BioProcess International eBooks

mRNA

NEGOTIATING NEW MANUFACTURING HURDLES

Jake Adams, Dan Stanton, Millie Nelson, and Cheryl Scott



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Messenger RNA

Negotiating New Manufacturing Hurdles

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Research institutions, drug developers, and contract service providers all are taking the lessons learned during the COVID-19 pandemic to develop and launch new biopharmaceuticals based on messenger RNA (mRNA). First in this eBook, an analyst from CRB explores how mRNA-based platforms could facilitate vaccine and therapeutic manufacturing — and what new complexities will arise. Writers from *BioProcess Insider* and *BioProcess International* follow in highlighting trends for establishing local/regional mRNA capabilities and strategies for purification of this still-nascent modality.

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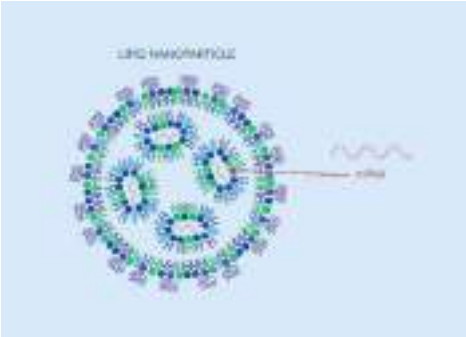





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RNA Is the New DNA

Manufacturing Trends Are Making Way for Emerging Therapeutics

Jake Adams

More than 30 years ago, when most other molecular biologists were putting money on the promise of DNA, Katalin Karikó was betting on a different horse. She was convinced that RNA — specifically, messenger RNA (mRNA), which holds the codes necessary for protein production — could be used to help assemble proteins upon uptake into cells. RNA does not integrate into a host cell's genome, as is the case with transfected DNA. Thus, RNA provides a means for transient protein expression in recipients. Karikó and her colleagues at the University of Pennsylvania even believed, most presciently, that mRNA could be used in vaccines.

Currently a senior vice president at BioNTech, Karikó now is being feted as a visionary whose company developed one of the mRNA vaccines that changed the course of the COVID-19 pandemic. The speed with which this clinically relevant but largely untested therapeutic modality was applied to treat a pandemic was astounding. Manufacturing enough mRNA to provide a global supply of COVID-19 vaccine ranks among the greatest achievements in the recent history of the biopharmaceutical industry.

Having watched mRNA's story unfold, research institutions and biopharmaceutical companies are taking lessons learned during the COVID-19 pandemic to develop and launch new prophylactics and therapeutics. Emerging applications include cancer vaccines, gene-editing tools designed to generate tumor-specific chimeric antigen receptor (CAR) T cells, and protein replacement therapies. Currently, nine RNA-based drugs have received regulatory approval, and many more have entered clinical trials (**1**). Among the 854 RNA therapeutics in development (from preclinical to preregistration stages), the most prevalent targets are cancers, rare diseases, infectious diseases, and neurological disorders (**2**).

What Types of RNA Are Used?

Once inside a cell, an RNA therapeutic either produces an antigen that will be recognized by the recipient's immune system or modulates protein production. RNA products can be divided into two broad categories depending on their function and size.

Products using **mRNA** tend to be long, and they encode information that cellular machinery translates into proteins. Liquid-phase chemical synthesis is used to make long mRNA

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molecules. One such method is in vitro transcription (IVT), which is used to manufacture large quantities of mRNA using readily available enzymes, nucleosides, and plasmid DNA (pDNA). IVT is fast and accurate, but it has limited ability to incorporate modified nucleosides.

Noncoding RNA drugs can be based on

- small interfering RNA (siRNA), which targets expression of a specific gene by binding to and thus inactivating mRNA
- antisense oligonucleotides (ASOs), which modulate mRNA translation
- microRNA (miRNA), which targets degradation of multiple mRNAs
- aptamers, which bind and alter the function of target proteins
- single-guide RNA (sgRNA), which is used in gene-editing platforms such as those based on clustered regularly interspaced palindromic repeats and associated protein 9 (CRISPR-Cas9) technologies.

Additive chemical synthesis is used to produce short noncoding RNAs and oligonucleotides. That process occurs in vitro with modified nucleosides that are designed to protect the resulting RNA from degradation, enhance its binding to other nucleic acids, and improve its stability in cells.

Advantages of RNA Drugs

Monoclonal antibodies (MAbs) and other protein biologics continue to provide therapeutic benefits for millions of people. Now, such products are being supplemented by RNA technologies to improve their tolerability, dosing, and efficacy. But how does RNA manufacture stack up against that for more-traditional biologics?

RNA-based therapeutics raise significant advantages for manufacturing compared with established biopharmaceutical technologies. In fact, the safety and effectiveness of the mRNA vaccines developed to mitigate the COVID-19 pandemic, along with the relative simplicity of the modality's manufacture, are encouraging influenza vaccine manufacturers to consider a transition from cell- to mRNA-based production processes. Those and other interested companies are hoping to leverage several key benefits, including speed to market, low costs, and smooth regulatory pathways.

Speed to Market: Development timelines are much faster for mRNA therapeutics than for other biologics. IVT is faster than cell culture, and large quantities of RNA can be produced with relative

Reinventing mRNA Production

Production of messenger RNA (mRNA) has come a long way, even since the onset of the COVID-19 pandemic, but it still involves lengthy optimization work. When transferring innovations from benchtop to industrial scale, scientists often need to optimize processes stepwise. To address that issue and speed up the transition from research and development (R&D) to market, Quantoom Biosciences designed the Ntensify platform, an RNA production system with an innovative approach for in vitro transcription (IVT).

Whereas traditional mRNA production approaches require multiple reaction vessels of different sizes, we propose reactor operation in repeat-batch mode in 20-mL increments. That small volume is appropriate because mRNA production is a biochemical rather than a biological process. Being independent of living organisms enables production to occur in a small platform process with predictable and controlled steps. Using an optimized IVT recipe, which we codeveloped with eTheRNA, subsequent purification is simplified into a single step, significantly improving product recoveries.

The automated Ntensify system comes with numerous benefits. Product development is accelerated by leveraging a preoptimized process at 20 mL without the need for further scale-up. The system is user-friendly, with both plug-and-play consumables and ready-to-use premixed reagents. Cost of goods (CoG) is reduced because the system generates high mRNA yields, works with affordable reagent premixes, diminishes workforce requirements, and operates within a small footprint.

The system is available in different models that address needs from screening and small-scale production for R&D purposes to good manufacturing practice (GMP)-compliant commercial production. This technology serves as the middle block in our comprehensive Nfinity platform, which connects mRNA production to upstream manufacturing of plasmid DNA and downstream encapsulation.

—Guillaume Roelandts, head of product management and marketing, Quantoom Biosciences (a Univercells company)

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ease. Moreover, pivoting to a different vaccine formulation or therapeutic indication is more straightforward and much less expensive for RNA-based processes because the requisite gene sequences can be altered easily to produce different antigens or noncoding elements. Despite sequence modifications, manufacturing usually involves the same raw materials and equipment across campaigns.

Low Cost of Goods (CoG): By eliminating cell-culture steps used during production of other biopharmaceutical modalities, RNA manufacturing processes reduce capital costs incurred for facility design and construction. IVT also generates higher product titers than those achievable with cell culture, enabling drug companies to operate at production scales suitable for single-use technologies while still producing hundreds of thousands of doses. Utility expenses are reduced by lower requirements for electricity, water for injection (WFI), and clean/sterilize-in-place (CIP, SIP) procedures.

Smooth Path to Regulatory Approval: RNA therapeutics and associated delivery systems are proven to be safe and effective. Rapid approvals for Pfizer–BioNTech's and Moderna's COVID-19 vaccines also underscore the fact that such products can be developed and taken to market faster than other biologics can. Having adequate safety data for a lipid nanoparticle (LNP) delivery system should shorten regulatory evaluation timelines for a vaccine with an mRNA sequence that has been altered to address a new variant of the same virus. That already has happened for the Pfizer and Moderna bivalent COVID-19 vaccines (**3**). Because changing the sequence of the mRNA contained within the same LNP should have little impact on the vaccine's safety profile or the manufacturing process, and because those elements already have received emergency authorization and/or approval, the regulatory burden should be greatly reduced, with drug efficacy being the main concern.

Obstacles To Face

The route to regulatory approval might be more straightforward for RNA products than for conventional biologics, but that doesn't mean the trip will be a cruise. For example, regulatory agencies have yet to establish guidelines on **purity levels** for RNA drugs. Impurity profiles for such products depend on which RNA-synthesis technique is applied. Manufacturers provide evidence to suggest that process-related impurities will not create efficacy or safety concerns, but regulators have not provided quantifiable guidance. Standardizing manufacturing processes and identifying allowable sequence-dependent impurity levels will go a long way toward clarifying the issue.

Other difficulties for manufacturing include the molecule's **in vivo and in vitro instability**. An early stumbling block for RNA as a therapeutic modality was its limited pharmacokinetic potential, due to degradation in vivo. Instability problems largely

The route to regulatory approval might be more **STRAIGHTFORWARD** for RNA products than for conventional biologics, but that doesn't mean the trip will be a cruise.

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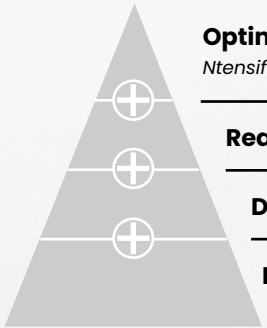

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have been overcome by including modified nucleosides in the RNA sequence and by protecting RNA during delivery with liposomes or LNPs. Even with LNPs, the in vivo instability of RNA means that therapies based upon it are only transiently effective, requiring redosing. Currently available mRNA vaccines have been burdened by cold-chain requirements, but RNA is relatively stable in vitro, both in liquid and solid forms. It is safe to assume that future mRNA therapies will be relieved of current ultracold-storage requirements as time-intensive shelf-life testing confirms the modality's stability.

The rapid increase in the production of RNA therapeutics has highlighted **supply-chain constraints**. For noncoding RNA, demand for heavily modified nucleosides and large volumes of pure acetonitrile have created supply stress. LNP-encapsulated mRNA therapeutics have created a large demand for the standard and customized lipids and cholesterols that make up the regulatory-approved LNPs. Many RNA therapy manufacturers rely on contract formulation and filling to take a manufactured drug substance to market, adding demand to an already constrained market for contract development/manufacturing organizations (CDMOs, CMOs). Overall, suppliers and contract fillers have been able to meet demand, but additional capacity will be needed to meet new demands and provide stability.

Tissue-specific delivery also remains difficult. Unlike vaccines, which usually do not need to target specific anatomical locations, the efficacy of therapeutic RNA depends on getting it to disease-associated tissue. Currently, intravenously administered RNA is difficult to deliver to tissues other than the liver.

RNA Delivery

RNA is negatively charged and hydrophilic; thus, it can be difficult to move it across cell membranes. Delivery systems protect RNA and facilitate its entry across cell membranes into the cytoplasm, where it either harnesses cellular translational machinery to make a protein or modulates protein expression. Viral vectors are a common approach for delivering DNA to a cell's nucleus, but such systems are expensive to manufacture and face payload limitations. Currently, researchers are pursuing alternative delivery systems that are more efficient to produce.

Encapsulation: Liposomes and micelles approximate cell-membrane structures, and they already have been used to deliver mRNA (**4**) and miRNA drugs during phase 1 clinical studies (**5**). Cationic polymers can be complexed with RNA as delivery vehicles. Such systems are being used in preclinical studies for RNA drugs (**6**). LNPs are spherical delivery structures that simulate viral vectors. Some LNPs have received US Food and Drug Administration (FDA) approval and have been applied in marketed products such as the currently available mRNA vaccines for COVID-19 and Alnylam's siRNA drug, Onpattro (patisiran).

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Conjugates: *N*-Acetylgalactosamine (GalNAc) is an example of a molecule that, when conjugated with siRNA, enhances therapeutic stability, encourages cellular uptake, and enables tissue targeting — in this case, selective binding to hepatic receptors. Currently, three GalNAc-conjugated siRNA drugs have received FDA approval (**7**). The success of GalNAc for liver targeting has prompted a flurry of exploration into other simple conjugates and modifications designed to increase specificity to other cell types, such as solid tumors and central nervous system tissues. Such developments are showing promise in preclinical and early clinical studies.

Needs for Internal and Outsourced Manufacturing

When industry experts were asked in 2021 about whether they intended to produce RNA in house or use a CDMO, 60% chose the latter (**8**). A possible explanation is that drug developers needed to produce relatively small quantities of RNA, making outsourced production a sensible strategy. One year later, companies involved in RNA production signaled a marked increase in the amounts of RNA that they intended to produce — and greater desire to manufacture that material internally (**9**).

A Dynamic Scale: The scale of RNA production that a company pursues depends on the type of therapeutic to be manufactured and its life-cycle position. Personalized medicines can require as little as microgram quantities of RNA for clinical trials, whereas large-scale commercial operations for some drugs, including vaccines, can necessitate production of more than 10 kg per year. Biopharmaceutical companies are planning to increase the scale of RNA production dramatically over the next five years. They also are targeting a wide range of scales, reflective of diversification in pipelines to include different RNA modalities (**9**).

Looming Capacity Constraints: Biopharmaceutical companies mounted a rapid manufacturing response to needs for mRNA-based vaccines against COVID-19. Whether such mobilization of resources continues at the same pace will depend on what direction the pandemic takes, whether boosters will be needed for years to come, and whether mRNA is harnessed to prevent other infectious diseases, such as influenza. Even if demand for SARS-CoV-2 vaccines does not increase, the many RNA therapeutics in the wings might require significant manufacturing capacity.

Demand for Contract Manufacturing: CDMOs will be needed to meet anticipated demand for RNA production. Several such companies have made sizable capital investments over the past couple of years to increase manufacturing capacity for large volumes (>100 kg/year) of both messenger and noncoding RNA. These financial commitments — and more expected over the next few years — should provide adequate manufacturing capacity globally. However, considering the ~80 oligonucleotide therapeutics in either phase 2 or 3 clinical trials (**10**), one blockbuster success story might be all it takes to alter the capacity landscape considerably.

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Even if demand for SARS-CoV-2 vaccines does not increase, the **MANY** RNA therapeutics in the wings might require **SIGNIFICANT** manufacturing capacity.



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Modular Facilities: RNA manufacturing does not require the large stainless-steel bioreactors used to make MAbs and other protein biologics. That factor has opened the door for modular facilities — complete with offices and warehouse space — that can be assembled rapidly when and where they are needed. Facilities can be built closer to patients, requiring smaller investments than what would be needed for more-traditional biomanufacturing infrastructure. Modular facilities can be designed to accommodate multiple mRNA products simultaneously. Such facilities also simplify supply chains and shipping. Thus, regional mRNA plants are likely to increase as companies seek to meet local demand.

The RNA Future Is Here

Given their adaptability, short development times, low manufacturing capital, low operating expenses, and smooth regulatory path, RNA-based therapeutics are being enthusiastically embraced by researchers and biopharmaceutical manufacturers. But these drugs would not be gaining traction if they didn't work — and not only as life-saving vaccines. Because they enable manufacturers to alter a gene sequence but continue to use the same processing equipment and delivery systems, RNA technologies represent a plug-and-play option for therapeutic production. We now can say confidently that the question is no longer whether RNA technology will bring forth the next wave of biopharmaceuticals, but rather, what it will be used to treat next.

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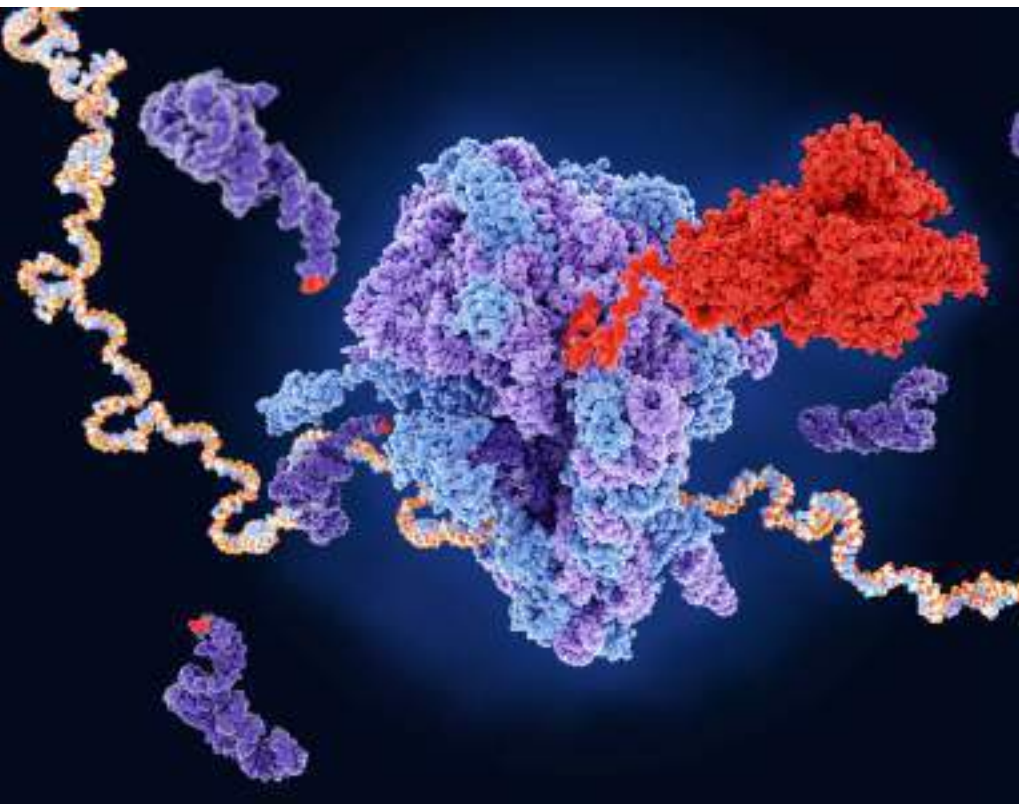

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Mapping Out mRNA Manufacturing Trends

Selections from *BioProcess Insider*

Dan Stanton and Millie Nelson, with Brian Gazaille

The emergency authorization, regulatory approval, and successful commercialization of Pfizer–BioNTech's and Moderna's respective mRNA vaccines for SARS-CoV-2 have ignited considerable interest in the modality across the biopharmaceutical industry. Drug developers and contract manufacturers alike are working quickly to establish mRNA capabilities. Often, such efforts emphasize regional and/or decentralized networks for cost-effective manufacturing operations. Below are recent articles from the *BioProcess Insider* that highlight the expanding geography of mRNA vaccine production.

Establishing Localized mRNA Manufacturing: From the United Kingdom to Rwanda

In June 2022, biopharmaceutical companies BioNTech and Moderna each announced expansion efforts for their messenger RNA (mRNA) manufacturing operations. BioNTech has initiated construction of a modular, scalable mRNA plant in Kigali, Rwanda, while Moderna has broken ground on a new facility in the United Kingdom.

BioNTech's facility will bring vaccine manufacturing capabilities to Africa. Initially, the plant is expected to produce ~50 million doses of mRNA vaccine per year, specifically to address the public-health needs of African Union member states. The 30,000-m^2 facility will be equipped with two BioNTainer modular cleanrooms to manufacture a breadth of mRNA-based vaccines, including the Pfizer–BioNTech COVID-19 vaccine (**1**). BioNTech's expansion follows an October 2021 announcement about deals that it struck with the governments of Rwanda and Senegal.

Tedros Adhanom Ghebreyesus (director-general of the World Health Organization) said, "The COVID-19 pandemic has exposed the need for significantly greater local production of vaccines and other essential products in all regions of the world, especially in Africa, which relies heavily on imported products and was left behind in the global rush for COVID-19 vaccines." He added, "I welcome BioNTech's efforts to establish manufacturing sites in Rwanda, Senegal, and South Africa, as well as its plans to commence clinical trials for its malaria vaccine candidates later this year [2022]."

*Written by **Dan Stanton**, this article originally appeared in* BioProcess Insider *on 23 June 2022; https://bioprocessintl.com/bioprocess-insider/global-markets/from-uk-to-rwanda-mrna-driving-localized-manufacturing-model.*

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Moderna has announced an agreement with the United Kingdom to establish an mRNA manufacturing facility for rapid pandemic-response capabilities and production of respiratory-virus vaccine candidates. Once it comes online in 2025, the facility is expected to produce vaccines against COVID-19, seasonal influenza, respiratory syncytial virus (RSV), and other pathogens for the UK market, with capacity for up to 250 million doses per year (**2**). Moderna has not disclosed the facility's location, cost, or funding source. But *Reuters* reports that Moderna has pledged a minimum R&D investment of US$1.35 billion in the United Kingdom (**3**).

Moderna also has announced plans to establish a $500 million mRNA plant in Kenya. The facility will be capable of producing up to 500 million doses of vaccine per year (**3**).

When the COVID-19 pandemic began in 2020, world governments scrambled to bring their citizens access to vaccines. The United States spent $18 billion on Operation Warp Speed to ensure sufficient vaccine manufacturing capacity, whereas EU countries funded billions of doses, particularly of Moderna's and Pfizer–BioNTech's COVID-19 vaccines.

Traditionally, vaccine production has been geographically concentrated. Merck & Co., for example, produces its global supply of human papillomavirus (HPV) vaccines from two sites in the United States. However, increased supply-chain pressure and global vaccine demand are encouraging governments to establish manufacturing and distribution networks inside their borders to secure advantages against future disease outbreaks. Large-scale mRNA manufacturing can be performed in relatively small facilities, emboldening governments and vaccine companies to invest in local production.

Normax Seeks To Slash mRNA Manufacturing Costs

Vaccine development and manufacturing company Normax Biomed seeks to reduce the costs of its mRNA vaccines through decentralized, modular facilities and a socially responsible financial model. Normax reported in July 2022 that it can produce and sell mRNA vaccines for US$4 per dose.

According to the company's mission statement, it is committed to decreasing mRNA vaccine costs while delivering sustainable returns for impact investors. As Peter Jensen (CEO and chairman of Normax) put it to potential investors, "We're on a social mission to redress global healthcare inequity caused by the profiteering pharmaceutical industry." He explained, "Currently, leading COVID-19 mRNA vaccine producers charge governments $8–35 per dose. At Normax, we can manufacture an mRNA vaccine for less than $2 per dose and charge just $4 per dose for large-scale delivery of mRNA vaccines on advance purchase agreements (APAs)." During large-scale campaigns, raw materials have cost Normax about $1.20 per dose, plus 80 cents per dose for manufacturing. "At high volumes, we believe that these [values] are relatively standard industry parameters."

*Written by **Dan Stanton**, this article originally appeared in* BioProcess Insider *on 21 July 2022; https://bioprocessintl.com/bioprocess-insider/global-markets/with-e300m-in-hand-normax-looks-to-slash-mrna-costs.*

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PC	Not Compatible	Semi-Compatible	Not Compatible	Not Compatible	Not Compatible
PP	Compatible	Compatible	Semi-Compatible	Compatible	Semi-Compatible
PE	Semi-Compatible	Semi-Compatible	Compatible	Compatible	Semi-Compatible
PS	Not Compatible	Semi-Compatible	Semi-Compatible	Semi-Compatible	Semi-Compatible

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Founded in 2020, Ireland-based Normax is developing a universal coronavirus vaccine (UCV) based on its mRNA technology. It also has launched the mRNA UCV Consortium as it expands into a global manufacturing network while operating on a contract development and manufacturing organization (CDMO) business model (**4**). The company is preparing to open five modular "vax factory" manufacturing facilities, the first of which will be located in Cork, Ireland. Jensen said, "It's still a small country, but it has a lot of talented people, and Ireland is a fantastic domicile to grow business." Normax will establish other facilities in Switzerland, the United Kingdom, the United States, and South Africa. "The capacity of each vax factory will be ~50–300 million mRNA vaccine doses per year, with dependencies as per industry standard variables."

Normax plans to develop up to 100 mRNA vaccines, which are to be manufactured by 100 modular vaccine factories. Luxembourg-based private equity group GEM Global Yield boosted that strategy early in July 2022 through a significant investment. "Following the recent €300 million capital commitment in Normax," Jensen explained, "we plan to deliver research and development (R&D) of mRNA vaccines for COVID-19, tuberculosis, human immunodeficiency virus (HIV), and malaria." He noted that "in the event of another public health emergency, we also are committed to delivering future mRNA vaccines in 100 days to help ensure that COVID-19 is the last pandemic we will all suffer through."

Jensen said that the support shown by companies such as GEM Global Yield is good news for investors who are seeking out fundamental value opportunities. "Our Normax core business of mRNA vaccine R&D and manufacturing can deliver sustainable returns on fundamental healthcare needs that biologically will last forever. Pathogens are inevitable, but diseases are not."



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BioNTech Buys Novartis Plant To Help Bring mRNA Manufacturing to Singapore

In November 2022, BioNTech reached an agreement to acquire a good manufacturing practice (GMP)-certified facility in Singapore from multinational pharmaceutical company Novartis (**5**). BioNTech will convert the site into the country's first fully integrated mRNA production facility. In a press release, BioNTech reported that the new site could produce up to several hundred million doses of vaccine per year after expansion is complete. The announcement came 18 months after BioNTech said that it planned to bring mRNA manufacturing to Asia by building a facility in Singapore.

A spokesperson for Novartis confirmed that the company "has entered into an agreement with BioNTech to sell its Singapore small-molecules site assets." The agreement stipulates that "Novartis will provide engineering and product transfer services to help BioNTech establish its manufacturing footprint in Singapore." The representative added that the sale follows a decision announced in 2020 to discontinue operations at the site through a phased exit by the end of 2023. "Throughout the process, Novartis continued to

*Written by **Millie Nelson**, this article originally appeared in* BioProcess Insider *on 14 November 2022; https://bioprocessintl.com/bioprocess-insider/facilities-capacity/biontech-buys-novartis-plant-to-bring-mrna-to-singapore.*

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explore options [that could] allow the site to remain operational, and the opportunity to sell the site asset and support the setup of BioNTech's mRNA vaccines manufacturing operations in Singapore turned out to be the best option."

BioNTech expects the Singapore facility to come fully online in late 2023, enabling the company to produce mRNA-based vaccines and therapeutics across Asia at clinical and commercial scales. Sierk Poetting (chief operating officer of BioNTech) said, "We are excited to be moving a step closer to expanding our global manufacturing network to Singapore, supporting production of mRNA-based vaccines and therapeutics for the Asia–Pacific region." The acquired facility provides an "opportunity to accelerate the establishment of a state-of-the-art mRNA manufacturing facility and thus to create capacity more quickly for potential clinical studies and commercial supply of our mRNA vaccines and therapeutics for the region."

BioNTech noted that operations at the Singapore site might include production of the COVID-19 vaccine that the company developed in partnership with Pfizer as well as new oncology product candidates, assuming regulatory authorization. The site also has the potential to expand its production focus to other modalities such as cell therapies.

BioNTech has made a string of investments to bring mRNA manufacturing to different parts of the world. In October 2021, the German biotechnology company signed a memorandum of understanding (MoU) with the Rwandan government and Institut Pasteur de Dakar in Senegal to build an mRNA plant in Africa. One year later, the company laid out plans to construct a research and manufacturing center for such products in Melbourne, Australia.

BioNTech will begin recruiting immediately for its Singapore facility, expecting to create over 100 jobs by 2024, with positions in engineering, operations, quality assurance and control (QA/QC), finance, supply chain management, and human resources.

Novartis's spokesperson clarified that sale of the site "does not include the transfer of Novartis employees to BioNTech," but rather creates "certain potential employment opportunities for Novartis associates from the Singapore small-molecules site, and it will enable the site to continue the rich pharmaceutical manufacturing legacy in the area." Novartis said that it remains committed to Singapore through its other facilities and operations. The decision to sell this particular small-molecules asset "is part of Novartis's strategy to reimagine manufacturing through an industry-leading transformation process to build a fit-for-future operating model."

RVAC and A*STAR Partner To Build mRNA Capabilities in Singapore

RVAC Medicines, a developer of mRNA platform technologies headquartered in Singapore, will work with the country's Agency for Science, Technology, and Research (A*STAR) to establish a joint laboratory. The facility will be located at A*STAR's Bioprocessing Technology Institute (BTI), and it will be used to advance a

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*Written by **Millie Nelson**, this article originally appeared in* BioProcess Insider *on 23 November 2022; https://bioprocessintl.com/bioprocess-insider/deal-making/rvac-and-astar-partner-to-build-mrna-capabilities-in-singapore.*

preclinical production platform that enables generation of raw materials and potentially clinical-grade assets for development of mRNA vaccines and therapeutics.

Sean Fu (CEO of RVAC) explained that the partnership will provide local innovators and worldwide manufacturers with access to a production platform for preclinical asset development. Fu said that his company is "committed to providing the staff, equipment, and technological expertise, with support from our R&D teams around the world, to this endeavor." The collaboration will leverage RVAC's capabilities in mRNA, drug-delivery technologies, vaccines, clinical development, and commercialization. He added that BTI's knowledge in analytical sciences and biotherapeutic processing will be critically important. "Combined with A*STAR's knowledge and experience, [our] partnership has the potential to continue the advancement of mRNA as a new class of medicines." RVAC already has developed an mRNA-based COVID-19 vaccine, which was used in a first-in-human clinical trial in September 2022.

Ng Huck Hui (assistant chief executive of A*STAR's Biomedical Research Council) emphasized that "public–private partnerships are key enablers in translating science into impact." Combining RVAC's expertise with A*STAR's multidisciplinary R&D capabilities in infectious diseases, artificial intelligence, analytics, and protein engineering "will advance the development of mRNA vaccines and therapeutics and contribute to better health outcomes for Singapore and beyond."

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Purifying mRNA

Downstream Options Continue To Multiply

Cheryl Scott

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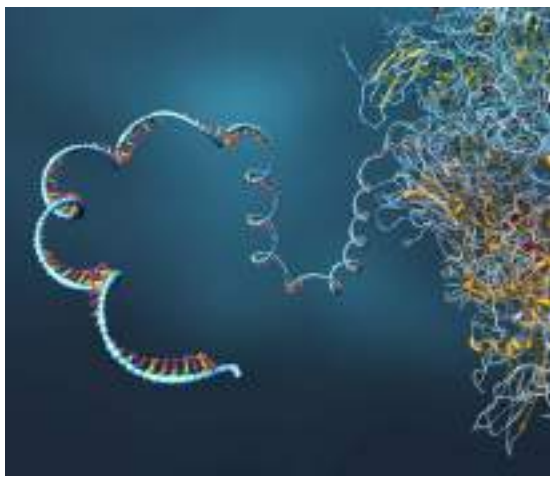

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With a synthetic production process that *technically* doesn't involve messy cells and culture media, mRNA manufacturing should have a much more straightforward downstream process than that of biologics such as antibodies and other proteins. And it does — to some extent. Consider, though, that plasmid DNA (pDNA) used as starting material is itself purified from a microbial cell culture. As with many other aspects of mRNA manufacturing, the reality of purification is much more complicated than might be expected:

> In vitro synthesis of RNA requires a purified DNA plasmid, purified RNA polymerase, metal-ion enzyme cofactors, and raw-material nucleotides. RNA in the completed transcription mixture is massively more abundant than the plasmid or enzymes. Purification should be simple. But it's not. Transcription does not always proceed to completion. In vitro transcription (IVT) mixtures commonly contain a substantial proportion of incomplete transcripts. Posttranscriptional variants include subpopulations of *double-stranded RNA* (dsRNA). This refers to species in which part of the sequence doubles back on itself and forms hydrogen-bonded bridges to complementary sequences on the same molecule and/or with other RNA molecules. When dsRNA is administered in vivo, recipient cells sense it as an invading virus. It must be absent from a final product to prevent triggering a toxic cytokine storm and to facilitate proper translation of the single-stranded RNA (ssRNA). (**1**)

Separation and Purification

Technologies for mRNA manufacturing have evolved at breakneck speed over the past few years, and purification is no exception to the trend. Following a similar path to that of protein therapeutics decades before, mRNA developers moved away from early laboratory-scale precipitation methods toward chromatographic separations for high-purity recovery, scalability, and reproducibility. Suppliers such as Astrea Bioseparations, Thermo Fisher Scientific, and BIA Separations (Sartorius) have stepped up to provide solutions for companies working on downstream mRNA processes to comply with good manufacturing practice (GMP) requirements. Options include affinity, anion-exchange, hydrogen-bonding, hydrophobic-interaction, and reversed-phase chromatography resins, monoliths, membrane adsorbers, and other media.

Thermo Fisher Scientific recently introduced a magnetic-bead–based option for mRNA production and purification processes, initially for research and development (R&D) scale (**2**). Dynabeads technology works "by holding onto the DNA segment (or template) where mRNA synthesis takes place." When synthesis is complete, the reusable beads can be pulled out of a reaction mixture magnetically in a capture step that initiates downstream processing. Ribosomal, transfer, and other small RNA molecules do not bind to the beads; only polyadenylated mRNA species are captured. After the beads are washed, mRNA can be eluted in 10 mM tris HCl buffer, with a neodymium magnet immobilizing the beads during buffer changes. "Pure mRNA" can be isolated within about 15 minutes, according to Marie Bosnes, a senior staff scientist in sample preparation for the supplier. Her company touts automatability, scalability, and minimized footprints among the benefits of this technology.

In 2021, Astrea Bioseparations partnered with Nanopareil, a fellow member of the Gamma Biosciences group, to introduce a nanofiber-membrane technology for separation of a broad range of biomolecules, including DNA and RNA (**3**). "Based on composite electrospun cellulose nanofibers with uniform and consistent composition and pore sizes, the matrix is physically or chemically functionalized for different separation modalities: e.g., ion-exchange (IEX), hydrophobic interaction (HIC), affinity, and steric exclusion." The company believes that this technology could present a product-capture alternative for developers seeking to minimize downstream processing costs.

BIA Separations jumped into the mRNA waters with both feet in 2020, showcasing its offerings in a number of BPI venues. Because a platform technology has yet to be established, much remains to be learned about manufacturing and purifying mRNA at commercial scale, said CEO Aleš Štrancar in an April 2021 webcast (**4**). He described production challenges and shared his company's efforts to devise flexible mRNA purification tools such as monolithic CIMmultus columns.

Analytical Methods

As with every other drug product, analytical methods are important for process validation and quality control (QC) of mRNA therapeutics. For example, worldwide regulatory agencies require proof that residual pDNA and other process-related impurities are minimized during downstream processing to below accepted limits for final drug substances. Some companies develop in-house quantitative polymerase chain reaction (qPCR) assays; others turn to suppliers for commercial kits. Thermo Fisher Scientific, for example, offers a multiplex real-time PCR assay for detection of residual plasmids based on targeting multiple antibiotic-resistance gene families (**5**).

In BPI's June 2021 issue, writers from BIA identified analytics as a bottleneck in mRNA production and processing (**6**). Process

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characterization is necessary for process optimization, which in turn helps companies find essential efficiencies in their manufacturing processes. Optimization begins with raw materials for mRNA synthesis: pDNA can come with residual host-cell proteins, endotoxins, and other contaminants from cell culture. Enzyme preparations used in IVT can include stabilizing additives that must be removed before the final drug-product formulation stage. Nucleotide and capping-reagent quantities must be determined and controlled precisely to achieve the highest transcription levels possible — without wasting money using excessive amounts of these expensive materials. The ratio of enzymes to plasmids must be kept in perfect balance. Buffer conditions, time, and temperature also need to be monitored closely.

In addition to PCR, electrophoretic methods have been a common choice for mRNA analysis. But they can be time-consuming and difficult to automate. Now, mRNA developers are turning to high-performance liquid chromatography (HPLC) and mass spectrometry (MS) technologies for more efficient options.

Toward a Purification Platform

The influx of money, interest, and time devoted to addressing the COVID-19 pandemic has advanced mRNA technology faster than most experts would have thought possible in early 2020. As scaling up processes revealed problems to be solved, developers have turned to suppliers for help. Whereas some established methods were adaptable from protein to nucleic-acid purification, such work hasn't been straightforward in every case. And just as the pandemic gave mRNA developers the means and motivation to leap forward with their vaccine (and therapeutic) programs, so too have these products presented the kind of challenge that helps technology suppliers make advances in their own capabilities. Although a decisive purification platform has yet to be established, the industry is working to define one — and making steady progress through creative and collaborative innovation.

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