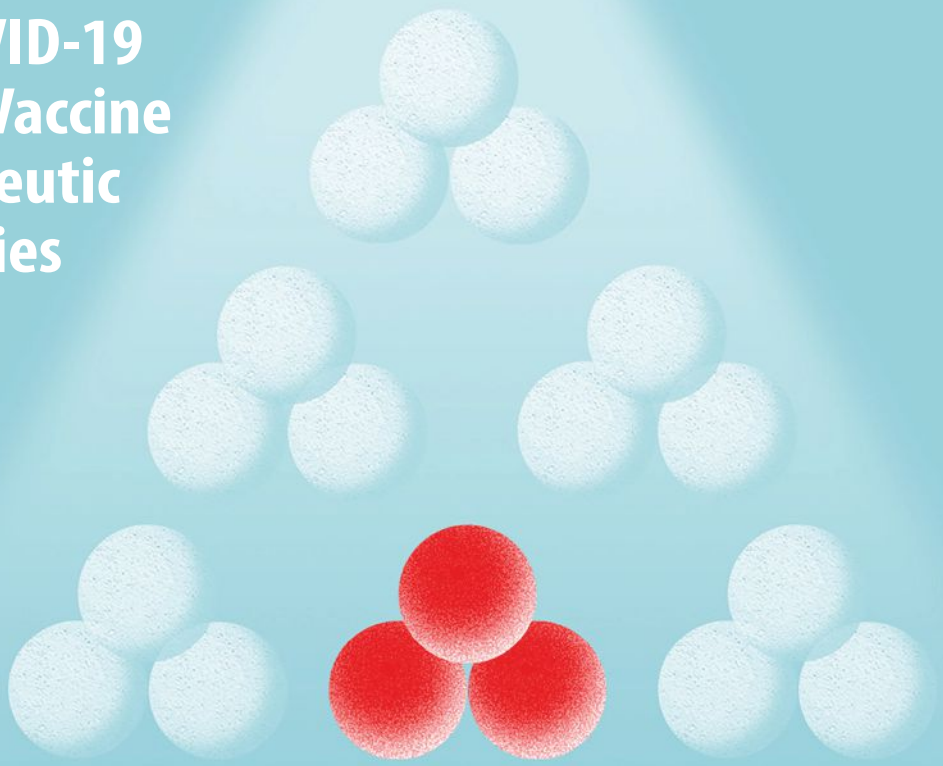
GEN Spotlight

mRNA

Ready to Make Its Mark

Beyond COVID-19 Lie Myriad Vaccine and Therapeutic Opportunities



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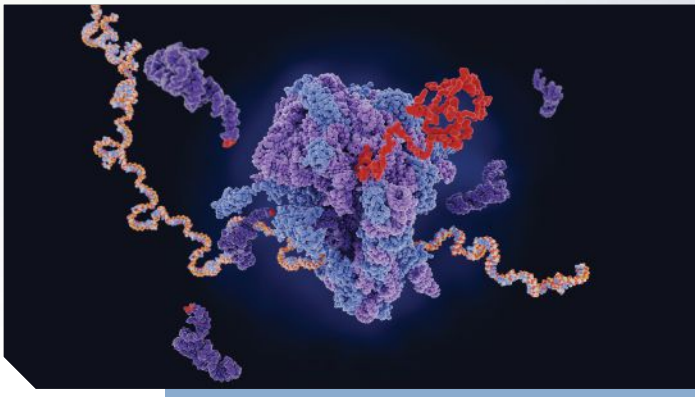
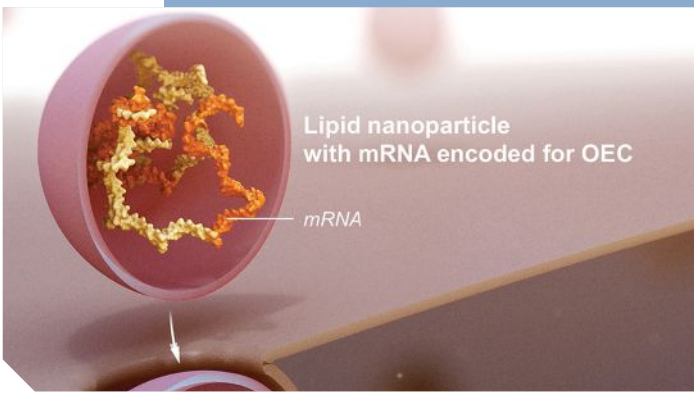
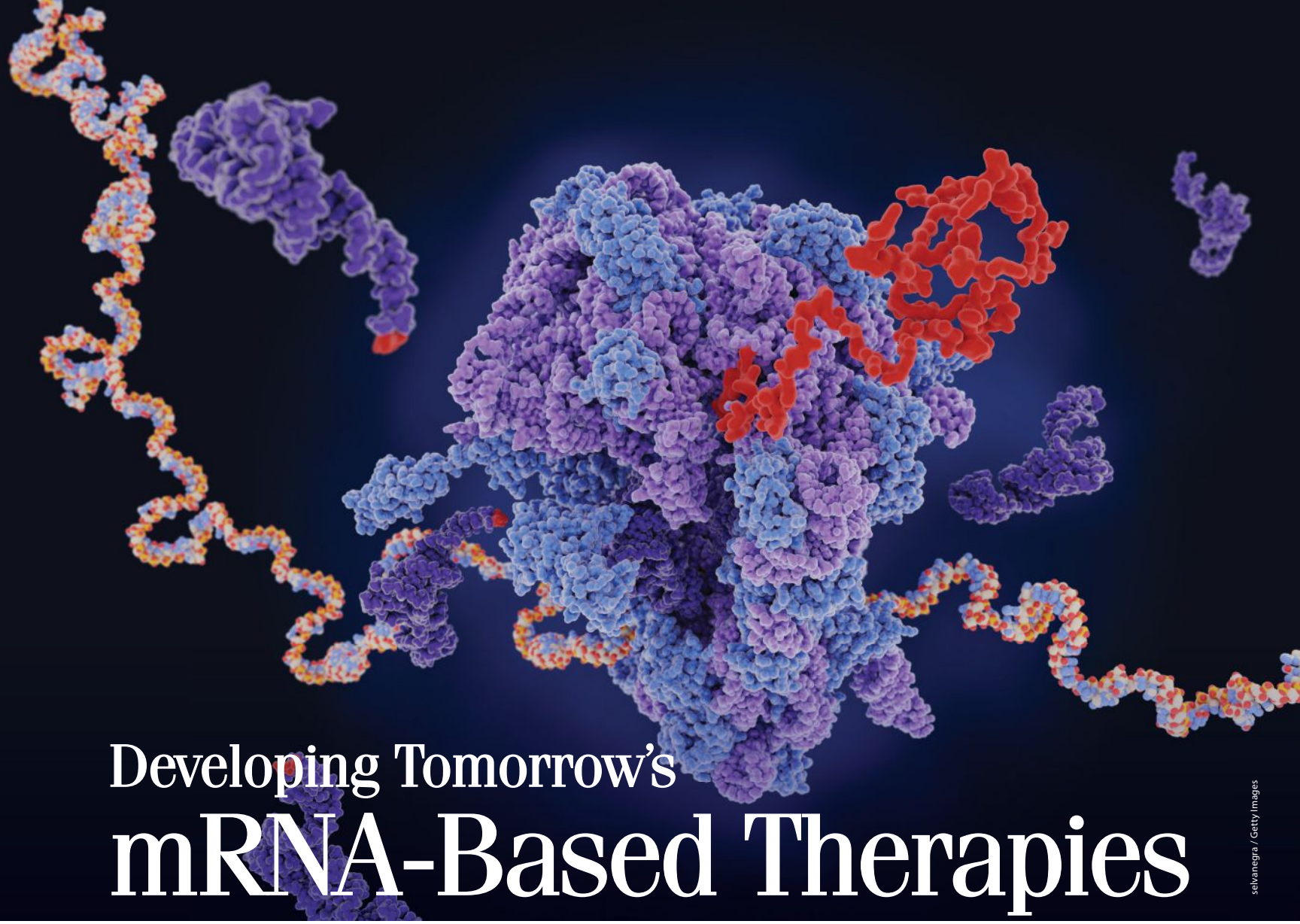






Developing Tomorrow's mRNA-Based Therapies

selvanegra / Getty Images

New technologies—such as circular mRNA, self-amplifying mRNA, programmable mRNA, and Cas13-encoding mRNA—point to more powerful mRNA-based treatments

By Mike May, PhD

During the COVID-19 pandemic, mRNA-based vaccines led the charge against the SARS-CoV-2 virus. These therapies would not have existed were it not for the painstaking mRNA research that began years before the pandemic. The lesson? It is important to be prepared. Let us remember that always, for we are bound to see more pandemics in the future.

More generally, we make a virtue of preparedness in all of our healthcare efforts. As the researchers interviewed here would no doubt agree, many of these efforts could benefit from applications of mRNA technology. The recently introduced mRNA-based vaccines represent just the start of what mRNA technologies can—and will—achieve.

Making ends meet

More than a decade ago, while spending a year working in a laboratory before entering medical school, a budding scientist got interested in circular mRNA, which (unlike linear mRNA) is in the form of a continuous, covalently closed loop. Today, that scientist—Robert Kruse, MD, PhD, clinical fellow, Harvard Medical School—recalls that he was "think-

ing about mRNA as a therapeutic and how to improve the properties." He wondered whether circular mRNA could outperform linear mRNA or even do things that linear mRNA couldn't.

Back then, no one knew if circular mRNA could even be translated inside a human cell. Kruse, however, showed that it was possible. (He did so while he was pursuing a medical degree and a doctorate in translational biology and molecular medicine at Baylor College of Medicine.) His work led to an early patent in the circular mRNA field.

But can circular RNA be made at scale as a drug? That turns out to be possible, too. In a test tube, autocatalytic, intron-splicing RNA from bacteriophages can yield "mRNA that will self-catalyze and create a circle by itself," Kruse explains.

The process was refined by a graduate student at the Massachusetts Institute of Technology. Indeed, that student—who is now better known as R. Alexander Wesselhoeft, PhD, founder and director of molecular biology at **Orna Therapeutics**—made the process efficient enough that most of the mRNA made in test tube reactions turns into circles that can then be used for vaccines or therapies.

Kruse observes that there is significant interest in circular mRNA for therapies. Some companies focused on circular mRNA—such as Orna Therapeutics and **Laronde**—are garnering sizable investment rounds. As he explains, circular mRNA is a promising therapeutic modality because the molecule's half-life is "three to four times longer" than linear mRNA's half-life. Consequently, circular mRNA gets expressed far longer.

An extended half-life could improve the use of mRNA in various applications. In vaccines, for example, a lower dose of circular mRNA might provide the same efficacy as a higher dose of linear mRNA, which could reduce the cost and potential side effects. Similarly, cancer therapies based on circular mRNA could allow less frequent treatments.

"Instead of giving linear mRNA to cancer patients every week, maybe circular mRNA could be given only twice a month," Kruse suggests. "That would be more convenient for patients, and it could be a lot less toxic for them."

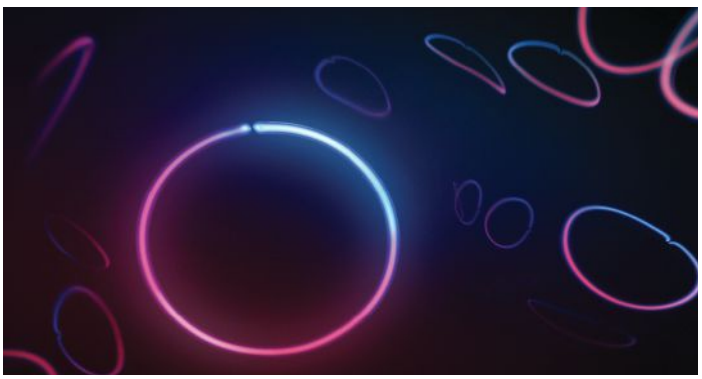
Still, Kruse notes that some of these applications remain theoretical. Although mouse studies show high levels of expression from circular mRNA, the same remains to be shown in humans. In addition, the tolerability of chronic dosing of mRNA therapeutics in humans also remains unknown. For both issues, Kruse says, "Scientists need to figure this out over time."

Making more mRNA

Besides increasing the expression of a desired protein, perhaps by using circular mRNA instead of linear RNA, it is possible to increase the level of a desired mRNA. One may simply increase the mRNA dose, or one may use a different kind of RNA, namely, self-amplifying mRNA (sa-mRNA). The second option has been researched for a number of years by scientists at **Seqirus**.

"In the case of sa-mRNA, the RNA goes into the cell, and there's an extra element on the mRNA molecule—replicase—that allows the RNA to replicate itself," says Ethan Settembre, PhD, vice president of research for Seqirus. "Each of those RNA copies creates the

Ethan Settembre, PhD
Vice President, Research
Seqirus



Recent discoveries that circular RNAs commonly occur in various species have prompted intense research efforts, such those supported by circRTrain, which created this image. Besides studying how circular RNA influences health and disease, researchers hope to find ways to use circular RNAs as biomarkers and therapeutics. For example, Harvard Medical School's Robert Kruse, MD, PhD, believes that circular mRNA therapeutics could be given at lower doses than linear mRNA therapeutics, since circular mRNA is more resistant to degradation.

Developing Tomorrow's mRNA-Based Therapies

protein of interest." So, the replication of mRNA makes more protein to generate an immune response.

The replicase comes from alphavirus. As Settembre says, "I often like to work with nature, rather than against it." In this application, nature's solution can increase the level of the mRNA so much that protein expression increases 80- to 100-fold, compared to using ordinary mRNA.

As a result, an sa-mRNA-based vaccine or therapy could be given at a lower dose and still achieve the same efficacy as an mRNA-based vaccine or therapy. If vaccines could have lower doses, vaccine manufacturers could produce more doses within a shorter period of time. According to Settembre, there is also "the potential to drop the reactogenicity of whatever the intervention is."

The effect of a therapy can also be extended over a longer period of time with sa-mRNA. In most existing mRNA-based therapies, protein expression is highest the first day and then drops over the following days. "For the sa-mRNA, the protein expression increases for about seven days after receiving an sa-mRNA-based vaccine," Settembre continues. "So, there is a somewhat prolonged expression."

The use of an sa-mRNA vaccine also triggers a stronger immune system attack. Like other mRNA vaccines, sa-mRNA vaccines can be used to create antibodies against a disease. In addition, sa-mRNA vaccines stimulate CD8-positive T cells, which are cytotoxic, to join the fight.

Getting mRNA deep in the lungs

Philip Santangelo, PhD
Associate Professor, Bioengineering, Emory University School of Medicine

Like any therapy, the method of delivery plays a crucial role in using mRNA-based methods. At Emory University School of Medicine, Philip Santangelo, PhD, an associate professor of bioengineering, studies ways to treat the lungs with mRNA-based therapies.

"Back in 2016," Santangelo recalls, "we proposed to DARPA using mRNA to deliver antibody-encoding mRNA to the lungs." At that time, he was trying to deliver the mRNA by using an intratracheal water spray instillation of mRNA-encapsulating polyethyleneimine-based nanoparticles.

To turn this concept into a clinical application, Santangelo started thinking about using a nebulizer to deliver the mRNA. The treatment, he explains, needs to get deep in the lungs, because "people die from lower respiratory infections, not upper infections."

In studies with rodents, Santangelo's team uses an Aerogen Solo or Aerogen Pro to deliver the mRNA formulation. "We made our own nose-only setups for delivery as they have very small dead volumes and allow us to screen formulations in vivo in rodents using very little material," he notes. "This is a huge advantage for screening formulations."

Tests in other animals require different approaches. In ferrets, for example, the researchers use a long tube with a nebulizer on the end. "For nonhuman primates," Santangelo continues, "we use a pediatric mask and nebulizer—very similar to what is used in humans."

Santangelo says that the mRNA used in the tests is "typically either formulated in lipid nanoparticles via microfluidics or with polymers via simple mixing prior to nebulization." He adds, "We have lyophilization protocols for our polymer."

Santangelo envisions using such treatments for a range of diseases, from bacterial and viral infectious diseases to various lung diseases, including pulmonary arterial hypertension and pulmonary fibrosis. Most recently, Santangelo and his team started treating the lung with mRNA that encodes for Cas13, which is a programmable RNase for targeting flu and SARS-CoV-2.

"We deliver an mRNA that encodes for Cas13 in addition to a guide targeted to the virus in question," he details. "We've seen that [using a nebulizer to deliver the polymer-formulated RNA] can be very effective for targeting RNA viruses in the lung." Changing the guide allows the treatment to be switched from influenza to COVID-19. Also, both guides can be deployed. Indeed, Santangelo reports

that his team is "making a version that targets both influenza and SARS-CoV-2 at the same time." That allows very efficient processing.

Cancer solutions from synthetic biology

Scientists and companies apply a wide variety of technologies, including mRNA, in hopes of developing new cancer treatments. "There are multiple advantages of using mRNA to treat cancer, because mRNA expresses more efficiently and is also safer than viral gene therapy vectors," says Tasuku Kitada, PhD, co-founder, president, and head of R&D at **Strand Therapeutics**. "When compared to DNA-based approaches, mRNA does not need to enter the nucleus." That's one less challenge when using mRNA-based cancer treatments.

The question is: How can mRNA be used most effectively against cancer? "Many companies are using mRNA to develop cancer vaccines or expressing cytokines, antibodies, and other factors that have anticancer effects," Kitada explains. "However, the problem with using these approaches is the risk of significant side effects." So, scientists at Strand use synthetic biology methods to develop mRNA-based therapeutics that reduce this problem. Kitada points out that Strand's technology allows "mRNA therapeutics to 'sense' biomarkers found in cancer cells and express proteins only inside cancer cells."

To create these very specific and targeted treatments, Strand scientists create programmable, long-acting mRNA therapeutics that will be delivered in nanoparticles. The result, says Kitada, is the ability to "precisely control the location, timing, and expression level of mRNA within the patient." Consequently, Strand's cancer therapies should be less toxic to patients.

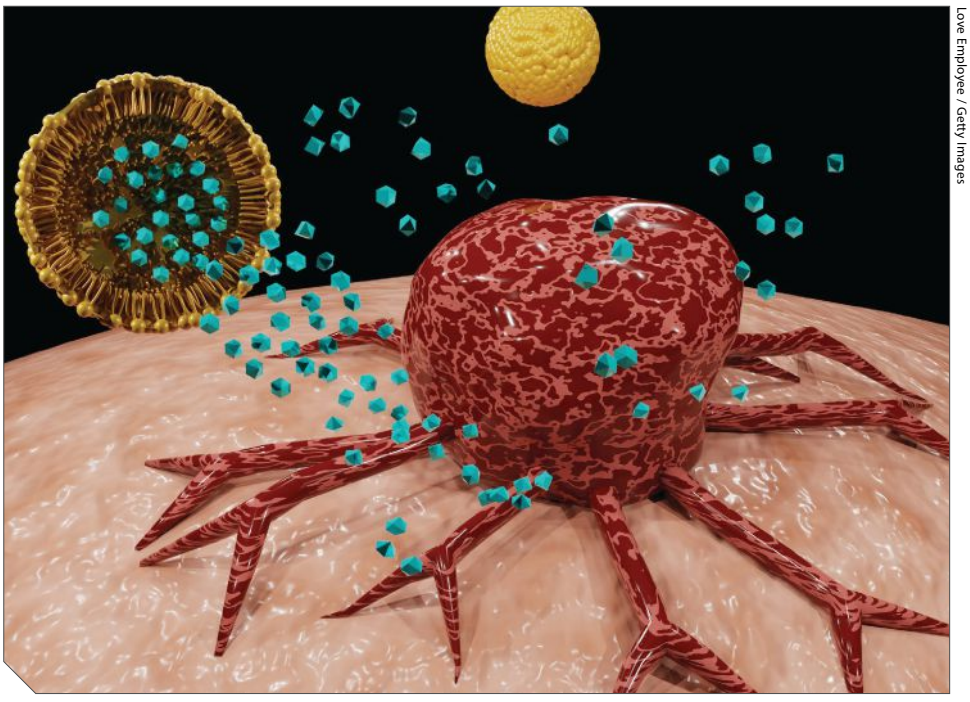
Current treatments under development include ones for solid tumors, such as melanoma and triple-negative breast cancer, as well as ones for blood-based cancers, including non-Hodgkin's lymphoma.

Tomorrow's treatments

Given the success of using mRNA against COVID-19 and the projects described here, many experts in healthcare expect even more powerful mRNA-based treatments ahead. Nonetheless, lots of work remains to be done, including much more basic research as well as an extensive number of clinical trials.

"It's still early days for the field of mRNA-based treatments," Settembre says. "There are so many improvements that we can make." GEN

Tasuku Kitada, PhD
Co-Founder, President, Head of R&D, Strand Therapeutics



Love Employee / Getty Images

Lipid-based nanocarriers may be used to deliver cancer therapeutics, as suggested by this image. One company that is using this general approach is Strand Therapeutics, which has developed a synthetic self-replicating mRNA for use with anti-PD-1. The company can program its mRNA therapeutics so that they express protein only when they "sense" biomarkers in cancer cells. Because the mRNA that is delivered is self-replicating, it lasts longer in the body, widening the therapeutic window.

With mRNA Technology, Vaccines and Therapeutics Stay on Message

The "m" in mRNA will reach the right audience—and have the desired effect—if better design, manufacturing, and delivery platforms become more readily available

By MaryAnn Labant

Messenger RNA (mRNA) technology is beginning to show its immense potential in medicine. Indeed, according to Sudha Chivukula, PhD, head of discovery biology, mRNA Center of Excellence, **Sanofi**, mRNA technology is inaugurating a new era in the development of vaccines and therapeutics.

"A true testimony for this technology will be revealed in the coming years as more and more clinical data from vaccine and therapeutic applications are made available," Chivukula declares. "Any infectious disease that can be prevented by a protein is a potential application for mRNA vaccines." He adds that mRNA technology has the potential to transform rare disease medicine, oncology, and personalized medicine.

Already, mRNA-based vaccine and therapeutic candidates are proliferating. Which ones should be prioritized?

Above. PCI Biotech specializes in photochemical internalization (PCI) technology, which enables the light-triggered release of molecules from endosomes. The molecules that are release may include nucleic acid therapeutics, which are notoriously difficult to deliver in large enough payloads inside a cell. In this image, a clinician uses laser light to maintain tight spatiotemporal control over the release of a cancer therapeutic.

To answer that question, developers must weigh various factors. The classic factors include potency, safety, and stability. With mRNA-based vaccines and therapeutics, however, there may be additional considerations. For example, delivery mechanisms may matter as much as mRNA sequences. Also, different mRNA platforms may vary with respect to flexibility (switching from one target to another, or even hitting multiple targets) and manufacturability (altering some processing steps while preserving others).

Weighing mRNA platform considerations

To narrow down the number of candidates to be evaluated in preclinical trials, developers can employ high-throughput automated robotic platforms. "Innate and adaptive responses to mRNA vaccines in animal models help to interrogate the immunological mechanism that underlie efficacy and reactogenicity," Chivukula elaborates. "New translational tools based on in vitro human immune system models could be deployed to better predict clinical outcomes."

Most common in pediatric vaccination, combination vaccines have successfully controlled several infectious diseases by combining antigens from separate and defined manufacturing process. While this adds complexity, it also creates opportunities to integrate both viral and bacterial antigens into a single vaccine dose.

Ideally, mRNA-compatible manufacturing platforms should be available, versatile, and broadly applicable, allowing for a simple swapping of the target gene sequence while largely maintaining the rest of the process. Such platforms accelerate vaccine development.

The "extras" that come with mRNA technologies are not limited to the flexibility and manufacturability issues considered thus far. Another extra is in the realm of immunity. Besides providing the target genes to train the immune system, mRNA is intrinsically immunomodulatory. Consequently, using mRNA can lead to augmented immune responses.

"mRNA vaccines mimic infection or immunization with live microorganisms and stimulate potent T follicular helper cell responses and germinal center B cell responses leading to generation of protective antibodies," Chivukula continues. "mRNA packaged in lipid nanoparticles can produce complex antigens in vivo, via engagement of endogenous ribosomal machinery, with proper post-translational modifications including assembly. All the mRNA requires is delivery into the cytosol for translation. No risk of host genome integration is incurred."

With mRNA technology, it is also possible to adopt in silico design approaches that could facilitate the development of multivalent vaccines. Such vaccines could be particularly effective against influenza.

"We recognize that addressing the broader consequences of influenza virus infection—such as severity of disease, extent of morbidity and mortality—is a challenge for any novel technology," Chivukula notes. "While it is a high bar to further improve our differentiated vaccines, we hope mRNA platform technology will help improve the effectiveness of vaccines against this persistent respiratory infectious disease."

Chivukula also observes that in silico design approaches could expedite vaccine development, helping developers "deliver high volumes on short timeframes." In addition to countering multiple strains, new vaccines for upcoming influenza seasons could be manufactured more expeditiously.

Changing the message

"With an mRNA platform, the only thing that changes is the 'm,' the message," says Brad Sorenson, president and CEO, **Providence Therapeutics**. "Our strengths are both in designing the mRNA and manufacturing it at high purity."

Founded in 2015, Alberta-based Providence focuses on identifying immunological targets and developing personalized cancer vaccines for glioblastoma, ovarian cancer, and breast cancer. Providence was set to begin clinical trials with a lead candidate in 2020, but then a different vaccine crisis arose, prompting the company to begin applying its technical expertise in mRNA to a SARS-CoV-2 vaccine.

"Our next step for our SARS-CoV-2 vaccine is to run a Phase III booster trial to address the endemic nature of the virus," Sorenson states. "Since there are now existing vaccines, emergency use authorization for approval is unlikely, and this has the effect of lengthening the path for regulatory approval. Based on this, we expect approval in early 2023."

The unstable mRNA needs protection during delivery,

With mRNA Technology, Vaccines and Therapeutics Stay on Message

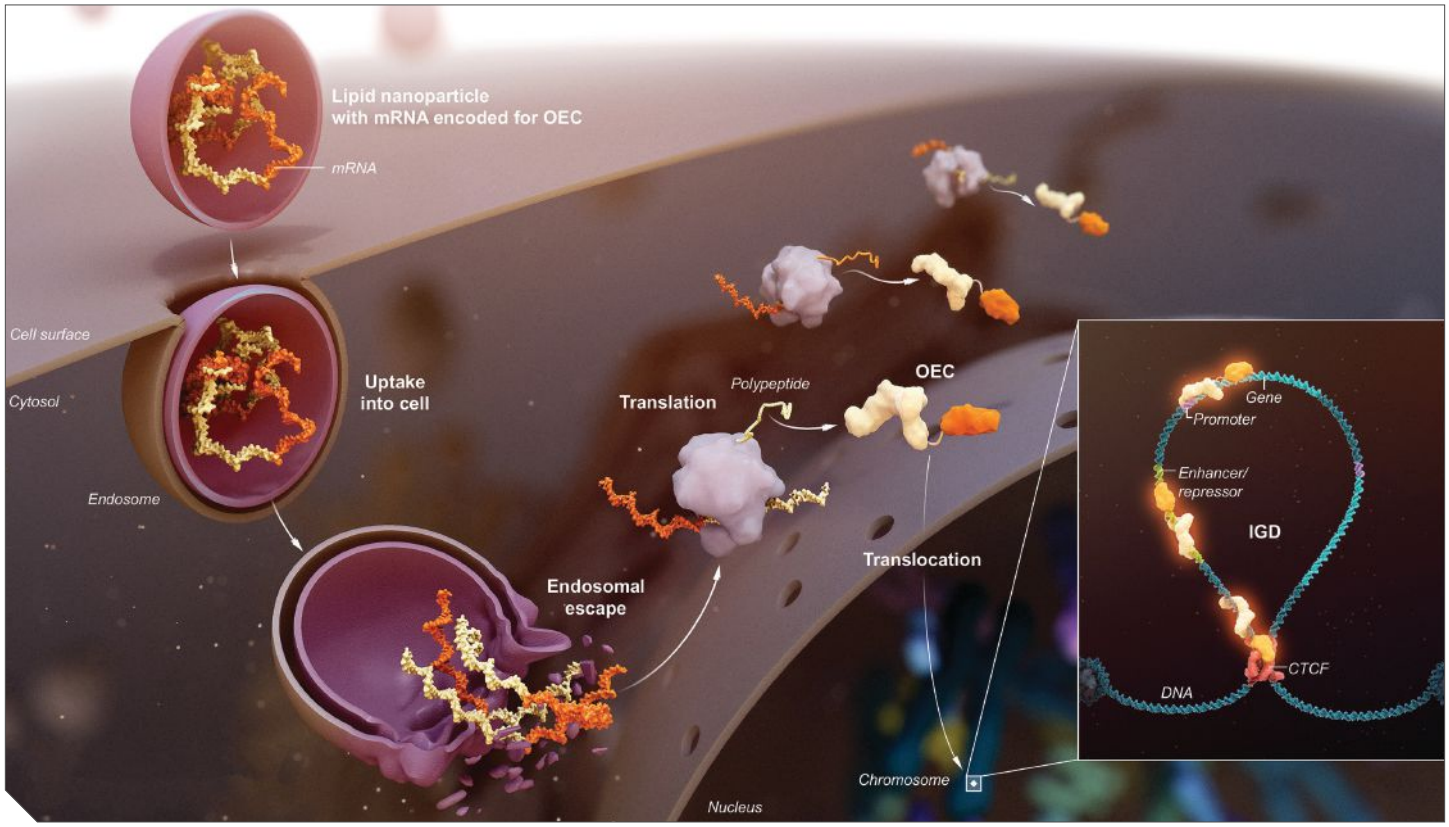
and lipid nanoparticles are typically used to encapsulate it for cellular delivery. To provide such protection, Providence started developing its own delivery technology. However, this technology would not have been ready for use with the company's COVID-19 vaccine, which needed to be developed extraordinarily quickly. So, delivery technology was licensed from **Genevant Sciences**. "Genevant's suite of technology showed that it worked for intramuscular (IM) injection," Sorenson notes.

Besides working on a vaccine for the parental SARS-CoV-2 strain, Providence is working on a vaccine for the Omicron variant as well as a long-term vaccine with a different mechanism to cover a wider class of coronaviruses. The long-term vaccine would also address variants. Preclinical work looks promising.

Providence is working with China-based **Everest Medicines** on COVID-19 vaccines. "We are looking for additional like-minded partners for full technology transfer to ensure our vaccine gets deployed globally," Sorenson remarks. "We also want to see more targets explored in general. Great academic scientists have identified targets and want access to mRNA platforms. We collaborate with them or support their research to design and manufacture the mRNA."

Harnessing epigenetics

"Epigenetics is nature's mechanism to control gene expression and cellular growth," says Mahesh Karande, president and CEO, **Omega Therapeutics**. "All of our genes along with their regulatory elements are located in loops of DNA called insulated genomic domains (IGDs)." He adds that



Omega Therapeutics has developed an epigenomic programming platform to design and engineer modular, programmable mRNA-encoded therapeutics called Omega Epigenomic Controllers (OECs). This image depicts the delivery, release, and functioning of an OEC. Inside the cell, the therapeutic mRNA expresses an OEC protein, which incorporates a DNA-binding domain and an epigenomic effector. The OECs target Insulated Genomic Domains (IGD) elements to influence gene expression, potentially treating or even curing disease.

at the base of each IGD loop there are CTCF proteins that insulate the IGD from "outside transcriptional machinery."

There are about 15,000 IGDs, and they act as fundamental regulators of the human genome. IGDs are ubiquitous in every cell, and they are distributed across the 23 chromosomes. "This is nature's operating system," Karande notes. "[It] has enabled us to create the OMEGA Epigenomic Programming Platform."

All the regulatory elements within an IGD, such as promoters, enhancers, and CTCF locations, have unique sequences that Omega refers to as epigenomic zip codes, or EpiZips. They can be used as drug targets to control gene expression. Although CTCF sequences are conserved evolutionarily, their flanking sequences in each IGD are unique. "All of the EpiZips that control single or multiple genes within an IGD can be targeted with high specificity, enabling the control of gene expression," Karande asserts.

Omega's epigenetic programming platform contains a proprietary database of thousands of EpiZips. To target the EpiZips, Omega deploys its aptly named Omega Epigenomic Controllers (OECs), which are modular and programmable mRNA medicines. OECs include a DNA-binding domain to home in on and bind to a specific EpiZip and an effector domain, which varies depending on the modulating effect required.

"We can target a gene with high specificity and tune it to the right level of expression with programmable durability to treat acute and chronic diseases," Karande explains. "We are not changing native DNA sequences but rather tuning the malfunctioning expression system and restoring it to normal levels. The approach is personalized to the disease, not the person."

An IND filing is expected this year for a therapeutic that addresses hepatocellular carcinoma by regulating overexpression of the highly autoregulated (and heretofore undruggable) MYC gene. "The beauty of our platform is that it is a deterministic, prospective, and programmable platform," Karande emphasizes. "Our ability to modulate disease is extremely broad.

"We tune genes pre-transcriptionally for controlled disease-specific duration. The specificity of targeting and the fact that our drug does not have to be continuously resident to have effect is why we believe our approach will lead to safer and more efficacious therapeutics than current modalities."

Treating the brain

RNA-based therapeutics against neurodegenerative diseases are being developed by **Biorchestra**, which bases its target discovery work on the research performed by Branden Ryu, PhD, the company's founder and CEO. Ryu discovered a novel brain regulator of inflammation, MiR-485-3p, that plays a role in common, neurodegenerative disorders such as Alzheimer's disease. To downregulate MiR-485-3p, the company developed an antisense oligonucleotide (ASO) as well as a novel nanoparticle for delivery of the ASO to the brain.

With CNS therapeutics, the holy grail is getting the drug to the brain.

The highly specific ASO is a relatively small molecule compared with other forms of RNA or RNA derivatives. "By combining designs of both the chemical elements and the bioinformatics elements, and by looking for potential overlap with known sequences, you significantly pare down the risks of off-target effects," says Louis O'Dea, MB, BCh, BAO, the chief medical officer of Biorchestra and the president of the company's North American subsidiary.

With central nervous system therapeutics, the Holy Grail is getting the drug to the brain. Some small-molecule drugs pass into the brain quite readily, but larger molecules like antibodies cannot. Although lipid nanoparticles are suitable for liver uptake, as they target the cholesterol receptor on liver cells, they have no affinity for the brain.

"We wanted to extend the lipid nanoparticle approach that facilitates the liver's uptake of RNA-based drugs," O'Dea

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RNA Leaders Explore New Frontiers in mRNA-Based Medications

Christoph Burgstedt / Getty Images

Encouraged by the success of mRNA-based COVID-19 vaccines, developers advance new technologies for leveraging or targeting mRNA

By Vivienne Raper, PhD

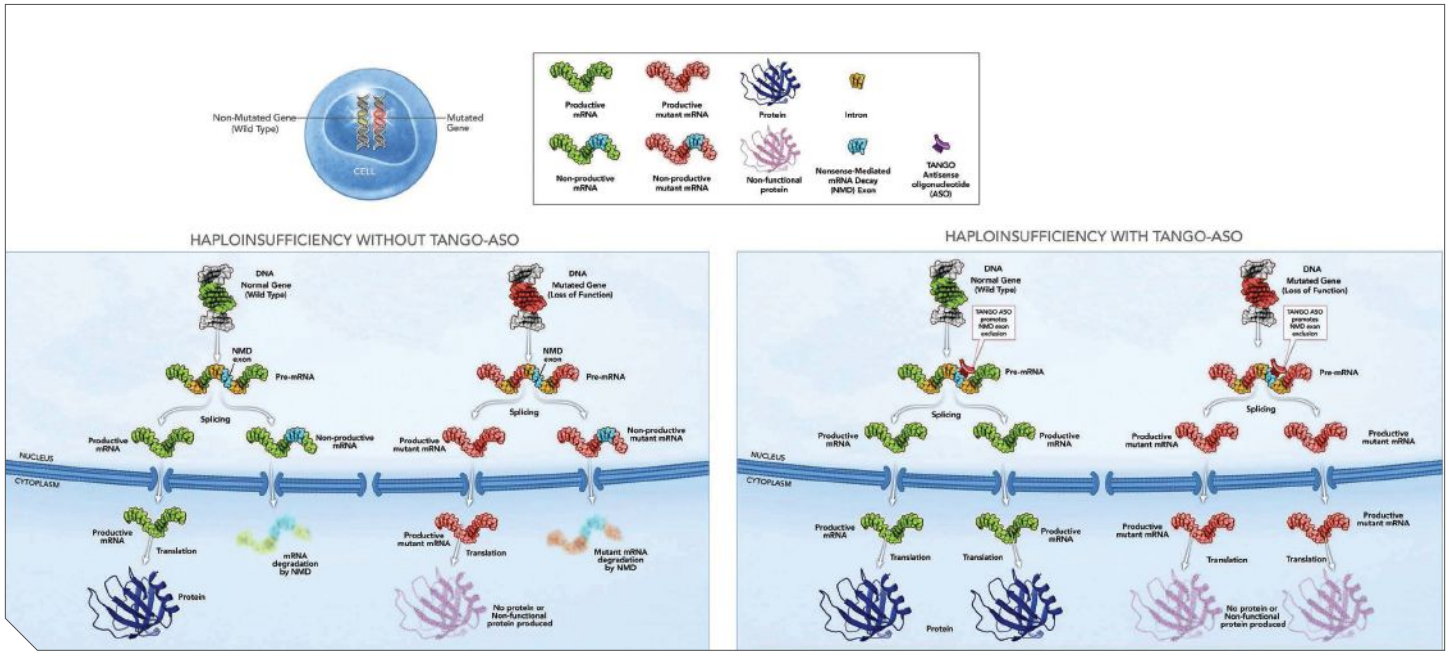
The history of mRNA-based medications is now well known. Early research revealed exciting possibilities. At the same time, it posed daunting challenges, such as mRNA's fragility. The molecule would break down before it could deliver its message to the right part of the body. Eventually, key challenges were addressed by technological advances. For example, carefully prepared mRNA came to be protected by fatty droplets (lipid nanoparticles). Indeed, the first mRNA-based vaccines to incorporate fatty protection were developed against the Ebola virus. However, mRNA didn't see worldwide commercial application until the COVID-19 pandemic led to the urgent development of vaccines by **Moderna** and **Pfizer-BioNTech**.

With billions of COVID-19 vaccines now delivered worldwide, mRNA technology commands interest. To share eagerly awaited news about the next generation of mRNA-based vaccines and therapeutics, the organizers of the RNA Leaders World Congress prepared a comprehensive program for their March 2022 event. The program covered subjects such as advances in delivery mechanisms, techniques for enhancing protein expression, and projects designed to bring mRNA-based and -targeted therapeutics to a wider group of patients. Outstanding presentations from the event are highlighted in this article.

Widening mRNA applications

Thanks to the COVID-19 pandemic, how mRNA vaccines work is widely understood. An mRNA vaccine delivers mRNA into muscle cells. There, the mRNA provides instructions for making the spike protein that is found on the surface of the COVID-19 virus. The protein is displayed on the cell surface, so the protein can trigger the immune system to produce antibodies against it. Subsequently, the immune system retains a memory and can respond quickly if it encounters a SARS-CoV-2 virus using a similar spike protein to enter body cells.

Messenger RNA has other applications, such as restoring the function of damaged tissue. "RNA therapeutics lends itself well to regenerative medicine. You need to inject it only once or twice to start a process to regenerate an organ or tissue," said



Stoke Therapeutics develops TANGO (Targeted Augmentation of Nuclear Gene Output) antisense oligonucleotides (ASOs), molecules that are designed to treat severe diseases that occur when a loss-of-function mutation occurs in one of the two copies of a gene. TANGO ASOs bind to premRNA, the precursor molecule to mRNA, to help a targeted gene—specifically, the functioning copy of that gene—produce more protein. Stoke hopes that its TANGO ASOs will treat and potentially reverse severe diseases such as Dravet syndrome.

RNA Leaders Explore New Frontiers in mRNA-Based Medications

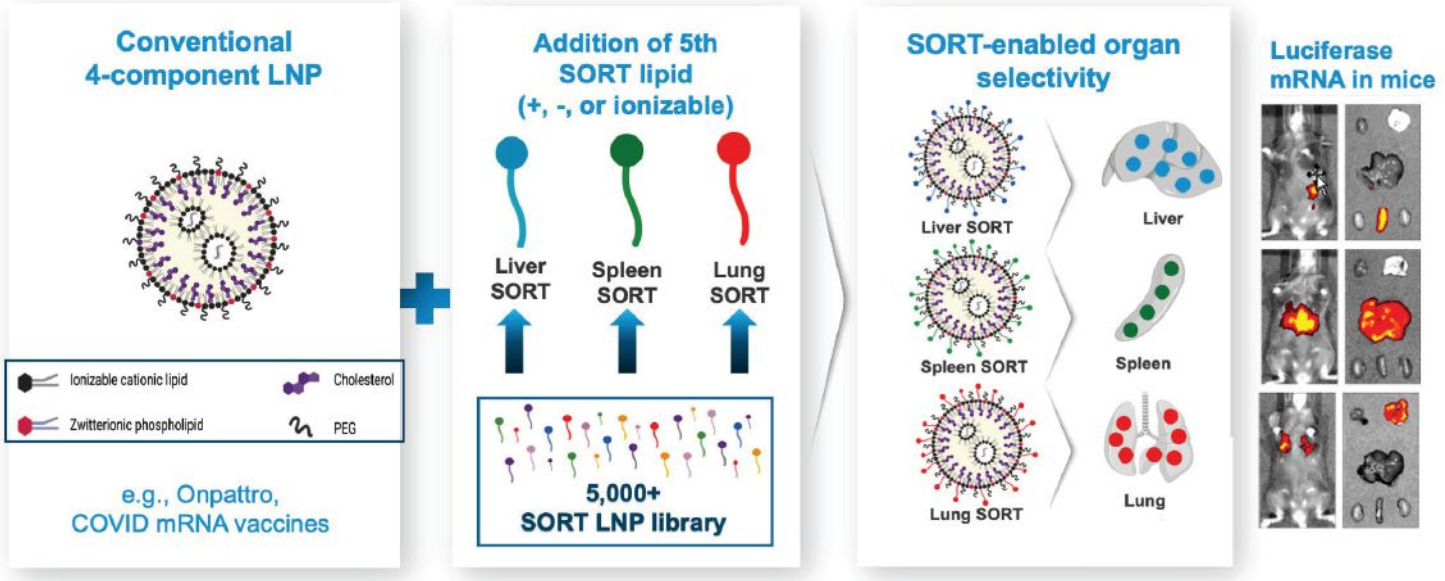
Klaas Zuideveld, CEO of **Versameb**, a company focused on the discovery and development of RNA-based drugs that can modulate protein expression and simultaneously attenuate multiple therapeutic targets.

At the RNA Leaders World Congress, Zuideveld presented on the company's successful preclinical work using mRNA to treat stress urinary incontinence (SUI). "SUI is a very large indication that affects lots of women," he pointed out. "Until 10 years ago, the mechanism of the disease wasn't well understood. And there are no therapeutic options whatsoever—just physical therapy and surgery."

SUI is caused by damage to the urinary sphincter. This damage, which often accompanies natural childbirth or results from aging, can prevent the urinary sphincter from closing properly. Versameb applies the mRNA directly to the urinary sphincter muscles. According to Zuideveld, the mRNA increases the expression of a naturally occurring protein and triggers the muscle's regenerative process.

The company uses a modified form of mRNA that is more effective than unmodified mRNA. That is, the modifications allow the regenerative process to be triggered with less mRNA. "We show a 20-fold increase in potency in our preclinical work," he asserted. "A small amount of mRNA injected into muscles leads to therapeutic levels of protein." (This observation followed the simulation of natural delivery in rats.)

A single injection of mRNA rebuilt within nine days the urinary sphincter of the rats to its pre-pregnancy functionality. Following this preclinical work, Versameb intends to perform a first-in-human study in patients with SUI. "Investigators and urologists are very excited about being to regenerate this muscle tissue," Zuideveld stated. "We're pleased to have [some of] these people as our supporters shaping



ReCode Therapeutics has licensed selective organ-targeting (SORT) technology from the University of Texas Southwestern Medical Center. The company is using SORT to generate lipid nanoparticles that incorporate five components instead of the usual four. The extra component is a selectable lipid that allows the lipid nanoparticle to target organs beyond the liver, such as the spleen and lung. The company say SORT enables new treatments for genetic lung diseases such as cystic fibrosis and primary ciliary dyskinesia.

our clinical program, and they are key to us starting a clinical trial before the end of the year."

Targeting mRNA with TANGOs

Another company is hoping to use mRNA technology to treat a serious disease caused by the loss of a single functioning copy of a gene. This company is targeting mRNA precursors with antisense oligonucleotides (ASOs), which are short, single-stranded RNAs that are complementary to mRNA. ASO are typically used to inhibit mRNA.

The company is **Stoke Therapeutics**. It has developed a technology platform—TANGO (Targeted Augmentation of Nuclear Gene Output)—to develop treatments that upregulate protein production. According to the company's website, TANGO ASOs bind to pre-mRNA, the precursor molecules to mRNA, to help target genes produce more protein.

"When we started the company in 2014, it was clear there are many diseases [in which there is one healthy copy of a gene and one mutated copy that fails to produce a protein]," said Huw M. Nash, PhD, chief operating and business officer, Stoke Therapeutics. "We were intrigued by the possibility of increasing expression of RNA to try to compensate for the missing protein."

At the time, many researchers were looking to use mRNA to synthesize the missing proteins, but this approach was largely unsuccessful because it triggered the immune system, Nash explained, and the applications of mRNA subsequently pivoted to vaccines. "We realized that ASOs could boost protein expression from the wild-type gene back to almost normal levels, and that if ASOs were applied at the right time, they could stop the disease from progressing."

The TANGO technology is unique, he explained, because most companies have focused on using ASOs to inhibit protein expression in diseases caused by a toxic protein, such as Huntington's. He claimed that companies that focused on boosting protein expression primarily targeted diseases where both functioning copies of a disease have been lost. In contrast, he emphasized, Stoke Therapeutics is focused on treating diseases caused by haploinsufficiency, the loss of a single functioning copy of a gene, which halves protein production.

Stoke Therapeutics currently has a lead candidate in Phase I/II studies for Dravet syndrome, a rare form of severe, drug-resistant epilepsy. "Although Dravet has antiseizure medicines, this is the first disease-modifying strategy," Nash asserted. "It can offer something important by replacing what's missing in these children." Stoke Therapeutics has a further candidate drug for autosomal dominant optic atrophy, which the company plans to move into Phase I/II studies in the second half of this year.

Boosting mRNA performance

With mRNA now regarded as a safe and proven technology, companies are now working to optimize the dosing and formulation. **ReCode Therapeutics** is a genetic medicines company targeting the underlying mechanisms of genetic lung diseases, such as cystic fibrosis (CF) and primary ciliary dyskinesia (PCD).

The company is aiming to encode a wild-type version of a healthy protein that can be incorporated into the lung. "We're also broadening beyond mRNA into gene correction of the lung," said Angele Maki, PhD, senior vice president and head of business development at ReCode. The company, she detailed, is aiming to make small corrections to the CF transmembrane conductance regulator (CFTR) gene to help restore lung function in CF patients.

To advance its program, the company has licensed a lipid nanoparticle formulation technology from the laboratory of Daniel Siegwert, PhD, an associate professor of biochemistry at the University of Texas Southwestern Medical Center.

"Most [lipid nanoparticle] formulations use four components, and [companies] alter the molecules that form each component," Maki said. "Ours is a five-component system, and the last one can be our choice of lipid."

By choosing whether to use an ionizable or charged lipid, users of ReCode's selective organ-targeting

RNA Leaders Explore New Frontiers in mRNA-Based Medications

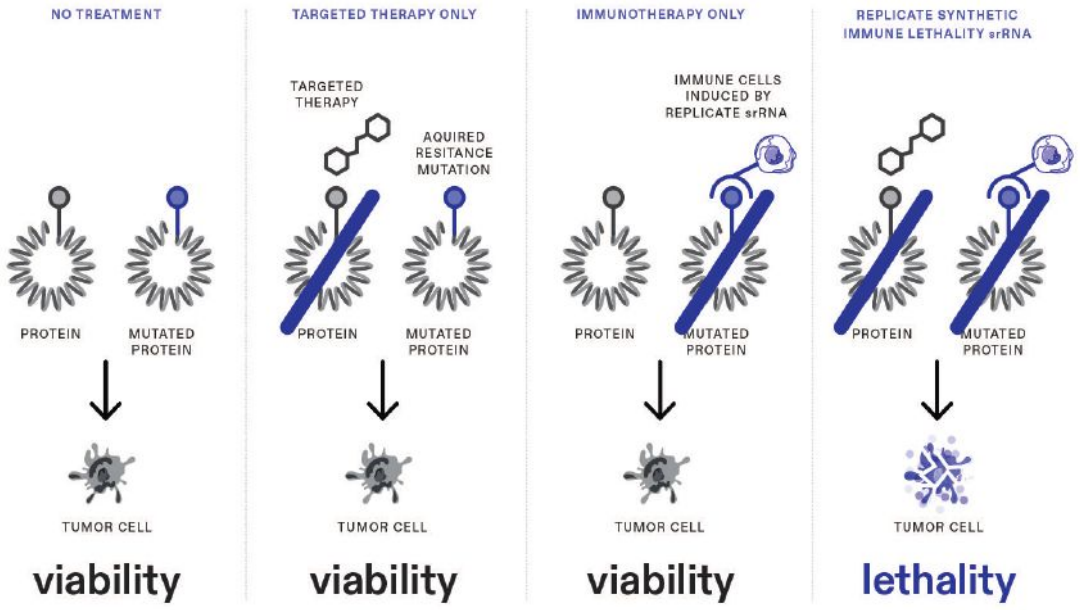
(SORT) lipid nanoparticle technology can target the lungs, spleen, or other organs beyond the liver. This differs from traditional lipid nanoparticles, which are taken up by the low-density lipoprotein (LDL) receptor in the liver. "When lipid nanoparticles go into the blood, they look like cholesterol," she explained, "whereas our SORT lipid nanoparticle is taken up by different receptors on target organs." The company hopes that its SORT technology can widen the applications of mRNA therapeutics and that, as gene therapy technologies improve, replacing the entire CFTR gene with a corrected copy will be possible.

Replicate Bioscience, meanwhile, has developed a self-replicating RNA (srRNA) platform with the aim of enhancing protein expression at lower doses than conventional mRNA therapeutics. "We think of conventional nonreplicating mRNA as an instruction manual the cell can use," said Nathaniel Wang, PhD, the CEO of the company. "That instruction manual falls apart after around two days."

The srRNA delivers a "copying machine" into the cell along with the instruction manual, Wang explained. "It delivers more protein for a longer time, and how much protein is produced is uncoupled from what is delivered to the cell." He added that this leads to a dramatic reduction in the dose of RNA that's needed—perhaps 1,000-fold or even 1,000,000-fold compared with a conventional mRNA.

The company, which was founded by srRNA experts, has addressed challenges unique to the manufacture of longer srRNAs. According to Wang, Replicate has succeeded through experimentation and the application of analytics technology, and that the company is confident about taking its technology into oncology and treating inflammatory disease. "We got funding only a year ago, and our pipeline already consists of a half a dozen products and multiple clinical entities," he asserted.

Among Replicate's prospective products is an srRNA therapy targeted to the mutations that a tumor is likely to undergo when it is treated with an aromatase inhibitor. "The tumor," he explained, "can mutate and be destroyed by the immune system, or it can revert to wild type and fall prey to the small-molecule drug." GEN



Replicate Bioscience develops self-replicating mRNAs that can overcome drug resistance in cancerous tumors. The graphic shows how drug resistance may arise whether a small-molecule drug or an immunotherapy is administered. (In the former case, a tumor may acquire drug-resistant mutations; in the latter case, a tumor may revert to wild type and keep growing.) If a small-molecule drug is administered along with self-replicating mRNAs that target common drug-resistant mutations, "synthetic immune lethality" may be achieved.

With mRNA Technology, Vaccines and Therapeutics Stay on Message

Continued from page 11

relates. "Our goal was to develop a delivery system uniquely focused on facilitating the brain's uptake of RNA-based drugs.

"Certain proteins are preferentially absorbed by the brain for its own benefits," O'Dea adds. "That understanding allowed us to attach a ligand to our nanoparticle that is recognized by the cells regulating entry into the brain. This ligand coating of the nanoparticle allows about 60-fold more of the drug to enter the brain.

"Our approach provides the potential to treat Alzheimer's disease. MiR-485-3p is also overactive in Parkinson's disease and amyotrophic lateral sclerosis. We have shown cognitive improvement in animal models of Alzheimer's disease, as well as functional improvement in animal models of Parkinson's disease and amyotrophic lateral sclerosis."

The overexpression of MiR-485-3p occurs in about 90% of Alzheimer's patients, leading to inflammation and beta-amyloid and tau protein overproduction. Biorchestra is not directly targeting these proteins but rather the process that causes their overexpression. The company's approach targets upstream processes and thereby reduces and clears both beta-amyloid and tau protein.

"Our central nervous system delivery platform can facilitate the work of other companies," O'Dea asserts. "We are open to discussing strategic partnerships to help patients access life-changing therapeutics more quickly."

Improving delivery

According to Anders Høgset, PhD, chief scientific officer, **PCI Biotech**, it is well known that problems with endosomal release represent a major barrier for the delivery of therapeutics, especially nucleic acid therapeutics. Even with the best systems used today (for example, lipid nanoparticles), only a few percent of the nucleic acid cargo will be released from the endocytic vesicles.

The company's photochemical internalization (PCI) technology enables the light-triggered release of molecules from endosomes. It has been used to enable the release of up to 50% of an endocytosed protein.

"Since the PCI endosomal release is triggered by illumination, delivery with PCI can be strictly regulated both in space and time," Høgset explains. "Delivery will only be induced in illuminated areas of the body after illumination occurs. PCI achieves targeted delivery of therapeutic molecules."

Problems with endosomal release complicate the delivery of therapeutics.

The photosensitizing molecule, fimaporfin, is designed to accumulate specifically in the membrane of endocytic vesicles. When illuminated, the fimaporfin molecule will take up energy from the light and be excited into a high-energy, unstable state.

The energy from this molecule's unstable state is then transferred to other molecules, most importantly to oxygen, to generate reactive oxygen species (ROS). The ROS will react with other molecules in the endosomal membrane, leading to membrane permeabilization so that the contents of the endocytic vesicles will leak into the cytosol. "Importantly," Høgset states, "illumination from a suitable light source is directed only at the site that the PCI effect is desired."

PCI Biotech's fimaVacc technology is used for immunotherapeutic purposes, especially to deliver antigens into the cytosol of antigen-presenting cells to increase MCH Class I antigen presentation and enhance cytotoxic T-cell responses. The antigen can be delivered in the form of peptides, proteins, or antigen-encoding mRNA molecules.

The company's fimaNAc technology is used to deliver various types of nucleic acids in all other therapeutic areas, such as regenerative medicine and treatment of various skin conditions. "The therapeutic aim of fimaVacc and fimaNAc is different," Høgset emphasizes. "Overall, PCI can increase the efficacy of nucleic acids, reduce their off-target effects, and achieve targeted, local delivery of therapeutics. We are open to collaboration in the nucleic acid therapeutic space." GEN

New Bead Technology Enables Commercial-Scale mRNA Purification

Affinity chromatography beads designed specifically for mRNA and FPLC eliminate toxic chemicals and purify tens of grams, removing a key bottleneck

By Gail Dutton

mRNA-based therapies catapulted to the forefront of public consciousness in the form of vaccines against the SARS-CoV-2 virus. After that success, mRNA therapeutics are now being developed for an ever-growing number of indications and applications that include cancer, cystic fibrosis, and infectious diseases, as well as gene and stem cell therapies based upon either gene replacement or gene editing.

However, the purification bottleneck must be solved before these therapies can be scaled up and produced in adequate quantities for clinical trials or commercialization.

"Traditionally, small-scale tools and products have been used to purify mRNA, such as reverse-phase high-performance liquid chromatography (HPLC), precipitation, and in some cases, cellulose-based chromatography," says Sirat Sikka, field application scientist at **Thermo Fisher Scientific** (Thermo Fisher). Those methods can be used to purify a few grams of mRNA and are adequate for bench work and some applications. Scale-up for clinical trials and commercialization, however, requires the ability to purify tens of grams or even tens of kilograms of mRNA.

"At Thermo Fisher, we understood the importance of mRNA and knew, even before the pandemic, that mRNA would be widely used," Sikka recalls. Since then, scientific and trade journals alike have cited mRNA therapeutics and vaccines as disruptive advances that can change the future of medicine and ease of manufacturing, and the ability to target pathways that otherwise are undruggable. Industry analyst Research and Markets predicts the global segment for mRNA therapeutics will grow from $46.7 billion in 2021 to $101.3 billion by 2026. That's a compound annual growth rate of 16.8%. (https://www.researchandmarkets.com/reports/5441159/mrna-therapeutics-and-global-markets-2021-2026).

Relieving the bottleneck

To be ready for such rapid growth in mRNA development, Thermo Fisher began developing a new affinity chromatography resin to isolate and purify mRNA long before the technology became a "household word." The team sought to develop a resin enabling improved recovery, increased purity, and enhanced reproducibility.

The POROS™ Oligo (dT)25 Affinity Resin—the resulting product—is a 50 µm poly(styrene-co-divinylbenzene) cross-linked porous bead functionalized with deoxythymidine (dT) strands that bind to mRNA via the poly-A tail (a chain of adenine nucleotides) that is on the three-prime end of all mRNA molecules.

One of the challenges is the size of mRNA. It is a large molecule —20 to 50 nm or greater in size that varies with construct length and solution composition—so there can be limitation to diffusion through the chromatography media and, therefore, hindrance to mass transfer, Sikka explains. Because the POROS™ beads have large throughpores the surface area available for interaction between the resin and mRNA molecule is increased leading to higher capacity. The large pores also result in a reduced mass transfer resistance, which helps to improve process efficiency and productivity.

"The POROS Oligo (dT)25 Affinity Resin minimizes the need to deal with organic solvents that are often used with HPLC systems," Sikka continues. "Using organic solvents in large volumes becomes an issue for manufacturing." She cites safety concerns regarding solvent disposal as well as the need to retrofit facilities to deal with them.

Instead of using toxic chemicals, after mRNA synthesis the column is loaded "with mRNA plus salt (for example, NaCl)." This neutralizes the negative charges on the RNA molecules so the poly-A tail can bind with the dT strands on the beads. Then, she says, "Elution can be performed using a low-conductivity buffer, or even water in some cases." Impurities and salt ions are washed away. With the sodium removed, the negative charges on poly-dT and the poly-A tail repel each other, freeing the purified mRNA and generating a recovery, typically above 90%, depending on the elution buffer and mRNA construct size.

From bench to manufacturing

The POROS Oligo (dT)25 Affinity Resin is designed for scalable purification processes,

so it is used to pack fast protein liquid chromatography (FPLC) columns. "The columns can be packed to multiple column size according to customers' needs, based on their process development and optimization," Sikka says. "We also have small-volume prepacked columns and Robocolumns. The 1 mL and 5 mL prepacked columns could be used with HPLC if needed, but that would only allow customers to purify very small sample volumes, may require re-plumbing and is usually not ideal for process development, so switching to FPLC is preferred. What customers mostly are looking for when they choose this resin is to scale-up purification, so they use it with FPLC systems."

Sikka says this affinity resin is a good option for scientists interested in developing a platform process that can be implemented for a variety of mRNA constructs. One of the benefits of using mRNA is that the same construct backbone could potentially be used to express different proteins. As a result, scientists can potentially use a platform process for multiple mRNA programs.

While researchers may switch out the gene of interest, "they could still be working with mRNA of comparable sizes," Sikka explains. "For example, depending on the protein they are trying to express, if the size range of all the constructs is between 4000 to 6000 bases, they could use this as the first capture step and develop a platform process." Working with much larger mRNA, such as self-amplifying could require some additional development.

As a platform technology, the first purification step with POROS Oligo (dT)25 would remove digested DNA template, nucleotides, enzymes, and buffer components. This could be the only step in the process before concentration and buffer exchange. If needed, a second chromatography step can be developed with POROS™ hydrophobic interaction chromatography (HIC) or anion exchange chromatography (AEX) resins to remove double-stranded RNA and uncapped or residual incomplete RNA transcripts.

"Starting with this resin during the research and discovery phase lets scientists continue using the same purification resin all the way to commercial manufacturing," she says. "This also eases the process of transitioning from one mRNA construct to another of similar size."

As she elaborates, "Once used in a process, the resin is already in the system and accepted by the customer's quality team." Additionally, scientists needn't redevelop their purification steps during each phase of scale-up, which minimizes the need to onboard a variety of chemicals and solutions or develop different buffer compositions, thus accelerating process development and reducing time to market. The resins are also reusable, which reduces the cost of goods.

"Importantly, POROS Oligo (dT)25 Affinity Resin beads are available for GMP production, and we provide the regulatory support package," Sikka says.

Transitioning to a new bead

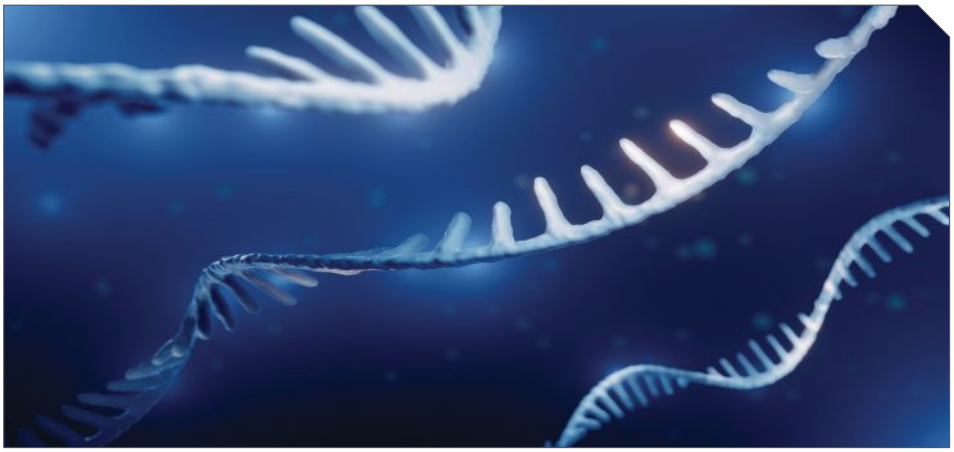
Switching to the POROS Oligo (dT)25 Affinity Resin is just a matter of ordering the prepacked columns if customers already use FPLC.

"A lot of our customers, however, are still at the research scale and are interested in scaling up," Sikka points out. "They don't necessarily have FPLC systems, and are trying to understand their options."

In those instances, she recommends ordering loose POROS Oligo (dT)25 Affinity Resin, which can be used in spin columns or microfuge tubes in a batch mode. If that works well for their purposes, they may consider investing in an FPLC for further purification optimization and scale-up.

"Thermo Fisher is very focused on the modern day," Sikka says, with solutions that address current and emerging purification challenges. Today, that means an intense focus on mRNA purification.

As interest in mRNA therapeutics continues to increase, the company's R&D is focusing on understanding the complexity associated with purifying self-amplifying mRNA, removal of product related impurities such as double-stranded RNA and abortive transcripts, and use POROS Oligo (dT)25 Affinity Resin and other technologies to resolve existing and emerging challenges. GEN



Before mRNA-based therapeutics can be produced in adequate quantities for clinical trials and commercial distribution, it will be necessary to remove a key bottleneck: mRNA purification. Existing methods usually purify just a few grams of mRNA, not the tens of grams or even the tens of kilograms needed. To improve mRNA purification, Thermo Fisher has developed the POROS Oligo (dT)25 Affinity Resin. Unlike alternate purification approaches, chromatography with a bead-based resin has excellent scalability. Notably, the resin selectively captures mRNA via the polyadenylated tail using simple salt and water purification steps. Artur Plawgo/Getty Images

ThermoFisher SCIENTIFIC

The way forward in mRNA purification

Find out more at **thermofisher.com/purification**