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VACCINE INSIGHTS

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RNA vaccines Part 1: exploring future potential

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RNA vaccines Part 1: exploring future potential

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VACCINEINSIGHTS

SPOTLIGHT

What's next for mRNA vaccines?

Jeffrey Ulmer
President, TechImmune LLC and
Chief Scientific Advisor, Immorna Biotherapeutics

"...the synthetic nature of the mRNA technology will streamline R&D timelines and reduce costs."

VIEWPOINT

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Vaccines have had a substantial impact on human health for centuries. Yet, several major challenges have persisted, including improving existing but suboptimal vaccines, addressing unmet needs, responding rapidly and effectively to emerging infectious disease outbreaks, and simplifying and accelerating vaccine research and development (R&D) processes in order to get life-saving products faster to people who need them. This has provided the impetus for developing new technologies for delivering vaccines, such as mRNA.

The remarkable successes achieved with severe acute respiratory syndrome coronavirus

2 (SARS-CoV-2) mRNA vaccines, as seen by their efficacy and the speed with which they were brought to bear on the pandemic, has demonstrated the potential for addressing all of the challenges listed above. The key attributes of mRNA that may enable this are 1) mRNA encoding the antigen is delivered into cells of the vaccinated individual, leading to expression of the antigen *in situ* similar to that which happens during live virus infection and resulting in the induction of potent and broad-based immunity, and 2) mRNA vaccines utilize synthetic production methods not involving cell culture (unlike all other types of vaccines), thereby markedly simplifying and accelerating the means by which vaccines can be manufactured and characterized.

CHALLENGES & SOLUTIONS

Despite these advantages, there are two main areas of challenge for broader application of mRNA vaccines – technical and logistical. First, the effectiveness of mRNA products for diverse disease indications will depend on several technical factors relating to gene expression and immune stimulation. For gene expression, the optimum kinetics, duration, and location of therapeutic protein production are likely to be quite different for a preventive vaccine than for a gene therapy application. The same is the case for the ideal magnitude and type of immune stimulation needed, where a cancer vaccine will require a much stronger innate immune stimulus than a protein replacement therapy. mRNA vaccines do not yet represent a 'plug and play' platform technology that can be directly applied to any new product. Rather, almost certainly each new product will require optimization, including the nature of the mRNA molecule, type of delivery system, design of the antigen insert, and methods for production and characterization.

These technical challenges will be addressed through a deeper understanding of the mechanisms of action of the different types of mRNA vaccines (conventional, self-amplifying, circular) so that rational approaches can be taken to increase their utility and best apply them to the appropriate disease targets.

However, overcoming technical hurdles is not enough. To achieve the maximum population benefit of effective vaccines they must be deployed quickly, broadly, and affordably. This will require large-scale on-demand production capabilities and infrastructure, both in the developed and developing world. Furthermore, to facilitate widespread distribution of mRNA vaccines to all regions of the world, increased stability will be critical – particularly at high ambient temperatures.

These logistical roadblocks will be addressed via increasing the number of vaccine doses available by production scale-up and/or reducing the dose of mRNA required for effectiveness, the discovery and application of new formulations and delivery systems to increase potency and thermostability, and investment in infrastructure and technology transfer of knowhow to establish research, development and manufacturing capabilities in the developing world, which can then be applied to diseases of local importance.

Besides broadening the use of the mRNA technology to other vaccine targets, exciting advancements are being applied to non-infectious diseases, such as:

1. Gene editing using mRNA to deliver CRISPR/Cas tools *in situ* to correct genetic abnormalities in live animals, as well as *ex vivo* using cell-based therapies;
2. Personalized cancer immunotherapies have been substantially facilitated due to the increased speed with which tailor-made mRNA products can be produced and administered to patients in a timely manner, and;
3. The early but encouraging development of circular RNA as a therapeutic modality, which owing to its inherent stability may lead to more durable gene expression and be particularly valuable as gene therapy.

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THE NEXT 10 YEARS

The success of SARS-CoV-2 mRNA vaccines has stimulated substantial interest and investment in the technology. In fact, more than 70 mRNA vaccine programs against SARSCoV-2 alone are in various stages of preclinical and clinical development, according to the World Health Organization [1]. The large resource and intellectual capital currently being applied, the large design space available, the strong incentives provided by a return on these investments, and the major human health benefits of broader implementation of the mRNA technology will be strong driving forces for success. As a result, within 5–10 years major advancements up to and including licensure of new mRNA-based products will be achieved for other infectious disease targets (both preventive and therapeutic), cancer immunotherapy (including off-the-shelf and personalized approaches), gene therapy, and gene editing. In addition to addressing these unmet medical needs, the synthetic nature of the mRNA technology will streamline R&D timelines and reduce costs.

It is unlikely that a 'one size fits all' solution using mRNA will be attainable. There is no question that mRNA has become an important part of the vaccine technology toolbox, but it will not in the foreseeable future obviate the need for established approaches, such as live attenuated and inactivated viruses, viral vectors, and protein subunits. Rather, innovation in the areas described earlier will be required to effectively address the many unmet medical needs awaiting enabling vaccine technologies. However, the recent announcement by Moderna on the protective efficacy of a mRNA vaccine to prevent Respiratory syncytial virus disease in older adults in phase 3 clinical trials [2] – to a degree similar to that previously reported by GSK and Pfizer with their protein-based RSV vaccines – is an encouraging sign that mRNA vaccine technology even in its present form will have some successes beyond SARS-CoV-2.

BIOGRAPHY

JEFFREY B ULMER, PhD spent more than 30 years in vaccines R&D at Merck Research Laboratories, Chiron Corporation, Novartis and GlaxoSmithKline. His most recent leadership positions included Global Head, External R&D; Head, Preclinical R&D; and Program Head, Technical R&D. His scientific focus has been vaccine technology platforms, including DNA and mRNA vaccines, viral vectors, and adjuvants. He received his PhD in biochemistry from McGill University and completed his postdoctoral training in the laboratory of Nobel laureate Dr George Palade in the Department of Cell Biology at Yale University School of Medicine. He has published over 210 scientific articles, is an inventor on 11 patents, and is a Fellow of the International Society for Vaccines where he serves as Treasurer. He is currently President, TechImmune LLC (Newport Beach, CA) and Chief Scientific Advisor, Immorna Biotherapeutics (Morrisville, NC).

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SPOTLIGHT

INTERVIEW

Developing mRNA influenza vaccines in the wake of COVID-19

Charlotte Barker, Editor, *Vaccine Insights*, speaks to **Raffael Nachbagauer,** Program Leader and Executive Director of Infectious Disease Development at Moderna, about his work to improve seasonal influenza vaccinations with the help of mRNA technology

RAFFAEL NACHBAGAUER oversees the influenza vaccine development portfolio at Moderna. Prior to joining Moderna, he was an Assistant Professor at the Icahn School of Medicine at Mount Sinai where his research focused on the immune responses to virus infections and vaccination, as well as the development of novel influenza vaccines.

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Q When did you first start working with mRNA vaccines?

RN: I was an Assistant Professor at Mount Sinai when I met Norbert Pardi from the University of Pennsylvania at a vaccine meeting in Europe in 2018, and we discussed collaborating. We submitted a grant, obtained funding, and were quickly

able to generate preclinical data in support of novel influenza vaccines. I saw mRNA as an interesting technology that enables a trial-and-error approach to vaccine design that takes much less time than using conventional platforms.

In late 2019, I was introduced to Andrea Carfi, the CSO for infectious disease research at Moderna, through a shared connection. We started a conversation about potentially working on seasonal influenza vaccination. I eventually joined Moderna in 2020 in the middle of the severe acute respiratory syndrome (SARS)-CoV-2 pandemic.

Q What motivated you to move out of academia and into an industry role?

RN: At Mount Sinai, I worked closely with GSK on a novel influenza vaccine. It was an interesting collaboration and exciting work, but for an academic career you need publications and funding, and neither of those is plentiful in vaccine clinical trials. I had the realization that if I stayed in academia, there would not be much opportunity to do this type of work, so decided to make the jump to industry.

Q What was it like joining Moderna in the midst of the pandemic?

RN: I joined the company just after the Phase 1/2 results for its COVID-19 vaccine (now known as Spikevax) were released, and the Phase 3 efficacy trial was getting starting. I joined to work on the influenza program and, with both the company and the whole world so focused on COVID, it was interesting to work on something that was under the radar.

We were a small company back then, and yet we were able to take the influenza program from having no preclinical data into a Phase 1 study within a year. To me, that was an amazing experience.

Q What does your role at Modena encompass?

RN: My role is to lead the influenza program. I work closely with the different functional leads including preclinical, clinical, commercial, manufacturing, and regulatory. The role has naturally evolved over time – I still check in with the preclinical lead regularly, but my interactions with my commercial and later-stage clinical colleagues have become more frequent as the program progressed.

My ultimate responsibility is to ensure that our program remains on course with our strategy, and we are aligned on what we are trying to achieve. What began as one seasonal influenza vaccine program has now become several different programs in an ongoing portfolio. It is part of our strategy at Modena to pursue different paths in parallel and see if we can combine them down the line.

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Q What are the advantages of mRNA compared with existing platforms and how are you harnessing these benefits in Moderna's seasonal influenza program?

"An obvious advantage of mRNA is that, unlike most current flu vaccines, it does not rely on eggs. Egg-based production is at risk from shortages; for example, the current avian flu outbreaks are causing supply issues."

RN: An obvious advantage of mRNA is that, unlike most current flu vaccines, it does not rely on eggs. Egg-based production is at risk from shortages; for example, the current avian flu outbreaks are causing supply issues.

Our mRNA-1010 seasonal influenza vaccine is the simplest version of an mRNA vaccine for influenza that you could make. It is a quadrivalent vaccine with the four strains that are recommended by the World Health Organization every year: two influenza A strains (H1N1 and H3N2) and two influenza B lineages (Victoria and Yamagata). This is the first program we have pushed forward because it was the most straightforward and could move quickly. This is the foundational piece of our influenza strategy, but it is only the starting point.

We are currently in two Phase 3 studies with mRNA-1010. We started a Phase 3 immunogenicity and safety study in the spring of 2022 and start efficacy trials in September. There is an established pathway for licensure of seasonal influenza vaccines based on immunogenicity, but it still requires for efficacy to be demonstrated after licensure.

One of the things that mRNA can do well is to make otherwise difficult-to-manufacture antigens. The current main target for influenza vaccines is hemagglutinin (HA), with a secondary target of neuraminidase (NA). By targeting two antigens, the virus cannot evade the immune response as easily. Current vaccines do contain some amount of NA, but they currently are not quantitively assessed as part of the vaccine release, so the sole focus is on the HA. The current manufacturing processes are optimized toward increasing HA. The NA is not as stable and tends to fall apart in most manufacturing processes.

By contrast, a specific mRNA can be delivered and presented to the immune system as a membrane-bound version of that protein in the same way as it would during a viral infection. We are evaluating this in the context of our mRNA-1020 and mRNA-1030 vaccines, which each contain eight mRNAs, targeting both HA and NA at different doses and ratios. We went into the clinic earlier this year with these octavalent vaccines and are currently awaiting the results of the study.

mRNA also has huge potential to provide faster, more responsive vaccines. Currently, there are announcements twice a year from the WHO on the new influenza strain vaccine targets. In February, a decision is made on what the vaccines in the northern hemisphere for September should look like. In other words, at the tail end of last year's season, we must decide what the

vaccine for the next season will look like. There is also an entire southern hemisphere flu season between, giving the virus many opportunities to change or for another strain to take over.

In the summer of 2022, when the FDA made the decision that the COVID vaccine updates for the fall should contain the BA.4 and BA.5 omicron variants, we were able to manufacture those vaccines and roll them out within 2 months. Ideally, we would do something similar in the future for influenza, which would allow the WHO and other recommending bodies more time to decide on the right strains to include.

H3N2 has the largest burden of influenza hospitalization and mortality, especially in older adults. There are a multitude of different clades within the H3N2 subtype. Right now, the WHO must pick just one of the clades that are circulating, which they think will make up the majority of circulating viruses in each given year. That is an incredibly difficult task. If there are a lot of clades circulating at similar levels in a given year, then it is an impossible task.

We hope that mRNA could allow us to include one or two additional clades. That would mean having a pentavalent or hexavalent vaccine instead of a quadrivalent one. This is what we are trying to explore in the mRNA-1011 and -1012 programs we are planning to start clinical studies for soon. If both of those work, we would be interested in exploring whether we can combine them, hopefully with broader coverage for HAs and good neutralizing responses against all circulating clades. It would also have NA as a second antigen to provide additional protection.

Q Are you hopeful of better immune responses with the mRNA vaccine compared with existing flu vaccines?

RN: For SARS-CoV-2, we know that mRNA vaccines have been very effective at eliciting protective immune responses in older adult populations. We are hoping that we will see something similar for influenza.

With our mRNA-1010 candidate, for the influenza A strains, we see about two-fold higher immune responses compared to a standard-dose influenza vaccine across all ages. Our hope is that we will also see better T-cell responses in those populations. We have also seen a better breadth of immune response with the mRNA vaccines, which makes us hopeful that, even in the context of our quadrivalent vaccine, we will see broader protection against the strains that are circulating.

We have recently released interim data from our Phase 3 immunogenicity and safety study, which confirmed strong immune responses for the influenza A strains, but the influenza B responses were lower than anticipated. In response, we have already updated our vaccine in a way that we believe could improve immune responses against the influenza B strains and we are aiming to confirm those improvements in an upcoming clinical study. Being able to quickly react to such results is another benefit of the mRNA platform being so flexible.

Q You have written previously on the prospects for a universal flu vaccine – has the pandemic helped or hindered efforts in that direction?

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"Our platform does have the flexibility to combine, and we have even started a flu/COVID combination program. We wish to identify the ideal combination to move forward and investigate what the immunogenicity and reactogenicity profile would look like in such a vaccine."

RN: The pandemic helped universal vaccine development in that many previously considered platforms were tested and evaluated in the context of COVID. mRNA was very clearly established as a useful platform for respiratory vaccines and hopefully other modalities in the future. It has been beneficial to have that proof of concept.

However, the pandemic also hindered efforts in that most influenza research in the last few years shifted gears toward COVID. There has not been a lot of progress in terms of further developing universal vaccine modalities.

In general, there are several challenges preventing universal vaccine targets from working in every single indication. Walking that line between what we know to be effective neutralizing responses versus relying on non-neutralizing responses can be difficult. Antibody-dependent cellular immune responses make up an important part of our general protection but may not protect older adults as effectively, which is the population where the largest unmet need remains. That is represented in our strategy of focusing on achieving the best possible neutralizing responses in older adults with higher valency HA and NA vaccines first. Down the line, mRNA vaccines have the flexibility and modality for additional targets. Our flu vaccine strategy is iterative, and we can continue to improve upon it in the future.

Q Could combining influenza and COVID vaccinations in one shot be viable?

RN: Our platform does have the flexibility to combine, and we have even started a flu/COVID combination program. We wish to identify the ideal combination to move forward and investigate what the immunogenicity and reactogenicity profile would look like in such a vaccine. We are also working on pediatric combination vaccines for respiratory viruses. Combination vaccines are already well recognized in the pediatric field as a means to improve compliance.

Q What are the most important challenges facing mRNA vaccine developers and how can these be addressed?

RN: One key challenge is the stability of mRNA vaccines and the requirement for long-term storage to be at freezing temperatures. However, the benefit of having a

platform technology is that there are continuous improvements so in the future I'm hopeful that we can make these vaccines more stable. There are some challenges, but I have an optimistic view of this platform technology that allows us to continuously evolve.

Q What's next for Moderna's infectious disease program?

RN: I will always be excited about influenza research and there are many things being planned in the context of influenza. Beyond flu, Moderna is also working on cytomegalovirus (CMV), in addition to herpes simplex virus (HSV), varicella-zoster virus (VZV), and Epstein-Barr virus (EBV). I am hopeful that mRNA vaccines could make a huge difference in all of these indications and allow us to improve public health.

AFFILIATION

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SPOTLIGHT

INTERVIEW

Leveraging mRNA technology to accelerate HIV vaccine development

Charlotte Barker, Editor, BioInsights, speaks to (pictured) **Mark Feinberg,** President & CEO, IAVI (formerly known as the International AIDS Vaccine Initiative) to explore how the nonprofit is bringing the latest advances in vaccine science to the fight against HIV and other diseases disproportionately affecting people living in low-income countries.

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Q How did you get involved in research on HIV?

MF: I was in medical and graduate school at Stanford University in the San Francisco Bay area when the first US cases of acquired immunodeficiency syndrome (AIDS) were reported nearby. At that time, little was known about human immunodeficiency virus (HIV)/AIDS, but it soon became clear that it was an unprecedented public health, cultural, and political challenge.

The initial stages of my thesis research examined how a then-newly discovered retrovirus, human T cell leukemia virus type 1 (HTLV1), causes leukemia. Many exciting new molecular biology techniques were pioneered at Stanford, and, when AIDS was also found to be caused by a retrovirus, I had an opportunity to apply these new tools I'd used to study

HTLV1 to study HIV in Bob Gallo's lab. It was a particularly exciting time to conduct this kind of research and generate new information at a time when rapid progress was being made in our understanding of a newly discovered virus that caused such an awful disease.

My postgraduate medical training at the Brigham and Women's Hospital and a postdoctoral fellowship with David Baltimore further strengthened my resolve to focus on HIV research and clinical care. I went on to join the faculty at UCSF and to also serve as an Attending Physician in San Francisco General Hospital's very active outpatient and inpatient services caring for people living with HIV and where new, compassionate standards of care for AIDS patients were developed. My time in San Francisco coincided with the peak of the AIDS crisis there and preceded the availability of effective antiretroviral therapies. Later, I served as a Medical Officer in the Office of AIDS Research at the US National Institutes of Health (NIH) and then joined the faculty at Emory University School of Medicine. While having a strong focus on scientific and clinical research, I also maintained active clinical responsibilities throughout this period and served as an Attending Physician at the National Institutes of Health Clinical Center and Grady Memorial Hospital in Atlanta. These experiences in conducting basic and clinical HIV research and providing clinical care for AIDS patients made clear to me that many aspects of HIV, from its complex biology to its public health severity and impact, lie at the interface between social factors, disease, equity, and discrimination. The time I spent caring for people with HIV infection and observing the challenges they faced were especially powerful motivators for me to work to develop solutions to prevent and treat HIV.

Q You've worked in pharma, academia, government, and now a nonprofit. What's your view on the role of those different entities in developing vaccines and how they should work together?

MF: I feel fortunate to have worked in those different settings. The different sectors have different perspectives, but a collective goal. At the same time, they also have different capabilities, limitations, and enablers, which can result in challenges and misalignments. However, having worked in a variety of sectors, I believe it is possible to create models that allow sectors to work together in synergistic and productive ways to fill gaps and minimize the negative impact of engaging in certain challenging endeavors.

My view is that success in the future of global health, especially in the space of pandemic preparedness, is going to depend on a more strategic and proactive alignment between the different sectors. In fact, that is an important part of why I left Merck to join IAVI – I wanted to focus on how to get those different sectors to work together as effectively as possible, and that was not realistic while working for a single, for-profit company. My experience has taught me that tremendous opportunities exist for better strategic alignment and collaboration across organizations, sectors, and geographies. By harnessing these opportunities, we can accomplish together what no one organization could accomplish on its own. This has been a major focus of my career and has become a major focus of IAVI. With our partners, we bring forward solutions against diseases that impact people living in low-income countries. I believe that is the pathway to future progress.

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Q What are IAVI's goals?

MF: IAVI was founded in 1996 with a specific focus on HIV vaccine development, and that remains core to our mission, but we have expanded our outlook over time. HIV vaccine development is highly complex, and while exciting progress is being made, it will be a long journey to an efficacious vaccine. However, the level of scientific creativity and innovation that has emerged in the study of HIV has led to tremendous advances in disciplines such as molecular virology, structural biology of viruses, and human immunology. IAVI developed a lot of world-class technical capabilities and partnerships during its work in HIV, and we came to appreciate that we could magnify our public health impact by broadening our portfolio and focus. We now work on vaccines and therapies for HIV, tuberculosis, Lassa fever, Sudan ebolavirus, Marburg virus, and other emerging infectious diseases.

> "...we hope RNA technology will allow us to take a promising concept – germline targeting – from the laboratory into the clinic more quickly."

Our current focus as an organization is a commitment to ensuring that the best scientific innovations can be applied to solve the challenges faced by people living in low-income countries who would otherwise be the last to benefit from them. This involves addressing the needs of low-income countries, engaging with communities at risk, and strengthening research capacity in those countries, especially when the diseases targeted are not commercially attractive to for-profit companies. We are committed to translating scientific innovation to public health impact and ensuring equitable, affordable access to the products we develop.

Q Where do you see the value of mRNA vaccines in IAVI's mission?

MF: One of the first things I did when I joined IAVI in 2015 was meet with scientists at Moderna. It was clear to me from my time at Merck that, while the mRNA platform had not yet been formally validated in human studies, it was very promising. We were working with Moderna well in advance of COVID, but COVID generated a tremendous amount of information and experience about the mRNA platform and its attributes in terms of flexibility and speed.

From our perspective, mRNA is not going to solve the challenges of the HIV vaccine field because those are related to the inherent properties of the virus, which make it difficult for the immune system to prevent, control, or eliminate the infection. Instead, we hope RNA technology will allow us to take a promising concept – germline targeting – from the laboratory into the clinic more quickly.

IAVI and its partners have made significant progress in eliciting broadly neutralizing antibodies (bnAbs) against HIV by vaccination, which have been shown to protect people from HIV if the titer is high enough and the antibodies and virus are well matched. Germline

targeting aims to guide the immune system to create bnAbs by activating specific B cell precursors with a series of immunogens defined by careful study of the subset of people living with HIV who develop these bnAbs. We use the tools of structural and computational biology to understand the viral targets of the antibodies and then recapitulate the evolution of bnAbs through a rationally designed series of vaccinations.

The process will require multiple sequential immunizations with different immunogens to 'nudge' B cells into producing broadly neutralizing antibodies, and testing multiple epitopes is time-consuming. For instance, the IAVI G001 study recently published in Science [1] demonstrates that it is possible to initiate the process of activating the relevant B cell precursors very successfully. However, using recombinant protein production, the process of going from idea to the clinic took about 3 years. To do the same thing using the RNA platform would take about 3 months. Keep in mind that we will likely need to elicit three different classes of bnAbs targeting distinct epitopes on the HIV Env glycoprotein, and that each class of bnAbs will require multiple immunogens administered in a defined sequence. This means we will need to conduct many early clinical studies and further optimize the immunogens based on the results of those studies. mRNA is a tool that will help us to move as expeditiously as possible through the requisite studies and iterations.

Whether or not the final HIV vaccine is going to be based on the RNA platform, I feel confident that the RNA platform is going to accelerate progress.

Q Tell us more about the ongoing collaboration between IAVI and Moderna

MF: We are working with Moderna and a wide range of amazing collaborative partners on several different activities, including studies using RNA-encoded immunogens to replicate and extend the results of IAVI G001. These include clinical studies (IAVI G002 and G003) in the USA and Africa to replicate the results of IAVI G001 using RNA and explore whether we can take the immune system further down the pathway toward making a broadly neutralizing antibody. If those studies validate the RNA platform, it will have a positive impact on the HIV field.

Support for discovery research for the HIV immunogens and clinical work comes from several partners with whom we work very closely. The Bill & Melinda Gates Foundation provides funding for research activities through the Collaboration for AIDS Vaccine Discovery, and NIH provides support through the Scripps Research Center for HIV/AIDS Vaccine Development. These funders provide guidance and feedback throughout all stages of the program. The NIH also provides support to the HIV Vaccine Trials Network, an important partner with expertise in clinical trial conduct.

The US Agency for International Development (USAID) is a key supporter of our HIV vaccine work and has provided funding for the IAVI G003 trial in Africa.

In short, this is truly a broad-based collaboration that is having synergistic beneficial effects across the HIV vaccine research field and advancing vaccine research in general.

Q What is next for IAVI?

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MF: For HIV, we are anticipating the results of the IAVI G002 and G003 studies and advancing other work in the germline targeting space with the goal of eliciting broadly neutralizing antibodies. There is exciting progress being made in preclinical studies that we hope to take into the clinic as soon as possible. We also have an active program advancing HIV broadly neutralizing antibodies as prophylactic agents in their own right, and we hope those will advance into initial clinical testing this year.

"We want to make sure that the best science can benefit everybody, regardless of their economic or geographic situation."

We have an active tuberculosis vaccine program that includes a major focus on advancing the clinical development of a live-attenuated *Mycobacterium tuberculosis* variant known as MTBVAC. We plan to initiate a proof-of-concept efficacy trial in adults and adolescents in the near future.

In the emerging infectious disease space, our Lassa fever vaccine is just coming to the end of a Phase 1 study, and we are preparing for Phase 2 evaluation, including a proof-of-concept Phase 2b efficacy trial. We are working with Coalition for Epidemic Preparedness and Innovations and the European and Developing Countries Clinical Trials Partnership on how to bring that product to licensure through a late-stage clinical evaluation planned for later this year. In addition, we have been actively involved in the Sudan ebolavirus response in light of the recent outbreak in Uganda. Fortunately, the outbreak has now been declared over. Recently, however, outbreaks of Marburg virus disease have occurred in Equatorial Guinea and Tanzania, countries where cases had never been previously reported. These developments have reinforced our commitment to driving our Ebola and Marburg virus vaccine programs forward expeditiously in preparation for future outbreaks and to continue to work with partners all around the world and further strengthen our engagement with stakeholders in Africa and elsewhere.

It is an exciting and busy time! Our work to apply the technologies that were put in place for HIV vaccine development to other targets is bearing fruit. We want to make sure that the best science can benefit everybody, regardless of their economic or geographic situation.

BIOGRAPHY

MARK FEINBERG, MD, PhD, is president and CEO of IAVI, where he leads a global team working to advance the development of vaccines and other biomedical innovations to protect against infection with HIV, TB, and other pathogens. Prior to joining IAVI, Feinberg served as chief public health and science officer with Merck Vaccines. In this role, he helped advance the development and global availability of vaccines against rotavirus, human papillomavirus, and other infectious diseases. He also played a key role in the coordination of a private-public partnership to expedite Ebola vaccine development. Previously, he spent more than 20 years in academia and government researching HIV/AIDS pathogenesis, treatment, and prevention, and the biology of emerging infectious diseases.

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VACCINEINSIGHTS

RNA VACCINES PART 1:
EXPLORING FUTURE POTENTIAL

SPOTLIGHT

Developing mRNA vaccines for malaria elimination

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"By targeting multiple stages of the parasite, both at the infection and transmission fronts, we hope to achieve a higher efficacy in reducing transmission at the population level, and ultimately local malaria elimination."

VIEWPOINT

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On March 16, 2023, Charlotte Barker, Editor, *Vaccine Insights*, spoke to Nirbhay Kumar, Professor, Global Health, Fellow (AAAS, AAM, ASTMH), Milken Institute School of Public Health, George Washington University. This article has been written based on that interview.

The approval of the world's first malaria vaccine in 2021 has been rightly celebrated as a breakthrough. However, while an approximately 30–40% reduction in hospital admissions is significant, the ultimate goal is to block transmission and eliminate the disease entirely – and the mRNA vaccine platform could be an important new weapon in our arsenal.

Despite the availability of a partially effective vaccine for at-risk children and infants and the knowledge that we have gained on the biology of *Plasmodium spp.* malaria remains one of the most impactful public health problems in the world in terms of human health and global economy. In 2021, there were an estimated 247 million cases of malaria, resulting in close to 619000 deaths [1].

The lifecycle of *Plasmodium spp.* are very complex and require multiple approaches to control transmission. Early efforts were based on trying to control the mosquito population and prevent bites. However, this proved to be challenging to implement, expensive to maintain, and requires major behavioral modifications, which are hard to sustain over the long term.

Anti-malarial drugs also continue to play a significant role in preventing severe illness and deaths, but drug resistance is a problem. Currently, there is only one combination drug being used for malaria treatment, artemisinin-based combination therapy (ACT) and the parasites are already beginning to show resistance in many different geographic regions. The situation will be very serious indeed if drug resistance leads to a lack of effective drugs to treat malaria.

While mosquito control and drugs are important, I believe effective vaccines for malaria are our greatest hope for the eradication of the disease.

Only one vaccine has been approved by the WHO for the prevention of malaria – RTS,S/AS01. The vaccine targets *Plasmodium falciparum* in its sporozoite form, which is injected by the mosquitos during the blood-feeding process. RTS,S prevents around 30–40% of hospitalizations for malaria in young children and infants, and a next-generation vaccine (R21) with a similar mechanism of action is reporting up to 80% protection in clinical trials. These levels of protection will be of huge benefit to people living in malaria-endemic regions; however, they will not be enough to eliminate malaria transmission at the population level.

My research career has been devoted to understanding the transmission biology of malaria infection and using that basic science knowledge to identify antigens that can be used to create transmission-blocking vaccines. Transmission-blocking vaccines alone do not necessarily prevent infection of the vaccinated individual – rather, they prevent transmission of the parasite to others in the community. To increase the functional effectiveness and acceptability of the vaccines to the public, we also aim to incorporate a component of the vaccine that will prevent infection by sporozoites.

Our approach has been to combine vaccines that will target infection of humans by *Plasmodium spp.* via mosquitoes to prevent severe illness, but also antigens involved in the transmission of parasites from infected people to mosquitos to reduce transmission to the next human host. By targeting multiple stages of the parasite, both at the infection and transmission fronts, we hope to achieve a higher efficacy in reducing transmission at the population level, and ultimately local malaria elimination.

For more than a decade, we have been working with DNA vaccines. DNA vaccines allow for relatively easy transport and storage, and our initial animal experiments have demonstrated excellent immunogenicity and efficacy in blocking parasite transmission. However, the requirement for specialized vaccine delivery methods, such as electroporation, can be a challenge for mass vaccination.

With mRNA vaccines coming to the forefront during the COVID-19 pandemic, we decided to apply this technology to our combination malaria vaccine approach. We recently published a preclinical study showing

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that even with very low doses of mRNA we achieved approximately ten times higher immune responses with mRNA compared with DNA vaccines targeting the same antigens [2]. The mRNA vaccines were 90–95% effective in blocking infection of mice by sporozoites and blocking *Plasmodium falciparum* transmission to mosquitos. We were particularly pleased to see no evidence of antigenic competition. In addition, mRNA vaccines can be rapidly developed in any combination of antigens, manufactured, and scaled up, with an excellent safety profile.

We are very encouraged by the results we've achieved in mice and the next step will be to establish that these data are reproducible in non-human primates. We are also developing combinations of antigens targeting *Plasmodium vivax* infection and transmission We are currently seeking funding and partnerships to expand on this exciting work and hopefully take the next step toward the elimination of a disease that kills more than half a million children a year.

BIOGRAPHY

NIRBHAY KUMAR, PhD, is a Professor in the Department of Global Health at the George Washington University Milken Institute School of Public Health. Prior to joining the Department of Global Health, Dr Kumar served as William G Vincent Endowed Professor and Chair of the Department of Tropical Medicine, and Director of a Vector-Borne Infectious Diseases Research Centre at Tulane University, New Orleans. A primary focus of research in the Kumar lab, funded by grants from the US National Institutes of Health has been in the area of Immunobiology of Malaria Transmission. Research in Dr Kumar's lab has played a critical role in the development and wider acceptance of the concept of malaria transmission-blocking vaccine to its present state as a key vaccine approach to achieve elimination and/or global eradication of malaria. Dr Kumar has published more than 190 research articles in peer-reviewed journals and has delivered numerous invited talks at various national and international scientific meetings, universities, and research institutions all over the world. Dr Kumar was elected fellow of the American Association for the Advancement of Sciences (AAAS) in 2007, American Academy of Microbiology (AAM) in 2012, and American Society of Tropical Medicine & Hygiene (ASTMH) in 2015.

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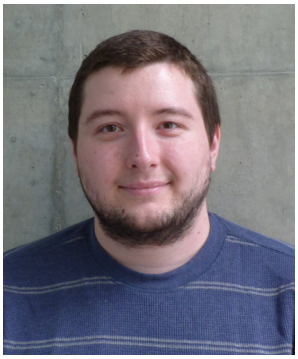
VACCINEINSIGHTS

RNA VACCINES PART 1:
EXPLORING FUTURE POTENTIAL

SPOTLIGHT

Expanding horizons with mRNA vaccines against bacteria

Nicholas K Clark, Senior Scientist, Discovery Biology, Sanofi mRNA Center of Excellence & **Leah Cole,** Director of Immunological Research, Sanofi



VIEWPOINT

"...mRNA offers an opportunity to develop vaccines against longstanding bacterial targets that have yet to be conquered and novel ones that might not have previously been considered."

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The rapid successful development of the SARS-CoV-2 mRNA vaccines Spikevax® and Comirnaty® offers the possibility that we might be entering a new golden age of infectious disease vaccines. The use of mRNA as a vaccine platform creates the potential to accelerate vaccine development and simplify vaccine production. Work is ongoing to develop novel mRNA design concepts and delivery systems for improved tolerability, potency, and thermostability, critical attributes for the successful launch of mRNA vaccines in a non-pandemic setting.

Viruses were the obvious first target for mRNA vaccines, since both mRNA vaccines and viruses hijack eukaryotic cell translational machinery to produce proteins [1]. In contrast, bacteria are larger and more complex than viruses and even obligate intracellular bacteria produce their own proteins rather than utilizing host cell machinery. Protein bacterial vaccines have previously been purified from the organism or expressed recombinantly in other prokaryotes. One example is Trumenba®, which is a vaccine for the prevention of meningococcal meningitis. This vaccine was approved in 2014 and is composed of two *Neisseria meningitidis* serogroup B factor H binding protein variants that are expressed as recombinant proteins in *Escherichia coli* [2]. Production of bacterial recombinant proteins in prokaryotes such as *E. coli* avoids the potential for eukaryotic posttranslational modifications such as glycosylation which could alter protein structure and therefore immunogenicity. As there are potential complications associated with prokaryotic protein production in human cells, the development of bacterial mRNA vaccines might be more complicated than their viral counterparts and may require adaption of the platform itself as well as ingenuity in candidate design. Despite these concerns, mRNA represents an exciting option for the development of novel bacterial vaccines.

While there is not yet clinical evidence supportive of bacterial mRNA vaccines, there are preclinical data that give credence to the idea that success is possible. Recent work from Tel Aviv University showed that a modified mRNA vaccine can protect mice against *Yersinia pestis*, the Gram-negative bacterium responsible for plague. Both purified and *E. coli*-produced recombinant versions of the F1 capsular antigen have previously been shown to be immunogenic and protective in the mouse model of bubonic plague [3] and anti-F1 IgG appears to be an immune correlate of protection [4]. Kon *et al.* designed multiple mRNA constructs for the F1 capsule antigen and the design variables included enrichment of guanine and cytosine content to increase mRNA stability and protein expression, conjugation of a human Fc to increase the stability and half-life of the protein, replacement of the bacterial signal peptide (SP) with an SP from human Ig kappa light chain to direct the protein toward the secretory pathway, and removal of the SP to avoid post-translational modification of the protein. The authors observed that a single dose of a F1 capsule antigen mRNA candidate with high guanine and cytosine content, human Fc conjugation, and a human SP was able to elicit uniformly high anti-F1 IgG titers and was fully protective against an otherwise lethal *Y. pestis* challenge [5]. The use of mRNA provided the opportunity to rapidly create and then assess multiple candidate designs, and the protective efficacy that had previously been observed with protein vaccine candidates was replicated with an mRNA vaccine candidate.

While antibodies are critical for protection against some infectious agents, cellular immunity, driven by $CD8^+$ or $CD4^+$ T cells, is critical for protection against other pathogens [6]. Use of mRNA enables the delivery of antigens designed to be preferentially presented by either MHC class I or MHC class II and therefore the response can be skewed towards either $CD8^+$ or $CD4^+$ T cells. Recent work from Ghent University assessed mRNA vaccine candidates against the facultative intracellular foodborne pathogen *Listeria monocytogenes*. Live replicating *L. monocytogenes*, but not killed bacteria, elicit cytotoxic $CD8^+$ T cells that resolve primary infection and provide long-lasting immune-mediated

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protection against a secondary challenge [7]. In the study by Mayer *et al.* the authors identified potential vaccine candidates using an immunopeptidomics approach. The sequences and abundancies of MHC class I-presented peptides from *L. monocytogenes*-infected HeLa and HCT-116 cells were used to identify immunodominant bacterial proteins. While virulence factors were identified, the authors selected *Listeria* proteins with no known toxicity or enzymatic activity for evaluation as mRNA vaccine candidates. These mRNA candidates were formulated in cationic lipid nanoparticles with the immunopotentiator α-galactosylceramide as an adjuvant to facilitate dendritic cell presentation of antigens on MHC class I molecules and activate invariant natural killer T cells. Immunization of mice with these modified mRNA candidates provoked $CD8^+$T cell responses and provided protection against challenge [8]. These preclinical data further support the idea that mRNA vaccines can be successful against bacterial pathogens.

The ability to design candidates to fit the constraints of eukaryotic expression and to elicit specific immune responses has spurred the movement of mRNA vaccine development beyond viral targets to bacterial pathogens. The continuing spread of antimicrobial resistance among bacteria is a global health threat that could be addressed through vaccines, and mRNA is a tool that can and should be used in this fight [9].

In conclusion, mRNA offers an opportunity to develop vaccines against longstanding bacterial targets that have yet to be conquered and novel ones that might not have previously been considered.

BIOGRAPHIES

NICHOLAS CLARK is an RNA biologist and Biochemist with an expertise in gene expression and the regulation of eukaryotic translation. He received his PhD from Brandeis University in the lab of Michael Marr studying translation under cell stress conditions.

LEAH COLE received her PhD from The University of North Carolina at Chapel Hill and continued her scientific education as a post-doctoral fellow at University of Maryland, Baltimore.

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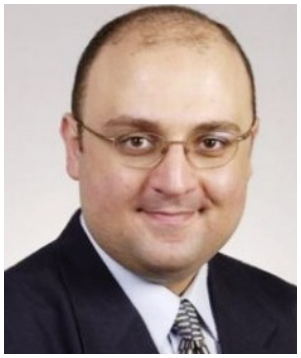
SPOTLIGHT

INTERVIEW

Pushing the frontiers of mRNA formulation & delivery



Charlotte Barker, Editor, *Vaccine Insights*, speaks to (pictured) **Mohamed EH ElSayed,** EVP & Chief Technology Officer, RVAC Medicines, Inc

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Q What is your role at RVAC and what does that entail?

ME: RVAC Medicines is a clinical-stage biotechnology organization focused on the development of mRNA-based vaccines and therapeutics, with a particular interest in bringing these potentially transformative therapies to emerging markets like China, the Asia-Pacific region, and beyond. I joined RVAC almost a year ago, after a career spanning 26 years in academia, industry, biotech, and consultancy where I worked on the development of different modalities including biologics, antibody-drug conjugates, and genetic medicines. At RVAC, my role is to build and lead the technology development organization accountable for delivery platforms.

In my opinion, despite the therapeutic advantages of mRNA and the success of the COVID vaccine, delivery remains a significant challenge. My organization focuses on

identifying and developing different types of delivery systems with differentiated safety, efficacy, and stability profiles.

Q What are some of those key delivery challenges for RNA vaccines and how is RVAC addressing them?

ME: One globally recognized challenge is the limited stability of mRNA, which dictates the need for an ultra-cold supply chain of -80°C. However, not all countries have the capacity for ultra-cold chain – or indeed any cold chain. We aim to identify the root cause of the instability of mRNA drug products and devise mitigating strategies to stabilize and retain the therapeutic activity of mRNA-based drugs at normal cold chain conditions of 2–8°C, and eventually at room temperature.

Another challenge is achieving optimal immune activation. The lipid nanoparticles that deliver mRNA vaccines improve their therapeutic activity by acting as an adjuvant. We are interested in understanding the role of the delivery system as a contributor to immune-mediated responses and hope this will open the door for modulation of the immune activation associated with the carrier, allowing us to dial up or down the immune response to suit the indication.

A third area that we are interested in is engineering nanoparticles to enable the delivery of mRNA to specific tissues or cells. In the case of vaccines, intramuscular administration anatomically facilitates the delivery of the mRNA to lymphatic tissues and antigen-presenting cells. However, for other routes of administration, there is a need to target the mRNA to a specific tissue and cell population, which requires a great deal of carrier engineering.

Q What formulation challenges are posed by different routes of administration?

ME: The goal of formulation is to overcome a series of anatomical, physiological, and enzymatic barriers that prevent the administered dose from getting to its target – in the case of mRNA, the cytoplasm. The microenvironment is different depending on the route of administration – different enzymes, pH, and even dynamic flow of fluids varies dramatically between intravenous, intranasal, or intramuscular administration. For example, in the intravenous route, you have serum proteins and high shear stress mediated by the flow in the blood circulation. This route potentially gives access to a wide range of tissues, but mRNA-loaded lipid nanoparticles are rapidly cleared by the liver.

Intranasal administration leads to a shorter residence time due to epithelial and mucosal barriers and high enzymatic concentrations that can degrade your molecule, so the window of action for your delivery system is much shorter compared to intravascular or intramuscular injection.

We design formulations with an eye toward overcoming the specific barriers facing each administration route.

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"Distributed manufacturing is another key capability for vaccine equity. Right now, vaccines are produced in sophisticated good manufacturing practice-certified manufacturing facilities."

Q Is it possible to reduce the amount of RNA given per dose without sacrificing efficacy?

ME: One approach to increase the potency of RNA vaccines or therapeutics is by using different mRNA constructs, for example self-amplifying RNA. The self-amplification component is encoded within the RNA, creating the machinery to make more copies of the mRNA within the target cell and increasing the translation of the desired antigen by several folds. However, the larger size of the self-amplifying RNA compared to conventional linear mRNA makes synthesis, purification, characterization, and encapsulation more difficult.

Similarly, circular RNA constructs are hypothesized to have a more durable protein expression compared to linear mRNA molecules due to their enhanced cytoplasmic stability. Ongoing research in academia and industry focuses on enhancing and stabilizing ribosomal loading of circular RNA molecules to increase their expression. The combination of these efforts can ultimately reduce the size and frequency of mRNA dose required.

Q Can formulation improve vaccine equity?

ME: Yes, it can, but you must start with the end in mind. One simple example is thermal stability – if you want to reach people around the globe, you cannot ignore the fact that many regions do not have the sophisticated supply chain to support ultra-cold or even cold distribution of vaccines. Starting with equity and access as key drivers for your business will automatically dictate a different formulation design to enable more universally applicable distribution channels.

Distributed manufacturing is another key capability for vaccine equity. Right now, vaccines are produced in sophisticated good manufacturing practice-certified manufacturing facilities. Is there a way to compartmentalize and miniaturize that manufacturing footprint into a series of modules that can be shipped around the world to facilitate on-demand, on-site manufacturing?

Both of these approaches are significant undertakings and cannot be tackled by a single entity but will require coalitions between private companies, governments, regulatory agencies, and global health organizations.

Q Final thoughts?

ME: I believe that mRNA is such a powerful modality that the only barrier to its utility is how effectively you can deliver it to different tissues and cells. Progress in pushing that delivery frontier will open a lot of new therapeutic indications.

BIOGRAPHY

MOHAMED ELSAYED is the Chief Technical Officer at RVAC. He is an accomplished leader with over 25 years of experience spanning academic, biotechnology, and pharmaceutical organizations. He is a recognized expert in the development of innovative delivery technologies for nucleic acids, biologics, bioconjugates, and small molecules. Prior to RVAC, Dr ElSayed held positions of increasing responsibility at Eli Lilly & Company where he established the Oral Peptides Delivery Platform and led the discovery and preclinical development of multiple oral biologics. Previously, Dr ElSayed was a tenured professor at the University of Michigan and served as an advisor for multiple biopharmaceutical companies. He is an inventor on six patents, has co-authored more than 175 research articles and conference proceedings, and delivered over 65 invited talks. He has received 25 honors/awards including Lilly Research Labs President's Scientific Recognition Award, US National Science Foundation CAREER Award, Coulter Foundation Translational Research Partnership in Biomedical Engineering Award, and US Department of Defense Award.

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VACCINEINSIGHTS

RNA VACCINES PART 1: EXPLORING FUTURE POTENTIAL

SPOTLIGHT

Opportunities and challenges in mRNA-LNP vaccine design

Rein Verbeke, Ghent University, Ghent University Hospital & University of British Columbia; **& Pieter R Cullis,** University of British Columbia

VIEWPOINT

"Future research should focus on how much of the mRNA-LNP dose drains into lymph nodes and spreads systemically, which immune cell types are involved in the uptake, translation, and presentation of the mRNA-encoded antigens, and which cellular interactions potentially contribute to adverse events..."

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In a record-setting 10 months, two highly effective vaccines, BNT162b2 (Comirnaty®) and mRNA-1273 (Spikevax®), were developed and granted authorization for emergency use against COVID-19, a medical achievement that has saved millions of lives. With this, a new platform revolutionized vaccine development, utilizing synthetic mRNA encoding a selected antigen and a lipid nanoparticle (LNP) as a delivery system [1]. Decades of research preceded the breakthrough of mRNA vaccines, where both mRNA design and lipid carrier were optimized based on the progress made in interdisciplinary areas such as RNA biology, biophysics in lipids, and immunology. Despite all these efforts, some basic features of this novel vaccine technology remain poorly understood. In this viewpoint, we will highlight some of the directions for future research which may help to develop the next generation of mRNA-LNP vaccines, with a special emphasis on the LNP design.

TAILORED LNP DESIGN FOR mRNA VACCINATION

The LNP technology was initially optimized and characterized for intravenous delivery of small interfering RNA (siRNA) to the liver, which culminated in the approval of the first siRNA product Onpattro® in 2018 [2]. Upon injection of these nanomaterials, interaction with blood components results in the desorption of PEG lipids from LNPs and subsequently allows the formation of a protein corona onto the LNP surface. Here, the binding of endogenous apolipoprotein E (ApoE) to LNPs has been shown to enable receptor-mediated uptake by the low-density lipoprotein receptor into hepatocytes, driving the specificity of gene delivery to the liver [3]. In recent years, there has been a strong focus to find new LNP compositions that can alter the amount and types of blood proteins that bind the particle surface, in particular to redirect LNP targeting, and thus to reach specific tissues beyond the liver. While it is widely recognized that protein corona formation is a critical factor for both the biological activity and targeting properties of mRNA-LNPs, this area is notoriously difficult to study, with breakthroughs being predominantly derived from empirical research.

The LNPs in the mRNA vaccines are largely adapted from those used for systemic (m)RNA delivery, but much less is known about their biological fate upon injection in the muscle tissue. Future research should focus on how much of the mRNA-LNP dose drains into lymph nodes and spreads systemically, which immune cell types are involved in the uptake, translation, and presentation of the mRNA-encoded antigens, and which cellular interactions potentially contribute to adverse events associated with the mRNA-LNP vaccines.

FORMATION & STRUCTURE OF mRNA-LNPs

The lipid composition of the COVID-19 mRNA-LNP vaccines contains four lipids: a cationic 'ionizable' aminolipid (ALC-0315 in the Comirnaty vaccine and SM-102 in the Spikevax vaccine), distearoylphosphatidylcholine, cholesterol, and a PEG lipid [1]. mRNA-LNPs are produced by rapid mixing of the lipids dissolved in ethanol solution with an acidic aqueous solution containing the mRNA content. This is followed by a step where ethanol is removed and pH is raised above seven, eventually forming uncharged mRNA-LNPs. Indeed, the ionizable lipid features a tertiary amine with an acid-dissociation constant (pKa) below seven, where its reversible (de)protonation capacity plays a key role in the encapsulation of mRNA strands, biocompatibility, and potency of LNPs. Intriguingly, the resulting mRNA-LNPs are more heterogeneous in shape and size compared to siRNA-loaded LNPs as visualized by cryo-transmission electron microscopy. Several studies have described LNPs forming protrusions of a bilayer membrane bleb containing an aqueous compartment, which appear to be filled with mRNA strands [4,5]. We believe that understanding the structure

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of mRNA-LNPs (including the function of these bleb-structures) and finding new ways to manipulate their morphological behavior may help in improving delivery potency, as well as enhancing both the biological stability and shelf-life of mRNA-LNP products.

ADJUVANT PROPERTIES OF mRNA-LNP VACCINES

Another gap in our understanding is how mRNA-LNP vaccines activate the innate immune system. Modification of mRNA with pseudouridines was necessary for the success of the COVID-19 mRNA-LNP vaccines, evidenced by higher tolerable doses and higher peak antibody levels in humans compared to the unmodified form of mRNA. These modifications allow mRNA to escape most (maybe not all) of the RNA innate sensing pathways, drastically reducing the inflammatory nature of mRNA. Interestingly, recent studies have focused on a potential intrinsic adjuvant effect of the ionizable lipid, as empty LNPs containing ionizable lipids were found to act as a powerful adjuvant for subunit protein vaccines [6]. This is leading researchers to screen for new ionizable lipids with better tolerability profiles and tailored adjuvant properties. However, the underlying mechanisms of the immunogenic and reactogenic effects of LNPs remain largely unknown [7]. In this context, one could also question whether mRNA-LNP vaccines could benefit from the addition or incorporation of other well-defined adjuvants, for instance, to extend the durability of antibody responses, to empower cellular immunity, and/or to achieve dose reduction. Particularly for vaccine development against diseases, such as bacterial diseases and cancer, this is worthy of investigation.

Taken together, we believe that addressing these questions is fundamental to gaining a better understanding of the pharmacokinetics and pharmacodynamics of mRNA-LNP vaccines, and could help in finding more effective and/or safer mRNA-LNP formulations.

BIOGRAPHIES

DR REIN VERBEKE received his MS in pharmaceutical sciences – drug development at Ghent University, Belgium in 2013. After graduating, he joined the Ghent Research Group on Nanomedicines (Ghent University) to start a PhD project under the supervision of Prof. Stefaan De Smedt and Dr Ine Lentacker. His PhD focused on mRNA-based vaccination for the treatment of cancer, with an emphasis on the design of mRNA lipid nanoparticles (LNPs) and the adjuvancy of mRNA vaccines. Based on his PhD, Dr Verbeke received the Belgian Industrial Research and Development (BiR&D) cross-disciplinary PhD Thesis Award and duo scientific career prize (together with Dr Heleen Dewitte) from the Belgian Royal Academy of Medicine. After a postdoctoral training at Karolinska Institute, Sweden (M. Karlsson lab), he continued his research on the validation and clinical translation of a novel mRNA nanovaccine (Galsomes) for applications in infectious diseases and cancer at Ghent University. In 2022, Dr Verbeke worked as a visiting postdoc at the University of British Columbia (Canada) in the lab of Prof. Pieter R Cullis. He has authored over 20 publications and is co-inventor on two patent applications.

PIETER R CULLIS, PhD, FRSC, FNAI (USA), OC, Director, Nanomedicines Research Group, Professor, Department of Biochemistry and Molecular Biology, University of British Columbia. Dr Cullis and coworkers have been responsible for fundamental advances in the development of nanomedicines employing lipid nanoparticle (LNP) technology for cancer therapies, gene therapies, and vaccines. This work has contributed to five drugs that have received clinical approval by the FDA, the European EMA and Health Canada. Dr Cullis has also co-founded eleven biotechnology companies that now employ over 400 people, has published over 350 scientific articles (h index 133) and is an inventor on over 100 patents. He has also co-founded and been Founding Scientific Director of two National Centre of Excellence networks, the Centre for Drug Research and Development (now AdMare) in 2004 and the NanoMedicines Innovation Network in 2019. These not-for-profit networks are aimed at translating basic research in the life sciences into commercially viable products and have given rise to numerous start-up companies. Dr Cullis has received many awards including the Order of Canada in 2021 and the VinFuture Prize (Vietnam),

the Prince Mahidol Award (Thailand), the Gairdner International Award (Canada) and the Tang Prize (Taiwan) in 2022. Two recently approved drugs that are enabled by LNP delivery systems devised by Dr Cullis, members of his UBC laboratory, and colleagues in the companies he has co-founded deserve special emphasis. The first is Onpattro which was approved by the US FDA in August 2018 to treat the previously fatal hereditary condition transthyretin-induced amyloidosis (hATTR). Onpattro is the first RNAi drug to receive regulatory approval. The second is Comirnaty, the COVID-19 mRNA vaccine developed by Pfizer/BioNTech that has received regulatory approval in many jurisdictions including Canada, the USA, the UK, and Europe. Comirnaty is playing a major role in containing the global Covid-19 pandemic with approximately 6 billion doses administered worldwide in 2021 and 2022.

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VACCINEINSIGHTS

RNA VACCINES PART 1: EXPLORING FUTURE POTENTIAL

SPOTLIGHT

INTERVIEW

Understanding and improving immune responses to RNA vaccines

Charlotte Barker, Editor, *Vaccine Insights*, speaks to **Justin Richner,** Assistant Professor, University of Illinois at Chicago, about unleashing the potential of mRNA vaccines by increasing the durability of immune responses

JUSTIN RICHNER earned his doctoral degree in 2011 from the University of California at Berkeley under the mentorship of Dr Britt Glaunsinger. He performed his post-doctoral studies at Washington University in St Louis with Dr Michael Diamond studying viral immunology and vaccine development. Dr Richner started his independent lab in 2018 and is currently an Assistant Professor in the Microbiology and Immunology Department at the University of Illinois Chicago College of Medicine.

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Q How did you get involved in the field of immunology?

JR: The overarching field throughout my scientific research, even from the undergraduate level, has been host–pathogen interactions. I began studying a bacterial

pathogen that infected an Arabidopsis plant, *Pseudomonas syringae*. That piqued my interest to go to graduate school at the University of California Berkley to study host–pathogen interactions, where I became interested in viruses and how they were able to infect host organisms.

After my graduate work, I became interested in how the host responded to the virus. I completed a post-doc with Michael Diamond at Washington University in St Louis, where we studied the host response to flaviviruses. I first established a project studying West Nile virus, and when the Zika virus outbreak occurred in 2016, we shifted the focus of my research to understanding the immune response on both the host and pathogen side of this relatively unstudied virus. I became interested in understanding the host response to these flaviviruses and how to develop medical countermeasures to combat these viruses. This was how I got into both immunology and vaccinology.

At the time, we were working with Moderna, who had a new vaccine platform in development. The Zika pandemic enabled a scenario where we could use this new technology to combat this emerging pathogen. That is where our work on the mRNA vaccine platform began.

Q What are the positives and negatives of immune responses to mRNA vaccines?

JR: In terms of immunogenicity, an advantage of the mRNA platform is that we can generate robust humoral immune responses as well as CD4 and CD8 T-cell responses. Now we have administered billions of doses of mRNA vaccines in humans, the high efficacy of the platform has been demonstrated. This efficacy has been seen in other diseases in smaller numbers, including in the Zika virus RNA vaccines in early-phase clinical trials.

On the other hand, we are seeing some levels of reactogenicity with the mRNA platform. These are minor adverse events, including classic immune responses such as malaise and low-grade fever, and seem to be slightly higher with RNA vaccines than with other platforms. There are also low levels of myocarditis in RNA vaccine recipients. That being said, these are very safe vaccines. The frequency of serious adverse events is very low, and we can work further on reducing the level of minor adverse events.

Another weakness we see is low immune durability with the SARS-CoV-2 mRNA vaccine. There have been many publications within this area showing antibody titers are declining. This is why regular booster doses are recommended.

Interestingly, if we look at data from Phase 1 human trials for the Zika RNA vaccines, we do not see the same reduced durability of the immune response. One big question in the field is whether we will see robust immune durability with RNA vaccines. Are the immune durability problems observed with the SARS-CoV-2 vaccines because of the specific biology of the virus, or will this occur globally across all RNA vaccines? The way that the spike antigen is presented in the SARS-CoV-2 vaccines will be different from other viral antigens. This will have an important influence on how we think about immune durability and understand the differences in how antigen presentation influences downstream adaptive immune responses. There is also a lot of work in the field on understanding the native immune pathways that are being induced by these vaccines.

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Q What do we know about the specific aspects of immunity in aged populations that pose a challenge for vaccine developers?

"There has ... been some evidence that immune durability is lower in older populations, meaning a more rapid decline of antibody titers than in younger populations."

JR: Aged individuals, in general, develop reduced immune responses to infectious diseases, as well as vaccines. This is well documented in influenza literature, where we see much lower vaccine efficacy in elderly populations versus younger healthy populations.

In general, age correlates with a higher increase in some markers of inflammation, including higher base levels of inflammatory cytokines. In the context of infectious disease or vaccination, younger people develop a robust rapid response that quickly goes up. The elderly seem to mount a much more limited response that does not reach the same magnitude as a younger response; we see a blunted and delayed adaptive immune response. There are multiple factors affecting this, including a lower frequency of naïve T cells, in addition to delayed activation of the T-cell response.

Intriguingly with the SARS-CoV-2 mRNA vaccines, we did not see this age-dependent decline in immune responses. The elderly developed robust immune responses to the mRNA vaccine platform, which was a pleasant surprise. The magnitude of the antibody titers and the T-cell response appeared to be equivalent in younger adults and the elderly after a two-dose vaccination schedule. It seems that the elderly can overcome this basal defect in their immune system, but it is still unclear why.

There has however been some evidence that immune durability is lower in older populations, meaning a more rapid decline of antibody titers than in younger populations. This is an area requiring further research to understand if it is unique to the SARS-CoV-2 antigen.

Q What questions remain to be answered about immune responses to RNA vaccines?

JR: One thing many people in the field are working on is understanding innate immune responses. Another advantage of RNA vaccines is that they do not require an additional adjuvant. RNA vaccines are considered self-adjuvating. The main component driving this self-adjuvant property is an ionizable lipid, which is a component of the lipid nanoparticle structure. There are several interesting studies showing these ionizable lipids are highly immunogenic and able to stimulate innate immune responses. The field is moving towards understanding the molecular pathways that are engaged by these ionizable lipids, and how these interface with the pattern recognition receptors to drive innate immune responses. The area is ripe for discovery.

Q What's next for your work and for the field as a whole?

JR: We are interested in developing novel flavivirus vaccines using this mRNA platform. We are currently working on an mRNA vaccine to combat both Dengue and Zika viruses. These viruses are co-circulating and are both transmitted by the same mosquito vectors.

For Dengue virus, there are replication-competent live attenuated vaccines; however, we know that some of the epitopes in these vaccines can drive antibody-dependent enhancement and lead to more severe disease in naive individuals. This was seen for the DENGVAXIA vaccine, which mimicked a primary Dengue infection and led to an antibody-dependent enhancement phenomenon. In our lab, we use the RNA platform to modulate the specific epitopes driving antibody-dependent enhancements to make a safer vaccine. We have previously taken the same approach to Zika virus vaccines. Importantly, this is not possible with a live-attenuated platform.

We are also working on understanding the innate immune properties of these vaccines and testing different lipid formulations to see if we can modulate innate immune responses to optimize vaccine immunogenicity and reactogenicity.

I still consider myself a virologist at heart, so I am most interested in understanding how we can inhibit viral infectious diseases. I am interested in how we can use this platform and the information we have about antigens to make vaccines, particularly against viruses that have failed to develop robust vaccine responses in previous attempts. A classic example is HIV; after decades of research, we still do not have a vaccine for HIV. There are other vaccines that fit the same mold, but we have not been able to generate good immune responses.

Influenza is a virus that is ripe for some new ideas in the vaccine field, due to the 9–12-month window we have to work in. If you could shorten this by several months, it could greatly increase the efficacy of the annual influenza vaccines. The mRNA platform could certainly help here.

Another benefit of the RNA platform is that we can rapidly generate new hypotheses and test them quickly in large numbers. As the field of vaccinology moves forward, it will be interesting to see how far RNA-based vaccines will overtake other platforms, and how far they will be limited by the durability of immune responses.

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