Part of the conference series
Breakthrough science and technologies
Transforming our future

Oligonucleotides therapeutics

Held on 8 February 2022

Conference report

Supported by AstraZeneca

THE ROYAL SOCIETY

Introduction

On 8 February 2022, the Royal Society hosted an online conference on *Oligonucleotide therapeutics: challenges and opportunities*. This meeting, supported by AstraZeneca, forms part of the Royal Society's Transforming our Future series.

The Transforming our Future meetings are unique, high-level events that address scientific and technical challenges of the next decade and bring together leading experts from the wider scientific community, including academia, industry, government, and charities. The meetings are organised with the support of the Royal Society Science, Industry and Translation Committee.

The conference series is organised through the Royal Society's Science and Industry programme which demonstrates the Society's commitment to integrate science and industry across its activities, promote science and its value, build relationships and foster translation.

The programme was organised by Professor Sarah Tabrizi FMedSci, University College London, Professor Peter Goodfellow FMedSci FRS and Dr Shalini Andersson, AstraZeneca. The speakers and panel examined recent advances in oligonucleotide therapeutics, explored opportunities and discussed some of the main challenges in pre-clinical optimisation, trial design, delivery, and manufacturing.

This report is not a verbatim record, but a summary of the discussions that took place during the day and the key points raised. Comments and recommendations reflect the views and opinions of the speakers and not necessarily those of the Royal Society.

Executive summary

Oligonucleotides are some of the most promising therapeutic mechanisms for a range of hard-to-treat diseases, in particular those caused by genetic mutations. They are short sequences of synthesised DNA or RNA that bind to complementary sequences in mRNA or proteins within target cells. Sub-types include anti-sense oligonucleotides (ASOs), aptamer RNAs and RNA interference (RNAi) oligonucleotides.

Whilst oligonucleotides' ability to modulate gene and protein expression was confirmed in the early 1970s, the last five years have witnessed remarkable developments in basic science, clinical delivery and manufacturing methods across academia and industry. A number of oligonucleotide drugs have been licensed since 2016. Many others are being developed and tested in pre-clinical studies and clinical trials.

"We are all here for one reason: namely that we are interested in helping the patient. So we will keep the patient front and centre."

Dr Muthiah Manoharan, Alnylam Pharmaceuticals

"Those of us who were in the field more than 20 years ago saw a lot of toxicities because high doses of siRNA had to be used to get an effect. I think chemistry really solves that. We can now use much lower doses and I think that has really helped to make oligonucleotide therapies clinically applicable."

Professor Annemieke Aartsma-Rus, Leiden University Medical Center

"The dream was that genome sequencing would lead directly to therapeutics through oligonucleotide therapeutics. The dream is now becoming reality."

Professor Peter Goodfellow, Abingworth

This conference discussed recent advances in the optimisation of oligonucleotide therapeutics and their delivery mechanisms. It celebrated achievements to date and explored strategies to overcome some of the current challenges associated with developing oligonucleotide drugs to accelerate their path to market.

Key points include:

- Oligonucleotide therapeutics have enormous potential to target a wide range of clinical indications. It is anticipated that an increasing number of generic and patient-specific drugs will offer cost-effective treatments for conditions with high economic and disease burdens including neurodegenerative and respiratory disorders.
- The UK government has invested substantially in research and development infrastructure and grant funding for oligonucleotide and other nucleic acid therapies. Artificial intelligence and computer modelling are likely to shape design and optimisation in future. Regulatory authorities seek to offer dedicated support with pre-clinical studies, clinical trials and licensing applications which should not conform to a 'one-size-fits-all' model.
- Advances in nucleic acid chemistry and cell biology have helped to refine delivery mechanisms, enhance potency and duration of effect and reduce toxicity. GalNAc conjugation in particular has transformed clinical outcomes for hepatic diseases. However, efficacy, toxicity and delivery challenges remain a barrier to the licensing of further therapies. Continued exploration of underlying chemical and biological mechanisms is required. This will be an international and multidisciplinary endeavour.
- A new clinical trial framework for oligonucleotides is needed. It should exploit the potential of biomarkers and should consider the role of disease progression over time. Organoid technologies and other in vitro platforms are likely to offer valuable alternatives to pre-clinical animal studies.
- It is essential to balance safety and efficacy in all phases of the drug development process. Future trials must also recruit participants in the earlier stages of disease to acquire more meaningful safety and efficacy data.
- The lived experiences of patients must be considered in the design of appropriate clinical trial endpoints, the optimisation of delivery mechanisms and the frequency of therapeutic administration.

Genetic based medicines for neurological diseases

Dr C Frank Bennett, founding member and Chief Scientific Officer at Ionis Pharmaceuticals, outlined the broad scientific principles behind oligonucleotide therapeutics, highlighted some key clinical achievements and challenges to date and offered an introduction to future developments in the field.

ASOs bind to specific mRNA or pre-mRNA sequences within cells via Watson-Crick base pairing. The binding process triggers a range of mechanisms which modulate the target RNA and limit gene expression associated with progression of disease. Mechanisms depend on oligonucleotide design and include RNA degradation, splicing modulation and disruptions to protein translation. ASOs' exceptional specificity and their ability to bind to currently undruggable targets – such as non-coding and toxic RNA sequences – make them promising alternatives to conventional small molecule and protein-based therapeutics.

Clinical achievements and challenges

The sequencing of the human genome in 2003 transformed understanding of the drivers of monogenic and polygenic disease, facilitating research into genetic-based medicines such as ASOs. Twelve ASOs are currently licensed for use in humans, mostly to treat rare diseases. One of the most successful ASOs is nusinersen (also known as Spinraza™). This drug received regulatory approval in 2016 and has been shown to improve or even avert symptoms in children diagnosed with spinal muscular atrophy, a rare and often progressive motor neuron disease. The ability of nusinersen to penetrate the central nervous system – including deep brain structures – has fuelled the development and testing of ASOs for other neurological diseases and neurodevelopmental disorders including Alzheimer's disease, Huntington's disease and amyotrophic lateral sclerosis (ALS).

Clinical trials and autopsies have provided robust evidence for the biological and chemical mechanisms underpinning ASO treatments. However, in many cases trials have been unable to demonstrate that these mechanisms translate into sufficient clinical benefit to secure regulatory approval. In 2021, Phase III trials of tominersen (to treat Huntington's disease) and tofersen (for ALS) closed early after failing to attain their primary efficacy endpoints. A complication of these studies is that trial participants are often in advanced stages of disease. More research is needed to understand the role of genetics not only in triggering symptom onset but also in the development of disease over time.

Future developments

The design of an ASO influences its mechanisms and thereby its efficacy. One approach is to modify the chemical properties of ASOs themselves. This can involve synthesising double stranded ASOs or modulating their sugar and phosphate moieties to enhance binding affinity, increase stability and reduce pro-inflammatory properties. A second option is receptor ligand conjugation, which links an additional molecule or peptide to the ASO to improve the efficiency of cellular delivery into tissues such as the liver, pancreas, and skeletal muscle. Recent murine studies show that the carbohydrate N-acetylgalactosamine (GalNAc) is associated with a 10-30-fold increase in ASO potency. Significant reductions in the cost of genome sequencing are likely to further intensify research and development into genetic-based therapies in the coming decade.

> "Today, patients can come in with an unknown disease and within a month [can] have a genetic basis for their disease... [and] potentially have a path to developing a therapy for their disease."
>
> Dr C Frank Bennett, founding member and Chief Scientific Officer at Ionis Pharmaceuticals

Basic science of oligonucleotides and how chemistry can solve some of the challenges faced

Professor Anastasia Khvorova, University of Massachusetts Medical School, outlined the fundamental role of nucleic acid chemistry in the design and optimisation of oligonucleotide therapies.

The chemical architecture of informational drugs such as oligonucleotides is universal, and defines tissue distribution and safety. Target interactions, which allow gene silencing to a high degree of specificity and selectivity, are defined by sequences. Compared with conventional small molecule therapies that require molecule-specific optimisation, informational drugs can be produced for different targets as long as the chemical scaffold for a particular tissue is optimised and validated. In recent years, informational therapies have led to profound improvements in clinical outcomes – especially in diseases of the liver – and are likely to play an increasingly important therapeutic role in the future.

Recent advances in nucleic acid chemistry have substantially influenced the design and optimisation of oligonucleotide therapies and their delivery mechanisms. This work should help enable an increasing number of drugs to be licensed in the coming years. Minor alterations to oligonucleotides' chemical structures can lead to major variance in efficacy, toxicity, and clinical outcomes. This process is exemplified by Phase III trials for two drugs – revusiran and inclisiran. Small differences in the relative composition of ribose and backbone modifications between the two oligonucleotides resulted in significant disparities in potency, toxicity, and duration of effect. Inclisiran has been described as a 'miracle drug', requiring only two doses per year and significantly slowing cholesterol build-up.

> "The foundation for observed unprecedented efficacy, duration of effect, specificity, and selectivity of current versions of oligonucleotides is defined by chemistry."
>
> Professor Anastasia Khvorova, University of Massachusetts Medical School

Stability and duration of effect

Chemical stabilisation techniques to enhance pharmacokinetic and pharmacodynamic response include:

- Modifying terminal backbones with phosphorothioate
- Modifying the 5'-phosphate of the guide strand with 5'-(E)-vinylphosphonate, where the bridging oxygen is replaced with carbon and fixed in a stereoconfiguration optimised for AGO2 interactions
- Modifying the stereochemistry around target single-nucleotide polymorphism (SNP) sites to influence the RNA-induced silencing complex (RISC) specificity
- Enhancing the size of oligonucleotide molecules to increase uniformity of uptake, prolong retention in cerebrospinal fluid, and enable penetration into deep brain structures

Recent studies have also shown that oligonucleotide endosomal escape facilitates slow release of siRNA, enabling therapeutic benefit for 6-12 months. This result means that similar clinical outcomes can be achieved by reducing dose frequency and concentration, which creates a more tolerable regimen for patients.

Specificity

Chemical modifications to the seed region, which reduce the affinity of the target interactions, can minimise off-target effects.

Selectivity

Huntingtin gene studies have demonstrated that selectivity can be achieved and even enhanced by designing oligonucleotides to silence only the mutant allele and not the wild-type (non-mutant) allele.
Current barriers to chemical optimisation include limited sequence space, the complex design process for custom precursors, and the high cost of infrastructure required to perform scaffold optimised informatics, rarely available in academic laboratories. This area is likely to be a future focus of collaboration between academia and industry.

Challenges and opportunities in targeted delivery and distribution

Professor Matthew Wood, University of Oxford and co-founder of spinouts PepGen and Evox, explained the challenges associated with delivery of oligonucleotide therapies and explored some of the technologies that are being developed in response.

The number of licensed oligonucleotide therapies has grown steadily in recent years, with eleven drugs receiving regulatory approval since 2016. Ten of these, however, target diseases of the liver or skeletal muscle. Nusinersen is the sole oligonucleotide therapy currently available to treat a neurological disorder and the only one that can be administered intrathecally using existing delivery technologies.

To increase the number of approved oligonucleotide therapies, novel delivery mechanisms which balance efficacy and safety are required. This is particularly challenging for neurological and neuromuscular disorders such as Duchenne Muscular Dystrophy (DMD) as treatments must target a diverse range of tissues including cardiac muscle and the CNS. For a delivery mechanism to be effective it first needs to enable the oligonucleotides to reach the diseased cells. It must then facilitate endosomal escape – the process whereby molecules leave the endosome and enter the cytosol – so that the oligonucleotides can penetrate the nucleus, mitochondria, or other targeted intracellular compartments.

Although still largely at the pre-clinical stage, research and development into innovative delivery mechanisms has received considerable investment. Mechanisms fall largely into two categories: bioconjugates and nanotechnologies.

> "These vesicles [exosomes] deliver protein and RNA. In other words they are almost perfectly suited to the delivery of gene-editing protein RNA complexes and so have wide utility."
>
> Professor Matthew Wood, University of Oxford

Bioconjugates

Bioconjugates link oligonucleotides to biomolecules such as proteins or nucleic acids. GalNAc conjugates, used mostly to treat certain liver and kidney diseases, are the most successful examples developed to date and offer hepatocyte-specific targeting. Alternatives include:

- Arginine-rich peptide conjugates which link to charge-neutral oligonucleotides. Recent studies in DMD mice demonstrate highly uniform delivery, homogenous restoration of dystrophin to muscle cells and a 10-100-fold increase in efficacy compared to non-conjugated oligonucleotides.
- Antibody conjugates which have achieved high levels of drug concentration within the brain and which have the potential to transport oligonucleotides across the blood-brain barrier.
- Lipid conjugates which have so far shown highly efficient delivery into the CNS and effective gene silencing through repeated dosing.

Nanotechnologies

Nanoparticle-based therapies are structurally complex, utilising a variety of lipids to facilitate endosomal escape. At present, few drugs are licensed for use in humans due largely to challenges around efficiency and toxicity of synthetic nanoparticles. However, recent murine studies have demonstrated that the proteins and RNA in exosomes – naturally-occurring nanoparticles that play a key role in intercellular transfer – can be modified and tailored using platform technology to facilitate efficient delivery of nucleic acid-based drugs, including oligonucleotide therapies. Mice receiving exosome-based nanoparticle therapy show marked localisation of the drugs in the cortex and silencing of the β-secretase gene which is linked to the development of Alzheimer's disease. In addition, drug exposure is up to 1,000 times higher in inflamed tissues. The therapeutic potential of nanoparticle-based oligonucleotide drugs for inflammatory diseases such as multiple sclerosis and colitis is therefore significant.

Exploring the safety of antisense oligonucleotides

Dr Ritwick Sawarkar, University of Cambridge, discussed safety considerations when developing antisense oligonucleotide drugs for use in humans, and emphasised that an increased understanding of specific cellular mechanisms is essential to better evaluate the safety and toxicity profile of these therapies.

Despite the enormous therapeutic potential of ASO therapies, safety and toxicity challenges frequently delay or prohibit regulatory approval. ASO drugs which exhibited favourable toxicity profiles during pre-clinical murine studies have in some cases failed to meet safety endpoints during clinical trials.

To overcome these difficulties stakeholders must collaborate to consider the balance between safety and efficacy from the beginning of the drug development process. Cell biologists are working to advance their mechanistic understanding of the events that follow ASOs' entry into cells. Two issues are currently of particular interest: on- and off-target effects on cellular processes, and the contextual health status of trial participants.

On- and off-target effects

Human cells contain complex networks of RNA-binding proteins (RBPs) which play an important role in RNA homeostasis. Studies suggest that ASOs bind to RBP nodes in these networks in various ways depending on the design and modifications of the ASO in question. In recent years several thousand RBPs with binding capacity have been identified. The binding of ASOs to RBPs can impair RNA homeostasis and disrupt cell signalling mechanisms including transcription, translation and splicing both on- and off-target, thus inducing toxicity. UV crosslinking and proteomics enable cell biologists to determine the precise mechanisms and cellular binding partners underpinning disrupted homeostasis and help understand how and why toxicity occurs. Areas of particular focus include sub-cellular localisation and changes in gene expression.

Contextual health status of trial participants

Clinical trial participants are typically in the later stages of disease. This means that ASO-associated toxicity is usually more evident and more acute. A recent study of Negative Elongation Factor (NELF) proteins in cells with protein misfolding revealed that RNA can bind to NELF in diseased cells but not in healthy cells, which may result in different types and degrees of toxicity based on the disease status.

A knowledge base to identify precise drivers of disease-specific toxicity and characterise optimal mechanisms for safe and efficient ASO delivery is being assembled by Dr Sawarkar's team in close collaboration with Professor Anne Willis. Both teams are based at the MRC Toxicology Unit in Cambridge, UK. This work will be instrumental in accelerating the design, development and licensing of less toxic ASOs.

> "We are starting to get a lot of exciting data. Hopefully in the next few years we will be able to collaborate with many of you to work on some of the ASOs which matter to you and use our mechanistic understanding of cell biology to identify mechanisms of efficacy and toxicity."
>
> Dr Ritwick Sawarkar, University of Cambridge

Living in the world of RNA therapeutics

Dr Muthiah Manoharan, Senior Vice President and Distinguished Research Scientist at Alnylam Pharmaceuticals, celebrated some of the recent successes in RNAi therapeutics and emphasised the importance of keeping patients and trial participants at the heart of drug development and decision-making.

Oligonucleotides modify gene expression through a number of mechanisms. One of the most established is RNA interference (RNAi), where small interfering RNA (siRNA) sequences – comprised of synthetic sense and antisense strands – enter cells. In the cytoplasm they bind to corresponding mRNA sequences and activate molecular scissors to block protein translation. Studies have demonstrated that in many cases only 100ng of RNAi therapeutic per gram of tissue is sufficient to induce long-term gene silencing.

Two decades of pre-clinical RNAi studies have resulted in four licensed drugs that act through the RNAi pathway, all of which target liver hepatocytes. It is expected that further RNAi therapies will be approved in the coming years to treat diseases of the eye and of the CNS. An improved understanding of the origin of off-target effects will be important to further enhance efficacy and facilitate the path to licensing.

Alnylam Pharmaceuticals has developed a three-pronged approach to maximise efficacy of RNAi therapeutics. This involves chemical modification of siRNAs, lipid nanoparticle (LNP) formulation of siRNAs for intravenous administration, and GalNAc conjugation to siRNAs for subcutaneous administration. The first RNAi drug, ONPATTRO® (patisiran), was licensed in 2018 to treat polyneuropathy in patients with hereditary transthyretin-mediated (ATTR) amyloidosis, a rare and incurable protein disorder caused by a mutation in the TTR gene in liver cells which can affect cardiac tissue, peripheral tissue, and the CNS. The Alnylam team first optimised lipid nanoparticles conjugated to the siRNA. They subsequently added a second-generation lipid to create an enhanced conjugate. This therapeutic demonstrated 90% long-term knockdown of the TTR gene, subsequently validated by the results of the Phase III APOLLO trial which likewise found high knockdown in all participants six months after dosing.

The other three licensed RNAi drugs all involve the GalNAc ligand conjugate and were approved between 2019 and 2021. They are GIVLAARI® (givosiran) for acute hepatic porphyria, OXLUMO® (lumasiran) for primary hyperoxaluria type 1 and Leqvio (inclisiran) for hypercholesterolemia. These therapeutics enable efficient delivery of RNAi into endosomes and demonstrate a 30-fold increase in potency compared to first- and second-generation antisense oligonucleotides. It is estimated that inclisiran may benefit up to 300,000 patients with high cholesterol over the next ten years.

Further RNAi therapeutics are being trialled in the clinic. These include vutrisiran, also to treat ATTR amyloidosis with polyneuropathy and cardiomyopathy. A single 50mg dose of vutrisiran has demonstrated 90% knockdown of the TTR gene over 12 months. Modifications such as thermodynamic destabilisation of RNA sequences and selective introduction of chiral phosphorothioate linkages provide additional opportunities to enhance pharmacokinetic and pharmacodynamic behaviour of RNAi therapeutics.

Advances in mechanistic understanding of the RNAi drugs and oligonucleotide therapeutics owe much to patients who participate in clinical trials. It is therefore essential that these populations remain at the heart of drug development and decision-making.

> "For the first time, patients who are suffering from [ATTR amyloidosis] have been given their life back."
>
> Dr Muthiah Manoharan, senior vice president and distinguished research scientist at Alnylam Pharmaceuticals

Building partnerships: an introduction to the MRC/UKRI Nucleic Acid Therapy Accelerator

Professor Nick Lench, recently appointed Executive Director of the new MRC/UKRI-funded Nucleic Acid Therapy Accelerator, outlined the mission of this multidisciplinary research institute and discussed the opportunities that it will offer to those at the forefront of research and development of oligonucleotide and other nucleic acid therapies.

The UK benefits from a well-established research base in nucleic acid therapies. This rapidly developing field has also received significant interest from SMEs and the pharmaceutical industry. To capitalise upon these opportunities, the UK Government has provided £30million from 2019 – 2024 through the Medical Research Council (MRC) / UK Research and Innovation (UKRI) Strategic Priorities Fund to establish the Nucleic Acid Therapy Accelerator (NATA).

NATA's mission is to rapidly overcome scientific and technological barriers to the development, manufacture and licensing of next-generation nucleic acid therapies. It will create opportunities to bring together national and international stakeholders from academia and industry to advance translational research and development. It will prioritise highly innovative and disruptive platform technologies that cannot be delivered without multidisciplinary and multisectoral collaboration.

At present NATA takes a 'disease and class agnostic' approach. This means that its specific research and development foci will crystallise over time and will remain responsive to emerging challenges and opportunities. Current areas of interest include the design and synthesis of oligonucleotides, ligand conjugation, and precision targeting.

£18million of the MRC award has funded the establishment of a translational research Hub within the Harwell Science and Innovation Campus in Oxfordshire. The Hub houses state-of-the-art biology and chemistry laboratories and the location enables NATA to collaborate with other institutes at the forefront of research and development such as the Rosalind Franklin Institute and the Diamond Light Source, the UK's national synchrotron. The remaining £12million will be awarded to two academic-industry consortia as part of the Manufacturing and Delivery Research Challenges in mid-2022. The successful consortia will address some of the most urgent challenges to delivery, development, and manufacture of nucleic acid therapies such as sustainability, immunogenicity and long-term safety. NATA will also enable knowledge transfer to researchers and SMEs through collaborative research, training and capacity-building in the UK.

> "The ability to provide both personalised and precision therapies to patients using these new innovative therapies will transform medicine."
>
> Professor Nick Lench, Executive Director of MRC/UKRI-funded Nucleic Acid Therapy Accelerator

Regulatory paths for oligotherapeutics

Dr David R Jones, Pharmaco-Toxicology Consultant and former Expert Pharmaco-Toxicologist at the UK Medicines and Health products Regulatory Agency (MHRA) discussed some of the challenges associated with pre-clinical animal studies and outlined some strategies to facilitate licensing of oligonucleotide therapeutics.

To date over 120 clinical trials for oligonucleotide drugs have been completed, and almost 40 are currently open to recruitment. Oligonucleotides – especially ASOs – have significant potential to target and treat a variety of diseases. However, until 2013 only one ASO was approved for use in humans. This can be attributed partly to various regulatory challenges.

Oligonucleotides are produced through chemical synthesis but also exhibit some mechanistic behaviours of biological therapies such as species specificity and tissue selectivity. It remains ambiguous whether pre-clinical testing should be guided by regulations governing biological or chemical pharmaceuticals. In many respects oligonucleotides are evaluated as biological therapeutics. However, single-species studies are usually sufficient to establish these therapeutics' long-term toxicity. Since oligonucleotide therapies typically demonstrate interspecies variance in toxicity most undergo pre-clinical studies in both rodents and non-human primates.

Animal models and studies

More than 90% of in vivo primary pharmacodynamic studies of oligonucleotide therapeutics reviewed by the MHRA utilised disease-based animal models. Conclusions drawn from these models – however carefully constructed – should be treated with caution. There are significant differences between the pathologies of clinical trial participants living with progressive disease and those of laboratory animals, which are often influenced by xenografts and diet or subject to chemical or transgenic intervention. Human and animal endpoints are rarely directly comparable. Pharmacodynamic studies for species-specific oligonucleotide drugs rely on surrogates. This presents a further complication due to interspecies variance in recovery from adverse events. Shortages of non-human primates for laboratory research delay approvals and increase drug development lead times.

The role of regulatory authorities

The MHRA plays an essential role in guiding pre-clinical and clinical studies and in approving therapeutics for use in humans. To facilitate licensing of oligonucleotide therapies it will be important to obtain high-quality predictive data and minimise unnecessary animal testing. The value of animal studies lies in the provision of sufficient pre-clinical data for swift progression to in-human trials, where the most relevant safety information is generated. Due to the wide range of oligonucleotide targets and toxicities there is no one-size-fits-all set of toxicology biomarkers. Pre-clinical safety data should be evaluated on a drug-by-drug basis.

To generate more realistic safety and efficacy data clinical trials are also beginning to enrol participants in earlier stages of disease. National regulatory bodies advise research teams at all stages of the drug development process. The MHRA offers particular support around innovative medicines and the transition from pre-clinical studies to in-human trials. Late-stage studies can seek guidance from the EU's Scientific Advice Working Party (SAWP) and the Committee for Medicinal Products for Human Use (CHMP). Ongoing dialogue between drug development teams and national and supranational regulatory authorities will be essential to realise the potential of oligonucleotide therapeutics in the coming years.

"Regulators are there to help. They are not there to put up roadblocks or hurdles."

"What's important to the patient is really and truly what matters and so clinical trials have to be designed better these days. They have to be more patient-centric. The choice of the patient has to be paramount."

Dr David R Jones, Pharmaco-Toxicology Consultant

PANEL DISCUSSION

Addressing key challenges to maximise the potential of oligonucleotide and other nucleic acid therapeutics

The panel discussion was chaired by Professor Annemieke Aartsma-Rus, Leiden University Medical Center, and featured Dr Steve Hood, Senior Research Director, Imaging Expertise Networks at GSK, Dr Muthiah Manoharan, Senior Vice President at Alnylam Pharmaceuticals, Dr C Frank Bennett, Chief Scientific Officer at Ionis Pharmaceuticals, and Professor Sarah Tabrizi, UCL Queen Square Institute of Neurology.

Improving the design of clinical trials

- There is considerable appetite for a paradigm shift towards science-led and patient-centric clinical trial design – especially for neurodegenerative disorders – to allow efficacy to be explored and established with greater confidence.
- Many trials enrol older participants in the later stages of progressive disease. This makes it more difficult to achieve efficacy endpoints, especially considering the placebo effects associated with participating in a trial. The use of high doses to maximise deep brain and tissue penetration may in some cases lead to inflammation and other toxicities.
- Certain clinical outcomes may not materialise for months or even years after a participant is recruited into a trial. This slows down drug development and optimisation towards potential marketing authorisation. Advanced imaging technologies and biomarkers – such as exosomes – could be used as surrogate endpoints in Phase I trials. These would offer a more reliable understanding of efficacy, minimise potential for a placebo effect and could facilitate rapid progression to Phase II and Phase III trials.
- It is essential to involve patients in the design of meaningful endpoints related to disease progression and quality of life rather than rely solely upon standard clinical outcome measures.
- If certain groups of participants display a promising biomarker response to a therapeutic, it can be instructive to conduct a stratified trial with this patient population.

> "Soon we may get more patient-specific ASOs than we currently have approved oligos for larger populations."
>
> Professor Annemieke Aartsma-Rus, Leiden University Medical Center

Modelling and development

- Pre-clinical animal studies have limitations, and shortages of non-human primates create additional challenges. It should be noted that preclinical studies are generally undertaken in animals with healthy brains and tissues. This does not allow useful comparison with progressive disease in humans.
- To generate meaningful pre-clinical data it is probable that some animal work may be superseded by organoid technologies and sophisticated in vitro platforms such as brain-on-a-chip, which may better replicate human CNS function.
- Patients often have a good understanding of their condition and should be consulted when designing the frequency, intensity and mechanisms of dosing regimens. Intrathecal administration is common and is well tolerated for CNS delivery if the frequency does not exceed every 3 – 4 months. Intraventricular administration and Ommaya reservoirs tend to carry a higher risk of infection and adverse reactions than intrathecal or subcutaneous injections.

- Improvements to delivery mechanisms – especially to the CNS – are one of the most critical areas for further development. It is anticipated that significant progress will be made in the next five years. A key factor will be an enhanced understanding of sub-cellular mechanisms. This will be achieved through complex modelling and intrathecal imaging.
- Delivery to deep brain structures is a particular challenge. Current modes of administration include intrathecal injections, and future opportunities include possible intraventricular administration. Even then it can be difficult to penetrate different cell populations within the brain. Myelinated tissue in the internal capsule (white matter) slows the transfer of oligonucleotides into the deep brain structures. An improved biological understanding of the impact of progressive disease on the blood-brain barrier is also needed.
- Considerable effort is being invested in developing extra-hepatic specific targeting ligands with comparable specificity and efficacy to the liver-specific GalNAc conjugates.
- Replacing native phosphodiester with phosphorothioate can enhance protein binding, reduce the impact of extracellular nucleases, and increase stability. In some cases, however, with certain sequences and ribosugar chemical modifications, too many phosphorothioates can elicit undesirable side effects.

"The limitations – and what we are learning from the patients – could be very much used in a positive way."

Dr Muthiah Manoharan, Alnylam Pharmaceuticals

"I felt energised by the potential excitement but also by the challenges of how we can improve CNS delivery because we have no treatments for neurodegeneration. It is [currently] costing us billions of pounds a year to treat dementia and other neurodegenerative diseases"

Professor Sarah Tabrizi, University College London

"When we talk about delivering oligos, we have to think that the patient's going to have to take it. So when we come up with our route of delivery it has to be patient friendly. We have to listen to the patient and see what they want to do."

Dr Steve Hood, GSK

- It is likely that advances in chemistry and peptide technologies will eventually enable diversified peptides to be conjugated with anionic (negatively charged) single- or double-stranded oligonucleotides. These conjugations have the potential to expand distribution and efficacy of oligonucleotide technologies and overcome some complexation issues associated with cationic peptides.
- Whilst most oligonucleotide drugs target a single gene, therapeutics targeting two or more gene isoforms simultaneously have begun to be tested in clinical trials. Advances in chemistry may also facilitate the development of oligonucleotides which affect only mutant genes and preserve the wild-type genes.
- The most successful oligonucleotide drugs are likely to have long half-lives, the potential for intrathecal or intraventricular administration every 4 – 6 months – which is usually manageable for patients – and sufficient potency to enable uniform penetration into deep brain structures and other tissues.

Manufacturing

- Each oligonucleotide sequence has unique properties and requires optimisation. Modification using phosphorothioate compounds is common but can involve trade-offs between stability and toxicity.
- At present only narrow patent claims are granted for oligonucleotide therapeutics. In the coming years many innovations will come off-patent. This is likely to benefit academic research teams conducting clinical trials. It is not expected to significantly deter commercial engagement because in many cases market exclusivity for rare disease therapies will persist.
- Although oligonucleotides are already more cost-effective to produce than some conventional drugs, there is an urgent need to find ways to manufacture and store them at pace, at scale and at lower cost.

The Royal Society is a self-governing Fellowship of many of the world's most distinguished scientists drawn from all areas of science, engineering, and medicine. The Society's fundamental purpose, as it has been since its foundation in 1660, is to recognise, promote, and support excellence in science and to encourage the development and use of science for the benefit of humanity.

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Registered Charity No 207043

Issued: April 2022 DES7994