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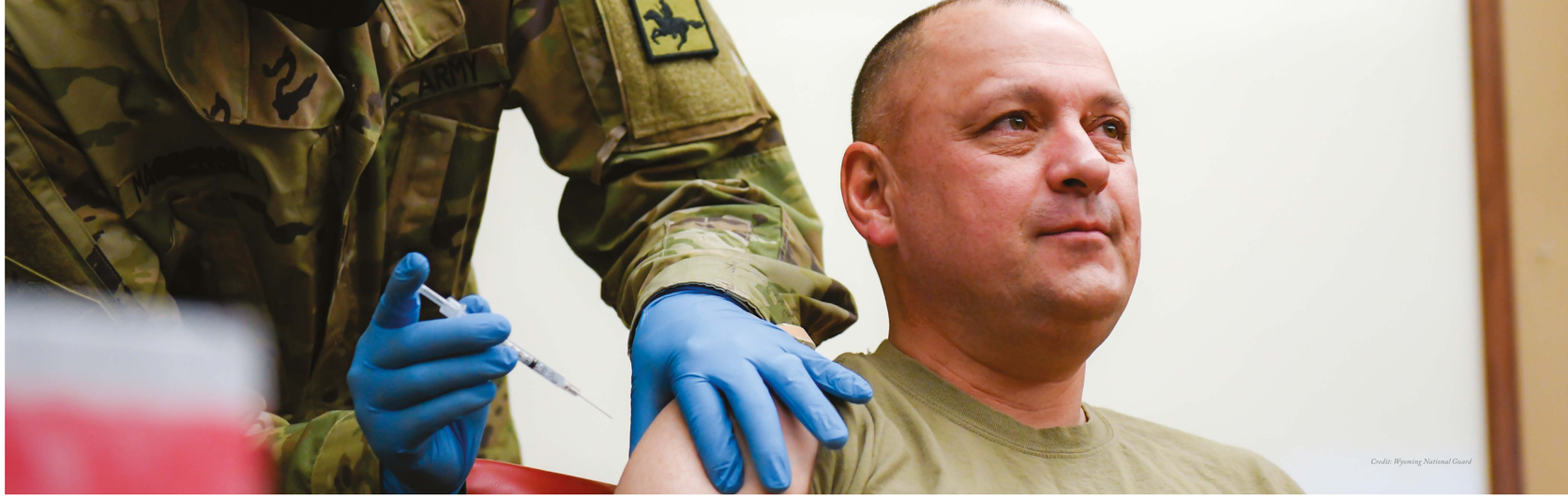
COVID-19: The Big Moments

It's hard to talk about mRNA without talking about the COVID-19 pandemic. Here's our picks of the top three defining moments of the pandemic in 2021.



Credit: Wyoming National Guard

The Rise of Omicron

The tail end of 2021 was marked by a surge in cases of the Omicron variant. On November 26, the WHO classified the strain as a variant of concern (1), but data from South Africa showed that patients only displayed mild symptoms (2). Nevertheless, travel restrictions and bans were enforced worldwide to prevent viral transmission. By the end of the month, many countries had announced their intentions to initiate booster campaigns. The UK was one of them, with its government offering additional vaccine shots to all adults as the country grappled with increased case numbers (3).

Let's Get Boosted

Ensuring long-term protection against SAR-CoV-2 has been a primary aim since the early days of the pandemic, but conversations about the importance of boosters in maintaining immunity intensified during 2021. By late February, Pfizer and BioNTech had launched a study to assess the potential of a three-dose-vaccine regimen in providing robust protection against variants of concern (4). Their work later showed that booster doses could successfully extend protection, pushing leaders worldwide to consider initiating programs for additional shots within their borders.

As some countries announced their intention to roll out booster COVID-19 shots for their populations, the WHO's Director-General, Tedros Adhanom Ghebreyesus, issued a plea requesting that governments focus their attention on vaccinating the unvaccinated rather than those with some protection against the disease.

"I understand the concern of all governments to protect their people from the Delta variant. But we cannot accept countries that have already used most of the global supply of vaccines using even more of it," said Adhanom Ghebreyesus in an August 2021 UN address (5).

Despite the concern about vaccine equity, international booster programs began in September to help combat the spread of both the Delta and Omicron variants. The WHO later recommended vaccine doses for immunocompromised patients regardless of vaccine status, as well as recipients of Sinopharm or Sinovac vaccines over the age of 60 (6).

Public Health Inequity Rages On

The international community has (for the most part) agreed that international access to COVID-19 vaccines is essential for curbing infection rates; however, ensuring equitable access has proven to be a sticking point for governments and healthcare organizations worldwide. Though the year began with a positive start, with many countries committing to sending vaccines to developing economies through initiatives like COVAX (7), the global community fell short of its targets.

At September's UN General Assembly conference, South African President Cyril Ramaphosa said, "It is […] a great concern that the global community has not sustained the principles of solidarity and cooperation in securing equitable access to COVID-19 vaccines. It is an indictment on humanity that more than 82 percent of the world's vaccine doses have been acquired by wealthy countries, while less than 1 percent has gone to low-income countries (8)."

The statement came only a few short weeks after COVAX announced that, because of restrictions, manufacturing challenges, and regulatory approval timelines, it would lower its vaccine supply forecast for 2021 from 2 billion doses to 1.425 billion (9).

Throughout the year, conversations about IP sharing persisted with many drug developers urged to share their vaccine blueprints with others under a TRIPS waiver (10). Though many refused to engage with the data sharing process, some organizations took matters into their own hands to help improve vaccine access – with some big pharma companies pledging to improve manufacturing infrastructure in low- and middle-income countries.

Some big pharma companies have pledged support specifically to Africa. Moderna, for example, is working towards the development of an mRNA vaccine manufacturing facility in the region – with five countries including South Africa and Rwanda suggested as potential locations. The company intends to create a site with the capacity to produce up to 500 million doses per year (11). Pfizer and BioNTech also signed a deal with South Africa-based biotech, The Biovac Institute, to produce its vaccine for African Union member states. The manufacturing operation is expected to begin this year.

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MANUFACTURE

The New Kid on the Vaccine Block

Exploring the manufacturing challenges of mRNA vaccines

Pharma has been investigating the potential of mRNA-based therapeutics for years but, prior to the pandemic, none had ever reached the market. The COVID-19 vaccines developed by BioNtech/Pfizer and Moderna both use mRNA – and moved from idea to commercialization in less than 12 months. Amélie Boulais, Head of Market Entry Strategy, Viral Based Therapeutics, Sartorius, gives the rundown on mRNA vaccine manufacturing, including how vaccines have been developed so quickly. But the biggest question is how much can the manufacturing process be scaled up? And are mRNA vaccines a viable option for vaccinating the world?

Were you surprised at how quickly vaccines against SARS-CoV-2 were developed and approved – particularly the mRNA vaccines?

Yes and no. When I first heard that the industry was aiming to develop and launch the vaccine in 12–18 months, I knew it would be challenging; the average time to develop a vaccine is 10 to 12 years – but we made it! The latest technologies combined with a willingness to collaborate were the keys to success.

Although mRNA vaccines are new, they have clear advantages as a platform technology. Manufacturers were starting with an unknown pathogen and, with mRNA, as soon as the sequence of the antigen was identified, developers were able to move forward quickly. With the mRNA approach, the process is the same for all potential indications, which makes it a true platform technology. For example, there is no did not need to find out how to produce the virus nor how to purify it. In fact, this platform is so efficient that BioNTech said they can produce a vaccine candidate against a new variant in just six weeks!

Another factor in the success of the mRNA vaccines was the speed at which companies could recruit patients and carry out trials, which is usually the most time-consuming stage of vaccine development. However, because COVID-19 was – and is – so prevalent, investigators were able to conduct trials much more quickly and were able to determine the efficacy within just a few months.

Pfizer and Moderna were also able to get ahead by initiating production of their vaccine before their clinical trials were complete, partnering with CDMOs to increase capacity and hasten delivery.

Overall, I am not surprised that the first approved vaccines were based on mRNA; as noted, mRNA platforms provide a clear advantage when it comes to development time. But viral vectors are also a promising platform – and they made it to the market very quickly, too, as we've seen with the Johnson & Johnson and AstraZeneca (originally developed by the University of Oxford) vaccines.

How does the mRNA vaccine manufacturing process work?

There are three steps in mRNA manufacturing. First, the target DNA sequence (coding for the antigen) is inserted into a DNA plasmid (pDNA). The production and purification of pDNA relies on *E. coli* fermentation, which is often outsourced.

Second, mRNAs are produced by an enzymatic reaction called in vitro transcription (IVT). Here, nucleotides, enzymes, and plasmid DNA (pDNA) that encodes the antigen are mixed together – the pDNA is used as a template, from which the mRNAs are produced. Then, the mRNA is purified to remove IVT reagents, pDNA, and other contaminants. These purification steps include a mix of chromatography, tangential filtration, and filtration.

Finally, the mRNAs are usually encapsulated into lipid nanoparticles – this allows mRNA to enter the cells once injected to patients while also improving stability) – which are also purified and concentrated using tangential flow filtration and chromatography.

What are the main challenges associated with mRNA vaccine manufacturing at large scale?

A common challenge for all vaccines is ensuring safety and efficacy at scale, but mRNA processes are very different compared with other vaccine types, and with that comes very specific challenges. First, these processes are very new and expertise lies in the hands of only a few players. Now that we are facing the need to produce billions of doses, partners need to rapidly acquire expertise.

Second, classical technologies used for vaccine production and purification are not always adequate for mRNA processes. What is most suitable reactor for the IVT? A bioreactor or a mixing bag? What about the purification steps? Chromatography or precipitation? And which type of chromatography matrices? What is the best analytical solution to characterize and monitor the process? These are still relatively open questions.

Third, the processes have been scaled up very quickly, which means they work but can be further improved. For example, choosing the right storage conditions for the lipid nanoparticles is a challenge we've heard from our customers. We also know that the productivity of IVT processes could be improved with a better understanding of the interaction between reagents. We also know that contaminants can interact and form stable aggregates, and some of these aggregates can bind to mRNA and interfere with purification. Our new colleagues from BIA Separations are working to address this challenge and we are learning from them as they develop solutions for these purification steps.

Why are mRNA vaccines so sensitive to storage conditions?

mRNAs are not stable molecules; they are very fragile and sensitive to degradation. Though a lot of work has already been done to improve their stability, there remains room for improvement. mRNAs are usually encapsulated as lipid nanoparticles to ensure entry into the cells, and this can also impact overall stability. Table 1 shows the storage conditions of the top three mRNA vaccine contenders.

New formulations and alternative excipients may improve the situation, but it simply wasn't possible to overcome the issues in 2020 given the time pressures. The main focus has been on safety and efficacy.

Could any shortages of raw materials delay the rollout of mRNA vaccines?

The most critical shortages have been related to the reagents used for the IVT. Also, lipid nanoparticles have never been produced at such a scale before, which has led to shortages. The quality of the raw material is critical, and lot-to-lot inconsistency and contamination make the purification process of mRNA much more complex.

There are also shortages of pDNA because. I noted earlier that it is used as a template for production of mRNA, but it is also used as a raw material for the production of viral vectors, which are used in cell and gene therapy; in fact, this is where pDNAs are used most often. The need for pDNA is increasing, and this is creating a bottleneck in the production of advanced therapies. The sudden needs in COVID-19 mRNA vaccine production is not helping!

What are the advantages and disadvantages of outsourcing vaccine production?

Much depends on the context. In the middle of the COVID-19 pandemic, there was no other choice; the companies licensing the vaccines do not have enough capacity to fill demand, nor the time to build new facilities. Outsourcing allowed them to ramp up production quickly. Another general advantage of outsourcing vaccine production is the reduced financial risk. Companies that outsource this step do not need to build a facility to produce their vaccine before they know it will be financially viable.

The disadvantage of outsourcing is the need to rely on a third partner and a loss of control over your process. Traditionally, CDMOs are not involved as much in vaccine manufacturing because the vaccines are all so specific and require a unique expertise. But mRNA and viral vector platforms are enabling companies to use CDMOs. And we will likely see CDMOs constructing their own mRNA platforms to support developers in the future.

For companies like Moderna, which have chosen to outsource their vaccine production, what are your top tips to ensure the relationship runs smoothly?

First, select a partner that has experience in the area they're being tasked with handling (whether it's vaccine production, packaging, and so on). Note that this first tip is general – and it is highly challenging with mRNA vaccines because no one has experience! Second, find out whether the partner is able to produce enough vaccine in time. Do they have all the equipment they need? Do they outsource some activities or perform everything in house? Companies must conduct a risk assessment. Third, verify their track record in vaccine production. Have they already been inspected by a major health authority? And finally, define the communication and project management needs upfront to avoid problems down the road.

Ultimately, the industry will have to produce billions – perhaps tens of billions – of doses of vaccine to tackle the virus across the planet. Broadly speaking, what will it take for this to happen?

It will take time. We are seeing some unexpected collaborations, like Sanofi partnering with Pfizer to help them produce their COVID-19 vaccine; but we're still not producing vaccines fast enough. We are also closely following the evolution of the virus and its variants, and we don't yet know how long the vaccine will protect us – and whether it will have to be a seasonal shot or not. There are many uncertainties!

REFERENCES AVAILIABLE ONLINE

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Cracking mRNA's Manufacturing Code

With complex workflows to navigate, pharma companies must find trusted partners to rely on as they navigate the challenges of mRNA manufacturing

While mRNA is now widely known for being a central component in vaccines for COVID-19, a group of dedicated scientists and researchers have been studying the potential of this molecule for decades in hopes of developing novel cancer therapies. Despite steep challenges, the researchers persevered, which has led to advances in a host of mRNA-based therapies that are now in clinical trials. The biggest success, however, has been the development and approval of several mRNA-based vaccines against the SARS-CoV-2 virus. In fact, the pandemic played a crucial role in "cracking the code" of mRNA technology – and mRNA changed the course of the pandemic.

It's time to take those years of research and recent learnings and help scientists discover the next mRNA breakthrough. Before that can be accomplished, however, the industry must make certain investments to help alleviate logistical and supply chain bottlenecks and address the unique needs of mRNA-based manufacturing. Today's mRNA manufacturers require holistic solutions that can help reduce project risks, stabilize costs, maximize capacity, and help speed time to market.

As a provider of end-to-end solutions for mRNA, Cytiva has recently moved toward enabling the development, manufacture, and delivery of mRNA-based vaccines and therapies through its large-scale mRNA manufacturing workflow offerings. From plasmid DNA (pDNA) template manufacturing to mRNA synthesis and mRNA–lipid nanoparticle formation, mRNA solutions from Cytiva help enable sequence-to-patient manufacturing with a fast and standardized process.

Through its Enterprise Solutions division, Cytiva provides flexible start-to-finish solutions that are configured to support different product modalities. These include modular solutions for both pDNA and mRNA manufacturing by way of Cytiva FlexFactory™ platforms and KUBio™ facilities.

Whether you are just getting started, or want to grow to large-scale production, Cytiva brings a breadth of offerings to help you evolve or scale up your mRNA manufacturing.

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FEATURE

Reaching for the Sun

The mRNA field is experiencing rapid growth in the wake of pandemic success, but what more needs to be done to ensure it truly flourishes?

By Maryam Mahdi, Deputy Editor of The Medicine Maker

Every success story starts the same way: with an idea. Whether those behind the idea enjoy early success or face setbacks along the way, innovators believe their work will ultimately help drive society forward. Take the likes of Apple or Tesla; without a certain degree of tenacity and determination, their impact on the global community may have not been as significant. The same is also true for mRNA vaccine developers. Though the field is still relatively young, the early efforts of the academics and companies driving progress have captured global attention.

mRNA-based vaccines and therapeutics were already gaining momentum prior to the pandemic, but COVID-19 vaccine success stories have injected additional excitement and hope – with new investment following close behind and research now blossoming throughout the industry. But what will happen next? COVID-19 provided companies with the right conditions to flourish; without the typical regulatory and legal restrictions in place (and with an unprecedented level of cross-industry collaboration), companies were able to accelerate drug development and get their product into the arms of patients quickly.

As the pandemic dust begins to settle and pharma (and the world) returns to some semblance of its former normality, mRNA developers will undoubtedly face a new level of scrutiny – and there will be those who question whether mRNA products will find long-lasting success in the industry. To move forward, companies will have to look back at the learnings they've gained to date.

"Simply put, mRNA allows the body to become its own drug factory. But to deliver mRNA into cells, we must rely on lipid nanoparticles."

The COVID-19 effect

The COVID-19 pandemic has shaped the way stakeholders both in industry and beyond view mRNA vaccines. Though some skepticism lingers within the general public, the uptake of mRNA vaccines as prophylactics against SARS-CoV-2 has been unprecedented. By mid-August 2021, more than 4.84 billion doses of COVID-19 vaccines (many of them based on mRNA) had been administered (1). But confidence in new vaccines was hard-won. Companies, government agencies, and regulators all had to ensure that people understood how the drug development process – a process well known to be notoriously long and riddled with challenges – was expedited to bring a new technology like mRNA to market in the space of a year.

Although mRNA seems new to the general public, those in industry circles are well aware that the R&D behind it has been years in the making. Amélie Boulais, Head of Market Entry Strategy at Sartorius says, "Researchers have been studying mRNA as a potential vaccine platform for indications such as infectious disease and cancer for almost 25 years. Before the pandemic, human trials were already underway for mRNA-based vaccines to prevent HIV, influenza, and Zika virus. This is because the antigen can be sequenced and manufactured very quickly, which makes it a practical solution from a commercial point of view."

Of course, the early mRNA pioneers couldn't have predicted that COVID-19 would emerge as a global healthcare crisis, but the scientific framework they developed allowed them to rapidly switch gears when the pandemic began. Prior to the pandemic, there was a lack of evidence to show the efficacy of mRNA in patients but this quickly changed as vaccine rollout programs began. Boulais says, "We were just waiting for the proof that mRNA could work in the real world. And we now have it. The success of the mRNA-based COVID-19 vaccinations created interest across the industry. There is a lot more funding available for companies seeking to enter the mRNA space, and now we are starting to see companies big and small developing mRNA-based vaccines. BioNTech and Moderna are pushing forward with mRNA-based vaccines for a variety of indications and creating a strong pipeline towards immunotherapy and even personalized therapies. Other major players in the field, such as Sanofi and GSK, are investing in mRNA too. Meanwhile, dozens of startups are popping up looking to discover novel uses for mRNA in vaccine development."

The growing interest sparked by COVID-19 will mean that mRNA-based products will have a greater influence on future drug pipelines. But what effect will this have on the use of more conventional products as the field continues to mature?

Weighing up the benefits

Though traditional vaccines are some of the best and most widely available pharmaceutical interventions used today, it is not an easy road for pharma to travel. If vaccines survive the "valley of death" – the translational gap between bench and bedside – developers must still face multiple challenges related to their manufacture. Historically, vaccines have been associated with high costs and low returns – and therefore considered unattractive to drug developers.

"Most conventional (viral vector) vaccines against viral diseases are made from viruses grown in chicken eggs or mammalian cells. The process of collecting the virus, adapting it to grow in the lab is lengthy and can take months to produce by growing weakened forms of the virus," explains Stefan Randl, Vice President of Research, Development and Innovation at Evonik. "In contrast, mRNA vaccines can be constructed quickly using only the pathogen's genetic code. It takes roughly a week to generate an experimental batch of mRNA vaccine. Producing and scaling up production is also relatively simple because the technology requires a standard production platform."

Simply put, mRNA allows the body to become its own drug factory. But to deliver mRNA into cells, we must rely on lipid nanoparticles (LNP). Once inside the cell, mRNA interacts with cellular machinery to "manufacture" the antigen and subsequently trigger an immune response. "They do this without integrating into the human genome making them particularly safe to use," adds Randl.

The mRNA technology available today also offers a potential solution to overcoming mutations in viruses, adds Dieter Schinzer, Director of the Institute of Chemistry at the University of Magdeburg. Referring to the latest COVID-19 vaccines he says, "When compared with classic vaccines, the flexibility of mRNA-based products shines. They can quickly adapt to mutations due to their mechanisms of action. They are more easy to produce and, for the most part, are cost-efficient."

But mRNA isn't without its limitations. Randl says, "Though players began to invest more heavily in the field at the start of the 2000s, the

immunogenicity of these products has slowed progress and hampered commercial success. mRNA is not very stable and has to be delivered to cells. If not, the right proteins will not be produced. The fact that there were only a handful of companies working in the space in these early days meant that it took longer for solutions to be devised."

Knowledge of the structures formed by lipid–nucleic acid complexes in the form of LNPs as well as of the effect of particle size, lipid composition, and distribution on biological activity, are also essential for the design of products with improved transfection efficacy. Aurel Radulescu, senior scientist at the Jülich Centre for Neutron Science in Forschungszentrum Jülich – a German interdisciplinary research center – uses a small-angle neutron scattering diffractometer to analyze scatter data of various molecules, including mRNA. He says, "If the industry aims to expand the use of mRNA from vaccines into other therapeutic areas, new methods of delivery will have to be considered. Great progress has been made in achieving efficient and tolerable LNPs for the delivery of mRNA for intravenous and intramuscular administration, but challenges remain with subcutaneous self-administration. If this is improved it opens up the possibility of patient self-administration and, therefore, long-term treatment of chronic diseases."

Storage conditions add an additional layer of complexity to the use of mRNA-based therapeutics. Boulais even argues that it is the "greatest limitation." "In developed countries, where cold chain infrastructure is in place, storage is less of a problem, but this just isn't the case for low- and middle-income countries (LMIC), where these facilities are lacking. However, we are starting to see improvement in this space. For example, the Moderna COVID-19 vaccine has now improved stability and can be stored refrigerated between 2-8°C for up to 30 days prior to first use. Therefore by working both on the LNP and the formulation itself this challenge might be soon overcome," she says.

"The challenge of logistics and cold chain with mRNA is difficult to overcome quickly. Considerations will have to be given to the individual circumstances of countries' governments as well as the support available from the public and private sectors," says Randl. "If rectified, equitable access for patients could be achieved. But, for now, it is apparent that this will be a long-term goal for all parties involved. And patients in LMICs will have to continue to wait for fair and equal access to these innovative products."

Even if the right supply chain conditions were in place today, the limited number of high-tech facilities to produce mRNA-based products introduces another barrier for companies worldwide. "Only a few enterprises worldwide have the technology to provide the required lipids at very high purity for the formulation of these new vaccines. Existing facilities will have to ensure that they can supply the quantities of raw materials and vaccines needed to keep up with industry demand," Schinzer explains.

There's also a lack of equipment specifically made for mRNA. Boulais says, "Many processes today have been scaled up and developed very quickly, and due to the COVID-19 rush must be re-examined to identify areas for improvement," she says. "Due to the fast development, in the near future we expect to see further optimization in processes to increase efficiency and reduce the cost of goods solds (COGS). We also expect innovation coming into the space can serve the different applications that mRNA might have in the future. The industry will learn from trial-and-error on a massive scale as different industry players test the limits and capabilities of mRNA in different areas of biopharmaceutical design and production. The very nature of mRNA technology offers much potential to unlock a new pipeline of drugs for some of the world's most challenging diseases."

As the industry looks ahead, Randl believes that regulators – although very supportive thus far of mRNA-based vaccines for COVID-19 – may also have more questions for mRNA drug developers in the future. "The FastTrack designation given to COVID-19 vaccines was essential for lessening the impact of the pandemic, but I believe that companies will have to do more to understand the unknowns about mRNA. We have to anticipate certain questions from regulatory agencies. How toxic are they? How well are they degraded? We have to be willing and ready to answer them as we look to develop lifelong treatments for patients


Stefan Randl, Evonik


Radulescu

using them. But although there may be more scrutiny, many of these questions should become easier to answer as the field advances."

mRNA: From building blocks to skyscrapers?

The field is currently experiencing a growth spurt so we can expect to see more mRNA-based products filling pipelines as innovation continues and funding continues to pour into the space. "Many in the sector are working to develop new delivery approaches for LNPs to cater to the growing spectrum of products being developed today," says Boulais. "The delivery of nanoparticles is still an important issue for us to solve. The first trials using these products only began in 2014 so there's plenty of room for growth."

For Schinzer, it will be important for companies to take a closer look at the nanoparticles themselves. "mRNA-based therapies are like building blocks. They can quickly be adjusted to meet new or emerging needs. At Corden Pharma International, plant-based cholesterol is used as an alternative to the animal-derived cholesterol currently used to produce LNPs," he says. "A product of non-animal origin, quite simply, avoids any potential animal source of contamination and has environmental benefits. Plant-based cholesterol uses a solid, renewable base of biomass as starting material and this will be important to overcome lipid shortages."

Another consideration for the industry is the specificity of LNPs. The current generation of products are not tissue-specific. According to Boulais, this is fine for today's needs, but it will need to change as the industry begins to explore its potential for advanced therapies. "If we are to use LNPs and mRNA-based products in the gene therapy sector, for example, a lot of basic science will be needed to modify current designs," she says. "I expect that in the near future we will begin to see progress here. Beyond these nanoparticle designs, manufacturers will also have to work to enhance facility design for mRNA-based vaccine production. From a manufacturing point of view, the lipids are usually dissolved in very high ethanol concentration, (typically around 98 percent). This makes the facility design challenging and lots of precautions need to be in place to ensure environmental and safety measures."

Though all these aspects of the future mRNA-based products are important, Randl and Radulescu make the case for continued collaboration. The mRNA-based COVID-19 vaccines came as a result of collaboration – and collaboration will be important as the field continues to grow and companies explore what else can be done with mRNA. Both Evonik and the Jülich Centre for Neutron Science have entered multi-year agreements with academic and industry partners to help push forward new projects.

"Evonik has entered a three-year deal with Stanford University in the US to develop a polymer-based drug delivery system to ensure the safe delivery of mRNA into cells, particularly as companies begin to further their applications for cancer immunotherapy and gene therapies," says Randl. "Though we have our own developments underway, we were keen to tech scout to find other promising work and support its growth," he says. The team came across the work of a research group at Stanford led by Robert Weymouth, Robert Eckles Swain Professor of Chemistry, who had developed a platform called "Charge Altering Releasable Transporters." The technology enables the delivery of mRNA into cells with a transfection efficiency rate greater than 99 percent.

"We initiated this collaboration as a strategic step to ensure mRNA technologies can be used fully and most effectively, and the platform developed by scientists at Stanford is promising because it is flexible and adaptable," says Randl. "The goal is to develop a technology for delivering mRNA to tissues and organs that goes beyond the current possibilities of LNPs."

Meanwhile, Radulescu and his colleagues at the Jülich Centre for Neutron Science have entered into a very different kind of agreement. As an academic group, their collaboration with AstraZeneca will see their research, which focuses on the use of neutron scattering studies, used to enhance mRNA-based products design for better

subcutaneous drug delivery. "Our collaboration with AstraZeneca started in 2015 when scientists from AstraZeneca visited our center in Garching to carry out structural investigations on newly proposed LNPs as delivery systems for mRNA therapies," says Radulescu. "We provide beam-time for neutron scattering experiments and expertise for the analysis of the scattering data collected during such experiments. We're now aiming for structural characterizations of new formulations to increase the therapeutic application opportunities."

These efforts are seemingly steps in the right direction for mRNA drug developers. But are the teams optimistic about seeing their efforts realized? "Absolutely. Without a doubt, mRNA technology holds a promising future," says Boulais. "Years of research and development using this technology are what made the COVID-19 vaccine possible – and the future of this technology will only continue to evolve as more companies continue to explore its potential."

It seems only a matter of time before the next mRNA breakthrough emerges.

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BEST PRACTICE

Top Tips for mRNA Sequencing

mRNA has become a superstar thanks to the success of mRNA-based COVID-19 vaccines, but its importance was known long before the pandemic. Bellal Moghis from the Diagnostics and Genomics Group at Agilent Technologies tells us about the potential of mRNA – and the challenges of working with and analyzing mRNA.

What attention was on mRNA prior to COVID-19?

mRNA has been key to understanding molecular pathways that underlie disease susceptibility or drug response. For this reason, scientists have used techniques such as quantitative real time-polymerase chain reaction (qRT-PCR) to understand how genes are regulated within a molecular pathway, or microarrays to understand how multiple genes are modulated in response to cellular changes. Insights from these experiments have allowed scientists to develop therapeutics to target faulty gene pathways that lead to disease. Now that high-resolution technologies such as next-generation sequencing are more accessible, mRNA is being considered at the individual sample level, allowing us to realize the benefits of personalized medicine. Most recently, just prior to the COVID-19 pandemic, mRNA was used to increase the clinical utility of diagnostic genetic tests (1). By conducting mRNA sequencing alongside DNA sequencing, some variants of unknown clinical significance can now be elucidated, improving diagnostic yield and aiding treatment.

What are the challenges of analyzing mRNA?

mRNA sequencing has long been a core workflow in many labs; however, it has a unique set of challenges. mRNA degrades significantly faster than DNA, so it is often frozen at -80oC for long-term storage. Before mRNA can be used in a sequencing workflow, its quality must first be assessed using reliable, automated benchtop capillary electrophoresis systems. Because genes are differentially expressed, experimental design is another important consideration to ensure that all genes of interest are detectable and quantified properly, with scientists required to choose between sequencing the global gene expression profile of a sample or enriching certain genes to study them in isolation. Once the samples have been sequenced, the scientist is then tasked with analysis, which can be cumbersome for new users. Luckily, there are several analysis software solutions on the market today that guide users and aid in interpretation.

How are technologies for mRNA sequencing advancing?

Sequencing technologies are becoming more accessible, allowing scientists to leverage large mRNA data sets to aid in clinical and diagnostic research. Though there is much more to be done in understanding how mRNA can provide clearer insights to disease, the information we currently have is already being used in labs across the world to gain more clinical utility from sequencing data. It is my belief that cancer diagnostics and treatment management has advanced significantly from these studies. Today, targeted treatments can be prescribed based on the mRNA profiles of cancer patients – namely, the presence of fusion genes that have been shown to directly cause cancer, such as BCR-ABL fusions found in some types of leukemias. As clinical research scientists continue to make advancements in understanding how mRNA can be used as an effective biomarker for cancers, pharmaceutical companies and molecular diagnostic providers can continue to develop effective diagnostic tools.

What are your top tips and best practices for sequencing mRNA?

Although there are many commercially available kits to guide new users through the process of sequencing mRNA, here are a few important considerations:

- As mentioned above, RNA is less stable than DNA, so sample preparation must be handled with care. Decontaminating workspaces during RNA isolation is a critical step to avoid introducing RNase, an ubiquitous enzyme that rapidly degrades RNA, into your sample. Although storage of your RNA will require a -80oC freezer, thawed RNA in use should remain on ice throughout library preparation.
- Assessing the quality of your RNA sample is key to ensure that your time and resources are not wasted preparing a sample that is destined for failure. There are many solutions that can help you understand both the quality and the quantity of your starting material, so take advantage!
- It is important to consider different approaches (either mRNA capture or ribodepletion) based on the quality of your samples. Since mRNA capture typically requires a 3′poly-A tail, mRNAs that are fragmented and missing the 3′poly-A tail may be missed. Ribodepletion, in which the highly abundant ribosomal RNAs are removed from the sample and the remaining mRNA is sequenced, is often considered in these cases.
- Maintaining consistency between sequencing runs is critical for comparing samples run in different batches. Like qPCR experiments, the preparation, sequencing, and analysis of samples should be normalized.

How excited are you at the future potential of mRNA?

The impact of mRNA as a diagnostic tool or, in the case of vaccines, a medicine, will continue to improve outcomes in cancer, genetic disorders, and infectious disease. By quickly producing RNA vaccines in vitro to specific antigen targets, precious time and lives can be saved. The potential of RNA vaccines is immense and in its infancy, but the highly positive results from the COVID-19 vaccines have shown the world what is possible. And, in the future, even more cutting-edge solutions will be available.

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DISCOVERY & DEVELOPMENT

Can mRNA Make a Difference in the Fight Against Malaria?

Evelina Angov, Chief, Laboratory of Molecular Parasitology, Walter Reed Army Institute of Research, believes mRNA vaccines could play an important role in the treatment of other infectious diseases – especially malaria, a disease that affects up to 200 million lives each year. Here, we ask Angov about her thoughts on mRNA-based therapies – and how the Institute is helping in the global goal of eradicating malaria by 2050.

What role can mRNA vaccines play in treating malaria?

A highly effective malaria vaccine would go a long way toward the goal of malaria eradication. mRNA vaccines' advantage over traditional approaches is the rapid transition from target discovery to manufacture. Similarly, this approach can be used to deliver more complex, multi-antigen vaccines by combining sequence variants and targets, which would broaden immunity.

What is the Walter Reed Army Institute of Research working on?

Recent successes of mRNA vaccine delivery for SARS-CoV-2 have propelled the long-neglected platform to the forefront of infectious disease research. In our recently published paper (see, https://go.nature.com/37Vex9x), we selected the immunodominant coat protein of the invasive stage of the malaria parasite, circumsporozoite protein (PfCSP), as the target to evaluate for the protective potential of mRNA malaria vaccines in mice. LNP encapsulation was used to protect and deliver the mRNA to the cell translation machinery and to supply adjuvant activity. We explored the effect of several factors, such as formulation, dose, number, and interval of immunizations, in two mouse strains, and showed the protective potential of a PfCSP mRNA-LNP against lethal, rodent-malaria transgenic parasites.

As people living in low- and middle- income countries are most vulnerable to malaria, what considerations will have to be made when developing suitable mRNA therapeutics?

Firstly, safety is paramount for any target population. But the product profile of a successful mRNA vaccine in these areas will potentially need to address narrower cold chain and storage capabilities, as well as a price-point compatible with fiscal sustainability. The advantages of mRNA are that the transcript (coding) sequences can be optimized rapidly to adjust for variants, mutations, or other modifications, formulations are fairly stable and fieldable under conventional deployable conditions, and manufacturing is rapid, and more cost-effective by comparison with small molecules or recombinant protein technologies.

What's next?

Though we are enthusiastic with the findings that we reported, we want to explore improvements to the malaria coding sequence (transcript) to see if we can enhance immune responses, prior to moving into a more representative animal model, such as the non-human primate. Outside of the improvements to the PfCSP mRNA transcript sequence, we are also evaluating mRNA as a viable immunoprophylactic modality to limit infection and disease. This is an area of research and product development that can greatly benefit from overcoming the traditional challenges and development costs of recombinant antibody-based products.

What is your outlook on the future of vaccines?

I have been a scientist in this field for 26 years, and really feel that we are living in an exciting time for malaria vaccines and vaccines in general. Despite numerous challenges, there is great progress toward the development of protective vaccines against pre-erythrocytic malaria infection. There are new vaccine platforms and technologies that have never been so available or accessible. Though we can easily advance to preclinical animal studies, the stable and reliable resources and funding to advance malaria vaccine candidates into the clinic and beyond phase I remains elusive, and we will need this type of support if we are to capitalize on our current advantage and ultimately push through to a victory against malaria.

REFERENCES AVAILIABLE ONLINE

SITTING DOWN WITH

Igor Splawski

Chief Scientific Officer at CureVac

How did you become interested in mRNA-based therapeutics?

It feels like I've always had an interest in discovering the genes behind diseases and disorders – certainly, it was the focus of my work starting with my PhD in 1992. I continued my gene identification research after the completion of my doctorate at the Howard Hughes Medical Institute, Children's Hospital Boston, and Harvard Medical School, where I became an assistant professor.

In 2005, I joined Novartis. While using the learnings from human genetics, I completely switched my focus to biologics. Most of my years there were spent working on ophthalmology and cardiovascular disease. Towards the end of 2011, I reignited my former interest – my "unfinished business" – by starting a group specializing in mRNA. The group began with one person but, at its peak, numbered around 50. Our primary goal was gene replacement with mRNA, where we showed several proof-of-concept studies in vivo and expression in non-human primates. Moreover, that's when I met some of my current colleagues from CureVac – the first ever mRNA technology company, founded in 2000 with the aim to successfully harness mRNA for medical purposes.

Last July, CureVac hired me as their Chief Scientific Officer. The work has proved extremely satisfying. To me, few things are more rewarding than working at the edge of knowledge in a whole new field of science, knowing and seeing that our work makes great contributions to health, medicine, and society.

How do you think mRNA research will evolve post-pandemic?

Most mRNA trials before COVID-19 involved only a small number of individuals. Now, millions have been vaccinated with mRNA. The push to make this happen produced a great deal of research and learning. We have the opportunity to capitalize on the work, and explore all kinds of interesting applications based on the data we have acquired.

Though many companies will continue to explore infectious diseases, new data can and will be used to develop cancer vaccines and treatments for indications where there is a need to express intracellular proteins, inhibitors, or modulators.

"Disruption" is a hard buzzword to avoid; mRNA therapies have certainly changed the industry for good. The fact that more people are getting involved is exciting; outsiders are now joining the field, and industry veterans are learning from them. We can expect input from engineers and physicists, sociologists and ethicists, chemists and IT experts – all will have a say in the future direction of the field. These collaborations are so important because they connect us to forward-thinking minds outside the pharmaceutical industry. There is scientific talent in other industries that we can tap into and help grow (and vice-versa!).

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