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VACCINE INSIGHTS

SPOTLIGHT ON
RNA vaccines: formulation and production

Guest Editor
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RNA VACCINES: FORMULATION AND PRODUCTION

SPOTLIGHT

Spotlight on RNA vaccines: formulation and production

Iulia Oita

"This month's Spotlight…features experts in the mRNA vaccine field discussing the different aspects of its status, challenges, and prospects…"

FOREWORD

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The mRNA vaccine field remains one of the most dynamic areas of novel drug development, with compendial guidance being released at an unprecedented speed and new RNA formats, delivery approaches, and components constantly emerging. Product quality analytics are also evolving, allowing developers to question the quality attributes listed in the draft pharmacopoeia chapters and better understand what factors are critical to producing high-quality mRNA medicines. Together, these developments offer promising potential for improved product design and more efficient manufacturing processes.

This month's Spotlight on 'RNA vaccines: formulation and production' features experts in the mRNA vaccine field discussing the different aspects of its status, challenges, and prospects, covering topics from DNA raw materials to manufacturing, formulation development, IP, and advanced analytical approaches.

Lawrence Thompson, Associate Research Fellow at Pfizer, provides an objective overview on DNA starting materials. Although the field has diversified, with promising developments like cell-free DNA materials, the high costs remain one of the biggest challenges for mRNA vaccine developers. Another significant challenge is establishing the right control plan, guided by available analytics and full understanding of the specific quality requirements when DNA materials are used for *in vitro* transcription (IVT), as well as their impact on the final RNA quality.

Adam Brown, Senior Lecturer (Associate Professor) at University of Sheffield, explores valuable lessons RNA vaccine developers could learn from the successes and challenges faced by traditional biologics. These insights could help avoiding 'reinventing the wheel' and stimulate growth in the field. One of the most important lessons is proactively expanding the current manufacturing toolkit to be able to support the evolution toward ever-more complex RNA modalities. This seems particularly important when looking into the evolution of protein modalities from small, simple molecules like insulin, to large, complex tri-specific fusion proteins. Another important lesson is related to large-scale data collection and knowledge sharing as drivers for process understanding, paving the way for innovative solutions, and ensuring that manufacturing is not the limiting step for therapeutics with so much promise.

Jesse Erasmus, Director of Virology at HDT Bio, shares a biotech perspective on challenges and opportunities in developing new RNA vaccine formulations, especially when targeting non-enveloped viruses and mucosal responses. The key ingredients in his recipe for pandemic preparedness include a robust platform, finding novel RNA delivery methods, and a better understanding of mechanisms of action of mRNA vaccines. He identifies tolerability, durability, and accurate modeling of human immune response as the main roadblocks in the RNA vaccine fields and he emphasizes the importance of safety studies for viral proteins encoded in mRNA vaccines.

Finally, Dan Shores, Partner at Rothwell Figg, offers advice on how to navigate the mRNA-LNP intellectual property (IP) labyrinth. The viewpoint paints a clear picture of the intricate ligation landscape in mRNA and LNP technology while also providing some guidance for navigating it. Given the complexity of this constantly evolving field, it is crucial to identify the right path forward, taking the impact of IP into account from the earliest stages of development. For companies with creative and knowledgeable chemistry teams, crafting proprietary lipids is one interesting way out of the lipid nanoparticle IP labyrinth.

BIOGRAPHY

IULIA OITA is CMC Head of Analytics at Ziphius Vaccines, Ghent, Belgium, where she has been since 2022. She is a pharmacist by training and received her PhD in Pharmaceutical Sciences from Vrije Universiteit Brussel, Brussels, Belgium in 2012. She has worked for over 15 years in the pharma industry, involved in pre-clinical and clinical analytical development of small and large molecules. In her current position, she coordinates outsourced and internal analytical activities.

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VACCINE INSIGHTS

RNA VACCINES: FORMULATION AND PRODUCTION

SPOTLIGHT

INTERVIEW

Understanding DNA starting material for mRNA production

Charlotte Barker, Commissioning Editor, *Vaccine Insights*, speaks to **Lawrence Thompson**, Associate Research Fellow, Pfizer, about advances and challenges in DNA starting materials for mRNA vaccine production, including gaps in guidance, the growing role of synthetic DNA starting material, and the transition of next-generation sequencing techniques into GMP environments.

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Q What is the main focus of your work?

LT: Currently, I am an analytical R&D lead in the mRNA vaccine space, focusing on DNA starting materials and mRNA drug substances. I contribute to the control strategy, method platform and quality attributes. As part of my role, I try to gather diverse perspectives and maintain a 30,000-foot view of the field.

Q What are the key challenges associated with mRNA manufacturing, specifically relating to raw materials?

LT: The cost of raw materials and reagents poses significant challenges. These components are critical because it is an *in vitro* process that requires various enzymes and other components, all of which must function correctly and in balance.

The goal is to build a robust platform, but another challenge lies in the increased analytical demands needed to delve deeper into the molecules. Everything must be managed *in vitro*, including the processes that cells normally manage on their own.

Another emerging challenge is managing timelines and seasonal strain changes. With seasonal products like COVID-19 and influenza vaccines, we must develop a new product each season.

Q What is the current regulatory guidance on quality limits for DNA starting material? Is there a need for more guidance?

LT: Initial guidance documents focused on DNA in vaccines (plasmid DNA specifically). As advanced therapies, including cell, gene, and mRNA therapies, began to reach maturity, guidance documents for these new modalities began to appear and included short sections around the DNA starting material used in advance therapy production.

Over time, the DNA starting material landscape has become so diverse that guidance in this specific area was needed. The *US Pharmacopeia* identified this need and in 2020 paneled an expert committee, of which I am a member, to begin authoring a general chapter on plasmid DNA starting materials. It is in the late stages of development. At present, the panel is reviewing and responding to comments from the general public.

In many instances, developers are using plasmid DNA as the starting material. Plasmid DNA has a long history in terms of manufacturing, analytics, etc., so authoring a guidance around its use as a starting material, although challenging, was a tractable endeavor. However, there is a significant gap in guidance regarding synthetic DNA starting materials, and for good reasons. Although only a handful of companies have developed mature products in this area to date, many developers are now diving in. Since everyone's synthetic process is different, the control strategies will also vary, which is both interesting and challenging.

Q What is the role of cell-free DNA templates going forward?

LT: Unlike plasmid DNA-based processes, which require cell banks, fermentation, lysis, flocculation, and chromatography and can take a few weeks, cell-free systems can be faster

"...it is essential to read extensively, seek advice from those with experience, and engage with regulatory bodies early on."

and more flexible. The challenge with synthetic processes is that there is no cellular control of the nucleic acid quality (i.e., DNA repair machinery). We encounter similar issues to those we see with *in vitro* RNA production, including the cost and control strategy for incoming reagents, as well as questions around fidelity.

Cell-free systems are maturing as a platform. There are several clinical studies underway that use synthetic cell-free DNA templates. The outcomes will be key to broad adoption. Despite the challenges, I believe that, in the next few years, cell-free systems will become a big part of the market.

Q What are the key considerations and common pitfalls when developing a control strategy for DNA starting material?

LT: Firstly, some developers set very tight specifications initially when those strict criteria may not be necessary. This can significantly increase the costs of goods while achieving no benefit to drug substance quality. This error often occurs when guidance documents designed for DNA as a drug substance are arbitrarily applied to DNA as a starting material. My advice for developers is to pressure-test the DNA starting material quality needed for their application and not follow past practices without questioning whether they are truly appropriate for their needs.

Another common pitfall relates to the type of materials used—whether GMP, 'GMP-like', or R&D grade. Regulatory bodies only recognize GMP. Anything else is just a label on a box. A vendor may sell a material labeled as 'GMP-like' but that is not a recognized quality level. It is what the vendor says it is. Developers must understand what each vendor means by this terminology and whether or not that is appropriate for their application.

One piece of advice to avoid these bear traps: it is essential to read extensively, seek advice from those with experience, and engage with regulatory bodies early on. Pre-IND and pre-BLA meetings, for example, are always valuable.

Q What is the impact of nicked DNA starting material on the quality of the drug substance and how does this differ between AAV and mRNA production?

LT: In my experience, the triple plasmid transfection process commonly used for producing AAV is much more tolerant of nicked DNA than IVT. If DNA has a nick when used

"I believe next-generation sequencing tools will become GMP-compliant because they are often sequence agnostic, meaning new reagents are not required for each test."

for IVT, the polymerase will likely fall off at that nick, directly affecting the integrity of the mRNA. However, if the plasmid is transfected into a cell, the nick will be handled differently—it might be repaired or processed in various ways.

There are different approaches to control the risk of nicked DNA. If you are working with a plasmid-based process, one strategy is to achieve the highest possible percentage of supercoiling. Supercoiled material is not nicked and can be controlled at the circular DNA level prior to linearization.

Additionally, it is vital to develop a representative small-scale IVT process, which allows for testing different starting materials and assessing the impact. This can be extended to all starting materials—having this small-scale process is essential for setting quality limits for the material.

Q How can next-generation sequencing (NGS) and Sanger sequencing be applied in plasmid DNA quality testing?

LT: Sanger sequencing is excellent for release testing and provides a high-level poly-A analysis. NGS offers both short-read and long-read sequencing, which are useful for analyzing impurity and purity profiles, respectively. Additionally, some companies are developing new direct RNA sequencing tools that could serve as multi-attribute methods for detailed RNA analysis.

Many people in the nucleic acids industry, regardless of a specific area, are already using these tools. However, the key question is whether NGS will remain solely as a characterization tool or transition into GMP environments like Sanger sequencing. Personally, I believe NGS tools will become GMP-compliant because they are often sequence agnostic, meaning new reagents are not required for each test. Instead, standard off-the-shelf kits can be utilized. The challenge (and power) lies in the bioinformatics aspect, which must be developed independently.

Q How can developers ensure a consistent and high-quality raw and starting material supply chain?

LT: You can either outsource the process or handle it in-house. Some companies, including Pfizer, have chosen to do a majority of DNA starting material production in-house for greater control. Even though vendors may offer quality agreements, the responsibility ultimately falls

upon the user to ensure the materials will work. The best strategy is always to take some material from the vendor, run it through the process, and test it to ensure it performs as expected.

Regarding raw materials, their quality has improved over time, and the DNA requirements have become more flexible. While many aspects have loosened up since the COVID-19 pandemic, the materials are still expensive. However, increased competition among suppliers should help drive prices down.

Q What advances will make mRNA vaccine production faster, better, and cheaper in future?

LT: Firstly, increased competition in the raw material space is needed to decrease the costs. Secondly, synthetic starting materials need further development, which could help speed up many processes. Thirdly, as mentioned previously, NGS tools could become integral in GMP settings.

More broadly, several new RNA-based modalities are on the horizon, which may offer new opportunities (and challenges) for manufacturers. There is growing interest in circular RNA as an alternative to mRNA vaccine and therapeutics. If it proves effective, circular RNA has great potential due to its stability and other advantages. Finally, self-amplifying RNA is also a promising modality, potentially reducing costs due to its ability to replicate in the human body. It will be interesting to see how these advances will develop.

BIOGRAPHY

LAWRENCE THOMPSON is an Associate Research Fellow and Group Leader in Analytical R&D within BioTherapeutic Pharmaceutical Sciences at Pfizer, Chesterfield, MO, USA. He is an analytical CMC SME for Pfizer's adenoviral and plasmid DNA based immunotherapeutics, mRNA drug substances, and nucleic acid starting material pipeline (used in rAAV and mRNA production). Prior to joining Pfizer, he worked at a couple of small biotech companies developing of serum-based cancer diagnostics. He received his PhD in Biochemistry from Vanderbilt University, Nashville, TN, USA and did his post-doctoral work at the University of Tennessee, Knoxville, TN, USA. His work has generated numerous peer reviewed publications and presentations at scientific conferences.

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VACCINE INSIGHTS

RNA VACCINES: FORMULATION AND PRODUCTION

SPOTLIGHT

History is the best teacher: what can RNA manufacturers learn from the challenges and successes of traditional biologics?

Adam Brown
University of Sheffield

"To help RNA achieve its therapeutic potential, it is important to future-proof RNA manufacturing by ensuring flexibility and adaptability as the field evolves."

VIEWPOINT

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The field of RNA-based vaccines and therapeutics is rapidly evolving. However, despite its reputation for speed and ease, RNA manufacturing can be complex, and looks likely to become even more so as the field evolves. To ensure that manufacturing does not become a bottleneck in bringing new products to market, the industry can learn valuable lessons from the successes and missteps of traditional biologics, including the importance of future proofing and the need for data sharing. On September 27, 2024, **Charlotte Barker**, Editor, *Vaccine Insights*, spoke to **Adam Brown**, Senior Lecturer (Associate Professor), University of Sheffield, about optimizing RNA manufacturing. This article has been written based on that interview.

For many years, our research laboratory at the University of Sheffield has worked on biopharmaceuticals, from protein to viral vectors, with a focus on upstream processing and developing the biological components involved in those processes. As an academic group, we are quite unusual in our focus on technology development, functioning more like an R&D laboratory. We are particularly drawn to solving 'wicked problems'—challenges that the industry does not have the time or coalescence of skills to address.

As the mRNA field expanded in the wake of the successful COVID-19 vaccines, companies began to approach us seeking help to set up their RNA manufacturing platforms. RNA production is often viewed as a completely different process to monoclonal antibodies or other traditional biologics since RNA is produced via a cell-free IVT process. However, there are enough fundamental similarities that our hard-won expertise with protein and AAV production has proved very useful in approaching problems in RNA manufacturing.

When viral vectors first emerged, one of the key questions was whether we could transfer the knowledge from protein manufacturers instead of 'reinventing the wheel'. However, despite attempts to transfer knowledge, platform developers did not always take into account what had been learned the hard way over 40 years in protein manufacturing. Similarly, with RNA, we see both opportunities and challenges. While RNA manufacturing differs from traditional biologics, learning from past trends in biologics manufacturing can guide more efficient RNA development.

BE PROACTIVE, NOT REACTIVE

One key lesson is that complexity will almost certainly increase rapidly as the RNA field matures. Just as therapeutic proteins have evolved from small, simple molecules like insulin, to large, complex tri-specific fusion proteins, we are already seeing a shift in R&D from simple linear mRNA to self-amplifying and circular RNAs. Conjugates linking RNA to different molecules will likely emerge, and future innovations are unpredictable.

Some in the industry advocate for RNA production to be fully platformed, standardized, and cell-free. While that may be effective for certain applications, we should take the lesson from other modalities and plan now for future complexity, including modalities we cannot yet envisage.

Exciting new RNA products could offer huge benefits for patients, but the current toolbox for manufacturing is small. To ensure that manufacturability does not become a bottleneck for evolving product designs, it is critical to future-proof manufacturing processes and introduce more flexibility to our toolkit.

Consider a new product format in development that shows great promise, clinically. But when you run an initial manufacturability test, it barely works. What are your options? For a protein product, there are already a wide range of tools available—for RNA, much less so. The goal is to have potential solutions ready, so you are not reacting to challenges but proactively solving them before they become bottlenecks.

For example, T7 polymerase is currently ubiquitous, but I would like to see a wide

range of polymerases that manufacturers can choose from according to the specific properties of the product. Similarly, I would like to see a range of tools for all aspects of the process, for example algorithms for DNA template design that optimize both manufacturability and therapeutic efficacy. My group is even looking at cell-based RNA production methods—an anathema to some! While the cell-free nature of the RNA production process is seen as an advantage, these methods may be unable to accommodate the manufacturability challenges of ever-more complex molecules.

SOLVE PROBLEMS BY SHARING DATA AND KNOWLEDGE

As someone working across proteins, viral vectors, and RNA manufacturing, I have noticed that RNA developers are less likely to speak openly about challenges and failures in the production process. RNA manufacturing has a reputation for being 'easy' (compared with the cell-based processes of traditional biologics), which may increase reluctance to discuss problems. RNA is also an emerging field and, as such, the formal and informal networks found in more mature fields have not yet fully formed. However, I believe that the RNA industry would benefit greatly from adopting the more open culture found in areas like CHO cell-based protein production. Naturally, companies cannot share proprietary or sensitive information; however, open discussion of common production challenges and collaboration to improve non-competitive technologies have been a real asset to traditional biologics, and it would be great to see this re-created for RNA.

The emerging field of nucleic acid production can also take lessons from where traditional biologics industries have fallen short. While data sharing has been happening for a long time in these fields, companies have not always collected enough or the right type of data to enable the large data sets necessary to progress our collective knowledge. Even in the most mature biologics field, CHO cells, we typically lack the large-scale transcriptomic, proteomic or metabolomic data that would help us to understand the process at a new and deeper level. If that data had been collected and shared historically, progress might have been faster. Large-scale data sharing—thousands of sequence variations—would be necessary to answer the key question of how RNA sequence affects manufacturability. By putting in place systems now for data collection and sharing at the community level, the whole field could benefit.

A BRIGHT FUTURE

From product design to process improvements, new components and product quality analytics, a lot is happening in the RNA field, and we are seeing significant progress. This phase is especially exciting for engineers because we are witnessing rapid improvements in yield and quality, which bring clear economic and therapeutic benefits.

Additionally, we are seeing new RNA formats and products emerging. For example, the technology behind innovations like self-amplifying RNA is now part of the molecular toolbox we can use in patients. Learning from traditional biologics manufacturing will help avoid bottlenecks and optimize processes for RNA products. To help RNA achieve its therapeutic potential, it is important to future-proof RNA manufacturing by ensuring flexibility and adaptability as the field evolves. Plus, by prioritizing data sharing and transparency, developers of RNA-based products can address challenges more effectively, paving the way for innovative solutions, and ensuring that manufacturing is not the limiting step for therapeutics with so much promise.

BIOGRAPHY

ADAM BROWN is an Associate Professor of Biopharmaceutical Engineering in the Department of Chemical and Biological Engineering at the University of Sheffield, Sheffield, UK. His research group is focused on developing technologies that improve the manufacture and performance of protein, DNA, mRNA, cell, and viral vector products. Engineered biological components from his lab are translated into the 'real-world' via commercialization activities and collaborations with biotechnology companies.

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VACCINE INSIGHTS

RNA VACCINES: FORMULATION AND PRODUCTION

SPOTLIGHT

INTERVIEW

Developing new RNA vaccine formulations to target non-enveloped viruses and boost mucosal responses

Jesse Erasmus, Director of Virology, HDT Bio, joins **Charlotte Barker,** Commissioning Editor, *Vaccine Insights*, to discuss developing an RNA vaccine for enterovirus D68, leveraging mucosal immunity, and strategies for improving tolerability in RNA vaccine formulations.

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Q What is the overarching theme of your work?

JE: My interest in exploiting viruses to benefit human and animal health. I started by studying alphaviruses and became fascinated with how we can engineer them, leveraging their functions to develop vaccines, therapeutics, platforms, and diagnostics.

Q What made you decide to move from academia to biotech? What insights have you taken from the move?

JE: In grad school, I focused on developing a vaccine platform using host-restricted alphaviruses that cannot replicate in vertebrates. The goal was to create vaccines and diagnostics, but a major hurdle was the complexity of producing these vaccines, establishing cell lines, and navigating the manufacturing and release processes.

That challenge led me to shift my focus to RNA vaccine technology. I moved to Seattle and worked at a nonprofit biotech, bridging the academic and biotech worlds. I continued writing grants and securing funding, and briefly rejoined academia as an acting Assistant Professor at the University of Washington.

I learned that establishing yourself in academia typically requires building a research program around a deep area of expertise. While collaboration becomes easier once established, initially, you need to be able to drive the program on your own. However, my work has always required a broader range of expertise than one person can achieve alone. For example, during my postdoc, I collaborated closely with a material scientist, Dr Amit Khandhar (now also at HDT Bio), to develop a proof-of-concept for our vaccine platform. To take it further, we needed expertise in immunology, clinical trials, regulatory matters, and manufacturing. Biotech provided an environment where we could bring together these diverse areas of expertise to achieve our goals—something that would have been much harder to accomplish in the academic world.

Q What are your main areas of focus as Director of Virology at HDT?

JE: On the discovery side, we focus on platform development—looking for better ways to manufacture RNA, improve its quality, and understand which aspects of the manufacturing process contribute to immune response and safety. Another key area is finding novel delivery methods, whether through formulation innovations or using devices to explore alternative delivery routes that could enhance safety and immunogenicity.

We are also working to better understand mechanism of action. Dr Taishi Kimura, a talented senior scientist on our team, brings core expertise in innate immunology. He helps us understand the host response to our vaccine platform, which in turn allows us to improve it.

Another major focus is pandemic preparedness. Viruses and the diseases they cause are my true passion, and we want to be ready for the next pandemic. During COVID-19, we were one of the first to publish a paper on an RNA vaccine, but as a smaller group, we could not move fast enough to compete with the bigger players. Now, we are working on prototyping vaccines for viruses from various viral families with pandemic potential, establishing proof-of-concept in animal models.

"We are definitely interested in continuing work in the influenza space, including vaccination of livestock in a one health approach, but vaccinating animals poses challenges."

Finally, we aim to establish clinical proof-of-concept to de-risk the technology, putting us in a better position to secure investment in other areas, like oncology and cancer immunotherapy. Before using private funds for these areas, we seek non-dilutive grant funding to gather clinical data in infectious diseases, which supports the platform.

Q Given your focus on pandemic preparedness, I imagine you are keeping a close eye on H5N1 avian influenza?

JE: Yes, we recently conducted a short study on vaccinating against bovine H5N1 with our collaborators at the US National Institute of Allergy and Infectious Disease who posted a preprint on the work. They had an isolate of the virus, and we wanted to see if the stockpiled vaccine, based on the 2004 Vietnam strain of H5N1, would be effective. We took the hemagglutinin (HA) from that strain and encoded it into our vaccine platform. We also made a version to match the currently circulating bovine H5N1 strain. After vaccinating and challenging mice, we found that the Vietnam 2004 HA did not provide cross-protection against the bovine H5N1, whereas the HA matching the bovine strain gave 100% protection.

This highlights a potential need to update the stockpiled vaccine for humans. We are definitely interested in continuing work in the influenza space, including vaccination of livestock in a one health approach, but vaccinating animals poses challenges. It is one of the reasons we might eventually face a pathogenic virus that could spread more easily between humans. Unfortunately, there is not much being done to mitigate this on the animal side.

Q Turning to your recent article describing a vaccine for EV-D68 (1)—what makes non-enveloped viruses a tougher target for RNA vaccines?

JE: If you look at the product pipelines for major mRNA vaccine companies, most of their infectious disease targets are enveloped viruses. The exception is Moderna, which is now developing a vaccine for norovirus—the first non-enveloped virus in their pipeline. One reason for this focus on enveloped viruses is that they tend to cause more significant diseases. However, non-enveloped viruses should not be overlooked, as they cause a wide range of

"To drive a robust immune response to EV-D68 we found that, in addition to expressing the viral capsid protein, we needed to co-express another viral protein, 3CD."

diseases. Noroviruses, polio, coxsackieviruses, and enteroviruses are all non-enveloped viruses that are important to target.

Another reason enveloped viruses are considered 'low-hanging fruit' is that, like RNA vaccines, they exploit the host cell machinery to express and secrete their antigens. These antigens are naturally trafficked to the cell surface or another compartment and can be easily released from the cell without the need for lysis or causing a lytic release of viral particles. It is straightforward to co-opt host cells, such as muscle cells, to express these secreted proteins, which can then interact with the immune system.

For non-enveloped viruses, the production process is different as these viruses do not use existing secretory pathways to release progeny viruses. With recombinant protein-based vaccines, viral antigens are expressed in cell lines, and the cells are lysed to harvest and purify the proteins that are produced inside the cells. However, with RNA vaccines, we do not have that luxury—we cannot manipulate host cells in the same way to purify antigens. Since non-enveloped viruses do not incorporate the host membrane or bud from cells, we had to find another way to release their antigens. Our approach was to exploit the virus's natural functions. In the case of enterovirus D68, we used viral proteins that the virus itself relies on for assembly and release, harnessing these mechanisms to facilitate the process.

Q What were your key findings from the EV-D68 study? Were there any surprises?

JE: Well first off, I'd like to give a shout out to Dr Nikki Warner, the scientist on my team who got all the animal models and assays up and running for this project, allowing her to interrogate the impact of RNA vaccine designs on protective immune responses against this virus. Enterovirus (EV)-D68 is a respiratory virus that has increased greatly in prevalence in the 21st century. It usually causes symptoms similar to the common cold but in rare cases can cause hospitalization, paralysis, and even death. Although the virus has been relatively quiet in recent years, it has the potential to reemerge and evade immunity rapidly, meaning a vaccine would likely need annual updates or a cocktail approach, similar to SARS-CoV-2. EV-D68 was selected by a panel of experts during an NIAID meeting as a virus with pandemic potential.

To drive a robust immune response to EV-D68 we found that, in addition to expressing the viral capsid protein, we needed to co-express another viral protein, 3CD. This protein acts as a protease but also plays several other roles, including processing and assembling the capsid and

potentially helping with its release from RNA-transfected cells *in vivo*. We were able to show that this combination of antigens works, producing robust neutralizing antibody responses when we delivered the RNA vaccine in a mouse model.

One of the main surprises was during a study where we compared two different formulations. We delivered the same RNA with either a lipid nanoparticle (LNP) or with LION, our proprietary cationic emulsion that carries the RNA on the nanoparticle surface, and observed significant differences in mucosal immunity in the upper respiratory tract of mice. Since EV-D68 is a respiratory virus, we challenged animals in the nose, examining protection in both the upper and lower airways—similar to studies on SARS-CoV-2 vaccines. As with SARS-CoV-2 vaccines, the LNP formulation induced strong protection of the lower airway, but poor protection in the upper airway, especially in the nasal cavity. When we delivered the RNA with LION, however, we saw strong protection in both the upper and lower airways. We had hints of this outcome in our SARS-CoV-2 work from previous studies with hamsters and non-human primates. There, we noticed better protection in the upper airway, though we had not directly compared it with LNP. In this study, the side-by-side comparison confirmed that LION induces better protection in the upper airway than LNP, which was surprising [1].

The field seems to be moving toward mucosal immunization, aiming to induce immune responses at the site of infection. However, there are plenty of examples of viruses that do not infect the same mucosal surface where the mucosal immune response is detected. This study confirms that intramuscular peripheral administration can drive mucosal immunity, and we plan to explore this further in future projects.

Q What are the next steps to build on the findings from that paper?

JE: We need to carefully consider the safety implications of encoding and expressing viral proteins, which may have unforeseen impacts on the host. For example, a paper was published recently showing that the SARS-CoV-2 spike protein, while primarily functioning as a surface antigen to mediate attachment and entry into host cells (and therefore an excellent vaccine target), also has other, lesser-known impacts [2]. The study found that the spike protein binds fibrin, potentially contributing to the clotting issues observed in some individuals during SARS-CoV-2 infection or after vaccination.

This highlights the need to fully understand the functions of all the viral proteins we are encoding in RNA vaccine platforms as we prototype for various viruses. We need to know exactly what these proteins do and whether they could lead to pathogenic side effects. If these proteins are found to have potentially harmful functions, we must develop ways to mitigate these risks. This is not just a concern for EV-D68, but for any virus we target in the future.

For EV-D68 specifically, if we want to advance that program and turn it into a product, there will need to be more interest from a funding perspective. Right now, the virus seems to have gone into stealth mode, and has not reemerged as predicted, so interest has waned.

However, we plan to continue submitting grants to the federal government so that when interest in EV-D68 revives, we can pick up where we left off and push the program into clinical development. Meanwhile, we will keep prototyping for other viruses, ensuring that we are prepared to advance them into the clinic when the time is right.

We are also planning more work on the impact of delivery vehicle on mucosal immune responses. We are also planning to evaluate the impact of alternative delivery routes on this phenotype to see if it can be improved—or possibly worsened—compared to intramuscular delivery. Additionally, we are examining this in the context of other viral diseases with higher urgency, aiming to understand these mechanisms and develop products and therapeutics accordingly.

Q More generally, what are the main roadblocks in the RNA vaccine field right now? What needs to happen to allow them to be overcome?

JE: The biggest roadblock to the widespread adoption of RNA vaccines is tolerability. For example, if you look at the seasonal flu vaccine market, there are well-established products with favorable safety profiles that induce decent immune responses. These vaccines are given to healthy individuals, for whom the risk of getting the flu is relatively low. In this context, a vaccine with a better tolerability profile will always be preferred over one that may cause reactogenicity.

Moderna's data on their trivalent or quadrivalent flu vaccines show non-inferiority in immune response compared to licensed vaccines, but the tolerability is significantly worse. This is one reason why many RNA companies are focusing on areas like cancer immunotherapy, where poor tolerability is more acceptable. If we want to break into the seasonal flu market or target healthy individuals, we have to improve tolerability, and that is a major focus for us at HDT.

Beyond tolerability, there are two other key areas: durability and mucosal immunity. As I mentioned, we are already working on mucosal immunity, but the durability of the immune response remains a big question. There is debate about whether the durability seen in COVID-19 vaccines is due to the antigen or the platform itself. Some data suggest that self-amplifying replicon RNA (repRNA), which is what we use at HDT, may drive a more durable immune response than conventional mRNA. This is supported by studies showing that humans vaccinated with repRNA have more lasting antibody responses, though studying durability is time-consuming and costly.

Finally, a major roadblock is the challenge of accurately modeling human immune responses. We do a lot of research in animal models, but the data does not always translate well to humans, even when using non-human primates. We need better models that can predict both innate and adaptive immune responses in humans.

"At HDT, we are exploring alternatives to lipid nanoparticles, and we expect that other approaches, though still in their infancy, will gain momentum in the near future."

Q How do repRNA-based vaccines compare to mRNA vaccines in terms of tolerability?

JE: Many companies in the space argue that repRNA can offer better tolerability due to its dose-sparing effect, as it requires less RNA for a similar immune response. However, repRNA is more complex to manufacture and inherently has more double-stranded RNA, which can lead to increased innate immune reactivity. Recent efforts include working with base-modified repRNA. For instance, we published that pseudouridine, used in conventional mRNA vaccines, negatively impacts repRNA by inhibiting antigen production, likely through interference of self-amplification. Therefore, the same base modification used to make conventional mRNA more tolerable cannot be used to increase tolerability to repRNA. As such, the dose-sparing effect of repRNA comes at the expense of tolerability, effectively lowering the tolerable dose ceiling, keeping us within a similar therapeutic window. New modifications, such as 5-methylcytidine, show promise, but it remains uncertain whether they will significantly improve tolerability.

At HDT, we are focusing on improving tolerability by targeting the specific biodistribution of the RNA and reducing delivery to innate immune cells, which initiate the initial immune response that drives reactogenicity. We are also working on reducing double-stranded RNA and refining manufacturing processes. These combined efforts are essential for enhancing the overall tolerability of repRNA vaccines.

Q How will the field look in 5–10 years?

JE: There will likely be significant advancements in manufacturing. We should see a better understanding of the critical quality attributes of these materials and advances towards decentralized, automated, end-to-end continuous manufacturing. This is especially important for pandemic preparedness, where decentralized manufacturing will be crucial.

Novel formulations will probably be needed, and right now there is a huge focus on LNPs, with many companies developing their own novel compositions. At HDT, we are exploring alternatives to LNPs, and we expect that other approaches, though still in their infancy, will gain momentum in the near future. Additionally, we anticipate major breakthroughs in cancer immunotherapy within the next decade.

Q What would be top of your wish list for new innovations, technologies, or tools in the field?

JE: There is really just one thing that stands out to me right now, and that is achieving truly unbiased direct RNA sequencing. While there has been a lot of progress in this area, the technology still has inherent biases. If we could sequence all the RNA molecules in a drug product without bias—understanding their sequence, length, and all the other parameters—then we would have a one-stop solution for assessing RNA material quality. It is still a way off, but it would be incredible if we could access such a capability.

REFERENCES

1. Warner NL, Archer J, Park S, *et al.* A self-amplifying RNA vaccine prevents enterovirus D68 infection and disease in preclinical models. *Sci. Trans. Med.* 2024; 16(759), eadi1625.
2. Ryu JK, Yan Z, Montano M, *et al.* Fibrin drives thromboinflammation and neuropathology in COVID-19. *Nature* 2024; 633, 905–913.

BIOGRAPHY

JESSE ERASMUS is the Director of Virology at HDT Bio, a biotech company in Seattle, WA, USA. Jesse received his PhD from the University of Texas Medical Branch (UTMB), Galveston, TX, USA and completed his postdoctoral training at the Infectious Disease Research Institute and the University of Washington, Seattle, WA, USA. His research is focused on the development of platform technologies, beginning with the first application of insect specific viruses as platforms for vaccines and diagnostics at UTMB and more recently with the development of non-viral delivery of replicating RNA as a gene-expression platform for vaccines and therapeutics.

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VACCINE INSIGHTS

RNA VACCINES: FORMULATION AND PRODUCTION

SPOTLIGHT

Navigating the mRNA-LNP patent labyrinth: advice for developers

Dan Shores
Rothwell Figg

"It is crucial that companies confer with their patent counsel early, and deeply consider their formulations in the context of the patent landscape..."

VIEWPOINT

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On October 3, 2024, **Charlotte Barker**, Commissioning Editor, *Vaccine Insights*, spoke to **Dan Shores**, Partner, Rothwell Figg, about intellectual property challenges in the mRNA-lipid nanoparticle space and potential patent strategies for emerging biotech companies in the field. This article has been written based on the interview.

Despite the success of mRNA-LNP vaccines during the COVID-19 pandemic, those who operate in the field face significant intellectual property challenges, including challenges relating to navigating the highly complex patent landscape directed to mRNA, lipids, and lipid nanoparticles. Here, I provide an introductory framework for biotech and academic developers operating in the space to consider.

I have been practicing patent law in the USA for 20 years and help biotech companies position themselves in emerging spaces. One of my favorite growth spaces, especially over the past several years, is the mRNA-lipid nanoparticle (LNP) space.

While the COVID-19 pandemic caused widespread tragedy for millions of families worldwide, it also ushered in the first-ever regulatory approvals of therapies based on mRNA technology.

COVID being but one of numerous potential other indications that mRNA could address, the promise of this technology is wide ranging, and that makes it very exciting to work with companies that are constantly innovating in this area to utilize this technology to treat human disease.

A major (and predictable) development in the space is the patent litigation activity that occurred following significant sales of the COVID-19 vaccines by Pfizer/BioNTech and Moderna. Starting in 2022, several market players filed patent infringement lawsuits against Pfizer/BioNTech and Moderna, seeking to recoup damages based on the alleged use of patent holder technology in the commercial vaccine products. I provide an overview of the patent litigation landscape in Figure 1.

It is critical for any company developing technology in the mRNA-LNP space to be aware of the intricate intellectual property (IP) and patent litigation landscape and equipped to navigate it properly.

FREEDOM TO OPERATE

It is smart to critically consider patent issues as early as possible in the development of therapeutic candidates. It should be a line item in the very first development project agenda. That is because many mRNA payloads or lipids may be covered by existing third-party patents or patent applications, and this can pose serious freedom-to-operate issues. No developer or investor wants to invest heavily in regulatory approval or clinical trials for a candidate only to later learn that they are using a product that is covered by, for example, a direct competitor that will refuse to license.

Therefore, well before the IND stage, it is important to confer with your patent counsel and compare the therapeutic that is being developed against the patent landscape to identify potential IP issues. Items that are relevant to this analysis include patent expiration, location of manufacture and sales, conduct in relevant jurisdictions, and applicable safe harbors,

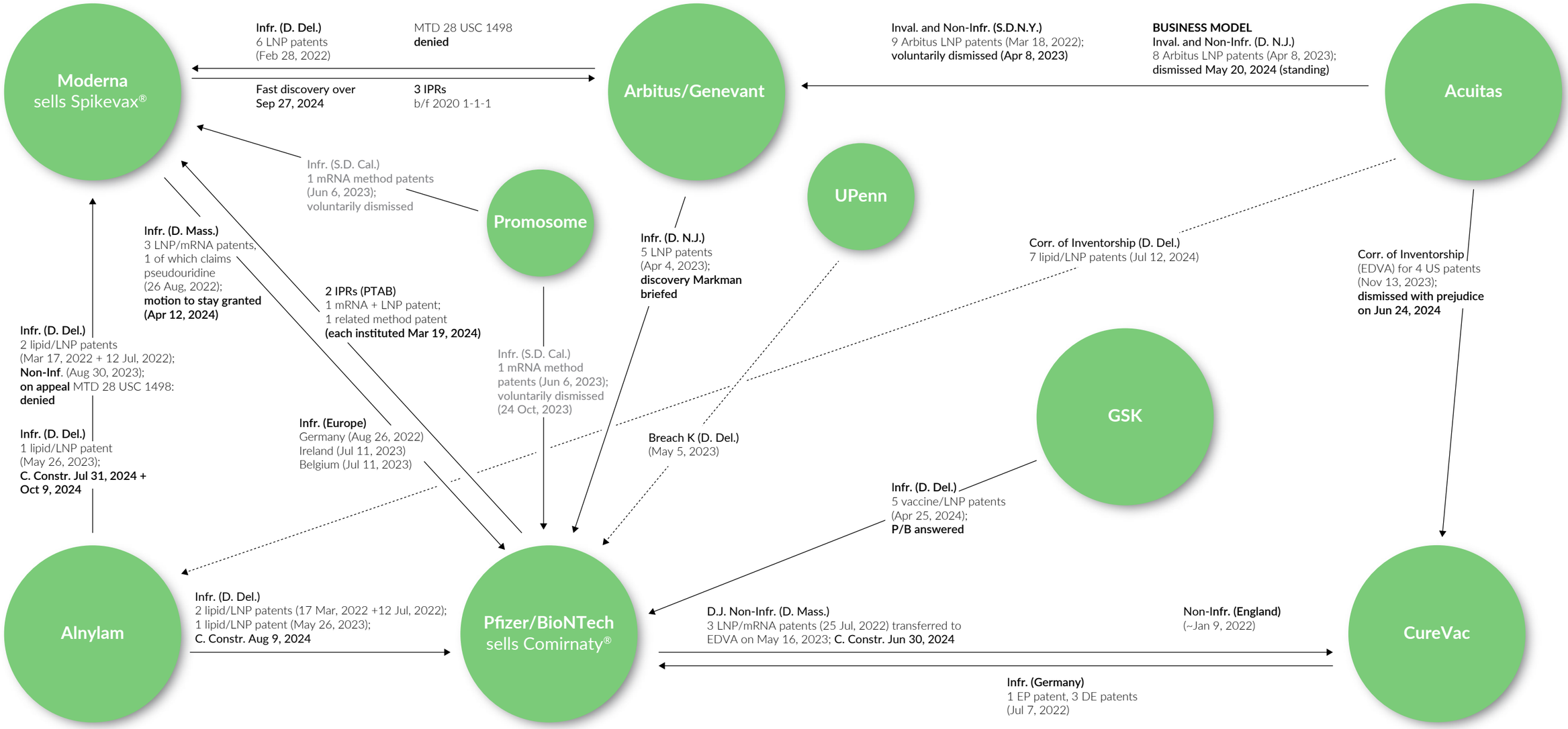
INNOVATION AND PROTECTION

Let's say you have a promising product in development but on examining the IP landscape, you find out that your cationic lipid infringes a patent—what are your options? One option is to approach the patent owner for a license, which may not be possible. Another option would be to choose an alternative lipid that is either off-patent or being offered on better licensing terms.

Another option is to innovate and develop a new lipid. This has a potential three-fold benefit. First, it builds your company's IP, which adds value to your business in numerous ways, including potential in-licensing revenues. Second, it may help avoid infringement issues and provide freedom to operate with regard to the cationic lipid being utilized. Third, if the cationic lipid can be successfully utilized in your formulation, you would avoid having to license a cationic lipid from a third party and save on that expense.

FIGURE 1

Patent litigation landscape for mRNA and LNP technology.



This landscape is based on publicly available information, is not exhaustive, and is in a constant state of flux.

SOME FINAL THOUGHTS ON THE EVOLVING IP LANDSCAPE

The current IP landscape in the mRNA-LNP space is quite complex. As the field continues to evolve with the advancement of new technologies, such as self-amplifying and circular RNA products, the challenges in navigating this landscape are likely to intensify.

Some patents directed to delivery systems (e.g., LNPs) and related formulations are broad but aging, yet lipid and delivery system IP (versus payloads) will likely remain the baseline IP issue going forward. That is because payloads are often unique on an indication-by-indication basis, and the LNPs can have (generally speaking) universal application. High-performing lipids and LNPS—especially those that perform well in the clinic and have low toxicity—will be in demand and this will drive increased patenting around these advanced lipid systems.

It is crucial that companies confer with their patent counsel early, and deeply consider their formulations in the context of the patent landscape, in order to fully understand the issues and develop an effective strategy before investing in a development path that may have significant patent-related risks.

BIOGRAPHY

DAN SHORES helps innovative companies build patent portfolios, negotiate strategic collaborations, conduct due diligence, litigate complex patent disputes, analyze patent landscapes, and develop and implement effective IP strategies. His clients operate in a wide range of spaces including biotech, tech, medical device, and other sectors. Examples of these technologies include mRNA; lipid nanoparticles; CAR-T therapies; CRISPR; oligonucleotides; genetically engineered swine organs for xenotransplantation; artificial intelligence for drug discovery; probes for tissue investigation; small molecule pharma; and numerous other technologies. He is a registered patent attorney licensed to practice before the United States Patent and Trademark Office and is admitted to practice law in Massachusetts and in the District of Columbia. He is a member of the Bars of the Supreme Court of the United States, United States Court of Appeals for the Federal Circuit, and United States District Court for the District of Massachusetts.

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