NUCLEIC ACID INSIGHTS

OLIGONUCLEOTIDES: INNOVATION IN ENGINEERING AND DESIGN

SPOTLIGHT

For the many and the few: bringing ASO therapies to both mainstream and nano-rare indications

INTERVIEW

"...we have a 100% success rate to date in the patients that have been treated long enough to evaluate for benefit."

Roisin McGuigan, Commissioning Editor, *Nucleic Acid Insights*, speaks to **Stanley Crooke**, Chairman, Founder and CEO, n-Lorem, about his long career and pioneering role in the antisense oligonucleotide (ASO) space, how RNA-targeting therapies are set to enter the mainstream, and his latest focus: making personalized, free-for-life ASO therapies available to nano-rare patient populations.

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Q Could you tell me a bit about your career, from entering the oligonucleotide space to founding the n-Lorem Foundation?

SC I have been involved in basic research and drug discovery and development for many decades now. I did my MD–PhD and house staff training at Baylor College of Medicine and was on the faculty for over 25 years. In the first 5 years of my career, I led the building of the first broadly successful line of cancer programs at

Bristol Myers, and then was president of R&D at what is now GSK. In 1989, I founded Ionis Pharmaceuticals in order to create a new, more efficient drug discovery technology: antisense oligonucleotide (ASO) technology. Having succeeded at doing this, in 2020 I retired.

Then, in response to the desperate need of patients with extremely rare mutations—what I have dubbed nano-rare—I established n-Lorem as a non-profit to provide experimental ASO treatments to patients with nano-rare diseases.

Q How has the antisense field evolved over the time you've been active in this space, and what would you pick out as the biggest milestones to date?

SC We spent 30 years creating the technology and advancing it. The fact that it exists is an extraordinary achievement, particularly since it was to a large extent done in a single company. At Ionis, we advanced the chemistry and the understanding of the molecular mechanisms by which these drugs produced their pharmacological, toxicological, and immunological effects, and we learned how to manage that. To date, there are now 17 RNA-targeted drugs that have been approved. That includes the first blockbuster, which is our antisense drug for spinal muscular atrophy, Spinraza®.

I fully expect additional drugs to be approved this year. At Ionis they have ASOs to treat very large cardiometabolic indications that are completing: including an 8,000-patient cardiovascular outcome trial, a 5,000-patient Phase 3 trial, and still other large clinical trials. In the next couple of years, I anticipate that the technology will take its place in the mainstream of therapeutic options for patients.

However, after scaling up for 30 years at Ionis, in the last 5 years at an-Lorem we have been scaling down. This is because antisense technology is today the only technology that can address the needs of a meaningful number of these patients with nano-rare mutations.

Q Turning to the present, could you tell me a bit more about the current key activities and goals of the foundation?

SC The history of the foundation begins around 2017, when I was CEO and lead scientist at Ionis. I was visited by two sets of parents of two boys with mutations in SCN2A, that encodes the voltage-gated sodium channel alpha-subunit Na(V)1.2. Both of these boys were severely affected, as one might expect, with severe seizures, movement disorders, autonomic dysfunction, and a wide range of autistic symptomatology. In short, they were desperately ill, and progressing.

In that meeting, I had to tell these parents that the indication was simply too small for Ionis to pursue. However, I realized that the technology is efficient enough that I could probably make an ASO for them and give it to them for free. As I learned more about nano-rare mutations and the syndromes they cause, I began to recognize that with genomic sequencing we were identifying many of these patients. While it wasn't what I originally planned for my retirement, it seemed like something that I had to do.

I founded n-Lorem with the mission to industrialize a process via which we can respond to the needs of these patients by discovering, developing, manufacturing, and providing

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ASOs. Our aim is to do this one patient at a time, with a personalized ASO for each patient, and to do that for free, for life.

That is our mission and we have been fortunate enough to raise sufficient funds to grow dramatically to respond to the need that we have—and I certainly didn't expect the demand that we have experienced. Further, we know that that demand is going to continue to grow exponentially every year.

Today we are privileged to be able to say we have a 100% success rate to date in the patients that have been treated long enough to evaluate for benefit. We have also observed pristine safety and tolerability, and that sets the stage for doing more tomorrow. And more is required, as every day more and more patients are identified and come to us for help.

Q You have published some very positive clinical data recently. Can you discuss some of those key clinical milestones to date, and what's next?

SC When I founded n-Lorem I thought by this stage we would have maybe a handful of applications, and might be treating a patient. In fact, we've now processed more than 330 applications for treatment and accepted more than 160 patients, with a wide range of different diseases and mutations. We are capable of filing about 20 INDs per year, and we have filed in four divisions of the US FDA: two neural divisions, the eye division, and cardiorenal. Although the clinical studies we conduct are not registrational studies, in our view, we now have significant evidence of benefit in patients with eye disease and kidney disease. In the CNS we've seen truly profound benefit in a wide range of patients, with dramatic reductions in seizures, significant improvement in movement disorders, substantial recovery of functions lost, and even the acquisition of new skills that the patient never had before. This causes us to rethink entirely how plastic the CNS is and how much we can recover from developmental delays. We've also seen significant benefit in autism symptoms, in patients with evidence of autonomic dysfunction, and improvement in pain syndromes.

Notably, we have done all this while having no ASO-related serious adverse events to date—we think that record can be continued, given the qualities of this technology and our experience in using it. It is very important to us to avoid imposing additional hardship on these patients in the form of drug-related side effects.

Another critical component of our efforts is the work that we did with the FDA, mostly in 2019, before I founded n-Lorem. This led to the unique guidance that the FDA has issued for ASOs for nano-rare patients, which supports us treating these extremely sick patients directly with only in vitro data and a single 3-month rodent toxicity study. That makes it cost-effective, and we can get to patients in 15–20 months. Most of these patients need that, because they are progressing rapidly to death or to loss of organ.

We are also investing in the future of antisense technology. I personally led most of the work that resulted in many of the advances in understanding the mechanisms by which

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these work and then used that to make better ASOs. The technology is still continuing to advance at a rapid pace. We are working in a number of areas to make better ASOs for our patients, both for nano-rare patients and for more prevalent diseases.

We think that our mission is galvanic, and have been gratified by the response to it. I'm very pleased with the amount of funds that we have been able to raise, and excited by the progress that we've made in moving toward establishing more sustainable financing models. We are poised to do a lot more and do it a lot better in the coming years.

Q Where are the key opportunities as you see them to improve targeted delivery of ASO therapies?

SC There are still many opportunities to broaden the reach of the technology and of course, a large fraction of current research efforts are focused on targeted delivery to different organs. From my perspective the GalNAc conjugation was a major step forward, but it was fairly obvious. The liver's job is to scavenge molecules from blood, so we just asked the liver to do what it does.

What gives me more optimism about targeted delivery is that we showed that with a GLP-1 conjugated ASO, we could get ASOs in pharmacologic concentrations in pancreatic β cells. We get no ASO in the absence of the GLP-1 conjugate, and that receptor is not a scavenger receptor, but actually a G protein-coupled receptor.

Many of the programs are also focusing on transferrin to improve delivery to skeletal muscle and elsewhere, and there are many broader opportunities for targeted delivery involving different targets.

The work we have done to understand the mechanisms of cytotoxicity has yielded what we think of as the probable third-generation chemistry, in which we point-modify at specific sites with specific modifications to reduce the potential for cytotoxicity.

We are also learning a great deal about why some phosphorothioate-modified ASOs activate innate immunity, and that's an area of active research in my group. We are making real progress in learning to control that. There are multiple new chemistries coming along, some of which look potentially interesting. Of course, we've looked at many thousands of different chemical modifications through the years. You have to accept that for every 1,000 modifications you look at, maybe one of them will have some value in the long term.

The entry of ASOs into the major cardiometabolic and CNS diseases in the next two to three years is going to be very important, because that will take RNA-targeted drug discovery and put it in the center of therapeutics, which is where it belongs.

These are a few areas of opportunity for the space, and there are many others. For example, we are learning that 60% of our patients require allele selective ASOs, and developing means to do that in a much better way. There are multiple mechanisms to upregulate translation that we have already shown, and perfecting those so that we can do a better job for loss of function mutations is another major effort that is ongoing at n-Lorem.

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Q A challenge often cited in the oligonucleotide space is moving towards more environmentally sustainable manufacturing. What are your thoughts on how the space can improve in this area?

SC It's important to emphasize that this is still an extremely young technology. Small molecule drug discoveries have been around for 125 years. Monoclonal antibodies are now in their 50th year. The industry has spent 40-plus years on gene therapy, and well over $60 billion, and fundamental advances are still needed and there are plenty of things to work on.

For ASOs the next steps are fully integrating manufacturing to include both manufacture at all kinds of scales, and formulation, and having more in-process quality control systems. I think there are a variety of firms that are working on that now.

There are also no exotherms, no high-pressure reactions, and no difficult chemical reactions. The main environmental issue we face is that we have still not found a substitute for acetonitrile. Handling acetonitrile and the waste from it is an expensive and environmentally sensitive challenge. However, in the grand scheme of manufacturing drugs, it's fairly modest compared to many other things I've dealt with in my career. Simplifying the manufacturing process and weaning it from acetonitrile to the extend that is feasible would be an important step forward. It's certainly not that we and others haven't tried, but it has proven tough. I am optimistic that it will get resolved.

Q How do you predict the ASO space will develop over the next 5 years, and what will be your own key priorities in that timeframe?

SC The technology's next step will happen in the next couple of years as ASOs take their place in mainstream medicine in cardiometabolic indications, and CNS diseases like Parkinson's and Alzheimer's disease. We will continue to advance new mechanisms that will broaden the reach of the technology and importantly, learn how to make these more agonist-like drugs by altering translation of specific target proteins. We know how to do that, so now we need to do it better.

Advances in allele selectivity that depend on understanding RNase H1 are important, particularly for our patients. For any situation in which you have a heterozygous mutation and you'd like to alter only the mutant form of the RNA and protein, targeted delivery is an important effort. I am particularly anxious to see us do a better job in the heart and in some immune cells. We still have challenges with treating solid cancers, for a variety of reasons. Better control of innate immune activation is something that we are actively working on.

At the same time as this is happening, at n-Lorem we are downscaling the technology to treat a single patient. To my mind, the breadth of the appetite that we have for antisense is truly extraordinary to think about: treating millions, and treating one. There's never been a technology that could lend itself to even thinking about that.

We have demonstrated we can do this, and do it repeatedly and safely. We've demonstrated that a non-profit model can work. Our next task is to take this to the next level and provide our services to the many patients who still need our help. I don't make light of those challenges, which are very real, but they are mostly about money. From a technical perspective we have crossed all the hurdles and shown that none of this is impossible. It

just boils down to raising as much money as we can for n-Lorem over the next few years, and expanding our reach.

n-Lorem is a non-profit organization creating individual antisense oligonucleotide (ASO) treatments for patients with nano-rare diseases, for free, for life—learn more about the foundation, and ways to support it, at www.nlorem.org.

BIOGRAPHY

Stanley T Crooke is Founder, Chairman and Chief Executive Officer of n-Lorem, Carlsbad, CA, USA, a nonprofit foundation focused on providing treatments for patients with nano-rare disease patients (1–30 patients worldwide), which he initiated in January 2020. Prior to n-Lorem, Dr Crooke founded and was Chairman and Chief Executive Officer and Lead Scientist of Ionis Pharmaceuticals. Early in Dr Crooke's career, he led the creation of the first broad anticancer program in the industry at Bristol-Myers. He then assumed responsibility for worldwide R&D (President) at SmithKline Beckman (now GSK). Dr Crooke has also contemporaneously led a successful academic career becoming a full Professor at Baylor College of Medicine, Houston, TX, USA and the University of Pennsylvania Medical School, Philadelphia, PA, USA where he trained a number of PhD students and won several teaching awards. Dr Crooke has been an active scientist throughout his career as well. He has received a number of awards, most recently, the Steven C Beering Award for Advancement of Biomedical Science, Indiana University School of Medicine, Prix Galien Roy Vagelos Pro Bono Humanum Award, the American Chemical Society's EB Hershberg Award for Important Discoveries in Medicinally Active Substances, the Lifetime Achievement Award presented by the Oligonucleotide Therapeutics Society, the Scrip Lifetime Achievement Award, and the 2019 Massry Prize. Dr Crooke received his MD and PhD degrees and house staff training at Baylor College of Medicine, where he currently serves on the Board of Advisors. In 2021, Dr Crooke has been named Distinguished Alumnus of both Baylor College of Medicine's Graduate and Medical schools and named one of the 20 of the most influential biopharma R&D executives by Endpoints News. He has published over 600 scientific publications, edited more than 20 books, has numerous patents, and led the development of more than 23 drugs that have been commercialized.

Stanley T Crooke MD PHD, Founder, Chairman and Chief Executive Officer, n-Lorem, Carlsbad, CA, USA

AUTHORSHIP & CONFLICT OF INTEREST

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