, non-alcoholic steatohepatitis (NASH), type 2 diabetes, obesity and rare diseases | Using its GalXC RNAi platform technology, Dicerna Pharmaceuticals partners with Novo Nordisk to develop RNAi therapeutics. Dicerna will receive an upfront payment of $175 million, but both companies retain rights to co-develop and co-commercialize products. |
| 07/10/2019 | Pfizer | Akcea Therapeutics | Cardiovascular and metabolic diseases | Akcea Therapeutics (affiliate of Ionis Pharmaceuticals) signs licensing deal with Pfizer to investigate antisense therapy AKCEA-ANGPTL3-LRx. |
| 25/02/2019 | Novartis | Akcea and Ionis Pharmaceuticals | Cardiovascular diseases | Akcea Therapeutics and Ionis Pharmaceuticals announces that Novartis has exercised its option to license AKCEA-APO(a)-LRx. This drug was developed using Ionis's ligand conjugated antisense technology platform. |
| 29/10/2018 | Eli Lilly | Dicerna Pharmaceuticals | Cardiometabolic diseases (and others) | Lilly and Dicerna announce RNAi licensing and research deal to develop and commercialize therapeutics, using Dicerna's GalXC RNAi platform. |

**Table 2 | Select metabolic and cardiovascular genetic medicine financings 2019–2020**

| Company | Technology | Summary |
|---|---|---|
| MiNA Therapeutics | Small activating RNA (saRNA) | In September 2020 MiNA Therapeutics completed a £23 million ($30 million) series A financing to advance its RNA-based therapeutics. |
| Omega Therapeutics | Gene therapy (epigenetic controllers) | In July 2020 Omega Therapeutics completed a $85 million series B financing to develop a pipeline from its epigenomic program platform. |
| Verve Therapeutics | Gene editing | In June 2020 Verve Therapeutics secured $63 million in a series A2 financing to progress development of its gene-editing therapies for heart disease. |
| Arrowhead Pharmaceuticals | Small interfering RNA (siRNA) | In December 2019 Arrowhead Pharmaceuticals, an RNAi-focused company, closed a public offering worth $266.8 million. |
| Shape Therapeutics | Gene editing (RNA editing) | In November 2019 Shape Therapeutics secured $35.5 million in a series A financing to advance its RNA gene-editing therapy platform. |
| Tenaya Therapeutics | Gene and cell therapy | In October 2019 Tenaya Therapeutics closed a $92 million series B financing to advance heart disease programs. |

developing epigenomic controllers in metabolic and other diseases, raised $85 million in a venture round last July.

## How to target the heart

Despite smaller patient pools, rare cardiovascular diseases are a natural fit for nucleic acid-based therapies, which enable well-defined causes of disease to be directly targeted to benefit patients who often have major unmet medical needs.

But, in some instances, tackling rare diseases can also be a strategic gateway to much larger populations. In the cardiovascular space, a series of large-scale genetics studies over the past decade has set the stage for therapeutic approaches aimed at the liver to modify lipid metabolism mechanisms that can lead to heart attacks. Sekar Kathiresan, CEO of Verve Therapeutics and a co-author on several of the studies during his time at Harvard Medical School, notes that the findings showed cohorts at higher risk for disease based on their mutations—but also a cohort with protective mutations that reduced gene function. This opens up the possibility of developing therapies for patients with a specific genetic risk factor, then testing them in broader populations with an unmutated gene.

According to Kathiresan, the studies helped identify eight genes that share three features marking their potential as therapeutic targets (Fig. 1). First, each is tied to at least one of three risk factors for cardiovascular disease—circulation levels of low density lipoprotein (LDL) cholesterol, triglycerides or Lp(a). Second, researchers have found resistance mutations in the genetic studies that reduced myocardial infarction and cardiovascular disease risk without negatively impacting health, suggesting that inhibiting their function will be safe.

The third factor is accessibility. Genetic therapeutics need to be delivered inside cells, and they cannot effectively reach every tissue, especially the heart. But the eight potential targets are found in hepatocytes. "The liver is the key place where cholesterol metabolism and synthesis happen," said Kathiresan. Importantly, it is also feasible to deliver siRNAs and ASOs to the liver specifically, with the leading approach being *N*-acetylgalactosamine (GalNAc) conjugation, pioneered by Alnylam. The company's Givlaari (givosiran), the first approved liver-targeting oligonucleotide drug, is a GalNAc–siRNA conjugate for acute hepatic porphyria.

## Two ways to target mRNA

Oligonucleotide therapies can reach the liver on their own. The only approved oligonucleotide therapy for a cardiovascular disease, Kynamro (mipomersen), directly targets the gene that encodes apolipoprotein (Apo)B-100 in the liver, reducing blood levels of LDL cholesterol and Lp(a). Originally developed by Ionis Pharmaceuticals and licensed by Genzyme, Kynamro is an ASO. But, because high doses of the drug are needed for a therapeutic effect, it carries a risk of hepatotoxicity, and is approved only in the USA for patients with the rare disease homozygous familial hypercholesterolemia.

Ionis's next-generation ASO therapies are conjugated to the GalNAc ligand by a linker, said Adam Mullick, Ionis's vice president of cardiovascular drug discovery. "The ligand directs it to a particular cell surface receptor, and thus allows us to dose [about 20-fold] lower and get the same effect," he said.

The linker gets cleaved once the therapeutic is taken into a hepatocyte, where the single-stranded ASO then targets a specific messenger RNA (mRNA) and recruits RNase H1 to degrade it. The approach is similar to an RNA interference (RNAi) therapy such as an siRNA, said Mullick, except, in that case, a double-stranded siRNA utilizes a different pathway involving the RNA-induced silencing complex to degrade the target mRNA.

At the front of the pack of siRNA-based therapies for cardiovascular diseases is the GalNAc-conjugated siRNA inclisiran, which is now under review at both the US Food and Drug Administration (FDA) and European Medicines Agency (EMA). The drug decreases LDL cholesterol levels by targeting PCSK9, a protein involved in lipid metabolism that is a prime example of a genetically validated drug target: gain-of-function mutations in *PCSK9* cause familial hypercholesterolemia, whereas loss-of-function mutations in *PCSK9* reduce LDL cholesterol and confer protection from coronary heart disease.

This compelling combination of genetic evidence has catalyzed efforts with multiple platforms to target PCSK9, including early efforts with first-generation unconjugated siRNA technologies. These were overtaken by two monoclonal antibodies—Regeneron's Praluent (alirocumab) and Amgen's Repatha (evolocumab)—which gained FDA approval in 2015. However, these antibodies need to be dosed more frequently than siRNAs. "RNA interference is often better than antibodies because of the catalytic process that

| Gene | Resistance mutation | Pathway |
|---|---|---|
| *PCSK9* | 1 in 50 | LDL |
| *NPC1L1* | 1 in 650 | LDL |
| *APOB* | 1 in 1086 | LDL |
| *APOC3* | 1 in 150 | TRL |
| *ANGPTL3* | 1 in 300 | TRL |
| *ANGPTL4* | 1 in 360 | TRL |
| *ASGR1* | 1 in 120 | TRL |
| *LPA* | 1 in 71 | Lp(a) |

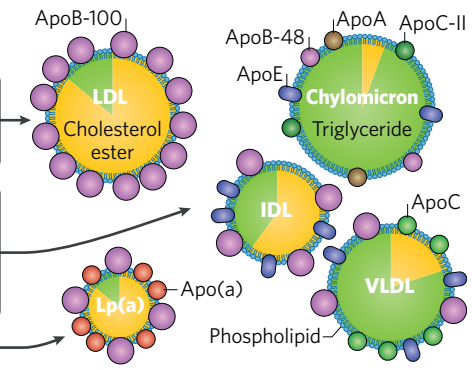


**Fig. 1 | Cardiovascular drug targets from human genetics.** Eight genes for which 'resistance mutations' have been linked to reduced risk of cardiovascular disease are shown, together with the related risk factor pathways. ApoA/B, apolipoprotein A/B; IDL, intermediate-density lipoprotein; LDL, low-density lipoprotein; Lp(a), lipoprotein(a); TRL, triglyceride; VLDL, very low-density lipoprotein. Adapted with permission from Verve Therapeutics.

you can generally use," said Chris Anzalone, CEO of Arrowhead Pharmaceuticals. A drug like inclisiran can cut LDL cholesterol in half with only two injections per year, whereas Praluent and Repatha injections are given every 2 to 4 weeks. There is also potential competition from two PCSK9-targeted ASOs; Roche spinout CiVi Biopharma is developing a GalNAc-conjugated ASO therapy, CiVi-007, which is currently in phase 2 testing and on November 13, Ionis announced promising phase 1 data for ION449, a PCSK9 antisense therapy it is developing with AstraZeneca.

Another platform battle is brewing over ANGPTL3, which Regeneron is targeting with another antibody, evinacumab. Reducing ANGPTL3 improves dyslipidemia in patients with high triglyceride and LDL cholesterol levels, which Anzalone estimates affects tens of millions of patients in the USA. After positive phase 3 data, Regeneron is initially seeking regulatory approval for evinacumab to treat homozygous familial hypercholesterolemia, with decisions expected from the FDA and EMA in the coming months, and trials in primary hypercholesterolemia are underway.

Mullick estimates that there are only about 300 familial hypercholesterolemia patients with genetically homozygous ANGPTL3. "We made a decision not to go after that very niche opportunity [at Ionis]," he said, noting that, as with Praluent, Regeneron's approach requires "extremely high levels of antibodies." This is why an ASO approach is compelling, he said. "It's a very precise approach. If you take an antibody that blocks the protein, that antibody is going to go everywhere." But he also noted that antibodies act faster than ASO therapies.

In 2019, Ionis-owned Akcea licensed its GalNAc-conjugated ASO against ANGPTL3, vupanorsen, to Pfizer. Under the deal terms, Ionis and Akcea received $250 million up front and could see milestone payments up to $1.3 billion, plus sales royalties. The drug candidate is in phase 2 testing for several cardiovascular diseases, as is Arrowhead's ARO-ANG3, a GalNAc–siRNA conjugate against the target for mixed dyslipidemias.

Arrowhead also has two other siRNA therapies in clinical development for undisclosed cardiovascular diseases, including ARO-LPA (now known as olpasiran), which lowers Lp(A) levels by targeting ApoA and was licensed to Amgen in 2016. Again, there is a competitor from another platform, with the GalNAc-conjugated ASO targeting ApoA that Novartis licensed from Akcea (now known as pelacarsen) entering a phase 3 trial for the reduction of cardiovascular risk in patients with elevated Lp(a) levels in 2019.

## Permanent changes

ANGPTL3 also may be a foothold for the next generation of genetic medicines: gene-editing therapies. In a 2010 *New England Journal of Medicine* paper, Verve's Kathiresan and colleagues including Kiran Musunuru, a cardiologist and genetics researcher at the University of Pennsylvania, identified a healthy family that carried two broken copies of *ANGPTL3* and showed they had lower LDL cholesterol levels and overall cardiovascular risk. Kathiresan says the research implied ANGPTL3 is a "spare part," and so a one-time therapy eliminating it would be beneficial.

In 2018, Musunuru and others at the University of Pennsylvania used CRISPR base editing to disrupt *ANGPTL3* in a mouse model of homozygous familial hypercholesterolemia, resulting in significantly reduced triglycerides and LDL cholesterol. The research was published in *Circulation*. Shortly thereafter, Musunuru and Kathiresan helped cofound Verve to develop gene-editing techniques for *ANGPTL3* and other targets that can reduce LDL cholesterol, Lp(a) or triglycerides. Investors followed.

Kathiresan said Verve's studies targeting ANGPTL3 in monkeys have shown the benefit of a one-and-done therapy. The company is aiming to select a lead candidate—which will include a specific configuration of a gene editor, plus a guide RNA—by the end of 2020. The therapy will be encapsulated in a lipid nanoparticle for specific delivery to liver cells via receptor-mediated uptake. The nanoparticles have the benefit of being more transient than the typical adeno-associated virus (AAV) vector-based delivery systems. "The longer it stays around, the more likely it might be to off-target edit," he said, adding that low levels of the nanoparticles have been detected in lungs and spleens.

Lipid nanoparticles do come with the risk of an inflammatory reaction, but it's the approach itself that raises some eyebrows. "The permanence of that scares me," said Anzalone. "RNAi allows you to get deep silencing in some cell types for a long period of time. But if you want to go off drug, you can go off drug with no harm, no foul."

Cardiovascular doctors tend to be conservative, Anzalone said, "And I think they should be. Because for most patients, even those with increased risk, this is not an acute risk."

Gene editing "is a great way of restoring a lost enzyme," said Mullick, and the risk–benefit ratio would warrant it in rare genetic diseases once the technology is established. But, as other modalities with established safety records seem effective at reducing gene expression in the liver, it may not be worth chasing cardiovascular targets like PCSK9 or ANGPTL3 with gene editing, he said.

According to Kathiresan, Verve's initial patient population will be a small subset of heart attack patients with familial hypercholesterolemia. "We're not going to start with the 12.5 million patients in the US with a heart attack," he said. "We're going to be able to come in with a genetic medicine for a genetic disease." The plan will be to expand to a larger population thereafter, as well as to develop therapies against targets that impact each risk pathway—LDL cholesterol, Lp(a), and triglycerides. "The benefit is additive," he said.

## Exploring other approaches

Even if this new wave of genetic medicines meets expectations, not every disease can be treated through the liver. A genetic therapy for cardiomyopathies or arrhythmias, for example, might require reaching heart muscle cells directly, said Kathiresan. "That's a much harder task than reaching liver cells with genetic medicines."

The paucity of therapies isn't from a lack of trying; a host of gene therapies have failed in clinical trials. But companies are still trying new approaches. For example, in May, rare disease company BioMarin announced a development partnership and licensing deal with DiNAQOR for the gene therapy company's lead asset targeting MYBPC3 in patients with hypertrophic cardiomyopathy caused by rare mutations in *MYBPC3*. DiNAQOR CSO Thomas Voit said the therapy will be delivered directly into cardiomyocytes via an AAV vector.

Stuart Bunting, a scientific fellow at BioMarin, said the ability of certain capsids to reach skeletal muscle tissue, such as the AAV9 vector Pfizer is using for its Duchenne muscular dystrophy drug PF-06939926 in phase 3 trials, bodes well for heart tissue. BioMarin staff scientist Matt Killeen notes that AAV9 is actually more tropic for heart muscle than skeletal muscle, and heart-specific promoters can further attune a precision medicine approach.

In February 2020, Asklepios BioPharmaceutical began dosing patients in a phase 1 gene therapy trial for congestive heart failure. The therapy, NAN-101, is delivered by AAV via an intracoronary infusion by cardiac catheterization, comparable to angioplasty, and activates protein phosphatase inhibitor 1 in cardiomyocytes.

Another company developing AAV-based gene therapies, Tenaya Therapeutics, raised $92 million in a series B round in 2019. The company is investigating therapies for severe genetically defined cardiomyopathies.

"I think we're on the cusp of a new era in cardiovascular medicine," said Killeen, "where we're bringing in some of the approaches and technological advances that are becoming more familiar in other therapeutic areas." Arrowhead's Anzalone agrees. "I view this as a real golden age for treating cardiovascular disease, because we the field are providing cardiologists with a whole new set of tools. It's a huge leap for the field, and we can see it happening in real time."

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