

# Strand and deliver

*The difficulties of delivering RNA interference therapeutics to their target cells brought the nascent research field to its knees, but a landmark drug approval offers hope.*

BY BIANCA NOGRADY

The commercial story of RNA interference (RNAi) harbours more plot twists and unexpected demises than television fantasy drama *Game of Thrones*.

As in all good dramas, there was someone — or something — that, just as things were looking promising, showed up to foil everyone's plans, in this case hindering efforts to safely get the RNAi into the right cells to go about its work.

Christopher Anzalone, chief executive of Arrowhead Pharmaceuticals, a company focusing on RNAi therapies, based in Pasadena, California, said: "The world saw this as a new panacea and the world goes crazy, the market goes crazy."

Then, about ten years ago, it dawned on people that getting the therapeutic strands of RNA into the right cells, so they could do their job of interfering with the target gene, was a greater challenge than first thought.

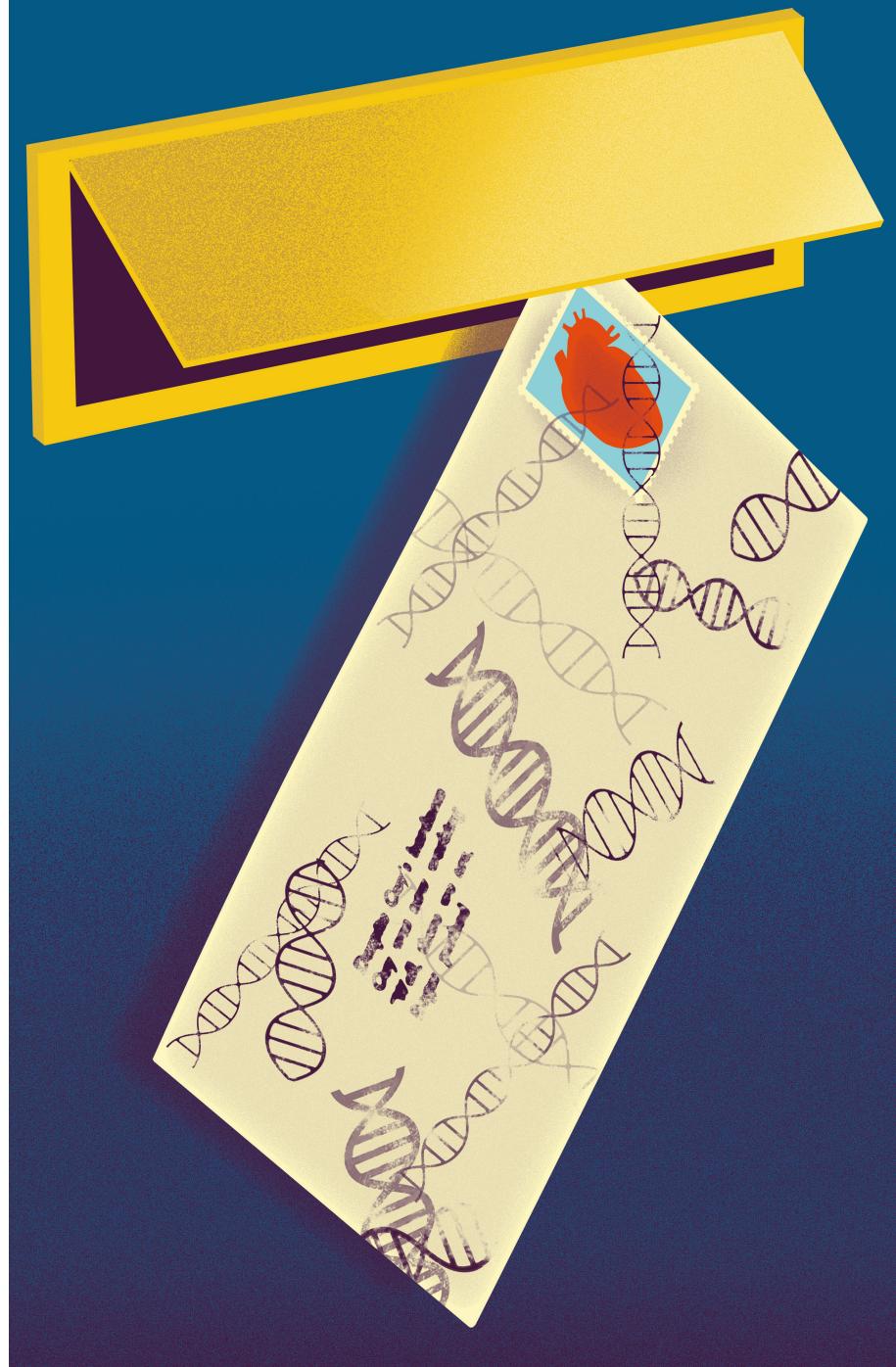
RNA is typically single stranded, so first researchers had to work out how to shield the therapeutic double-stranded RNA from degradation by the human immune system.

Second, they had to ensure that the RNA molecule would remain stable long enough to do its job.

After overcoming these hurdles, the researchers had to get the RNA into the target organ and cells, and once inside the cells, make sure the RNAi machinery was unleashed.

"The conundrum of RNA therapeutics has always been delivery," says Bo Rode Hansen, chief executive of Genevant Sciences, a company in Cambridge, Massachusetts, that focuses on delivery methods for RNAi.

It's too early to say that all these challenges



have been overcome. But US and EU approval in 2018 of the first RNAi-based therapeutic product — patisiran, which treats a hereditary nerve condition caused by the accumulation of an aberrant protein — is an encouraging sign.

## HIDING IN PLAIN SIGHT

The human immune system is on high alert for double-stranded RNA, which is a hallmark of some pathogenic viruses.

"We know that double-stranded RNA is a really important danger signal," says Ken Pang, paediatrician and biomedical researcher at the Murdoch Children's Research Institute in Melbourne, Australia. "The innate immune system is finely attuned to seeing double-stranded RNA as a signal that viruses are present, and it starts mounting an immune response."

The first solution that the industry devised to tackle this was nanoscale spheres of lipid

molecules to encapsulate the double-stranded RNA and hide it from the immune system. These lipid nanoparticles not only provide a shield, but also piggyback on existing cellular transport mechanisms to get the nanoparticle and its cargo into the cell. In the case of patisiran, the nanoparticle containing the double-stranded RNA is taken up by the receptor that normally transports low-density lipoproteins (LDLs) into the cell. Once inside the cell, the lipid nanoparticle dissolves, releasing its double-stranded RNA cargo into the cellular cytoplasm to silence the target gene.

A second approach is to engineer the double-stranded RNA so that it becomes invisible to the immune system. "The key concept here is that every base is chemically modified such that, while it's called an RNAi therapeutic, there's no pure RNA in the drug," says Kevin Fitzgerald, senior vice-president of research at Alnylam

Pharmaceuticals in Cambridge, Massachusetts, the company that makes patisiran. It turns out that if enough modifications are made to the RNA molecules, the immune system doesn't see them.

One advantage of this engineered double-stranded RNA is that it can be injected through the skin, whereas the lipid nanoparticle-based formulation has to be administered intravenously. What's more, the double-stranded formulation is stable at room temperature and has a long shelf life.

"You start to think what could you do with that in the less developed parts of the world, where practical cold chain is difficult, and dosing is difficult," says John Pasi, director of the Haemophilia Centre at Barts Health NHS Trust in London. "You start to think on a global scale about how this actually might have real value."

This engineered RNA approach is also being applied to a new RNAi therapeutic for haemophilia — a disease characterized by the lack of clotting agents in the blood. The drug, fitusiran, targets the production of antithrombin, a protein that inhibits clotting. By silencing a gene for antithrombin, fitusiran enables a rise in the production of the enzyme thrombin, which helps the blood to clot. Phase I and II clinical trials have shown that a fixed monthly dose of this subcutaneous treatment can significantly lower the number of some bleeding episodes that patients experience each year — down to zero in around two-thirds of patients. A phase II clinical trial found that 48% of people who were treated with a fixed monthly dose of this subcutaneous treatment experienced no bleeds at all during the observation period, according to Alnylam and pharmaceutical giant Sanofi.

Engineered RNA seems stable enough to survive even the hostile acidic environment of the stomach, possibly enabling oral delivery of RNAi therapeutics, say Alnylam.

Although the stability of double-stranded RNA means it is currently the dominant approach, single-stranded RNA is also a potential therapeutic candidate. Research suggests the other half of the double RNA strand isn't necessary for the interference action<sup>1,2</sup>, and single-stranded RNA can also be chemically stabilized to survive inside the body.

Lipid nanoparticles and engineered RNA molecules aren't the only options being explored in the quest to overcome RNAi's delivery challenges. Anil Sood, co-director of the Center for RNA Interference and Non-Coding RNA at the MD Anderson Cancer Center in Houston, Texas, is leading a group that is investigating a polysaccharide-based nanoparticle called chitosan to deliver small lengths of RNA into the cell, with the aim of inhibiting the growth of prostate-cancer cells. The group is also looking at using a form of high-density lipoprotein (HDL) to create a nanoparticle-delivery platform.

"We feel like that that's a neat approach because it uses a system within the body that already exists," says Sood. Their research

suggests reconstituted HDL could be an efficient way to get RNAi inside cells, and potentially better than the LDL-receptor uptake mechanism now used by patisiran's lipid nanoparticle. (High levels of LDL, sometimes called 'bad' cholesterol, can raise the risk of heart disease or stroke.)

HDL is taken into liver cells through a receptor called SR-B1. As well as being widespread in the liver, SR-B1 can also be found in tumour cells. That's significant because it has been difficult to find suitable RNAi targets for such cells.

### GOING BEYOND THE LIVER

If there was such a thing as low-hanging fruit in the fiendishly challenging landscape of RNAi, it would be diseases that involve the liver.

"The liver is a clearance organ, so between that and the kidneys it should be reasonably easy to get into those types of cells because the liver is trying to pull stuff out of the blood anyway," says Anzalone. Liver cells also have a well-known and characterized receptor — the LDL receptor — that can be used to transport sufficient quantities of RNA into those cells.

That has worked for lipid nanoparticles, but what about naked RNA, which would avoid the need for delivery mechanisms such as nanoparticles?

For this delivery challenge, researchers attach the engineered double-stranded RNA to a molecule called a ligand, which has a natural attraction to a particular type of receptor found on the surface of the target cell.

For the non-encapsulated version of patisiran, the RNA is joined to a ligand called *N*-acetylgalactosamine, or GalNAc, which has a high affinity for a liver hepatocyte receptor. The ligand drags the RNA to that receptor, and opens the door to the inside of the cell.

With this development, delivery to liver-based targets is now straightforward. "It's an organ that we're all very confident that we can make safe and effective therapies in now," says Fitzgerald.

The eye is another organ for which delivery is relatively easy, although the method — direct injection into the fluid inside the eye — might make some a little squeamish. Fitzgerald says this approach is ideal because it delivers the treatment directly to the target cells of the eye. Alnylam's preclinical work on eye delivery suggests that doses as small as three micrograms can be effective and have a long-lasting effect.

Similarly, the central nervous system has a backdoor that enables researchers to bypass the blood-brain barrier — a tightly-packed conformation of endothelial cells that protects the brain from potentially harmful molecules circulating in the blood. Drugs can be injected directly into the intrathecal spaces in the spinal cord. The cerebrospinal fluid circulates the drug throughout the brain and spinal cord, and the

ligand makes sure the drug reaches the target cells in the brain.

Targeting cancer with RNAi therapy is a daunting prospect, because the drug has to hit every single cell, says Fitzgerald. There's also the possibility that the tumour will adapt to the therapy by reducing the number of target receptors. This could be where the SR-B1 receptor — found in high numbers in many cancer cells — comes into its own.

### ESCAPING THE ENDOSOME

Once the double-stranded RNA has been encapsulated or stabilized, found its way to its cellular target and been transported inside the cell, there's still one last hurdle to overcome. When the cell takes in the double-stranded RNA complex, it forms a membrane around the RNA that then buds off — a process called endocytosis.

The double-stranded RNA must escape the membrane so that it can then reach the cellular machinery and interact with and silence its target gene.

Pang and his colleagues have identified a receptor, called SIDT2 in mammals, which is responsible for the release of viral double-stranded RNA into the cytoplasm as part of the normal immune response to viral infection<sup>3</sup>. But for some reason, the receptor doesn't seem to free RNAi therapeutics from their membrane vessels, or endosomes, in the same way.

Anzalone says that Arrowhead Pharmaceutical's approach relies on the fact that the engineered RNA is stable enough to survive inside the endosomes until a small number leak out naturally. RNAi is so efficient that only a few molecules need to leak out into the cellular fluid to produce the desired effect, he says.

Fitzgerald says endosomal escape can actually work in RNAi's favour, because it allows the double-stranded RNA to be released slowly, and the effect, therefore, to be extended and dosing to be less frequent.

### DELIVERING ON THE POSSIBILITIES

Delivery has been such a significant challenge that those working in the area of RNAi therapeutics might be forgiven for admitting defeat. And there's still a long, hard road ahead, says Fitzgerald. "Each organ is a little bit different and the challenges of delivery are a little bit different," he says. "As we go into each new organ, there will be some learning to do."

Despite this, enough researchers have clung to the vision of RNAi as a potent new therapeutic modality, and refused to give up. Their persistence and years of toil are paying off, says Anzalone, delivering treatments that "can change millions of lives".

**"The conundrum of RNA therapeutics has always been delivery."**

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2. Yu, D. et al. *Cell* **150**, 895–908 (2012).

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