NUCLEIC ACID INSIGHTS

LAUNCH EDITION

SPOTLIGHT

INTERVIEW

Pushing the boundaries of nucleic acid delivery

The limitations of current viral and non-viral gene delivery technologies are well documented, but the solutions to challenges such as tolerability and moving beyond the liver remain elusive. **David McCall**, Senior Editor, BioInsights, talks to **John Lewis**, Founder and Chief Executive Officer of Entos Pharmaceuticals, about a novel nucleic acid delivery technology that seeks to harness the best of both worlds.

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Q What are you working on right now?

JL: Entos is working on developing next-generation genetic medicines using our Fusogenix proteolipid vehicle (PLV) drug delivery system. We are interested in utilizing the system to partner with the world's leading pharmaceutical companies to develop next-generation genetic medicines. We are also interested in developing an internal group of genetic medicines for indications ranging across cancer, age-related diseases, rare diseases, and metabolic diseases.

Q Can you give us your high-level summary of the current state of the art—and its limitations—in nucleic acid delivery?

JL: We are in an age of incredible promise when it comes to genetic medicines. Through the COVID-19 pandemic, we learned that we have the ability to generate and gain approval

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for genetic medicines very quickly. In addition, we are able to sequence the human genome, we understand the genetic underpinnings and causes of most diseases, and we have developed in the lab tools to up-regulate, down-regulate, or even edit genes to cure diseases. The big challenge now concerns getting these potentially life-saving tools into the body and into specific cells in a safe, effective, and redosable manner. We do not yet know how to do that. However, we do have some commercially approved viral and non-viral proofs of concept.

On the viral side, we have adeno-associated viruses (AAVs) and some other promising viruses that can deliver genetic material—in this case, DNA—to effect either gene editing, the expression of genes, or even the knockdown of genes. However, viruses have significant limitations. For example, they elicit their own immune response, so you can only use them to treat one disease since they are not redosable. Furthermore, viruses pose a challenge in terms of cargo capacity. Some genes are much larger than the cargo capacity of an AAV, for instance. There are also manufacturing challenges and tolerability challenges when systemically dosing viruses. Sadly, we have seen some high-profile adverse events, including patient deaths, as a result of poor tolerability.

On the other side of things, we have non-viral approaches like the lipid nanoparticle (LNP), which is perhaps the 'poster child' of non-viral approaches given its successful application in the mRNA vaccines against COVID-19. The real challenge with LNPs has to do with the way in which they deliver genetic material. They rely on endocytosis and use ionizable lipids to escape the endosome. This escape causes damage to the cells. In addition, LNPs are very liver-tropic, particularly when they are administered systemically to the whole body. In these cases, they get taken up by the liver and cause dose-limiting toxicities in this organ. Therefore, they must be administered locally, such as in a vaccine, or if they are not administered locally, they can only be used to treat diseases in the liver. Lastly, since nucleic acids are delivered to the endosome in the case of LNPs, which is where all the immune sensors (like toll-like receptors and the cGAS-STING pathway) are located, they create quite a substantial immune response. In fact, in the case of DNA delivery with LNPs, the immune response is so potent that it is unfeasible.

Right now, AAV vectors and LNPs can only address perhaps 10% of the possible indications that could potentially be addressed with genetic medicines. The state of the art right now are genetic medicines that can deliver either RNA or DNA everywhere in the body safely, effectively, and repeatedly.

Q Tell us more about Entos' approach and platform—what differentiates it in the realm of nucleic acid delivery technologies?

JL: At Entos, we thought, 'What if we could combine the best aspects of viral and non-viral delivery, while also reducing the limitations of both platforms?'. We have taken the viral

fusion protein, which is the part of the virus that is most important for efficient cargo delivery by allowing the delivery platform to fuse with the target cells.

To give a bit of background, envelope viruses like HIV and influenza have a membrane on the outside, and they have fusion proteins that fuse with cells. However, they are very large and immunogenic, so they are not feasible for use in drug delivery. The fusion protein that we use at Entos is very small and is from the only non-envelope virus to make a fusion protein. One of Entos' co-founders, Roy Duncan, called it the fusion-associated small transmembrane (FAST) protein. It facilitates the fusion of a lipid particle like an LNP directly with the outside membrane of a target cell, completely avoiding endocytosis and endosomal escape.

This protein has allowed us to do two things with our Fusogenix proteolipid vehicle formulation. Firstly, we can completely change the way we formulate it because with the FAST proteins helping with the delivery, we do not need ionizable lipids, and we do not need the cholesterol that is in conventional LNPs. The FAST protein has also allowed us to be very safe, as it makes our formulation so much better tolerated than an LNP or AAV. Secondly, without cholesterol, our PLVs will go everywhere in the body without being liver-targeted. This enables us to deliver both RNA and DNA since we are avoiding all of the immune sensors in the endosome, making our formulation an endosomal escape-independent mechanism of delivery for nucleic acid medicines.

Q As you mentioned earlier, the Entos pipeline ranges across a wide variety of therapeutic areas and indications—could you go into more depth about the particular considerations for delivery in some of the target tissues involved, and the specific benefits that an approach such as Entos' can offer to each one?

JL: First, I will mention our vaccine program. During the pandemic, we developed a DNA-based COVID-19 vaccine. The reason we developed a DNA-based vaccine was because we had a good feeling that the RNA vaccines would work, but we knew they had significant limitations. The main limitation is that they need to be kept very cold—at -80 °C. In comparison, a DNA vaccine is perfectly stable at fridge temperatures, just like the influenza vaccine, so cold chain distribution is much cheaper and more straightforward. In addition, the cost of goods for making DNA vaccines is much lower than with RNA, as there are far fewer steps involved in making DNA. DNA vaccines also have much better durability of effect than their RNA counterparts. We are finding that RNA vaccines require frequent boosting to keep the levels of neutralizing antibodies up. DNA makes the antigen for a longer period of time, which should substantially increase the durability of boosters as well.

The first DNA vaccine for use in humans was approved during the pandemic. It requires a huge dose, between 4 and 6 mg of DNA, which is a much larger dose than that of RNA used in the currently approved COVID-19 vaccines. With the PLV platform, however, we are able to create a DNA vaccine that uses very similar dosages to the RNA vaccines, and which is delivered through the same route: intramuscular injection.

As far as our other programs, we have a partnership with Eli Lilly developing genetic medicines for central and peripheral nervous system diseases. We are able to introduce our platform through various roots of administration (e.g., intrathecal administration) and get great transit to the brain for approaches like gene editing.

We also have a great partnership with the Bill and Melinda Gates Foundation. In this case, we are addressing diseases like influenza, malaria, and HIV. The idea here is to create medicines that are analogous to the infused antibodies we have seen from Regeneron Pharmaceuticals, Eli Lilly, and AbCellera Biologics, where we infuse neutralizing antibodies to reduce the severity of or even prevent disease. We want to encode those antibodies in either DNA or RNA and deliver them in a PLV through intramuscular injection. This will dramatically reduce the cost associated with these medicines, making them more readily available worldwide to at-risk populations.

Q As the CEO of a biotech in the rapidly evolving and advancing nucleic acids space, what is your take on the current funding environment for the field?

JL: Most of the large pharmaceutical companies and many small biotechs are focused now on genetic medicines, particularly given the success of the RNA vaccines during the pandemic. So overall, despite a challenging financing environment, there is a lot of interest out there in genetic medicines in general.

I think we are almost but not quite at the point where people in the investor community are realizing that the delivery technology is really the drug. It does not matter how well you can edit a gene—if you cannot get it into the cell, then it is not a drug. Hopefully, over the next 2–3 years, more investors will realize that the delivery technology is the key to making these drugs safe, effective, and redosable, and will therefore fund more research into the delivery technology.

Q What targets will be next for nucleic acid therapeutics?

JL: Again, there are a number of targets in the liver, which can be addressed by local delivery. Because we can easily hit the liver, there are many liver programs underway. However, there are many targets outside the liver, too. As pharmaceutical companies realize the opportunity to hit tissues outside the liver, there will be a lot more programs developed.

One of the key initial target organs that we will likely see is the lung. There is a huge opportunity for genetic medicines to treat diseases like cystic fibrosis and eye diseases—for example, the first commercial AAV gene therapy was approved in the eye. However, there are still many diseases of the eye that cannot be addressed by current delivery technologies. Finally, Entos will also be looking to develop targets in extrahepatic tissues, like the kidneys and bone marrow.

Q What would you identify as some strategic keys for future success in these target areas?

JL: First and foremost, we need safe, effective, and redosable medicines that target extrahepatic tissues. Obviously, targeting is a big component of this as we need to be able to

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get an effective dose to the tissue and cells in order to generate a therapeutic response. However, we must also have a reasonable cost of goods.

A key area of innovation for all companies, including Entos, is developing formulations that have tropism for certain tissues like the lung, the kidney, and the bone marrow. There is a lot of innovation to be done in these areas.

Q How significant are IP and freedom to operate issues in this space, and how do you see this situation playing out as we move forward?

JL: There are certainly IP and freedom to operate issues with cargo. Companies developing state-of-the-art gene editing technologies are, in many cases, having to license those proteins and approaches from major institutes. On the delivery side, there is a lot of IP restriction in terms of both LNPs and AAV.

We have been able to carve out our own space, as we are not using a conventional LNP, and are therefore not reliant upon all of the foundational LNP patents. And since we are not using AAV, we are not relying on those patents either. We have great IP around the FAST proteins and their use for a wide variety of different genetic medicine approaches.

Again, the key to the IP challenge moving forward will be working with partners who have licensed key cargo technology and targets to create lifesaving medicines.

Q What will be some important next steps in innovation in nucleic acid delivery?

JL: The speed with which gene editing technologies have improved is really remarkable. What I love about these gene editing technologies is that we are curing disease. These are not long-term treatments for chronic diseases: we are able to effect cures.

I believe that the combination of novel genetic medicine approaches like ours with state-of-the-art gene editing techniques is where we are going to see some amazing results in the next 5 to 10 years.

Q It's January 2025—what is the one thing everyone in the nucleic acid space is talking about?

JL: They will be talking about gene editing. We now have in-human proof-of-concept that gene editing can work in the liver and the heart, which is really exciting. The next thing will be to apply these strategies to other tissues and other diseases more broadly. The other important topic people in the space will be discussing is, again, getting outside the liver to

functionally target important tissues that have significant disease, like muscles, lungs, and kidneys.

Q Lastly, can you sum up one or two key goals and priorities that you have for Entos over the foreseeable future?

JL: Over the next 2 years, it is our goal to develop more than a dozen internal programs that we would like to bring up to IND. Within 5 years, we want to have at least two of these product candidates in clinical trials. In 10 years, we want at least one of our medicines to be commercially available to patients.

BIOGRAPHY

JOHN LEWIS is a Professor in Oncology and the Bird Dogs Chair in Translational Oncology at the University of Alberta, and the Founder and Chief Executive Officer of several biotech companies, including Entos Pharmaceuticals. As a scientist, he pioneered the use of intra-vital imaging in the *in vivo* study of tumor cell invasion and metastasis to discover key targets for cancer therapeutics. Dr Lewis also develops novel nanotechnology, nanoparticle drug delivery technologies, and imaging-based treatments for chronic diseases, such as aging and cancer, as well as for early detection of cancers. As an entrepreneur, Dr Lewis translates scientific discoveries from the lab to the clinic to improve patient health and quality of life. Entos Pharmaceuticals is a clinical-stage biotechnology company developing next generation genetic medicines using its Fusogenix PLV nucleic acid delivery system. Dr Lewis trained at The Scripps Research Institute and received a PhD in Biochemistry from the University of Victoria.

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AUTHORSHIP & CONFLICT OF INTEREST

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