RNA in focus: Tara Raveendran

RNA therapeutics are now a clinical reality

The genomic era has ushered in the age of personalised medicine. However, to fulfil its promise, genomic medicine must expand beyond its current domain of predominantly diagnostic use and support the development of drugs that can specifically modulate the expression of disease-relevant genes. Nucleic acid-based drugs, which is to say those based on DNA and RNA, represent the newest wave of therapeutics able to interrogate the genomic axis. DNA-based gene therapy has been progressing at a rapid pace. In contrast, its chemical cousin RNA, due to its inherently more labile and transient nature, has proven elusive as a therapeutic modality.

The approval of five next-generation RNA-based drugs, the most recent based on a platform which has been in development for just over 20 years, heralds a coming of age of the science of RNA, and RNA therapeutics more broadly. In this article we offer a brief overview of the clinical development of RNA-based drugs, with an emphasis on recent progress.

By targeting nucleic acids instead of protein, RNA, like DNA, has the potential to broaden the range of druggable targets beyond the scope of existing small molecule and biologic medicines. Targeted therapy broadly works via two mechanisms: up-regulation (increasing) or down-regulation (decreasing) of disease-relevant proteins. While it has been known for over three decades that RNA can be used to modulate protein expression levels *in vivo*, the effective delivery of therapeutic RNA has proven a challenge – reflecting the molecule's unfavourable physiochemical properties including a negative charge and large molecular weight and size. The molecules are also unstable; in its natural state naked RNA has a plasma half-life of c.10 minutes.[1] Hence much of the early development efforts in the field were centred on (i) chemical modifications to improve the stability of the therapeutic agent and make it less immunogenic on administration, and (ii) the design of effective delivery methods to transport the therapeutic RNA to the target site of interest.

There has been significant progress on both fronts, with standard chemical modifications of RNA now widely used in the clinic, and a number of advances in the development of delivery systems for short interfering RNAs (siRNAs) in particular, including lipid formulations, nanoparticles and conjugates (chemically-linked functional groups). After years of iterative study, lipid nanoparticle technology and next generation GalNAc conjugates have been shown to be the most tolerable and efficient delivery technologies to date. GalNAc (n-acetylgalactos amine) is used as a targeting ligand in antisense oligonucleotides and siRNA therapies targeted to the liver.

However, drug candidates based on these platforms are only suitable to modulating genes that are expressed in the liver. Hence many organs currently remain inaccessible with available delivery systems. While theoretically by changing the conjugated ligand, one could change the target organ, this has yet to be validated in larger late-stage studies.

From the pipeline to the clinic

There are a number of RNA-based drugs on the market which are broadly based around two mechanisms, antisense and RNA interference (RNAi).

Antisense oligonucleotides (ASOs) are short synthetic oligonucleotides with RNA/DNA- based structures that can bind to RNA and reduce, restore or change protein expression via several distinct mechanisms. They are emerging as a novel therapeutic option to treat diseases with known genetic origin. ASOs are very stable at room temperature, highly soluble in water and used in saline solution, making them particularly amenable to therapeutic use.

Kynamro mipomersen, indicated for the treatment of homozygous familial hypercholesterolemia (HoFH), a genetic disorder characterised by high cholesterol, was one of the first US Food and Drug Administration-approved RNA-based ASOs. In the recent past two further ASOs have gained FDA approval – Sarepta's

Table 1: Selected RNA therapeutics – Approved & In late-stage development

Drug (Company)	Status	Indication	Mechanism (delivery vehicle)
Formivirsen	Withdrawn US (2002)	Cytomegalovirus	ASO (naked, modified)
Macugen	Approved (2004)	Macular degeneration	ASO* (naked, modified)
Kynamro (Isis/Ionis)	Approved (2013, US)	HoFH	ASO (naked, modified)
Spinraza (Biogen/Ionis)	Approved (2016)	SMA	ASO (naked, modified)
Exondys 51 (Sarepta)	Approved (2016, US)	DMD	ASO (naked, modified)
Onpattro (Alnylam)	Approved (2018)	hATTR amyloidosis	siRNA (lipid nanoparticle)
IONIS-HTTRx (Ionis/ Roche)	Filed	Huntington's	ASO (naked, modified)
Fitusiran (Alnylam)	Phase 3	Haemophilia	siRNA (Gal-NAc conjugate)
Inclisiran (Alnylam)	Phase 3	Hypercholesterolaemia	siRNA (Gal-NAc conjugate)
Givosiran (Alnylam)	Phase3	Acute hepatic porphyria	siRNA (Gal-NAc conjugate)
DCR-PHXC (Dicerna)	Phase 2/3	Primary hyperoxaluria	siRNA (Gal-NAc conjugate)

* binds protein as opposed to RNA — Source: Company data, Shore Capital

controversial Exondys 51 (eteplirsen) for Duchenne muscular dystrophy (DMD), and Biogen's Spinraza (nusinersen) for spinal muscular atrophy (SMA).

Eteplirsen works by a mechanism known as exon skipping, whereby the drug binds to exon 51 of the dystrophin gene and enables production of the truncated dystrophin protein (i.e. without this section), which slows the progression of the disease. Like eteplirsen, nusinersen also works by altering the splicing (processing) of pre-mRNA transcripts, but instead of promoting skipping over the mutated gene, nusinersen is designed to favour inclusion of the right exon in the SMN protein.

While both offer potentially disease-modifying benefits in conditions where there were previously no/limited therapeutic options, FDA approval for eteplirsen is conditional, with a requirement for post-marketing studies. This reflects concerns around the limited dataset of the pivotal trial which included clinical information from just 12 patients. The regulator also had questions around the clinical relevance of the endpoint which was performance on a six-minute walk.

The European Medicines Agency also had doubts about the medicine, recommending on 31 May that it be refused a marketing authorisation. Sarepta appealed against the decision but on 20 September the agency confirmed its negative opinion. Explaining the opinion, the EMA cited the small size of the study supporting registration. It also noted that Exondys had not been compared with placebo beyond 24 weeks, during which time there was no meaningful difference between the drug and placebo on the six-minute walk.

The second mechanism, RNA interference (RNAi), is a naturally occurring process used by cells to regulate gene expression in which genes are silenced or prevented from being translated into protein. With the FDA's approval of Alnylam's Onpattro (patisiran) in August, and encouraging safety data for the PCSK9-targeting inclisiran in over 1,000 patients, the RNAi mechanism is now clinically validated as a new therapeutic modality.

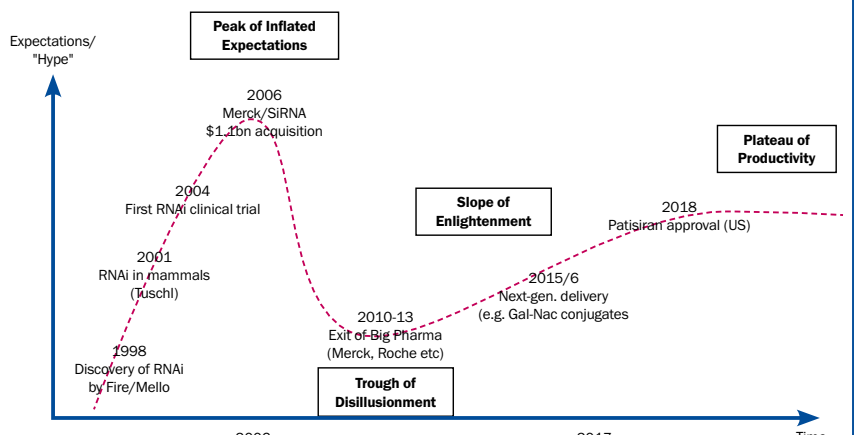
As the first marketed RNAi drug, patisiran has its shortcomings. It is based on an intravenously administered lipid nanoparticle (LNP) technology, which itself has gone through a number of iterations to improve safety and reduce off-target effects. Patisiran was approved on the basis of data from the APOLLO study in patients with hereditary transthyretin amyloidosis (hATTR)-related polyneuropathy.

Nearly a decade in development and twenty years after the discovery of RNAi, there has been significant progress in RNAi trigger design (safety and efficacy) and delivery technologies (i.e. conjugates). Hence much of the late-stage pipeline of RNAi drugs from Alnylam and others incorporates the newer liver-targeting GalNAc conjugate technology (for example Anylam's patisiran follow-on ALN-TTRsc02) which can be delivered subcutaneously. Looking further to the future, after the liver for which the current crop of RNAi candidates is being developed, the central nervous system (CNS) is the next big opportunity. Beyond that, some companies, including Alnylam and Arrowhead Pharmaceuticals, are referring to larger opportunities in the lungs and in cancer.

Box 1: RNAi unfulfilled expectations

The commercial fortunes of early RNAi pioneers (Merck-Sirna, Ionis, Alnylam, Silence-Atugen) have tracked the ups and downs of the so-called "*Gartner Hype Cycle*", riding up to the "Peak of Inflated Expectations" with Merck's $1.1 billion acquisition of the pre-clinical RNAi company Sirna in 2006, only to come crashing back to the "Trough of Disillusionment", with Merck exiting RNA-directed gene silencing at a loss a mere six years later. Technical challenges, including intra-cellular delivery, off-target effects/immunogenicity, and molecular stability as well as restrictive IP, (with Alnylam and Merck/Sirna exclusive holders of the foundational Tuschl patents), were ultimately responsible for the unrealised potential of RNAi. Fast-forward to 2018, and we have finally arrived at the "Slopes of Enlightenment" with the approval of the first RNAi drug Onpattro. The age of RNAi has arrived.

Fig 1: RNAi and the Gartner Hype Cycle



Source: Adapted from Haussecker et al 2012[2]

Final thoughts

After nearly two decades in development, RNA therapeutics are now a clinical reality. The approval of these RNA-based therapeutics marks the start of the next chapter in what has been a rollercoaster ride for this novel drug class. Recent advances continue to broaden the clinical potential of this nascent class of medicines, but off-target effects remain a concern even with second generation RNAi candidates. Further strides in delivery technology will be required to expand and broaden their application.

What the future holds for RNA-based medicines remains to be seen, but what is certain is that the field of RNA therapeutics is experiencing a resurgence and their application in targeted genetic disease as well as their potential to address cancers, infectious and chronic disease will continue to drive their development for the foreseeable future.

References: 1. Aum J (APPS Journal, Dec 10), Delivery of siRNA Therapeutics: Barriers and Carriers.

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