FEBRUARY 2024

Volume 1, Issue 1

NUCLEIC ACID INSIGHTS

BIOINSIGHTS

LAUNCH ISSUE

NUCLEIC ACID INSIGHTS

Volume 1, Issue 1

CONTENTS

Spotlight

Launch edition

FOREWORD

David McCall, Senior Editor, *Nucleic Acid Insights*

I am delighted to welcome you to *Nucleic Acid Insights*!
This new journal represents something old and something new for BioInsights. Over the past few years, we have increasingly covered the messenger RNA (mRNA), plasmid DNA (pDNA), and lipid nanoparticle (LNPs) fields through our three established journals—*Cell & Gene Therapy Insights*, *Immuno-Oncology Insights*, and *Vaccine Insights*. However, we feel the time is now right to give nucleic acids its own platform, allowing us to cover the aforementioned specific areas in greater depth, whilst also affording us the opportunity to explore the oligonucleotide space in earnest for the first time.

As pathways to clinical and commercial 'proof of concept' for emerging modalities go, that trodden by mRNA and LNPs in the form of the COVID-19 mRNA vaccines was unusual to say the least. However, what is perhaps most significant as the field now looks to the future is the fact that CAGR estimates generally point to double-digit growth throughout the course of the next decade, despite the inevitable major loss of revenue caused in part by the huge success of the COVID-19 vaccines themselves. While investment in expanding the reach of mRNA and LNPs into new applications and therapeutic areas will remain a key driver for this growth, there is of course much more to the nucleic acids field. We can look forward to more and more novel vaccines and therapeutics reaching the market, and building on the extensive knowledge base that has been built over the past two decades and more in both lab and clinic. Furthermore, there is a tremendously exciting and diverse range of applications beyond vaccines and therapeutics to consider—opportunities abound for RNA and DNA innovation in *in vitro* modelling, gene editing platform development, diagnostics, synthetic raw materials, and data storage to name but a few.

Nucleic Acid Insights is designed to fill a number of important gaps in the current range of media and publications for the field by drawing its many disparate strands together in a single open access online resource.

We cover all the component technology areas with BioInsights' established blend of independently peer reviewed, deep technical/scientific content and strategically-pitched commentary, allowing our members to delve into the data for specific tools and technologies whilst gaining a better understanding of how it all fits into the bigger picture.

We include all functions relevant to the space, from manufacturing to market access, and from regulatory affairs to preclinical/translational/clinical R&D.

As a translational journal, we look to the future through the eyes of our expert Editorial Advisory Board Members and contributors, providing usable insights to help shape and drive the development of the next wave of nucleic acid technologies and ultimately, speed delivery of their benefits to the patients who need them.

For our launch, we have aimed to offer 'status updates' on some key technology areas, and a glimpse of what lies ahead, through the perspectives of some of the foremost thought leaders working in and around nucleic acids today. Many thanks to all of those who have kindly taken part and to the members of our Editorial Advisory Board.

I hope you enjoy this inaugural edition of *Nucleic Acid Insights*!

NUCLEIC ACID INSIGHTS

LAUNCH EDITION

SPOTLIGHT

INTERVIEW

Wall Street analyst's perspective: taking the pulse of the nucleic acid-based prophylactic & therapeutic vaccine sectors

It is a challenging funding environment for all in the biotech world, but just how resilient are the nucleic acids field and its component technology areas such as mRNA and oligonucleotides, comparatively speaking? **David McCall,** Senior Editor, *Nucleic Acid Insights*, talks to **Hartaj Singh,** Managing Director and Senior Analyst, Biotechnology, Oppenheimer & Co., Inc., about the current and future issues, trends, and drivers shaping the prospects of the field.

Nucleic Acid Insights 2024; 1(1), 31–36

DOI: 10.18609/nai.2024.006

Q What are you working on right now?

HS: I have worked as a biotechnology analyst at Oppenheimer & Co., Wall Street for 7 years now, during which a number of waves of innovation have come through in the sector. Currently, these waves are focused on digital tools—specifically, AI and its application in biotechnology. However, post-pandemic, there has also been a significant focus on nucleic

acids and mRNA technology in particular. At the start of 2023, we published a white paper addressing this emerging trend [1].

These two areas stand out as the focal points of general interest in our current discussions with various stakeholders. Regarding mRNA, we have observed a considerable influx of private investment, while some noteworthy companies such as Moderna and BioNTech have entered the public domain. While smaller initial public offering opportunities are not yet prevalent, there is an expanding interest in this space.

Q How would you sum up how the financial markets have responded to both progress and setbacks in the nucleic acid-based prophylactic and therapeutic vaccine spaces over the past few years?

HS: Nucleic acid-based prophylactic vaccines, like the ones developed for COVID-19, have surprised many by creating significant value. Moderna's market capitalization increased from roughly US$10 to 30 billion, and BioNTech's from a few billion to $25 billion. Both companies now hold substantial cash reserves of approximately $10–15 billion each. This success can be attributed to their rapid development and introduction to market of prophylactic mRNA COVID-19 vaccines within a year of the start of the pandemic. As a point of reference, numerous industry professionals initially doubted the feasibility of such a quick development cycle. They were not necessarily skeptics, but they were uncertain whether nucleic acid technology had advanced to the extent necessary to allow this speed of progress. Nevertheless, nucleic acid-based prophylactic vaccines have provided significant benefits to society, both in terms of financial value and in saving human lives. It is estimated that hundreds of millions of lives have been saved by these vaccines over the last 3 years.

With therapeutic vaccines, however, the landscape is more complex. Moderna has recently seen promising Phase II results for a therapeutic vaccine for cancer, but overall, the promise of the field remains to be determined. Much more research is needed, and although there is consensus on the scientific and biological rationale for nucleic acid therapeutic vaccines, the challenge now lies in translating this knowledge into a commercial product.

Q How would you say the oligonucleotide and mRNA sectors in particular are bearing up at the moment, comparatively speaking?

HS: The current financing environment is obviously challenging, but having experienced several cycles in the biotech industry, I have developed a nuanced perspective. Currently, funding still seems readily available for companies demonstrating exceptional scientific advancements that translate effectively in clinical settings. Conversely, there appears to be a lack of funding for companies that resemble more of a science project, or that are several years away from presenting clinical data. This is especially true when either the regulatory path is uncertain or the causal biology behind the disease is not well understood.

To phrase it slightly differently, the financing environment is favorable for companies with robust scientific foundations that have successfully translated into clinical applications and possess some visibility toward a commercial product. In terms of mRNA, particularly given the success of Moderna and BioNTech, there seems to be a phenomenon akin to a gold rush.

A similar trend is emerging in the wider nucleic acid space, albeit more concentrated in private ventures at the moment. However, there is anticipation of a significant influx of public companies entering the market in the next 3–5 years, accompanied by substantial mergers and acquisitions activity within the next 5–10 years; let's say, although this will likely conclude before the end of this decade.

Q What will be some keys to sustained recovery and future success for biotechs in these areas moving forward?

HS: One broad factor influencing the landscape is macroeconomics. A simple aspect of that, widely agreed upon, is the decrease in interest rates. This results in a more financially liquid environment, fostering easier financing—a macroeconomic boost.

Another thing to consider, however, is the potential persistence of high interest rates, coupled with robust economic growth. Again, though, even in such an environment, companies excelling in both great science and translation into the clinic can secure financing. This emphasizes the critical importance, particularly in biotech, of companies possessing science that translates well into clinical applications.

In the realm of nucleic acids, Moderna and BioNTech have demonstrated the significance of lipid nanoparticles (LNP) in ensuring effective mRNA delivery into targeted tissues. It's worth noting that those companies that have made significant investments in optimizing LNPs likely hold a substantial lead of a few years over others in the sector. While LNPs are analogous to tiny fat droplets, their engineering complexity lies in encapsulating small mRNA molecules. When delivered subcutaneously, LNPs must traverse cell membranes, undergo controlled disintegration, and release mRNA—a series of technical challenges that many companies and contract manufacturers have yet to scale commercially.

Q Where and when do you expect to see RNA and DNA therapeutics having an impact?

HS: The most straightforward analogy for therapeutic cancer vaccines is that they are a turbocharger for the immune system. Much like attaching a turbocharger to a car engine for an extra boost, cancer vaccines can be administered alongside or following other therapies, providing a complementary benefit to ongoing treatments. However, addressing the challenges posed by the tumor microenvironment is crucial to success in this field. Dysfunctionalities within the immune system, often encouraged by tumors, make this a complex task. While progress is anticipated in cancer, significant scientific and translational work is still needed due to the dynamic and intricate nature of the tumor environment and immune system dysregulation.

In rare diseases, the outlook is optimistic but dependent on overcoming specific technical challenges. For instance, engineering mRNA to produce a missing enzyme regularly and in sufficient quantities poses a hurdle. Once these technical challenges are addressed, the prospects for mRNA applications in rare diseases are promising. The resolution of these hurdles is expected to trigger a swift scale-up of products into clinical trials and subsequent market entry.

"Looking forward, I think it is conceivable that a nucleic acid company could emerge as the first trillion-dollar biotech company within the next decade."

In infectious diseases, the success of the mRNA vaccines for COVID-19 make this a highly promising approach. Indeed, positive results have already been seen in mRNA vaccines for influenza and RSV, with Moderna planning to launch soon in the latter. The outlook is optimistic for mRNA vaccines targeting various infectious diseases globally, as evidenced by the increasing number entering clinical trials.

There is also potential in applying mRNA technology to immune-related conditions, with its ability to up-regulate or down-regulate the immune system. However, despite this intuitive appeal, further scientific and causal biology work is essential across various autoimmune diseases to realize the full potential in this area. This comprehensive approach aligns with my general viewpoint on the current landscape.

Q As you look across the field today, what specific technologies and modalities catch your eye in terms of their potential, and why?

HS: I have been considering this topic a lot lately. Over the past 30–40 years, there has been a noticeable shift in the creation of value. Up until the 1990s, a substantial amount of value was generated from small molecule drug development. However, with the advent of companies like Genentech, followed by Regeneron and Amgen, among others, there was a significant shift towards creating value using biologics. However, I believe we are now witnessing a resurgence in small molecule drug development. I recently spoke with the Chief Scientific Officer of Vertex, who highlighted cryoEM, a tool that enables mapping of the molecular structure of small molecules with exceptional precision. This advancement allows for the careful selection of molecules to target specific diseases and their underlying pathology.

Thanks to innovative hardware and software tools, small molecule drug development is experiencing a renaissance, with better design and more targeted therapies on the horizon. Companies utilizing cutting-edge tools are speeding up the process of bringing small molecules through clinical trials and getting them commercialized much faster than the average rate of 7–9 years. For instance, Vertex developed each of its three CFTR modulators for cystic fibrosis in less than 4 years.

Digital tools, including AI, play a crucial role. While the immediate impact may not be fully realized due to the abundance of data in biotech, there is potential for significant benefits. The initial steps involve structuring data and creating predictive models. Large, well-capitalized biotech companies, such as Vertex and Moderna, are already employing these tools extensively. Over time, the utilization of digital tools is likely to expand, with standalone contract manufacturers, clinical research companies, and healthcare software companies providing these tools. Although this diffusion might take 5–10 years, I believe that forward-thinking biotech companies will increasingly leverage digital tools for enhanced efficiency and innovation.

Q What are the obstacles to the future success of nucleic acid-based vaccines and therapeutics that Wall Street worries about the most?

HS: The primary challenge lies in mRNA manufacturing. Creating an mRNA that can endure *in vivo* is difficult. Moderna, for example, has implemented numerous modifications to the mRNA at both ends, enhancing its robustness and protein generation capability. This involves intricate scientific processes that require companies to develop exceptional proficiency.

Additionally, the core LNP adds another layer of complexity. The engineering and scientific understanding required are exemplified by Moderna's investment in advanced technology. In 2018, the Moderna CFO mentioned their use of a synchrotron, a sophisticated physics device, to delve into the atomic level for LNP generation. To illustrate the commitment, Moderna acquired its own synchrotron, underscoring the level of investment needed for success in this space.

It is crucial for investors in the public domain to grasp that despite the notable achievements of Moderna and BioNTech spanning over a decade, other companies are still in the process of overcoming similar obstacles. Much of what these companies have achieved is safeguarded by intellectual property. This intricate and protected knowledge base contributes significantly to their success.

Q Finally, what's your personal vision for how nucleic acids will shape healthcare moving forward?

HS: The potential for nucleic acid technology in the biotech industry is enormous. Currently, we have glucagon-like peptide companies specializing in glucagon-like peptide-1s for obesity nearing a market cap of $500 billion. Looking forward, I think it is conceivable that a nucleic acid company could emerge as the first trillion-dollar biotech company within the next decade.

The versatility of mRNA is significant in targeting diseases in ways that small molecules and biologics cannot. It is similar to uncovering a new frontier, allowing us to explore and address diseases more effectively. This expansion of possibilities, especially in rare diseases, oncology, immune-related conditions, and infectious diseases, could reshape the entire landscape of biotechnology on a global basis.

REFERENCE

1. Oppenheimer Inc. Oppenheimer innovation series: mRNA technology, Jan 2023.

BIOGRAPHY

HARTAJ SINGH is a Managing Director and Senior Analyst covering Biotechnology. Prior to joining Oppenheimer & Co., Hartaj was a Managing Director and Senior Biotechnology Analyst at BTIG Securities. Hartaj began his sell-side career at Lehman Brothers, and subsequently moved to the buyside covering biotechnology at Visium Asset Management and Tecumseh Partners. He began his career as a Clinical Trial Project Manager for ClinTrials

Research, and also worked as a Strategic Analysis Manager for Johnson and Johnson, both of which give him critical experience in clinical trial design. Hartaj has a BA in Biology from Case Western Reserve University, and also did extensive graduate work in computational neurobiology. He also holds an MBA from Duke University's Fuqua School of Business.

AFFILIATION

Hartaj Singh
Managing Director and
Senior Analyst, Biotechnology,
Oppenheimer & Co., Inc.

AUTHORSHIP & CONFLICT OF INTEREST

Contributions: The named author takes responsibility for the integrity of the work as a whole, and has given their approval for this version to be published.

Acknowledgements: None.

Disclosure and potential conflicts of interest: The author has no conflicts of interest.

Funding declaration: The author received no financial support for the research, authorship and/or publication of this article.

Article source: Invited.

Interview held: Nov 16, 2023; **Revised manuscript received**: Jan 17, 2024; **Publication date:** Jan 24, 2024.

NUCLEIC ACID INSIGHTS

LAUNCH EDITION

SPOTLIGHT

INTERVIEW

Harnessing the differentiated capabilities of mRNA for rare diseases

Having blazed a trail in the application of mRNA in the prophylactic vaccine setting, Moderna is now engaged in expanding the reach of this technology into the therapeutic sphere. **David McCall,** Senior Editor, *Nucleic Acid Insights*, spoke with **Paolo Martini,** Chief Scientific Officer of the International Therapeutics Research Centers at Moderna, about the potential to transform the lives of patients with rare diseases.

Nucleic Acid Insights 2024; 1(1), 21–27

DOI: 10.18609/nai.2024.004

Q What are you working on right now?

PM: I am currently involved in managing Moderna's international operations recognizing the global prevalence of rare diseases. Specifically, we focus on regions around the world that are affected by genetic disorders. Our strategy involves entering geographic locations where it is challenging to locate patients with rare diseases. We aim to target regions where there may be a concentration of affected individuals, allowing us to engage with a significant number of patients dealing with the specific rare disease we are addressing. Through these efforts, our goal is to extend our reach to as many rare disease patients worldwide as possible.

Q What for you are the chief high-level trends and strategic drivers shaping both the prophylactic and the therapeutic mRNA vaccine spaces as we move into 2024?

PM: The most evident application area for RNA currently is vaccines—however, beyond this, gene replacement offers a compelling route. While commonly seen as a chronic disease therapeutic option, RNA actually provides a secure way to replace mutated or absent genes in patients, potentially yielding the correct protein with physiology that aligns with the protein's natural half-life. What distinguishes RNA from gene therapy is its ability to mimic the physiological pulsatility of the substrate body. This mirrors the cyclical pattern of substrate levels rising followed by enzyme production to reduce the substrate, and so on. The body's intelligent process aligns well with the opportunities presented by mRNA, for example. While gene editing holds promise for the future, RNA stands out as a noteworthy alternative for the near-term in this area. Promising outcomes are emerging in patients with propionic acidemia, methylmalonic acidemia, and glycogen storage disease type IA in ongoing clinical trials for rare diseases. The initial results offer hope, and the outcomes of these trials will be crucial for the development of a viable solution until genetic engineering matures sufficiently.

Q Can you give us some further details of Moderna's current rare disease therapeutics R&D pipeline, and discuss the rationale behind its evolution over the past couple of years?

PM: I joined Moderna in 2015 and our focus was on addressing high unmet needs, which meant tackling diseases that had been previously largely untouched. This was due to challenges such as the complexity of triggering responses, as seen in propionic acidemia, for instance, where mutations in two different subunits make gene replacement difficult. mRNA presented a unique opportunity by allowing the combination of various RNAs in a single drug to address multiple components simultaneously.

Our approach was opportunistic based on the tools we had to hand, such as lipid nanoparticles designed with a preference for hepatocytes due to their affinity for the low-density lipoprotein receptor in order to optimally deliver mRNA to the liver. We attempted to maximize these tools and selected diseases characterized by high severity and mortality, aiming to correct the underlying disorders.

This focus led to the development of our pipeline, centering on diseases with critical needs. Unfortunately, conditions like methylmalonic acidemia and propionic acidemia often result in a lifespan of only around 6 years for affected children. There are rare instances of adults surviving with mild mutations, but these patients face numerous issues in their lives. I had the unfortunate privilege of witnessing the struggles of children with these diseases first-hand, including the frequent intubations due to metabolic decompensation, ER visits, cognitive disorders, and dietary restrictions limiting them to high-fat or high-carbohydrate

"What distinguishes RNA from gene therapy is its ability to mimic the physiological pulsatility of the substrate body."

diets. However, our ability to translate these experiences into actionable insights for the company allowed us to strategically build a portfolio aimed at making a meaningful impact. If our ongoing clinical trials prove successful, we may offer a chronically administered solution, providing some relief to patients. Ultimately, we envision a future where our efforts lead to solutions for these challenging medical issues.

Q Can you expand on the current state-of-the-art in the application of cutting-edge mRNA technology within the rare disease therapeutics space as you see it?

PM: When I first joined Moderna, many people said that utilizing RNA therapeutically was impossible due to the body's recognition of it as a virus. RNA was considered an unlikely candidate for therapeutic use at that time. However, by mutating uridine, we found a way to bypass the immune system, enabling the body to accept RNA.

The cutting edge with our current capabilities lies in changing RNA into a form that the body can accept and translate. There has been significant progress in harnessing the best characteristics of mRNA in the human genome, standardizing the RNA region and optimizing the operative frame. This optimization enhances the ability to translate. Furthermore, lipid nanoparticles exhibit superior distribution capabilities through the liver when compared to viral particles, as observed in animal models.

A crucial advantage of mRNA is its dose responsiveness. Predicting and administering efficacious doses early on in development is feasible, especially in rare diseases. Through modeling, this predictability allows for potential modifications. Another key feature is multiplexing; enabling the combination of multiple RNAs to construct more complex structures, which could be beneficial in vaccination.

RNA technology offers valuable therapeutic flexibility, particularly when combined with lipid nanoparticles, which demonstrate safety and tolerability recognized by regulatory bodies such as the US FDA. Despite being categorized under the gene therapy umbrella, lipid nanoparticles are comparatively better understood, reducing the need for extensive long-term safety studies.

While chronic therapy has many disadvantages, the potential benefits in diseases with limited survival probability are important. The future holds promise for developing user-friendly delivery systems that enable self-administration, but in the meantime, we can start to use the therapy and begin to see some potential benefit.

So, there are a lot of different elements that differentiate mRNA, including dose responsiveness, lipid nanoparticles, safety/tolerability, and the ability to multiplex. If these factors can align with the health authorities' concerns, emphasizing safety and tolerability in particular, it paves the way for a promising therapeutic drug with proven efficacy.

Q What should be the key areas of focus for research to further enable the growing pipeline of RNA therapeutic product candidates?

PM: What sets Moderna apart from other companies is our comprehensive approach to understanding every aspect of drug development. We put emphasis on understanding how

to produce the drug and how to transcribe RNA from a template whilst ensuring the integrity of the RNA and avoiding small fragments. We have optimized purification processes, considered codon optimization, and examined the speed of RNA transcription and translation by the ribosome. There are various other aspects we are looking at, including protein binding to RNA, RNA binding to other RNAs, and seeking improvements in RNA half-life to enhance protein production. However, the most significant challenge lies in delivery.

Ongoing research aims to refine delivery mechanisms, a complex task that requires extensive efforts. This holds the potential to broaden the spectrum of diseases that can be treated with RNA. Crossing the blood-brain barrier remains a challenge for central nervous system disorders due to the brain's protected nature.

Despite these challenges, Moderna is actively engaged in various aspects of delivery research. Learning from failures is integral to our approach as it acts as a catalyst for more research, helping us to identify critical aspects that can enable advancement, at Moderna, failure motivates us to invest even more effort—a distinctive characteristic that sets us apart.

Q Can you expand on how Moderna is aiming to address the key challenges facing mRNA therapeutic developers seeking to advance product candidates for rare diseases into and through the clinic?

PM: Patient-centricity is at the core of our approach and understanding patient needs and anticipating the future of a drug are crucial. Currently, we address only severe diseases because we have the necessary tools, but the ability to treat more conditions becomes desirable as patients recognize the broader potential. There may be milder diseases where traditional infusion methods could have challenges due to patient time constraints but again, optimization of delivery systems may eventually make it possible for self-administration.

Addressing certain organs presents ongoing challenges such as the central nervous system and skeletal muscle, due to their difficulty to target. Despite these challenges, there is still a great effort of work needed and the key is maintaining focus; when concentrating on a specific aspect, you can gradually build knowledge. Trying to tackle too many things at once can delay progress, as it disperses focus and understanding.

While progress is being made in some areas, there's still considerable work ahead. As mentioned, maintaining focus is critical—I have seen many companies attempting to cover too much ground at once. Tackling one thing at a time allows for a deeper understanding of the drug being produced and the potential responses it may elicit. An incremental approach contributes to a more comprehensive understanding and effective development of therapies.

Q What can we deduce from regulators' reactions to mRNA technology applied in the therapeutic setting to date, and what should be some corresponding important points of focus for drug developers?

PM: I have been pleased with our interactions with regulatory agencies thus far, particularly with the Medicines and Healthcare products Regulatory Agency in the UK—their deep understanding of lipid nanoparticle technology has been invaluable. Similarly, the FDA and several other agencies are increasing their understanding of RNA modalities due to an increased

"Similarly, the FDA and several other agencies are increasing their understanding of RNA modalities due to an increased awareness of their safety and tolerability driven by the increasing generation of patient data."

awareness of their safety and tolerability driven by the increasing generation of patient data. In the case of our propionic acidemia therapy, patients have been on treatment for over 18 months now. This real-world evidence is helping to contribute to the growing understanding of the therapy's safety profile. The regulatory agencies have shown a willingness to understand more about the space. This includes discussion on necessary drug approval processes in terms of toxicology studies for safety tolerability of the drug and species selection for testing.

The agencies are well-versed and prepared in the specifics of the diseases we are addressing, and now have a good understanding of RNA technology to go with it. RNA and the unique characteristics of its therapeutic application are constantly emerging themes in these discussions. Regulators are actively engaging to explore ways to streamline and expedite the approval process for RNA therapies.

Q Looking to the future, can you sum up your vision for how mRNA will impact the rare diseases therapeutic space moving forward?

PM: Based on the positive outcomes observed in some patients, which span safety, tolerability, and efficacy, I am hopeful that there is a significant opportunity within the field of rare diseases. It holds promise not only for patients with specific rare diseases, but also for those who are ineligible to receive AAV-based gene therapy due to the presence of pre-existing antibodies.

Assuming everything continues in the right direction, there is a bright future for mRNA therapy, particularly if the technology evolves to become more patient-friendly. It would also be great to see the continued success of gene editing in terms of correcting genetic abnormalities and utilizing the body's own regulatory mechanisms. However, in the meantime, mRNA therapy fills a crucial gap, especially for patients who cannot access certain other therapies. It may complement or even enhance existing treatments, addressing multiple aspects of a condition. This should still be approached with caution, but if the evidence aligns with expectations, mRNA therapy is likely to persist for a while until more permanent solutions emerge.

Q What is the one thing that people in the nucleic acids space will be discussing in January 2025?

PM: I am optimistic that in 2025, people will actively discuss and recognize the benefits of these therapies, laying the foundation and building anticipation for further developments in the future.

Q Lastly, can you highlight one or two key goals or priorities that you have for your work over the foreseeable future?

PM: The main focus will be on capitalizing on existing tools to maximize the efficacy of RNA therapy and to treat diseases effectively. Initially, the emphasis will be on diseases that can benefit from the existing system, allowing for effective treatment whilst also laying the groundwork for the development of new systems.

Ultimately, the clinical data will guide decision-making and shape the direction of the pipeline. The key goal is to progressively enrich the pipeline, ensuring that the therapies developed align with the needs of patients and the evolving landscape of rare diseases.

BIOGRAPHY

PAOLO MARTINI currently holds the position of Chief Scientific Officer, International Therapeutics Research Centers at Moderna. He is also the founder of Moderna Rare Diseases, a division of Moderna. His research career, spanning over two and a half decades, is marked by his contributions to drug discovery, focused on molecular mechanisms under-lying monogenic and multigenic metabolic and fibrotic disorders. His laboratory focuses on identifying novel mRNA therapies and applying translational approaches for drug development in rare diseases and hematologic disorders.

In his previous tenure at Shire Pharmaceutical, Martini served as the Senior Director of Discovery Biology and Translational Research. His research focus was on fibrotic diseases of muscle, kidney, skin, lung, bone marrow, and metabolic liver diseases with an emphasis on different therapeutic modalities for pathway modulation.

Prior to his role at Shire, Martini was an integral part of the discovery research team at EMD-Serono, where he concentrated on understanding the complexity of breast tumor tissues and related markers.

As an accomplished author, Martini has over 40 publications in peer-reviewed journals. Additionally, his work has been featured in numerous scientific magazines, covering a wide range of topics from oncology research to rare genetic disorders.

Martini lends his expertise to several esteemed organizations. He serves on the scientific advisory board of the Keystone Symposia and Certa Therapeutics, based in Melbourne, Australia. He is also a board member of the Institute of Life Changing Medicines, a US-based non-profit organization that focuses on Crigler-Najjar disease type 1.

AFFILIATION

Paolo Martini
Chief Scientific Officer,
International Therapeutics Research Centers,
and
Founder,
Moderna Rare Diseases,
Moderna

AUTHORSHIP & CONFLICT OF INTEREST

Contributions: The named author takes responsibility for the integrity of the work as a whole, and has given their approval for this version to be published.

Acknowledgements: None.

Disclosure and potential conflicts of interest: The author has no conflicts of interest.

Funding declaration: The author received no financial support for the research, authorship and/or publication of this article.

Article source: Invited.

Revised manuscript received: Jan 8, 2023; **Publication date:** Jan 11, 2023.

NUCLEIC ACID INSIGHTS

LAUNCH ISSUE

SPOTLIGHT

INTERVIEW

Delivering on the promise of mRNA cancer vaccines in the immuno-oncology clinical setting

The recent unveiling of highly promising data from the Keytruda in combination with V940 (mRNA-4157) development program has breathed new life into the cancer vaccine field. **David McCall**, Senior Editor, *Nucleic Acid Insights*, talks to **Dr Jane Healy**, Vice President of Early Oncology Development, Merck Research Laboratories, about this ongoing study and Merck's future plans to explore the full potential of mRNA technology in the oncology space.

Nucleic Acid Insights 2024; 1(1), 47–53

DOI: 10.18609/nai.2024.009

Q What are you working on right now?

JH: I am excited about our ongoing exploration of the next generation of therapies for cancer patients. Our focus includes a variety of approaches such as combinations with Keytruda (pembrolizumab), which has shown activity in several tumor types and has been under development for the past decade. But we are also exploring combinations with other therapies, including chemotherapy and backbone drugs that have approval, as well as some novel mechanisms.

"What sets mRNA technology apart, specifically when compared to peptide-based vaccines, is that cells take up that mRNA and translate it using their native machinery. This results in a more organic presentation on the cell surface."

Our primary goal is to reach as many cancer patients as possible, providing significant benefits and extending lives. Leveraging cutting-edge science, whether developed internally or in collaboration with partners, is crucial to achieving this goal.

We are actively exploring novel targets, alone or in combination with standard of care therapies and earlier lines of treatment, targeting different stages of diseases to assess our impact on survival rates. We aim to maximize patient benefit, potentially reducing the need for later treatment and minimizing associated toxicities. While immunotherapy remains a significant focus, we are also investigating combinations with therapies such as antibody-drug conjugates.

Our involvement extends to various fields including small molecule inhibitors such as a kirsten rat sarcoma virus (KRAS) G12C inhibitor, as well as molecules like the hypoxia-inducible factor 2 alpha (HIF2α) inhibitor, and steroidogenesis inhibitors like CYP11A1. We are open to exploring several mechanisms, recognizing that broadening the response to these therapies for patients with cancer is our goal.

Q Can you give us some more details on the data achieved to date from, and the current status of, the Keytruda in combination with V940 development program?

JH: Among all of the therapies currently under exploration in the field, V940 is the one that excites me most in terms of the potential impact on the future of oncology, pending successful data replication in confirmatory Phase 3 trials. In the oncology space, there have been decades of research into finding an effective vaccine against cancer. Finally, breakthroughs are being made, particularly with the promising Phase II data for V940 in combination with Keytruda in patients with resectable melanoma.

V940 is characterized as an individualized neoantigen therapy (INT), as it is designed using information from an individual patient's tumors to generate the therapy. The mutations identified in the sequencing of their tumors are used in the creation of the INT, focusing only on mutations predicted to be most recognizable to the immune system. These mutations are coded onto an mRNA molecule, with up to 34 different neoantigens included in a single INT. The mRNA is then encapsulated into a lipid nanoparticle to aid in delivery and minimizing the immune response associated with the mRNA.

What sets mRNA technology apart, specifically when compared to peptide-based vaccines, is that cells take up that mRNA and translate it using their native machinery. This results in a more organic presentation on the cell surface.

The use of the patient's own cellular machinery to present the antigen to immune cells enhances recognition and the cells' ability to respond to their normal cellular processes. Another advantage of mRNA over other traditional approaches is its ease of manufacture. Synthesizing RNA and its packaging is more straightforward, offering simplicity in terms of

the supply chain and patient delivery—a crucial factor considering the individualized nature of this therapy, and one that differentiates it from patient-specific cellular immunotherapies. After sending a sequencing sample to a lab, the mutations are identified, those predicted to be most recognizable to the immune system are ranked according to a proprietary algorithm, and then they incorporated into an MRNA, encapsulated in a nanoparticle, and sent back to the patient's doctor to be administered as part of their treatment regimen. Our target turnaround time for this process is approximately 6 weeks. Patients receive a total of nine doses of the INT every 3 weeks, in addition to any other standard-of-care therapies.

The Phase II trial is the first randomized trial with an INT that shows statistically significant and clinically meaningful benefit for patients. This represents a significant milestone in cancer vaccine development, particularly in the adjuvant setting for resectable melanoma, demonstrating a benefit over the active comparator standard-of-care, Keytruda.

The setting of melanoma for the trial was chosen because this is an immuno-oncology-sensitive tumor, making it a suitable candidate for assessing the impact of INTs. We know the sensitivity of melanoma to checkpoint inhibitors. The resectable setting was chosen for several reasons including that in the early-stage disease, there is less tumor heterogeneity, and since the tumor is resected, the immune cells do not have to contend with the immune exclusion and immune suppression characteristic of bulky metastatic tumors. Choosing a high-risk population for recurrence was also crucial—thus, Stage IV NED resected was chosen as the population to assess the rates of recurrence in patients. Logistically, this setting offered the advantage of a shorter wait time for patients as they had already undergone resection. The randomized study compared Keytruda, the standard of care in this setting, with a combination of V940 plus Keytruda. We focused on the difference in recurrence-free survival (RFS) between those two groups.

The study yielded positive results, showing a statistically significant improvement in RFS for the combination compared to Keytruda. The initial data presented a 44% improvement of RFS, and in our most recent data cut in December, with 6 months of additional follow-up, the trend remained consistent and even showed slight improvement. This reaffirmed our confidence in the positive outcome.

The combination of Keytruda plus V940 holds promise because while Keytruda activates T cells and helps reverse the exhaustion process, V940 continues priming, educating the immune system to respond more effectively to particular antigens present in a tumor. This combination, which addresses both activation and exhaustion, represents an exciting prospect. You will notice this combination included in many of our other trial designs compared to various comparators.

Q What are some of the key challenges or considerations when designing combination therapy clinical trials involving RNA-based cancer vaccines with checkpoint inhibitors, and how does Merck approach these?

JH: There are a couple of considerations, the first being the turnaround time for vaccine manufacturing. Given that each patient receives an INT tailored to their specific tumor, there is a lag period between sequencing the tumor and delivering the therapy to clinic. In our trial designs, we have addressed this by allowing the patients to start Keytruda or other

backbone agents by themselves while they are awaiting INT generation. This flexibility ensures that patients can receive INT treatment while continuing standard of care treatment.

Another important consideration is vaccines act on the immune system, not the tumor directly, so they take time to act. This is called a delayed treatment effect. In our Phase II trial, KEYNOTE-942, we observed that it takes about 40 weeks after the first administration of V940 to see separation of the RFS curves. This delay is a characteristic feature that is well described in the cancer vaccine literature—it takes time for the T cells to be primed and to then respond to the cancer. Our upcoming trials will carefully consider this delayed treatment effect in statistical analyses to ensure that assessments align with the agent's mechanism of action. It is a challenge, but one that can be managed with careful planning.

Additionally, the choice of the early treatment setting reflects our understanding of the drugs mechanism of action. Opting for IO-sensitive tumors in an early treatment setting allows for the assessment of whether the INT promotes efficacy in patients we believe to be most likely to benefit and guides potential expansion to other indications based on these data.

Q What can we deduce from regulators' reactions to mRNA technology applied in the therapeutic setting to date, and what should be some corresponding important points of focus for drug developers?

JH: Regulators have demonstrated positive feedback and a willingness to collaborate and engage with us on how to best develop and study this new class of therapies. This is evident in our announcement that we achieved both Breakthrough Therapy designation in the US and PRIME scheme designation in Europe for this specific therapy. We are working with them to aligning our plans to study it comprehensively and demonstrate its benefit to patients.

Our primary focus is on conducting the appropriate studies with proper designs to demonstrate the treatment benefits, aiming to make it available to patients as soon as possible. The collective commitment of investigators, regulators, and industry professionals is geared toward achieving this goal efficiently.

Q Looking to the future, what are some key next steps for the Keytruda plus V940 combination, both in terms of the current development program and potential future trials/target indications?

JH: There are several aspects we are currently exploring. Our immediate and primary focus is expediting the opening of trials to understand how this INT will benefit these IO-sensitive early-stage treatment settings. However, we are leveraging exploratory studies and biomarkers to identify which patients benefit most and which biomarkers are the most effective for this group. For instance, we have examined programmed death-ligand 1, KRAS, and serine/threonine-protein kinase B-Raf mutations in the melanoma population, as well as other biomarkers like tumor mutation burden.

The data for tumor mutation burden are currently inconclusive due to limited sample size, these investigations are crucial for understanding how the INT operates and benefits patients. Another aspect we will be closely assessing in terms of biomarkers is circulating tumor DNA. Particularly in the early-stage treatment setting, circulating tumor DNA can serve as a negative

prognostic marker for patients with a higher risk of recurrence. Understanding and monitoring the kinetics of the response over time will enhance our comprehension of its optimal usage.

In the personalized therapies space, such as neoantigen therapies, there are a lot of open questions. Exploring the potential role of boosters and addressing the emergence of new clones within different mutations are among the current unknowns. To answer these questions, we firstly need to see the data to establish efficacy in the initial setting and understand the initial effects—specifically, the long-term immunogenicity responses.

It is crucial to note that the safety profile of vaccines is significantly different to that of chemotherapy or small molecules. If we can better enhance the immune systems to provide long-term responses, it has the potential to bring transformative change in oncology and offer substantial benefits to patients.

Q Could you expand on any general future directions in oncology combination therapy studies at Merck?

JH: As mentioned, we are looking at several different mechanisms. As a company, we are excited about antibody-drug conjugates, given their specificity and targeted nature compared to traditional chemotherapy. This class has shown promising activity, and we are actively collaborating both internally and with partners like Kelun-Biotech and Daiichi Sankyo to investigate different payloads and targets. Our goal is to identify combinations with improved targeting that can enhance treatment efficacy.

Further, we are engaged in exploring small molecule inhibitors across various disease settings. For example, the belzutifan program is primarily focused on clear cell renal cell carcinoma, while the KRAS G12C program, at least for now, is focused on non-small cell lung cancer. We are exploring a wide diversity of mechanisms targeting a variety of different cancer types. As a field, it will be interesting to observe how we best combine all of these approaches in the future and determine the best utilization of these agents. Exploring biomarkers that aid in selecting patients likely to respond well and minimizing toxicity is a key aspect of our pipeline moving forward.

Q And are there any future plans for the Merck-Moderna partnership that you can share?

JH: Currently, our focus is on getting the Phase III trials up and running, strategically determining where the INT is going to provide the best benefit to oncology patients. Our goal is to work collaboratively with regulators and other stakeholders in the field to understand these therapies and optimize their utilization.

"Exploring biomarkers that aid in selecting patients likely to respond well and minimizing toxicity is a key aspect of our pipeline moving forward."

Our partnership with Moderna has been an exciting and productive collaboration. We are able to leverage Moderna's expertise in mRNA and vaccine technology, particularly evident during the COVID-19 pandemic. The validation of mRNA technology as a vaccine platform was remarkable. Now, taking this technology to the oncology space and combining it with Merck's expertise in oncology clinical trials, with Keytruda and indication-specific knowledge, has created a robust collaboration.

As we progress, the partnership may evolve to address future scientific questions. Right now, we are dedicated to advancing V940 and bringing it forward to benefit patients.

Q Finally, can you sum up your own vision for how mRNA will impact the immuno-oncology space moving forward?

JH: I am particularly excited about the potential impact of mRNA technology, specifically as a means of better priming the immune system to adequately recognize changes resulting from cancer mutations. This distinctive and unique aspect sets it apart and positions it to address challenges that other therapies may not.

Great promise lies in the combination of its potential to reach a diverse range of cancers and its compatibility with other agents. By combining mRNA technology with other mechanisms, we can aim to provide additive benefits by targeting various aspects of cancer pathology.

BIOGRAPHY

DR JANE HEALY is the Vice President and Head of Early Oncology Development at Merck Research Laboratories. She is a medical oncologist by training, and a physician scientist with a passion for innovative drug development aimed at improving outcomes for patients. Jane joined Merck in 2016 and rapidly assumed leadership roles of increasing responsibility. She led early clinical development for key pipeline candidates, vibostolimab (an anti-TIGIT [T cell immunoreceptor with Ig and ITIM domains] therapy), and favezelimab (an anti-lymphocyte-activation gene 3 inhibitor), steering these programs into current Phase 3 studies. Jane led the integration team for V940/mRNA-4157 (an individualized neoantigen therapy; in collaboration with Moderna), playing a crucial role in asset strategy and transition to Phase 3. Throughout her tenure, Jane managed early to late-stage development plans for new compounds including vaccines, small molecules, cell-based therapies, and antibody therapeutics. Prior to Merck, Jane specialized in the treatment of hematologic malignancies. Jane completed her residency in Internal Medicine at the Brigham and Women's Hospital, and subsequently conducted a fellowship in hematology/oncology at Duke University. She is externally recognized as a leader in oncology clinical development, and has served on industry panels for workshops led by Friends of Cancer Research and the Society of Immunotherapy of Cancer.

AFFILIATION

Jane Healy MD
Vice President,
Early Oncology Development,
Merck Research Laboratories

AUTHORSHIP & CONFLICT OF INTEREST

Contributions: The named author takes responsibility for the integrity of the work as a whole, and has given their approval for this version to be published.

Acknowledgements: None.

Disclosure and potential conflicts of interest: The author has no conflicts of interest.

Funding declaration: The author received no financial support for the research, authorship and/or publication of this article.

Article source: Invited; interview held on Dec 18, 2023.

Revised manuscript received: Feb 7, 2024; **Publication date:** Feb 14, 2024.

Scalable ultrafiltration/diafiltration process of clarified pDNA using T-series cassettes with Omega™ membrane

Angel Lorenzo, Manager, MSAT Field Team, and **Adam Armengol**, Manufacturing Supervisor, Akron Bio

Plasmid DNA (pDNA) is widely used in biomanufacturing but isn't simple to produce at high yield and GMP quality. High-salt buffers can compact pDNA and complicate ultrafiltration/diafiltration (UF/DF). Here we explore a scalable post-clarification process that enhances pDNA yield while minimizing RNA contamination.

SCALABLE UF/DF PROCESS OF CLARIFIED pDNA

pDNA is vital in biomanufacturing, playing roles in genetic engineering, transfection, viral vector production, and as a template for mRNA therapies. Supercoiled plasmids, crucial for therapeutic applications, face challenges in the UF/DF step due to plasmid compaction induced by high-salt buffers, resulting in low yields. A specialized process has been developed using polyethersulfone (PES) flat-sheet cassettes with Omega™ membrane, designed for high flux and minimal binding. These cassettes, scalable from benchtop to production, integrate into single-use systems, offering flexibility for optimal performance and increased post-clarification yields in pDNA manufacturing.

MOLECULAR WEIGHT CUTOFF (MWCO) SELECTION GUIDE FOR pDNA

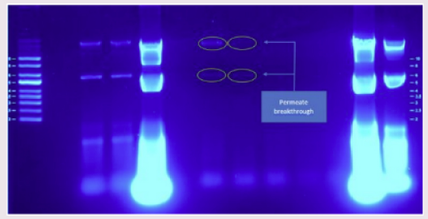
It's useful to start with recommended MWCOs based on pDNA size **(Table 1)**, but initial confirmation studies are always advisable to validate or adjust MWCO based on specific process considerations and characteristics of unique plasmids.

Table 1. Guideline for the selection of the initial MWCO based on pDNA base pairs.

MWCO	pDNA base pairs
1 kDa	5 to 16
3 kDa	16 to 32
5 kDa	25 to 50
10 kDa	50 to 145
30 kDa	145 to 285
50 kDa	240 to 475
100 kDa	475 to 1450
300 kDa	1450 to 2900

Figure 1. Agarose gel of sample aliquots using 100 kDa MWCO.

Lane 1: Supercoiled (SC) ladder
Lane 2: *(Empty)*
Lane 3: Post-depth filtration (3125 mL)
Lane 4: Post-0.2 µm filtration (4000 mL)
Lane 5: Post-UF retentate (250 mL)
Lane 6: Post-UF permeate (3750 mL)
Lane 7: Post-DF No. 1 permeate (400 mL)
Lane 8: Post-DF No. 2 permeate (400 mL)
Lane 9: Post-DF No. 3 permeate (400 mL)
Lane 10: Post-DF No. 4 permeate (400 mL)
Lane 11: Post-DF No. 5 permeate (400 mL)
Lane 12: Post-recirculation (pre-flush, 250 mL)
Lane 13: Recovery flush (152 mL)



During diafiltration, where unique buffer characteristics and conductivities can lead to compaction and passage into the permeate, it is particularly essential to verify the suggested MWCO selection.

Generally, plasmids utilized in biopharmaceutical processes range from 5000 to 20,000 bp.

100 kDa MOLECULAR WEIGHT CUTOFF: pDNA PERMEATE BREAKTHROUGH

During the study with a 5000 bp plasmid, sample aliquots were taken at each stage and run on agarose gel **(Figure 1)**. Notably, no plasmid was detected in the permeate following ultrafiltration at 100 kDa, as seen in Lane 6. This highlighted the suitability of the 100 kDa filtration for concentrating plasmids with sizes exceeding 5000 bp without alterations to the buffer composition. However, once diafiltration began, leakage became apparent in lanes 7 and 8, representing aliquots from the permeate of the initial two diafiltration volumes. This leakage issue was resolved in the subsequent third through fifth diafiltration volumes, with no further visualization of plasmid bands in lanes 9–11.

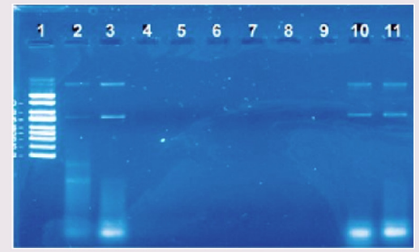
30 kDa MOLECULAR WEIGHT CUTOFF: pDNA RETENTION

An agarose gel from the 30 kDa study revealed no breakthrough of plasmid permeate at any stage of the filtration process, including diafiltration, using the same buffer as employed for the 100 kDa filtration **(Figure 2)**. Notably, RNA, visible at the bottom of lanes 2, 3, 10, and 11, is absent from all permeate lanes.

The study indirectly demonstrated that MWCOs of 100kDa and above can be effective for removing RNA, a desirable outcome for optimizing plasmid binding capacity in subsequent capture steps involving ion exchange.

Figure 2. Agarose gel of sample aliquots using 30 kDa MWCO.

Lane 1: Supercoiled (SC) ladder
Lane 2: Neutralized lysate
Lane 3: Post-clarification (3000 mL)
Lane 4: Post-UF permeate (2700 mL)
Lane 5: Post-DF No. 1 permeate (300 mL)
Lane 6: Post-DF No. 2 permeate (300 mL)
Lane 7: Post-DF No. 3 permeate (300 mL)
Lane 8: Post-DF No. 4 permeate (300 mL)
Lane 9: Post-DF No. 5 permeate (300 mL)
Lane 10: Final feed/retentate (195 mL)
Lane 11: Post-recovery flush (100 mL)



NUCLEIC ACID INSIGHTS

Nucleic Acid Insights 2024; 1(1), 29; DOI: 10.18609/nai.2024.005

In partnership with: cytiva

In the version of this FastFacts poster initially published, Adam Armengol was listed as the sole author. However, the poster was in fact co-authored by Angel Lorenzo. This error has been corrected in the HTML and PDF versions of this article as of March 6, 2024.

NUCLEIC ACID INSIGHTS

LAUNCH ISSUE

SPOTLIGHT

INTERVIEW

Targeting improved longevity and cancer through proteo-lipid vehicle delivery

The high cost of current advanced therapies remains a major barrier to their successful application in the more prevalent therapeutic areas and indications. **David McCall**, Senior Editor, BioInsights, talks to **Matthew Scholz**, Founder and Chief Executive Officer of Oisín Biotechnologies and OncoSenX, about novel approaches that can potentially unlock some of the largest unmet medical needs by patient population on a worldwide basis.

Nucleic Acid Insights 2024; 1(1), 55–65

DOI: 10.18609/nai/2024.010

Q What are you working on right now?

MS: I am currently involved in the process of developing genetic medicines aimed at improving health and longevity. One of our two lead programs focuses on building muscle in the body without exercise through the transient expression of follistatin, the main goal being to assist older people in becoming stronger. The second program is designed to selectively kill fat cells.

Q Tell us more about Oisin's platform and approach—what differentiates it in the nucleic acid therapeutics space, firstly in terms of the delivery technology?

MS: The delivery technology utilized is a proteo-lipid vehicle (PLV) developed by Entos Pharmaceuticals. Oisín was one of the first companies to employ this approach for nucleic acid delivery. The vehicle combines some of the best attributes of non-viral and viral delivery mechanisms: regarding the former, it is still lipid-based, manufactured using a microfluid system, and can be scaled up affordably. But it also incorporates a small viral fusion protein to enable cell entry. Moreover, it can also fuse with the membrane of any cell it comes in contact with and unlike traditional lipid nanoparticles (LNP), the vehicle requires neither interaction with a cell surface receptor nor endocytosis. This technology allows us to navigate around the 'Faustian bargain' that LNPs make with charge chemistry (they must have the potential to escape the endosome, but this often results in toxicity because the ionic lipids that LNPs require to escape the endosome are toxic to cells). Oisín's PLV is able to avoid this.

The decision to adopt this approach early on in R&D was driven by the need for a tool with the flattest possible biodistribution curve, as the aim was to target every cell in the body. The goal was to take targeting out of the realm of chemistry and into the realm of information, enabling precise targeting with the payload using mechanisms such as Boolean logic or promoters and repressors.

This approach is not only unique in the nucleic acid therapeutics space, but also in the longevity field. The partnership with Entos has proven highly productive, as shown by their significant growth and achievements, including a substantial deal with Eli Lilly and successful Phase 2 trials for a COVID vaccine. It has been exciting to be a part of.

I have had something of a professional obsession with delivery technologies over the years. I first started my career at Immusoft where we attempted to modify B cells and plasma cells for cell therapy. It quickly became apparent to me that delivery is the Achilles heel in genetic medicine—the challenge of striking a balance between overcoming the body's defenses and developing a practical therapy is substantial. When we started looking towards longevity and we were seeking an appropriate tool to use, we saw that the PLV vehicle was unique. We were also able to take advantage of it being in the nadir of the Gartner hype cycle for LNPs at the time—most people looked on it as 'just another LNP', but we felt it was something quite different.

Q What differentiates your approach to payload?

MS: At Oisín, our approach places significant emphasis on the payload for targeting compared to most other developers, especially those in AAV-based therapies who primarily focus on targeting the tropism of the virus rather than the payload design. This is particularly evident in *in vivo* therapies, where the goal is to deliver the payload to every possible target cell.

"...the fields of computer science and medicine are becoming increasingly intertwined, and this integration will yield far more sophisticated targeting logic in the future."

Again, the core thesis of our approach is rooted in the idea of targeting with information rather than chemistry, reflecting the body's natural processes. We recognize that every nucleated cell in the body shares the same DNA but expresses different genes. Our initial senolytic therapies aimed to exploit the transcriptional differences between a healthy cell and a senescent cell.

Senolytic therapies face the issue of needing to selectively target harmful senescent cells among an ocean of healthy cells while causing minimal collateral damage. The solution we devised enables targeting both healthy and senescent cells using the delivery technology, but engineers the payload so it only activates in the senescent cell. This approach avoids the risk of generic toxicity that could harm the wider environment. The payload encodes for a caspase, which initiates programmed cell death.

Leveraging this technology allows us to pursue new strategies—for example, there is a genetically engineered mouse model called 'INK-ATTAC' that enables scientists to selectively ablate p16 cells at different times throughout the life of the animal. This model provided valuable data on the benefits of p16-based senolytics; however, its translational applications were limited—humans aren't genetically engineered mice. Oisín aims to develop clinically viable methods to achieve similar outcomes. The ability to selectively and safely ablate cells with genetic medicine, and control expression and activation of the payload, is crucial.

Our oncology-focused work with OncoSenX also reflects this broader theme of evolving payload strategies as well. I believe that the fields of computer science and medicine are becoming increasingly intertwined, and this integration will yield far more sophisticated targeting logic in the future. My real interest isn't just in expressing proteins, it is in manipulating the control logic of life.

Q Can you expand on the rationale for going after diseases of aging with this type of approach?

MS: In essence, the rationale behind our focus on aging is that it is a universal experience—every individual is inevitably impacted by the progression of time and the effects of entropy. My first company was primarily focused on rare diseases. Over time, I became increasingly interested in how to leverage advanced genetic medicines in very large patient populations.

When we decided to establish a company with aging as its primary target, we had some challenging criteria for the tools that we needed to use. They had to be scalable to a global population level, and had to have the ability to be administered repeatedly (i.e., safely and affordably),

"Follistatin, as a target, aligns with this approach by addressing frailty and sarcopenia, both diagnosable conditions with recognized ICD codes."

and they had to facilitate precise targeting. Early on in company development, we assessed the evolving academic landscape of the aging and longevity field, and contemplated where we thought the science was likely to go. We then identified tools that would enable us to build therapeutics in those areas when the corpus of scientific knowledge had sufficiently progressed.

In the case of follistatin, our interest in muscle-building stemmed from how profoundly physical strength affects health as we age. While the FDA may not classify aging as a disease, and the true mechanisms of aging remain elusive, we can still target age-related diseases. Thus, our therapeutic focus lay with addressing the parts of the body that deteriorate first with emphasis on healthspan rather than just lifespan. We observed that such interventions can lead to an increased lifespan—however, more importantly, they contributed to animals living in better health for longer before dying fairly abruptly. I believe this is something we want ourselves; to live in good health and not suffer a slow, painful decline. Follistatin, as a target, aligns with this approach by addressing frailty and sarcopenia, both diagnosable conditions with recognized ICD codes.

Physical strength becomes increasingly crucial with age, influencing lifestyle and everyday factors ranging from social enrichment, to metabolism, to resilience against physical injury, stress, and sickness. We are primarily looking at nursing home patients with frailty as a starting point. Moreover, viewing this from an insurance/Medicare standpoint, there is potential for lobbying efforts to encourage these therapies as it would be more beneficial from a payer standpoint to try to eliminate frailty and sickness rather than pay for treating their consequences. I believe that if you have therapies that meaningfully intervene in these processes, then the math is straightforward; it is cheaper to use it than to not.

Q What are some of the specific challenges that need to be addressed to successfully target senescent cells?

MS: The primary challenge in targeting senescent cells lies in the fundamental ambiguity surrounding the definition of the cells themselves. I don't believe that the field has yet characterized all the senescent cell populations in humans and we probably do not fully understand the role of each one. Early iterations, such as ours and transgenic mouse approach, focused on cells that overexpress p16, a tumor suppressor associated with damage repair. While clearing these cells shows a clear benefit, p16 is not a perfect marker for senescence.

There is also the issue that the goal is not to break or remove the senescence pathway itself, as it plays an essential role in processes like wound healing. Instead, the goal is the point-in-time

removal of specific cell populations. As the field matures, there is optimism that we can improve targeting. For example, our discovery of synergies between targeting p16 and p53 suggests that more effective combinations may emerge. As we start finding more effective ways to target these cells—specifically with learnings from the field of synthetic biology or through the *de novo* design of promoters—you will see cheaper and more effective products emerge.

Another challenge in this space is the absence of reliable methods to measure senescent cell burden in humans. Current approaches such as tissue staining from biopsies are impractical and invasive. This is not unique to senescence but is a problem across the whole field, meaning there is a lack of good biomarkers for aging. This makes it difficult to assess the effectiveness of interventions in humans within practical timelines.

Our other lead program that focuses on killing fat cells, was informed by another transgenic mouse model 'FAT-ATTAC' This program is also effectively leveraging transcriptional targeting. While killing fat cells generally is something that can be done effectively, my goal for this project is to be able to selectively ablate visceral fat cells in particular, and target the therapy locally for therapeutic use in some rare diseases. While the metabolic and transcriptional differences suggest an opportunity for precise targeting, we have yet to identify a safe and specific gene for more broad visceral fat targeted applications.

Q Can you expand on how you are working through these issues preclinically to set Oisín up for a successful IND?

MS: The decision to prioritize follistatin as our lead program was driven by the fact that it had a much clearer regulatory path. Initially, we explored senolytics as a potential low-hanging fruit in the longevity space, developing and building a tool to kill them. However, the regulatory space proved to be more complex, especially in tying development to a specific Phase 3 endpoint in a patient population that the FDA would approve.

In contrast, follistatin's appeal is that it is a self-protein, which minimizes potential risk, and that there is already an extensive knowledge base for it. When a person exercises, follistatin expression is naturally increased by around ten-fold transiently, giving it a large therapeutic window. It is also known that making a follistatin overexpressing transgenic animal does not lead to apparent ill effects. Clinical trials, involving both recombinant proteins and gene therapies involving follistatin, provide a robust foundation for its regulatory journey. Our approach benefits from the outcomes of these earlier trials, allowing us to assess any potential challenges that lie ahead for us. The key challenge remains in identifying the ideal initial patient population, and this should become more apparent over the course of our regulatory interactions.

Given our focus on longevity, targeting frailty represents a compelling goal. There are many routes you can take with this, one of which is sarcopenia broadly to compensate for the loss of lean muscle mass. Moving forward, however, our focus is on refining the dose regimen and identifying the optimal initial patient population.

Q What is your vision for future applications of Oisín's technology, and for the potential of nucleic acid therapies in general, in the aging space?

MS: There are several noteworthy points to make here, one being the growing recognition of nucleic acid therapies by the pharmaceutical industry.

I believe tools like ours will prove essential for addressing aging. The treatment of diseases associated with aging cannot be based solely on pills and small molecule drugs; we need more comprehensive solutions. Looking at the trajectory of Oisín's technology, we see a broad range of applications in promoting longevity including more advanced applications like partial reprogramming. The current academic proof-of-concept approaches may not be viable in the clinical setting—however, a tool such as ours could be used to advance this field via direct epigenetic modifications.

Looking further ahead, other interesting applications of the technology include either replacing mitochondria or improving mitochondrial function, and removing cross-linked proteins - an area that is currently underexplored. Dealing with extracellular damage more broadly poses significant challenges and, in my opinion, is an area that is not receiving sufficient attention. Additionally, the ability to modulate extracellular matrix proteins for applications such as regenerating collagen, holds promise for the future.

Each of these prospects requires some level of scientific progress and regulatory process to identify and address reasonable therapeutic targets, but I think this is likely the trajectory of future developments in the field.

Q Turning to Oisín's spin-out, OncoSenX, can you tell us about the application of this technology in the oncology arena and the specific opportunity there?

MS: The inception of OncoSenX was driven by the recognition of significant transcriptional overlap between senescence and cancer. This observation came from the finding that many transcriptional senescence targets are also tumor suppressors. Our entry into the field was somewhat unconventional but proved to be more successful than we initially anticipated.

This led to extensive work on suicide gene therapies and their combination with immunotherapies such as checkpoint inhibitors. The objective was to debulk tumors and stimulate the immune system to eliminate the remaining cancer cells. This approach underwent non-human primate studies, and we had our first regulatory interaction with this kind of therapy.

A notable development has been our program to generate CAR-T therapies within the body, which aligns well with our platform and targeting strategy. While CAR-T therapies have only been used in a few thousand people in the US to date, the demand for them is significant and many more patients could benefit from them. Aside from the high cost, patient access is

"Having a transient CAR could open the possibility of going after aggressive targets where integrating approaches have previously failed."

primarily constrained by supply, making hundreds of thousands of autologous cell therapy products just isn't viable. Alternatives such as allogeneic CAR-T cell therapies have seen some progress, but still face significant underlying issues. However, if both cell therapy and virus are both removed from the equation, the cost of goods could drop by roughly 100-fold. This would potentially make these therapies accessible on a global scale.

The tool we have developed administers the PLVs IV. The T cells in the patient are targeted and provided with a CAR construct *in vivo*. One unique aspect of this is that the construct is not integrated, allowing it to wear off as the cells divide. This addresses the FDA's recent scrutiny of oncogenesis and insertional mutagenesis from the pseudorandom integration of virus-based CAR-T constructs. Furthermore, as our product is not immunogenic, redosing is possible until the desired effect has been achieved.

The lack of integration is a game-changer as it overcomes certain primary limitations. With OncoSenX, we can seamlessly integrate into any existing CAR therapy just by dropping the construct, behind our targeted promotor, and encapsulating it in a PLV. Another interesting application would be in solid tumors—something that has eluded the field to date largely due to toxicity limitations. Having a transient CAR could open the possibility of going after aggressive targets where integrating approaches have previously failed.

Q Where else might Oisín's/OncoSenX's platform be applied, moving forward?

MS: I believe that the CAR space will remain a fruitful hunting ground for a while and is likely to evolve into a large market. Once we demonstrate we can bypass the *ex vivo* manufacturing limitations, the field will see significant growth. CAR-T therapies can also be utilized beyond cancer treatments, expanding their potential applications. For example, I think modulating immune responses for autoimmunity can be an incredibly powerful use of this technology.

There is some overlap in these focus areas—for instance, CAR-T therapies can be designed to target senescent cells. It is unclear whether it is more effective than using the suicide gene for our purposes, but the option is there. Autoimmunity tends to worsen with age, and with our bodies becoming a more pro-inflammatory environment, the likelihood of acquiring one of these conditions increases. Autoimmune diseases exhibit varied patterns: ignoring the known genetic ones present around the time of birth, some are early-onset, some onset around puberty, and others onset around upper middle age. It is not a Gaussian distribution of onset for these

conditions. I believe that there is a reason behind these patterns especially in upper middle age. This chronic sterile inflammatory environment that increases with age may make the immune system more prone to attacking the body by mistake. I would describe it as having a conversation in a loud stadium—when your immune cells are communicating in a noisy inflammatory environment, they are more likely to make mistakes.

With OncoSenX, we have delved into exciting areas, particularly in academic collaborations. We are exploring exotic new suicide genes, pairing them with other approaches. Our collaborations have included things like pairing suicide genes with radio immunotherapy and experimenting with the immunomodulatory aspects of our approach.

One early experiment involved producing CD40 ligand in the tumor, a potentially toxic substance. However, when localized to the tumor, it becomes dose-limiting by default. As soon as it produces enough to stimulate the immune system and trigger a reaction, it gets killed and therefore has minimal systemic effects. I believe these tools will play a crucial role in future—for example, in the context of brain cancers where selective ablation of a cell without harming the surrounding healthy cells is essential.

Q The current pace of innovation in the payload space—e.g., in the emergence of synthetic alternatives to plasmid DNA—is fierce. What new opportunities might this present?

MS: I am a huge fan of this—I have been waiting with bated breath for my DNA printer! My involvement in the space started at Immusoft where we discussed the idea of deploying a DNA printer on a space station or on Mars with the space medicine people at NASA Ames. The concept involved wirelessly sending treatment to space, printing out the DNA and utilizing our platform to upload it to the astronauts, effectively turning the astronauts into their own bio-factories.

While developments in DNA printers have seen great progress over the years, I have yet to see one that I think is at this level. But what makes these printers incredibly valuable for our work is their potential to shorten the iteration cycle. The current process involves cloning plasmid DNA, which involves significant preps. The time required to do this and the limitation of only being able to test a few versions at a time prolongs our iteration cycle. If you want to explore many permutations, the entire process has to be repeated over and over. With good synthesis technology, you could generate numerous versions simultaneously. This would lead to a significant reduction in the build-test cycle time for groups like ours.

An example of the exciting possibilities we are exploring involves building an optically inducible expression construct, utilizing near-infrared light to activate transcription. It would enable exciting applications such as placing these elements in muscles and activating transcription with near-infrared LEDs, to achieve precise control of the dose.

Optogenetic promoters have the potential to make expensive processes more cost-effective, managing the challenge surrounding variable dosing in genetic medicine. However,

constructing a system like that requires extensive R&D, which slows down the process significantly. In our lab, the motto is 'Clone first, ask questions later,' highlighting the need to overcome the bottleneck caused by waiting for DNA. This is where a DNA printer would help to overcome a lot of these challenges.

In the therapeutic space, a primary cost driver for therapies like ours is nucleic acids, with DNA and RNA being relatively expensive. Reducing the cost of DNA is crucial for making the therapies more affordable, especially when considering therapeutic areas with patient populations as large as age-related diseases and longevity. Ideally, we would have processes and therapeutics that are cheap and can be delivered at scale. The potential of cell-free systems to achieve this is promising but all the current options I'm familiar with have their challenges, and no solution is the clear winner yet.

While progress in this area is evident, it remains challenging to predict the long-term impact on both R&D efforts and the viability of these kinds of products. When assessing and modeling future therapies, the cost dynamics pose a significant challenge. The shift in technology and potential plateaus complicate future predictions further.

If confined to a virus-based or manual process, there are real limitations on scalability. For instance, Bluebird Bio's shift to suspension culture for making lentivirus significantly reduced costs and made human therapies more practical, but there is a limit to how far this can be pushed. The synthetic biology world has done a lot in optimizing microbial cultures and this knowledge could be applied to therapeutics. However, a lot of retooling may be required.

In the gene therapy space, a shift to non-viral approaches seems likely, although certain viral applications may persist. Scaling up *E. coli*-based production to meet demand for significant quantities of DNA, in the order of kilograms, is challenging but also necessary and attainable. The potential for therapies like ours to use more DNA than the entire field currently does is not far-fetched. Overcoming limitations in DNA availability is a unique challenge for all of us due to how much we can safely administer.

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

MS: I am optimistic that we will witness the development and emergence of a more advanced DNA printer soon, similar to office printers, where they become more reliable, widespread, and affordable. Similarly, on the nucleic acid front, I am hopeful and believe that there will be development with respect to reliably and cost-effectively producing larger constructs.

On the downside, however, I think we will see an increase in potential adverse events from gene and cell therapies. As more people receive these therapies and life expectancy rises, a more realistic view of the risk-reward profile will emerge. This might lead to the demise of certain technologies as the field navigates these vectors, particularly indications with very large patient populations.

An interesting aspect worth considering is the impact of the durability of mRNA therapies on the landscape of vaccination. It could be argued that some of these may prove to be better suited to tolerizing against allergens than protecting against infectious agents. There is biology being learned that I believe will have serious positive ramifications for what we build next.

A significant shift that has taken place in recent years is the FDA's willingness to entertain gene therapies in healthy patients. It makes me wonder if this precedent may pave the way for elective or cosmetic gene therapies. Previously, this idea would have been quickly shut down—however, there has now been precedent set for giving genetic medicines to healthy individuals. This precedent will likely affect the development of longevity-related treatment. If healthy individuals can receive genetic medicines for prophylactic purposes, it raises the possibility of using similar approaches to slow down or address the diseases of aging. While this may not be widely discussed in the field today, I find it fascinating and I am optimistic there will be positive progress.

Q Can you highlight one or two key goals, priorities, or milestones for both Oisín and OncoSenX in the near future?

MS: For Oisín, our current focus is on advancing two programs, the fat-killing and muscle-building programs, towards IND. We are also trying to pursue a business development deal. Our main goal for Oisín is to effectively evolve into a major player in the longevity space, effectively becoming a big pharma that addresses the complexities of aging. The objective is to initially build and out-license therapies, and eventually amass the resources to take our innovations to market ourselves.

On the OncoSenX side, our goal in 2023 was largely oriented around business development. We aim to continue encouraging developers of CARs to adopt our technology, with the ultimate goal of entirely replacing the *ex vivo* process that is prevalent in the industry.

BIOGRAPHY

MATTHEW SCHOLZ serves as the Chief Executive Officer and co-founder of Oisín Biotechnologies and its oncology-focused spin-off, OncoSenX—two biotech firms advancing a revolutionary genetic medicines platform. A serial entrepreneur with expertise in immunology, gene therapy, oncology, and computer security, Matthew has spent the past 15 years bridging the gap between computer science and biology. He has founded or co-founded five biotechnology companies, raising millions in investment capital and grants, assembling world-class teams, and negotiating licensing agreements with top-tier academic institutions and pharmaceutical companies. Immusoft, his first biotech venture, created the first engineered B-cell therapy to ever enter human trials. Matthew has been instrumental in the development of several advanced therapeutics, including three first-in-class treatment modalities. He has extensively worked on innovative treatments for infectious diseases, such as antiviral gene therapies and vectored immunoprophylaxis for HIV, Ebola, and a COVID-19 vaccine. Matthew is the author of dozens of patents covering innovations in

areas such as novel transfection technologies, immune cell programming, *in vivo* production of therapeutic proteins, immunomodulation, senolytics, longevity, and oncology. A frequent speaker and presenter at universities, associations, and scientific events, including his *alma mater*, the University of Washington, Matthew is also a mentor for TKS, an organization committed to training intelligent high school students to tackle complex problems. He has served as a mentor for Thiel Fellowship recipients, a program that grants awards to some of the world's brightest scientific minds under the age of 20, and is a mentor and speaker at Global Biotech Revolution's GapSummit in Cambridge, UK, assisting young scientists in bringing their ideas to the industry.

AFFILIATION

Matthew Scholz
Founder and Chief Executive Officer,
Oisín Biotechnologies,
and
OncoSenX

AUTHORSHIP & CONFLICT OF INTEREST

Contributions: The named author takes responsibility for the integrity of the work as a whole, and has given their approval for this version to be published.

Acknowledgements: None.

Disclosure and potential conflicts of interest: The author is the CEO and on the Boards of Oisín and OncoSenX, and on the Board of Immusoft. OncoSenX has licensed technology from Oisín. He is listed as an author on Oisín, OncoSenX and Immusoft patents. He owns stock in Oisín, OncoSenX and Immusoft. Oisín owns stock in Entos and has a license to Entos tech.

Funding declaration: The author received no financial support for the research, authorship and/or publication of this article.

ARTICLE & COPYRIGHT INFORMATION

Article source: Invited.

Interview conducted: Dec 8, 2023; **Revised manuscript received**: Feb 11, 2024;

Publication date: Feb 15, 2024.

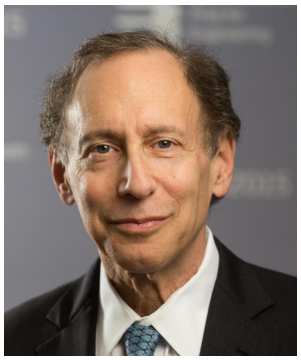
NUCLEIC ACID INSIGHTS

LAUNCH EDITION

SPOTLIGHT

INTERVIEW

Pioneering nucleic acid breakthroughs for the world



The COVID-19 mRNA vaccines amply demonstrated the potential of nucleic acid-based medicines to reshape healthcare on a truly global basis. **David McCall,** Senior Editor, *Nucleic Acid Insights,* speaks to **Robert Langer,** David H Koch Institute Professor, Massachusetts Institute of Technology, about building on his enormous legacy as a founding father of nucleic acid delivery by enabling worldwide patient access to novel vaccines and therapeutics alike.

Nucleic Acid Insights 2024; 1(1), 37–41

DOI: 10.18609/nai.2024.007

Q What are you working on right now?

RL: I am currently working on quite a few things. Dan Anderson, a Professor of Chemical Engineering at the Institute for Medical Engineering and Science at the Massachusetts Institute of Technology (MIT), and my former postdoc fellow, is my collaborator on work in lipid nanoparticles, including developing new types. We are also investigating ways to use AI to help better design lipid nanoparticles and target them to different cell types, as well as uncovering novel methods of administration such as delivery via inhalation.

I also work closely with Giovanni Traverso, an Associate Professor in the Department of Mechanical Engineering, on the oral delivery of nucleic acids. And I am working with Ana Jaklenec on a variety of things, including ways of delivering nucleic acids via microneedles. In addition, Ana and I are developing portable 3D printers to produce microneedles in a globally accessible way, and finally, we are working on ways to better stabilize mRNA.

"...a standard flu vaccine might be 40–50% effective, but we see that mRNA vaccines can be as much as 94% effective in the case of Moderna's COVID-19 vaccine."

Q Can you give us your high-level summary of the current state of the art in the nucleic acids-based prophylactic and therapeutic vaccine spaces—and its limitations?

RL: It is remarkable to think about what has happened with mRNA vaccines over the past few years. Today, they can certainly be made rapidly and in a way that works very effectively—a standard flu vaccine might be 40–50% effective, but we see that mRNA vaccines can be as much as 94% effective in the case of Moderna's COVID-19 vaccine. The state-of-the-art in that sense has taken a giant leap forward. Furthermore, before the pandemic, we had never seen vaccines being approved in less than a year from the start of development.

That being said, I think there are many improvements still to be made to mRNA vaccines. I would like to see the vaccines having a greater durability of response depending on the disease. You also want to see better tolerability—even though the side effects have been exaggerated by some (in the sense that according to the published literature, the mRNA vaccines have fewer side effects than the standard flu vaccines) we would someday like to see no side effects at all. Vaccines classically have been one of the safest ways to do things in healthcare. I would also like to see the field leverage other routes of delivery, including pulmonary and nasal delivery.

The same is true for DNA vaccines; we also want to see those become safer and more effective.

Q As the field strives to get beyond the liver in terms of targeted nanoparticle-enabled drug delivery, what do you view as the most promising approaches and target cells/tissues in this regard, and why?

RL: It depends on the type of nucleic acids being used. For many nucleic acids—namely those that are small enough, such as siRNA—you may not need nanoparticles for delivery. Instead, you may be able to modify these and bind them to something to improve their stability. This is something that Dan and I have worked on for siRNA. However, if a nanoparticle is required for delivery, then the approach taken will depend on what you are trying to do and which nucleic acids are being used. In our work, we have looked at targeting the heart, immune cells, and the brain.

It is possible to get these drugs past the liver, as there are ways to disguise them. The problem is then getting them to other places in the body. One of the keys for me that we have been working on is finding new receptors in other cell lines. Dan and I have been collaborating on some work with other targets. By identifying new receptors and specific tissues, there will be more opportunities to reach different and novel targets moving forward.

Q Looking to the future, what will be some important next steps or new directions in innovation in nucleic acid delivery for you?

RL: Many areas of study could prove useful, such as trying to introduce nucleic acids through less invasive routes. As mentioned earlier, if we could better stabilize nucleic acids and change the duration of these medicines, that would be valuable.

For vaccines, other routes of administration such as the dermal route or the nasal route will require different formulations. Over the years, our lab has worked on delivering things through just about every route in existence, including the eye, the nose, the lungs, the vagina, and the skin. We are continuing our work on expanding these delivery routes. Looking at it historically, every single one of those routes has been important for some specific molecules. Hopefully, cancer vaccines will be an area to watch in 2024.

Q On that topic, as a co-Founder of Moderna, you must have been particularly encouraged by the recent promising data for mRNA-4157/V940 in combination with Keytruda—what is your vision for the impact that nucleic acid vaccines can have in the therapeutic setting, both in cancer and beyond?

RL: The 3-year follow-up data for melanoma treatment with Moderna's mRNA-4157/V940 in combination with Keytruda was recently released, and the findings have been nothing short of spectacular. Data continue to show an improvement in recurrence-free survival and a reduction in risk of recurrence or death of 49% compared with Keytruda monotherapy.

Keytruda is an effective drug on its own, so seeing improvements of this scale is excellent. The hope is to use this kind of vaccine in as many cancers as possible, although I do not see nucleic acid vaccines as being limited to just cancer. Any disease could be a target. Respiratory syncytial virus vaccination is one target on the immediate horizon for Moderna. The pipelines from Moderna and other companies are applying nucleic acid vaccines to many, many different diseases.

Q As a pioneer in the field, do you have a message for new arrivers in the rapidly expanding space of nucleic acid delivery?

RL: Looking back at my career, we were the first to develop delivery systems for nucleic acids, back in the mid-1970s. When we first looked to do that, people did not believe that it could be achieved. Many people said that delivering large molecules with tiny particles was impossible. When I give commencement speeches today, I tell the young people to dream big dreams. Dream things that you hope will change the world. If you do that, you should expect a lot of criticism, but don't give up easily. This has been key in my own life and work.

Q Lastly, what are some key priorities for your work over the foreseeable future?

RL: One of my biggest goals is to continue the work we have been doing in collaboration with the Bill & Melinda Gates Foundation to develop accessible medicines for the developing world. In addition to the microneedle patches I mentioned earlier, we have also developed so-called self-boosting vaccines. This is work conducted in collaboration with Ana Jaklenec—we have been working on a way to give one injection in circumstances that may currently require multiple injections. This means that patients would not need to come back for a second, third, or fourth injection, thus increasing compliance.

We are working on things across the board that will not only be as good or better than what currently exists, but that will also be cheaper and easier to use. The focus on improving both patient compliance in general and the ability to manufacture and deliver these products to patients on a truly global basis are cornerstones of these activities. Drug delivery in nucleic acids and many other molecules has made a tremendous impact on healthcare to date—the more we can do to enable the developing world to feel this impact, the better. The Gates Foundation has been very helpful in working to achieve this goal.

BIOGRAPHY

ROBERT LANGER is one of eight Institute Professors at the Massachusetts Institute of Technology (MIT); being an Institute Professor is the highest honor that can be awarded to a faculty member. He has written over 1,500 articles, which have been cited over 414,000 times; his h-index of 320 is the highest of any engineer in history and the second highest of any individual in any field. His patents have licensed or sublicensed to over 400 companies; he is a cofounder of a number of companies including Moderna. Langer served as Chairman of the US FDA's Science Board (its highest advisory board) from 1999–2002. His over 220 awards include both the United States National Medal of Science and the United States National Medal of Technology and Innovation (he is one of three living individuals to have received both these honors), the Charles Stark Draper Prize (often called the Engineering Nobel Prize), Queen Elizabeth Prize for Engineering, Albany Medical Center Prize, Breakthrough Prize in Life Sciences, Kyoto Prize, Wolf Prize for Chemistry, Millennium Technology Prize, Priestley Medal (highest award of the American Chemical Society), Gairdner Prize, Hoover Medal, Dreyfus Prize in Chemical Sciences, BBVA Frontiers of Knowledge Award in Biomedicine, and the Balzan Prize. He holds 42 honorary doctorates, including those from Harvard, Yale, Columbia, and Northwestern universities, and has been elected to the National Academy of Medicine, the National Academy of Engineering, the National Academy of Sciences, and the National Academy of Inventors.

AFFILIATION

Robert Langer PhD
Institute Professor,
David H Koch Institute,
Massachusetts Institute of Technology

AUTHORSHIP & CONFLICT OF INTEREST

Contributions: The named author takes responsibility for the integrity of the work as a whole, and has given their approval for this version to be published.

Acknowledgements: Photo source: Bob QE Prize© 2015 Queen Elizabeth Prize for Engineering Foundation

Disclosure and potential conflicts of interest: Langer R received support for the present manuscript from Combined Therapeutics, Evox Therapeutics, Geneleap Biotech, Hopewell Therapeutics, Marble Therapeutics, and Moderna.

Funding declaration: The author received no financial support for the research, authorship and/or publication of this article.

ARTICLE & COPYRIGHT INFORMATION

Article source: Invited; externally peer reviewed.

Revised manuscript received: Jan 31, 2023; **Publication date:** Feb 2, 2024.

NUCLEIC ACID INSIGHTS

LAUNCH EDITION

SPOTLIGHT

The evolving realm of nucleic acids: past, present, and future

Chris Mason
University College London

"...we now need to challenge ourselves to transform the power of nucleic acid technology to enable everyone under the sun to potentially benefit."

VIEWPOINT

Nucleic Acid Insights 2024; 1(1), 43–46

DOI: 10.18609/nai.2024.008

With over 30 years in translational R&D, **Chris Mason** has focused his career on addressing the critical question of how to enable people worldwide to have access to cell and gene therapies. On November 16, 2023, **David McCall**, Senior Editor, *Nucleic Acid Insights*, spoke to Chris about trends in the nucleic acids field. This Viewpoint article is based on that conversation.

EVOLUTION OF THE NUCLEIC ACIDS FIELD

Nucleic acids have transformed biotechnology and healthcare and are now showing disruptive potential outside of traditional life sciences, such as computing. In biotech, DNA and RNA technologies have enabled groundbreaking advancements in medicine, agriculture, industrial bioprocessing, and environmental sustainability. Healthcare has seen a revolution in precision medicine, with nucleic acids facilitating early disease detection, personalized medicine, cell and gene therapies, and the rapid development of vaccines. This integration of nucleic acids across varied domains underscores their unparalleled potential to address some of the most pressing challenges in science and technology today. However, with only a few exceptions, nucleic acid technology has yet to benefit the global population. I believe that we now need to challenge ourselves to transform the power of nucleic acid technology to enable everyone under the sun to potentially benefit. Similar revolutions have occurred with cars and semiconductors, so why not in our most fundamental building blocks of life?

In my own field of gene therapy, where I include all nucleic acid therapeutics (including oligonucleotides and mRNA vaccines), our reach has become much greater over time. A few years ago, our goals were mainly limited to treating inherited monogenic diseases. However, the COVID-19 mRNA vaccines changed the world for billions of people, helping the field to expand from rare and ultra-rare diseases and towards universal treatments for everyone on the planet. The magnitude of their impact cannot be overstated, but it is only a start.

I am also excited by new areas far beyond healthcare, such as DNA computing. Presently highly experimental, the power of DNA computing over silicon-based computing lies in its potential for massively parallel processing, extraordinary data density, and energy efficiency. DNA computing can theoretically perform complex computations leveraging the unique properties of DNA to store and process information. This makes it especially suited for solving certain types of applications, such as optimization tasks, pattern recognition, and complex mathematical problems, which would be impractical or too time-consuming for traditional computers.

Gene editing is also part of the nucleic acids spectrum. It impacts many areas in addition to the therapeutic sphere itself, including the ways in which we discover and develop new drugs. The recent news surrounding the approval of Vertex/CRISPR Therapeutics Casgevy is incredibly exciting for patients today. I am proud that the UK Medicines and Healthcare products Regulatory Agency was the first regulatory agency to grant this landmark approval. The greatest milestone to date, though, is that the predicted adverse off-target effects have yet to materialize.

In 1992, when I initially became involved in gene therapy, the first in-human trials were just beginning. In 1998, Novartis' Vitravene for the treatment of cytomegalovirus retinitis became the first oligonucleotide drug to be approved by the US FDA. Since then, many variants of oligos and gene therapies, including CAR-Ts and AAV vector-based therapies, have reached the market. Over the last 30 years, the space has evolved from having no proof of concept to having dozens of approved products on the market, with billions of patients treated, when we include the COVID-19 vaccines.

A SHIFT IN SCOPE

Nucleic acid therapeutics are not the universal solution for all medicine. If a small molecule drug can do the job well with minimal side effects and cost, then using a small molecule drug is still sensible. Avoiding unnecessary interventions is crucial, particularly when alternative, less intrusive measures such as lifestyle modifications can give positive outcomes.

Gene therapies have ethical, technical, and safety considerations, therefore their use

requires the benefits to clearly outweigh the risks. I believe the prevention of disease constitutes a cure, through early pre-symptomatic diagnosis and appropriate intervention with the right risk-benefit profile. Early diagnosis is critical. We as a field need to establish ultra low-cost, comprehensive testing of both genetic and epigenetic factors. Then, it is hoped that there will be therapies that can be administered easily to the patient with appropriate risk-benefit for the disease before symptoms ever occur. This could be of use with the aforementioned inherited monogenic diseases where ideally, it would be a case of a 'one-and-done' treatment. A patient diagnosed with hemophilia at birth or even in utero could be treated immediately and before irreversible damage is done.

However, many other diseases may require multiple or repeat dosages, as demonstrated by Krystal Biotech's Vyjuvek, the first-ever redosable gene therapy. Gene therapies that can be readministered easily, at a low cost, and potentially in a convenient home setting, hold enormous potential for the field. With redosing as an option, this will also allow us to develop gene therapies for a much broader range of diseases. This approach could even be used to treat certain symptoms. For example, research into the epigenetics of pain and common pathways of inflammation and fibrosis may yield treatments to be delivered a few times a year to treat chronic pain and other common sequelae of the majority of diseases at the symptom level. Gene therapy would not be about cure in those instances; it would be about improving the quality of life for patients living with a wide variety of diseases or conditions. But in order to do this, the drugs must be very safe, predictable, scalable, and low-cost in order to make them accessible globally.

Speed is another factor to consider. During the COVID-19 pandemic, we saw that the regulators can move at speed when necessary. They will also need help from governments, other funding bodies, clinicians, and patient groups to do this for gene therapies, but it is critical that we see a faster way of getting therapies to patients emerge, because these patients are waiting.

IMPERATIVES FOR THE FUTURE

Presently, my own key area of interest is how to enable the delivery of these nucleic acid drugs in a reliable, low-cost way. Others are working hard on therapeutic cargoes.

My role is to enable the delivery of these fantastic therapies to ensure tangible benefits for patients, so they are not confined to the pages of science journals. When considering the future needs of the community, it is evident that standards are needed. Industries are propelled by standardization, enabling seamless transitions between companies and processes, and enabling regulators not to have to be constantly learning novel processes and assays. Standardization of manufacturing would free drug developers to do what they do best, namely pre-clinical, clinical, and commercialization; not manufacturing. Standardization has previously driven the development of the monoclonal antibody and recombinant protein industries. Currently, a notable gap in our field lies in the lack of collaborative efforts across different sectors, including gene therapy, mRNA vaccines, and oligonucleotides. In the face of similar challenges, collaborative efforts aimed at standardizing manufacturing processes, assay procedures, and other CMC aspects will enable faster progress in the wider field. The first step would be to unify the sector under a common banner such as nucleic acid drugs or therapeutics.

Finally, a recent comment by Dr. Peter Marks, Director, CBER, FDA nicely sums up where we are as a sector, "We still have not made the quantum leap forward that we need to in our ability to manufacture cell and gene therapies to help reduce the cost and improve accessibility". But more importantly, it highlights where we need to focus…

BIOGRAPHY

CHRIS MASON is a clinician-scientist with over 30 years of cell and gene therapy experience spanning R&D, clinical medicine, bioprocessing, and business. He is a Full Professor of Cell and Gene Therapy in the Advanced Centre for Biochemical Engineering, University College London. Chris is also a gene therapy serial entrepreneur, including a Founder and Board Director of OriBiotech Ltd, a company focused on next-generation fully automated cell therapy bioprocessors, a Founder and former Chief Scientific Officer at Avrobio Inc., and a Board Director of Krystal Biotech Inc. (NASDAQ: KRYS). Mason was instrumental in the founding of the Alliance for Regenerative Medicine (ARM) and is a current Board Director. He is on the Advisory Boards of several companies, as well as the UK Cell & Gene Therapy Catapult, the Canadian Centre for the Commercialization of Regenerative Medicine (CCRM), and the University of California San Diego (UCSD) Gene Therapy Initiative. Chris is the Senior Editor of the journal *Cell & Gene Therapy Insights*.

AFFILIATION

Chris Mason
Professor of Cell & Gene Therapy,
Advanced Centre for Biochemical Engineering,
University College London

AUTHORSHIP & CONFLICT OF INTEREST

Contributions: The named author takes responsibility for the integrity of the work as a whole, and has given their approval for this version to be published.

Acknowledgements: None.

Disclosure and potential conflicts of interest: Mason C is a member of the Board of Directors for Krystal Biotech and OriBiotech. Mason C holds stock/stock options in Krystal Biotech and OriBiotech.

Funding declaration: The author received no financial support for the research, authorship and/or publication of this article.

ARTICLE & COPYRIGHT INFORMATION

Article source: Invited. This article is based on an interview, held Nov 16, 2023.

Revised manuscript received: Feb 7, 2024; **Publication date:** Feb 14, 2024.

NUCLEIC ACID INSIGHTS

LAUNCH EDITION

SPOTLIGHT

INTERVIEW

Charting a course for the expanded application of mRNA vaccine technology

A pioneer in the development of mRNA technology, CureVac has spent more than two decades in exploring its potential in both prophylactic and therapeutic vaccine settings. **David McCall,** Senior Editor, *Nucleic Acid Insights,* discusses the promise of next-generation approaches with CureVac Chief Development Officer, **Myriam Mendila.**

Nucleic Acid Insights 2024; 1(1), 1–8

DOI: 10.18609/nai.2024.001

Q What are you working on right now?

MM: I came to CureVac about 9 months ago. Prior to that, I spent 6 years with Novartis as Chief Medical Officer in Oncology, and before that, I dedicated 15 years to Roche-Genentech. Throughout most of my career, I have focused on oncology products.

mRNA technology has always held great interest for me. Even during my teenage years in biology classes, learning about genetics, DNA replication, transcription into mRNA, and translation into proteins always fascinated me. Today, the realization that we can use this technology in medicine appeals to me immensely. mRNA serves as a command to the cell to produce something, for example a protein or signaling peptide that can fight a disease, so the possibilities are myriad. That is what excites me about this technology and is precisely

why I joined CureVac; to deliver on the potential that mRNA holds. I hope to contribute to advancing the science around mRNA technology for medical purposes, accelerating our clinical programs and bringing these innovations to patients as quickly as possible.

Q Can you give us some more background on CureVac and details of the current R&D pipeline?

MM: CureVac was founded in 2000 and has been pioneering mRNA technology since then, making significant strides in manufacturing mRNA, enhancing its stability and effectiveness in cell transfection, and leveraging its ability to express encoded antigens.

CureVac has also been developing formulations for mRNA delivery. Upon direct injection of mRNA into the bloodstream, the ribonucleases in the blood swiftly degrade it—we have therefore been focusing on carriers such as lipid nanoparticles and lipoplexes to effectively deliver mRNA to cells. I have observed substantial progress in this regard, particularly with the second-generation mRNA backbone. This progress includes enhanced RNA stability, improved protein translation and expression, and increased immunogenicity.

Turning to the R&D pipeline, CureVac is strategically focused on three major therapeutic areas. Firstly, in infectious diseases, we have a collaboration with GlaxoSmithKline. Here, we are developing vaccines for COVID-19, influenza, and a combination of the two. Promising Phase I data from our second-generation mRNA backbone with monovalent vaccines in both COVID-19 and influenza showed that with low doses of mRNA, a significant immunogenicity in patients could be induced at a reasonable tolerability. The COVID-19 vaccine development program has since advanced into Phase II, with a trial that completed enrollment at the end of October 2023. For influenza, while the initial published Phase I data was for a monovalent vaccine, we have since generated a multivalent vaccine that addresses all four influenza strains recommended by the WHO. The platform has evolved to address multiple antigens with the potential to encode up to eight constructs. With the multivalent influenza vaccine, we conducted another Phase I dose escalation study, which had a successful readout. This led to the commencement of a Phase II study at the end of October 2023. This study is progressing well and the data from both Phase II studies should be available at the beginning of next year, triggering the decision of whether to progress to Phase III.

Secondly, we are active in oncology. We acquired a company, Frame Therapeutics, in 2022, which enriched our antigen discovery machinery. Since then, research has been focused on finding the right antigens to encode for a cancer vaccine. Current work concentrates on initiating and preparing for the next round of Phase I trials.

A Phase I trial with our cancer vaccine platform for glioblastoma began in summer 2023—however, this is in effect more of a proof of principle trial designed simply to show that our mRNA backbone works within the setting. The real innovation will come with the enhanced platform based on the antigen discovery research that has been initiated with the

"mRNA serves as a command to the cell to produce something, for example a protein or signaling peptide that can fight a disease, so the possibilities are myriad."

neoantigen compositions and constructs. Phase I clinical trials are being prepared in different cancer indications to hopefully enroll patients within the next 18 months.

The third strategic pillar of the pipeline is molecular therapy where mRNA is used to encode for a missing enzyme or signaling peptide or deficient proteins in order to replace the missing element and reconstitute its function. While specific projects cannot be disclosed, we are optimistic about bringing new treatments or indications to the clinic in the upcoming years.

Q What for you are the chief high-level trends and strategic drivers shaping both the prophylactic and the therapeutic mRNA vaccine spaces as we move into 2024?

MM: Starting with the prophylactic vaccine space, the one key trend is the remarkable effectiveness that continues to be shown by mRNA vaccines. In some areas, they have demonstrated greater efficacy than currently approved medicines. The mRNA platform allows us to address and generate prophylactic vaccines in areas where today's vaccines fall short.

The second notable trend is the versatility of mRNA. Due to the ability to design proteins by using the genetic code combined with an efficient manufacturing process, mRNA can be employed to develop vaccines against pathogens where other technologies have struggled, including bacterial and fungal pathogens. Companies that are invested in mRNA platforms are venturing into these and other new areas, addressing unmet needs.

A crucial lesson from the COVID-19 mRNA vaccines is the need for enhanced durability. Many companies are actively working on improving the duration of immune responses, aiming to provide extended protections beyond the currently observed 6–7 months. Additionally, while a potent medical tool in some region of the world, mRNA vaccines face logistical challenges in delivering these benefits to certain other geographical areas due to the requirement for a –60–80°C cold chain. Efforts are underway to improve stability, allowing mRNA to be stored in fridges for extended periods, ideally in pre-filled syringes. This will not only increase convenience but also broaden the reach of mRNA vaccines on a global basis.

In oncology, recent data from current mRNA therapeutic vaccine candidates has sparked new hope. Traditionally, cancer vaccines have faced challenges with many peptide vaccines failing to deliver significant benefits in Phase III studies. However, recent data, including those from randomized Phase II studies in early cancer settings like melanoma, have shown promise. Within the cancer setting, experiments with mRNA technology are increasing—specifically, exploring its use in combination with other immunotherapies to enhance treatment responses. However, the potential applications extend beyond vaccines, including encoding CAR-T and T cell receptors, tumor-suppressing factors, and antagonists to tumor-promoting factors.

Q Can you deeper on the current state-of-the-art in the application of cutting-edge mRNA technology with next-generation COVID-19, as you see it?

MM: It is evident from the data that mRNA vaccines have proven to be significantly more effective than other vaccine platforms and this was particularly highlighted during

the COVID-19 pandemic. The mRNA platform showed the potential to deliver high efficacy, achieving levels of protection for around 90–95% of vaccine recipients—a figure rarely seen with the current standard, mainly peptide-based vaccine technologies.

Explaining why the mRNA platform surpasses peptide-based platforms is challenging, but in the context of a pandemic or other viral infections with continuous mutations, the mRNA platform allows for faster adaptation to emerging viral strains. This adaptability enhances patient protection and has a more substantial potential impact on the health system due to its flexibility.

The first-generation mRNA vaccines, although developed rapidly and demonstrating efficacy, revealed areas for improvement, such as durability and reactogenicity. The latter, while manageable, was a particular consideration. The second-generation mRNA backbone now focuses on addressing these gaps. At CureVac, our second-generation backbone has shown the ability to induce immunogenicity at very low doses, potentially improving tolerance. This advancement allows for the combination of different mRNA vaccines, such as for COVID and influenza, thus streamlining the vaccination process.

So, the ongoing evolution of mRNA vaccines aims to reduce dosages, enhance effectiveness, improve reactogenicity, and enable the combination of multiple viral antigens in a single vaccine. The technology's potential extends to bacterial infections and parainfluenza, offering a unique advantage in creating comprehensive vaccines. Looking further ahead, efforts are concentrated on improving efficacy and tolerability, facilitating combinations of vaccines, and enhancing convenience through pre-filled syringes. This collective focus on formulation and backbone improvement aims to make vaccinations more accessible and convenient.

Q And moving to the therapeutic setting, as an oncology specialist, can you expand on what mRNA can bring to the cancer therapeutic armamentarium?

MM: As we have discussed, one significant approach involves using mRNA as a cancer vaccine, where it constructs and codes for cancer antigens, initiating an immune response in patients. This marks the starting point for us and others in the field. In parallel, we are exploring ways to augment the immune response by combining mRNA cancer vaccines with other immunotherapies such as checkpoint inhibitors, which are standard of care in most solid tumors.

In the cancer vaccine space, our exploration extends to both monotherapy and combination with data showing successful combinations of mRNA with CAR-T or adoptive T cell therapies. Combining mRNA with CAR-T cells has allowed for the *in vivo* expansion of CAR-T cells, addressing challenges related to yield and persistence.

In preclinical studies, we have observed that booster vaccines, administered approximately 6 months after CAR-T cell infusion, can re-expand the pool of CAR-T cells *in vivo*. This is particularly promising as it offers a potential solution to the challenges CAR-T cells face in both solid tumors and hematological cancers.

Beyond therapeutic vaccines, addressing the immunosuppressive tumor microenvironment is crucial. We are actively researching factors that can help overcome this suppression, exploring the possibility of encoding them alongside an mRNA vaccine. This dual strategy involves stimulating the immune system while mitigating immunosuppressive effects within the tumor.

"...one significant approach involves using mRNA as a cancer vaccine, where it constructs and codes for cancer antigens, initiating an immune response in patients."

Another exciting project that is explored in research is encoding T cell receptors using mRNA. This approach allows for the design of receptors based on known antigens, presenting a novel way to address cancer. While still in the theoretical stage, it highlights the versatility of the mRNA platform.

Lastly, optimizing mRNA technology for precise cell targeting remains a priority. This involves evolving the delivery mechanism to access cells that may be challenging for other therapies to reach. While we are still in the early stages, many are invested in this particular area or research.

Q What considerations and challenges do you see in incorporating mRNA technology into the cancer therapeutic space, whether in the combination therapy or monotherapy setting?

MM: Considering cancer vaccines, several challenges need to be addressed. The first is the identification of the right antigens or neoantigens for mRNA to encode because in the oncology space, a given patient may have hundreds of antigens. Sophisticated methodologies are essential to determine the antigens that elicit a robust immune response and are worthy of being encoded on the mRNA.

CureVac has taken a different approach by delving deeper into patient genome sequencing from tumor tissue, combining whole genome sequencing with mRNA sequencing to identify any possible genomic alteration in the patient's tumor that could be immunogenic. By doing this, new classes of antigens and neoantigens that are not uncovered by conventional methods like UV4 exome sequencing can be discovered.

Another one of the challenges involves determining the future direction of cancer vaccines and deciding if they should be 'off-the-shelf' with shared antigens across different cancers, or personalized for each patient. CureVac is pursuing both approaches given the current uncertainty as to which might prove to be superior. Personalized cancer vaccines, while promising, face supply chain constraints and may not be applicable to patients with advanced disease due to the extended manufacturing turnaround times. The critical challenge there is to expedite and streamline this process—currently, sequencing, analysis, prioritization, and manufacturing may take up to 6 weeks.

The final challenge revolves around delivering the vaccine to the right cells *in vivo*. Current mRNA lipid nanoparticles reportedly reach only a small proportion of target cells—around 3–5%. As the goal is to target immune cells, it is vital to enhance the efficiency with which the right cells are reached, and to make sure this happens in sufficient quantities. This optimization is crucial for increasing antigen expression, subsequently boosting immunogenicity throughout the body where tumors are located.

Q What can we deduce from regulators' reactions to mRNA technology applied in the therapeutic setting to date, and what should be some corresponding important points of focus for developers?

MM: In infectious diseases, following the success of the COVID-19 vaccines and given their well-characterized safety profile, regulators take a standard approach to mRNA vaccines. The emphasis lies on demonstrating various quality criteria for the manufacturing process. Clinical development follows a relatively standard path, requiring proof of immunogenicity, vaccine efficacy, and comparisons to existing products on the market. However, as the next generation of vaccines is developed, the focus has shifted towards areas not fully addressed during the pandemic, such as biodistribution and other features.

In oncology, on the other hand, mRNA-based therapeutics such as cancer vaccines fall under a different categorization, alongside gene therapies and cell therapies. This leads to more stringent regulatory requirements, and authorities tend to adopt a conservative stance due to the novelty of the technology. Generating substantial data with which to approach regulators becomes crucial, and a platform approach is employed to eliminate the need to repeatedly generate new information with each product redevelopment. This approach is intended to promote faster development programs in the future.

Development with mRNA cancer vaccines has slowed down recently due to the regulations. The goal is to continue working on refining the platform, leveraging the well-tolerated safety profile.

Q What is your vision for the future impact of mRNA on both prophylactic and therapeutic vaccine spaces?

MM: Looking to the future, the potential applications of mRNA technology are highly promising. With regard to prophylactic vaccines, there is hope for developing new vaccines against diseases that have been challenging to address, such as malaria and HIV, as well as in bacterial infections where traditional approaches, including antibiotics, have fallen short. The versatility of mRNA offers a glimpse into a future where we can address medical challenges and needs that were previously unmet.

In oncology, following the success of immunotherapies like checkpoint inhibitors in the last decade, I would say that mRNA is seen as the next significant advancement. Engaging the patient's immune system is believed to be crucial for achieving long-term cancer control. While targeted therapies and chemotherapies have demonstrated effectiveness, they often provide transient benefits. The belief is that by instigating a robust immune response, mRNA-based cancer vaccines can play a transformative role in cancer therapy, particularly in earlier disease settings.

Further to this, I foresee increased research interest and activity in the use of mRNA technology to prevent cancer in individuals deemed to be at high risk based on genomic alterations in their germline DNA. This highlights the potential for mRNA to offer a comprehensive approach to addressing the complexities of this disease. In summary, the future holds enormous promise for mRNA to emerge as a powerful tool in areas where traditional medical approaches have faced challenges.

"The belief is that by instigating a robust immune response, mRNA-based cancer vaccines can play a transformative role in cancer therapy, particularly in earlier disease settings."

Q Lastly, can you sum up one or two key goals or priorities, both for your own work and for CureVac as a whole, over the foreseeable future?

MM: My personal goals are also corporate goals because they are closely aligned. My first goal is to move our vaccine programs forward, especially in influenza where we aim to transform the field. Currently, the influenza clinical development program is going smoothly, and I want us to continue towards generating better vaccines in both influenza and combination settings for the convenience of patients.

My second goal is to bring our cancer vaccine candidates into patients as soon as possible. We have great technology in the design space, so it is all about execution. The science is there, the technology is there—let's execute it and get it into patients.

BIOGRAPHY

MYRIAM MENDILA joined CureVac from Novartis, Switzerland, where she served as Chief Medical Officer in the Oncology Business Unit with responsibility for the worldwide medical affairs function. In this role, she was a member of several governance boards, including the Oncology Leadership Team, the Scientific Development Leadership Team, and the Development Committee Novartis. In the past, Myriam has held different leadership positions of increasing responsibility within global medical affairs, US medical affairs, global product development, and global product strategy at Roche/Genentech. Prior to that, she led Global Medical Affairs at Roche HQ in Basel. Myriam holds an MD from the Medical School Hanover. She has research papers in journals, including *AIDS*, *The Lancet*, *The Journal of Clinical Oncology*, *Annals of Oncology*, and *The Lancet Oncology*.

AFFILIATION

Myriam Mendila PhD
Chief Development Officer,
CureVac

AUTHORSHIP & CONFLICT OF INTEREST

Contributions: The named author takes responsibility for the integrity of the work as a whole, and has given his approval for this version to be published.

Acknowledgements: None.

Disclosure and potential conflicts of interest: Mendila M has stock/stock options in CureVac and Novartis.

Funding declaration: The author received no financial support for the research, authorship and/or publication of this article.

ARTICLE & COPYRIGHT INFORMATION

Article source: Invited.

Revised manuscript received: Dec 7, 2023; **Publication date:** Jan 11, 2024.

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, *Nucleic Acid Insights*, 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.

Nucleic Acid Insights 2024; 1(1), 9–15

DOI: 10.18609/nai.2024.002

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

"Right now, AAV vectors and LNPs can only address perhaps 10% of the possible indications that could potentially be addressed with genetic medicines."

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

"One of the key initial target organs that we will likely see is the lung."

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 intravital imaging in the *in vivo* study of tumor cell invasion and metastasis to discover key targets for cancer therapeutics. 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, 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. Lewis trained at The Scripps Research Institute and received a PhD in Biochemistry from the University of Victoria.

AFFILIATIONS

John Lewis PhD
Professor in Oncology and the Bird Dogs Chair in Translational Oncology,
University of Alberta,
Canada
and
Founder and Chief Executive Officer,
Entos Pharmaceuticals

AUTHORSHIP & CONFLICT OF INTEREST

Contributions: The named author takes responsibility for the integrity of the work as a whole, and has given his approval for this version to be published.

Acknowledgements: None.

Disclosure and potential conflicts of interest: The author has no conflicts of interest.

Funding declaration: The author received no financial support for the research, authorship and/or publication of this article.

Article source: Invited.

Interview held: Nov 9, 2023; **Revised manuscript received:** Dec 19, 2023; **Publication date:** Jan 11, 2024.

NUCLEIC ACID INSIGHTS

LAUNCH EDITION

SPOTLIGHT

A brave new world of next-generation DNA applications in the biotherapeutics field

Floris Engelhardt
Co-Founder and CEO, Kano Therapeutics

"Three different steps are enabling progress in the DNA space: parallelization, acceleration, and enablement."

VIEWPOINT

Nucleic Acid Insights 2024; 1(1), 17–20

DOI: 10.18609/nai.2024.003

On November 20th 2023, David McCall, Senior Editor, *Nucleic Acid Insights*, spoke to Floris Engelhardt, Co-Founder and CEO of Kano Therapeutics, about recent evolution in DNA design and synthesis, and its application in the biotherapeutics space. This Viewpoint article was written based on that conversation.

THE EVOLVING SCOPE OF NUCLEIC ACIDS

Over the past decade, two major changes have shaped the field of nucleic acids as we now know it: DNA writing and DNA reading. The transition from first-generation DNA sequencing (e.g., Sanger sequencing) to nanopore sequencing and next-generation sequencing methods that allow rapid whole plasmid sequencing has completely turned the field around.

During my PhD, which began in 2015, the process of sequencing involved ordering primers, sending the sample out to a vendor, and receiving Sanger sequencing results 24 h later. The problem was that the reads were short—around 800 nucleotides. Every read needs an individual primer, which can become expensive when several kilobases of plasmid DNA need to be sequenced. Today, nanopore sequencing is becoming an easily obtainable commodity; a plasmid can be easily sequenced for US$15, so things can be sequenced repeatedly, if needed. In addition, DNA cloning has become much easier; the enzymes work at different temperatures and cloning can be performed on much smaller or larger scales.

Prevalent thinking in the field has also changed considerably over the past 10 years. In the early 2010s, companies worked simply to make more mass of DNA because that was the huge production bottleneck in the market. Now that suppliers are able to meet the market need for DNA mass, new companies are driving the market based on making products by rationally designing DNA, enabled by far greater access to large numbers of designs. This in turn is driving the creation of more complex nucleic acids that facilitate a variety of product features. For example, there are DNA storage companies like Cache DNA, and DNA nanotechnology companies like Capsitec and Plectonic Biotech. Similarly, at Kano Therapeutics, our manufacturing arm is used to design better products, rather than just to make more DNA. We want to make access to functional therapeutic payloads for cell and gene therapies possible.

DNA synthesis has also evolved. Initially, chemical DNA synthesis dominated the landscape through companies such as Integrated DNA Technologies (IDT) and Twist Bioscience. In 2016, the field of enzymatic DNA synthesis with terminal deoxynucleotidyl transferase was launched, with molecular assemblies and DNA script. Throughout this period, microbial DNA production resulted in double-stranded DNA but today, microbial production can create single-stranded DNA. In 2018, I recall being offered the opportunity to purchase 40 clonal genes with a length of close to 2 kb for under $10,000 by a DNA vendor, which was a pretty good deal at the time. In 2023, 5 years later, one vendor offered to synthesize the same sort of length (1.8 kb) with a 96-well plate, for free.

ADVANCES AND POTENTIAL BENEFITS OF DNA IN NOVEL BIOTHERAPEUTICS

Three different steps are enabling progress in the DNA space: parallelization, acceleration, and enablement.

Firstly, as there is so much access to DNA today, we can start thinking about the parallelization of design optimization. More DNA—and more types of DNA—means more experiments can be run in parallel for the same cost, allowing us to collect more data simultaneously.

That data leads to acceleration. The field is now moving towards the utilization of AI. The overarching problem with biology and AI is the large amounts of high-quality data required to achieve a good output. It is the case with nucleic acids in particular that the more quality data coming in, the more we can analyze to establish the right machine learning outputs. That acceleration gives us an understanding of the biology, immunogenicity, and spatiality of DNA interactions.

Enablement means moving into various applications. Companies including Strand Therapeutics are developing longer-lasting RNAs for genetic medicine. At Kano Therapeutics, we are working on longer genes in site-specific insertions. Ultimately, more data means better decision-making, and better decision-making enables the exploration of more complex disease areas and mechanisms.

REMAINING CHALLENGES IN THE USE OF COMPLEX DNA STRUCTURES

We do not yet fully understand the impact of DNA structure on function. Everyone thinks of DNA as just a four-letter code with a simple one-dimensional sequence and a functional outcome. But the reality is that this structure is not a 1-D molecule; it is a 3-D molecule that interacts with itself and its environment, all of which influences its function. Uncovering that part is important in developing a better understanding of DNA biology and improving genetic medicine.

The oft-used term 'non-viral delivery' suggests that delivery is the only issue at hand. Non-viral systems do include delivery, but they also include editing and payloads. These three components together contribute to the complexity of enabling non-viral approaches for genetic medicine. You can make an AAV vector that is great in terms of transducing different cells, but we must think about the other systems that we want to deliver, and build the components together. We are moving beyond AAV or lentiviral vectors that contain everything needed, and to enable that next layer of complexity, we need to think about non-viral systematic approaches. Non-viral delivery is unlikely to be solved with a lipid nanoparticle alone. For example, the specific DNA payload used in connection with the delivery tool can make a large difference—single-stranded DNA has lower toxicity and higher efficiencies, for example. But we will only get non-viral delivery approaches off the ground if we understand all these systems and make them work together perfectly.

THE ROLE OF NOVEL TECHNOLOGIES IN THE FUTURE OF DNA-BASED THERAPEUTICS

Collaborative drug development is a huge opportunity for the entire field of medicine. The systems that we are building to tackle more complex diseases are sophisticated, so new players are needed to investigate specific high-risk unexplored areas. Working collaboratively enables the building of horizontal knowledge layers that connect various use cases and accelerate learning cycles across different fields. We can connect fields like *ex vivo* cell therapy for cancer immunotherapies, genetic medicines for monogenetic disorders, or DNA vaccines that normally do not talk to each other. This will allow us to build an umbrella of DNA innovation to enable novel therapeutic approaches.

It is important to mention that the key step within single-stranded DNA genome editing is homology-directed repair. Homology-directed repair, which CRISPR insertions are based on, is the cutting of DNA, and through that cut, another piece of DNA is inserted, before the cell's own repair mechanisms seal the gaps. This leads to a novel piece of genetic material inserted at a site based on your rational design.

Single-stranded DNA has huge potential in the treatment of monogenetic disorders like hemophilia, Duchenne muscular dystrophy, and cystic fibrosis. Additionally, the non-viral gene delivery field is being revolutionized with non-viral chimeric antigen receptor T cell therapies, regulatory T cells, and natural killer cell therapies, and other *ex vivo* methods in which new functionalities are added to cells efficiently, especially in human patients. We must focus on bringing synthetic biology into the human body, where the cost of losing a human cell is much greater than the cost of losing a bacterial microbe.

A colleague recently compared the nucleic acid field to the early years of oil discovery, where oil was viewed as a natural resource that simply allowed us to power engines. Back then, no one thought about the follow-on applications, such as plastics, that would be based on that early discovery. Similarly, we think nucleic acids will go way beyond genetic storage material for cells or cloning, being used in nanodevice applications, for data storage as computing mechanisms, and in novel therapeutic areas. Pointing out these new use cases and helping people to understand where the field is going is important.

BIOGRAPHY

FLORIS ENGELHARDT's career in nucleic acids began with an undergraduate thesis in 2013 on DNA binding protein kinetics, which established a persisting love for the DNA field. Her PhD involved a study of DNA origami with Hendrik Dietz, developing three-dimensional nanostructures using DNA. This, in combination with her postdoctoral studies at MIT, sparked a vision that led to the establishing of Kano Therapeutics, a biotech startup enabling the correction of gene-length stretches of DNA using circular single-stranded DNA.

AFFILIATION

Floris Engelhardt
Co-Founder and CEO,
Kano Therapeutics

AUTHORSHIP & CONFLICT OF INTEREST

Contributions: The named author takes responsibility for the integrity of the work as a whole, and has given their approval for this version to be published.

Acknowledgements: None.

Disclosure and potential conflicts of interest: Engelhardt F received a non-dilutive grant as an activate fellow from 2021–2023. Engelhardt F is CEO, Co-Founder, and board member of Kano Therapeutics. Engelhardt F has founder equity stocks in Kano Therapeutics. Engelhardt F recieved a prize sponsorship at Lab Central.

Funding declaration: The author received no financial support for the research, authorship and/or publication of this article.

ARTICLE & COPYRIGHT INFORMATION

Article source: Invited.

Revised manuscript received: Jan 8, 2024; **Publication date:** Jan 15, 2024.