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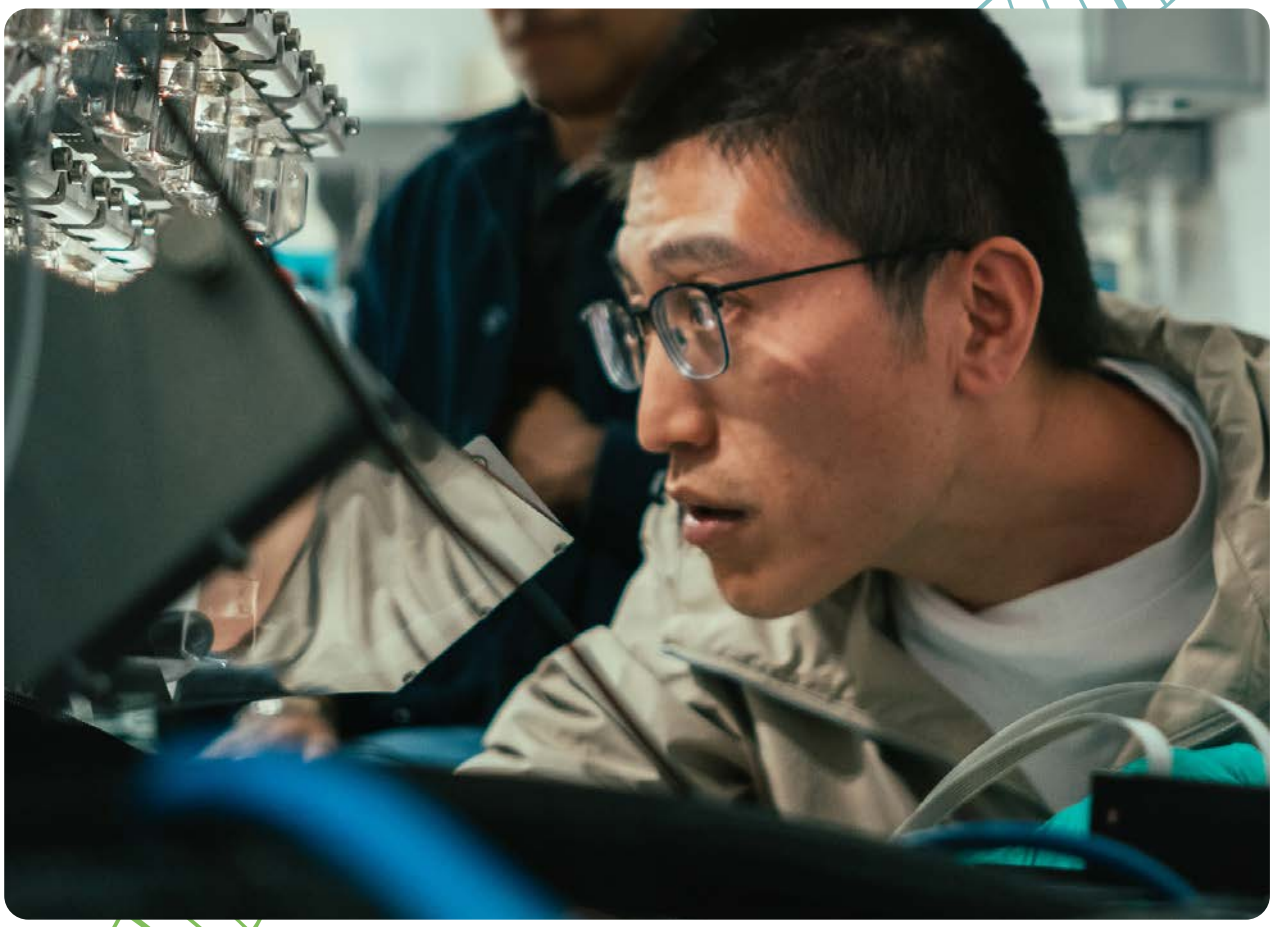
Next-Gen Gene Synthesis Using Cell-Free Cloning

Speeding Up Genetic Medicine Development with Cell-Free Synthesis of Next-Gen DNA



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Streamline Your mRNA Pipeline From Start to Finish

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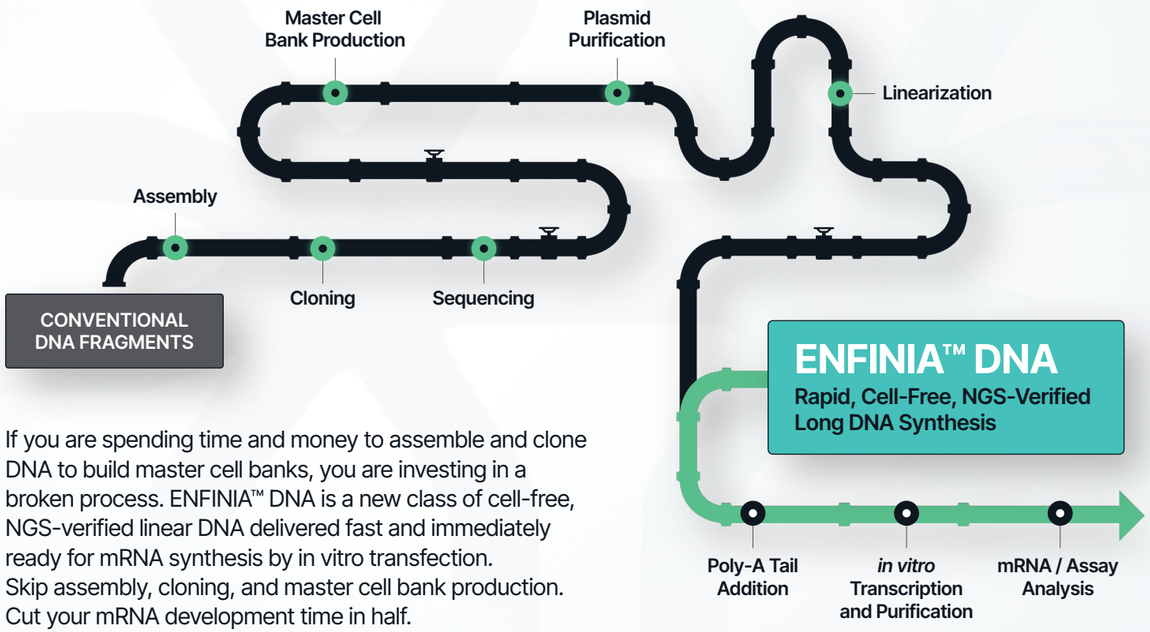

If you are spending time and money to assemble and clone DNA to build master cell banks, you are investing in a broken process. ENFINIA™ DNA is a new class of cell-free, NGS-verified linear DNA delivered fast and immediately ready for mRNA synthesis by in vitro transfection. Skip assembly, cloning, and master cell bank production. Cut your mRNA development time in half.

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Next-Gen Gene Synthesis Using Cell-Free Cloning

Speeding Up Genetic Medicine Development with Cell-Free Synthesis of Next-Gen DNA

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INTRODUCTION

The COVID-19 pandemic and the development of mRNA-based vaccines brought attention to the need for faster healthcare innovations to meet medical challenges. Since those first FDA approvals, companies have raced to apply this technology to prevent and treat many illnesses, including influenza, diabetes, HIV and various cancers.

One ubiquitous and indispensable biomanufacturing input ripe for improvement is a scalable, turnkey solution for high-quality synthetic DNA. DNA serves as the programmatic code for every living cell and plays a crucial role in developing all biopharmaceuticals, including mRNA vaccines.

Surprisingly, conventional DNA synthesis has seen little innovation. It relies on a slow, continuous build process to transform low-quality oligonucleotides and fragments into longer DNA through cloning. In the early stages of biopharmaceutical development, the iterative design-build-test-learn (DBTL) cycle is crucial. This cycle involves repeated testing and redesigning of synthetic DNA constructs to identify potential therapies. However, traditional methods often take weeks, especially for complex DNA constructs, delaying the optimization of candidate therapies and significantly slowing progress.

To stay competitive, biopharmaceutical firms must adopt innovations like Next-Gen Gene Synthesis using Cell-Free Cloning to speed up therapeutic development. Elegen's innovative cell-free synthesis platform produces ENFINIA™ DNA, which is sequence-perfect, double-stranded linear DNA up to 7,000 bp, shipped in a week. This high-quality, high-complexity, NGS-verified DNA requires minimal cloning and is ready for immediate use in therapeutic workflows. With accuracy nearly equivalent to clonal DNA, ENFINIA DNA significantly reduces the weeks typically needed for cloning, purification, and linearization of plasmid DNA for IVT workflows.

This innovation in DNA manufacturing offers unparalleled scalability and cost-efficiency compared to traditional methods, revolutionizing the discovery and development of mRNA therapies to enable rapid scale-up of therapeutic candidates for preclinical and clinical studies. By eliminating bottlenecks in genetic medicine workflows, this technology not only accelerates the development process but also ensures the delivery of full-length, high-quality DNA free from cellular contaminants. Looking beyond mRNA biotherapies, this capability is particularly crucial for individualized therapy, where the accelerated production of safe and effective treatments, devoid of cellular contaminants, can significantly impact patients' time-to-treatment options.

ELEGEN

Elegen's Cell-Free DNA Synthesis Speeds the Discovery and Development of Genetic Medicines

Ezumelmages / Getty Images

By MARIE DAGHLIAN

Synthetic DNA is the backbone of genetic medicine development. However, DNA manufacturing hasn't changed in the past 50 years.

Elegen is changing that paradigm by rapidly producing high-quality synthetic DNA without using bacterial cells. With its proprietary technology, Elegen delivers complex DNA sequences of up to 7 kilobases in half the time of conventional manufacturers, NGS-verified to ensure high accuracy and purity.

A Novel Approach to DNA Manufacturing

Elegen Chief Scientific Officer Marc Unger leads the team behind Elegen's innovative technology. They intend to disrupt the status quo for DNA manufacturing by employing innovative

technologies to bypass the age-old process of cell-based molecular cloning. Unger explains, "The conventional method is to start with oligonucleotides, on the order of 60 to 90 bases of DNA, and then assemble them into pieces several hundred bases long. And then if you want DNA thousands of bases long, further assembly is required. Today everyone does this by molecular cloning DNA in bacteria, and that's how it's basically been done since the early 1970s."

It's a long and tedious process that includes sequencing DNA isolated from many clones to identify a clone containing sequence-perfect DNA.

Molecular cloning involves assembling the synthetic DNA into a vector that is then transfected in bacteria. The bacteria grow and spread as colonies across the surface of an agar plate. Each colony comes from a single bacterium and therefore a single clone acts as a representative of the DNA sequence replicated within its colony. Clones from each colony are sequenced to determine which colony contains DNA with a perfectly correct sequence. The selected colony is grown further before DNA is isolated from it, purified, and sequenced again prior to shipping to a customer.

"That's the way everybody has done it until now, but there are a lot of problems and steps associated with this way of manufacturing DNA," says Unger. "Elegen has innovated the DNA production process to selectively amplify a single, sequence-perfect molecular species, but without the bacteria. This allows us to avoid several steps and the problems associated with them."

Those problems include extraneous DNA that, if expressed, could be toxic to cells. Also for certain complex sequences, it's difficult for the bacteria to actually propagate the DNA or do so without making mistakes or errors.

"Elegen's speed advantage comes from eliminating the operational complexity of working with bacteria — thus reducing the number of steps to manufacture the DNA of interest," says Unger. "Another benefit of eliminating bacteria from the workflow is that there's no need to purify the DNA before using it."

The DNA assembly and purification process can take several months for long multi-kilobase sequences of DNA. Unger says Elegen's process cuts that time drastically. "The product we have on the market right now, ENFINIA DNA, is up to 7 kilobases in length. We ship that in 6 to 8 business days, and it's NGS verified with a 99.999% per base accuracy."

Accelerating Genetic Medicine Development

Following the launch of ENFINIA DNA in 2023, Elegen has seen a large interest in its products and technology coming from the biopharma space. "It's our contention that the production of DNA for a genetic medicine is often the rate-limiting step," Unger says. "If I want to produce enough of an mRNA vaccine to treat millions of people, I may need a gram or tens of grams of DNA." The same can be said for screening through hundreds or thousands of mRNA vaccine designs—the supply of DNA quickly becomes a bottleneck.

Marc Unger, PhD, Chief Scientific Officer, Elegen

The development of gene therapies often involves the use of viral vectors, long DNA constructs—sometimes up to 15 kilobases. If they assemble this DNA construct hierarchically from smaller pieces of low-quality DNA, it could take several weeks to months to perform multiple rounds of assembly and cloning.

"Part of why researchers approach Elegen is because they can get longer DNA faster at very high quality," Unger says. "And then if they are making even longer constructs, say 15 kilobases, they can buy three 5 kilobase pieces of really high-quality DNA from us and assemble them in one step. That's a lot faster than building from many small pieces that require cloning."

Fewer steps means the customer can explore many more constructs in the same amount of time, saving costs as they go from *in vitro* screening into mice until the final drug candidate is identified.

Even when Elegen's customers clone their DNA, they rarely have to pick more than a few colonies because the error rate of ENFINIA DNA is very low. "You pay a significant time and cost to build DNA the conventional way," says Unger. "For larger constructs especially, you're going to pay that multiple times over in the course of making a single construct. With ENFINIA DNA, researchers can do this faster and with fewer steps."

Opportunities Ahead: From IVT-Ready DNA to Bypassing Master Cell Banks

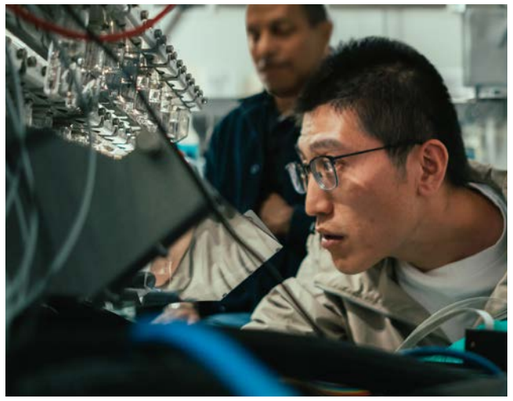
For companies developing mRNA vaccines and mRNA therapeutics, Elegen is already developing a new capability to streamline their workflows. mRNA is conventionally produced by *in vitro* transcription (IVT), which requires the addition of a poly-A tail of 30 to 160 bases to the 3' end. "Right now, ENFINIA DNA is not available with the poly-A tail, but that's coming," says Unger. "With this enhancement, our customers will be able to use ENFINIA DNA directly in their IVT reactions to more quickly produce mRNA."

Elegen is also developing a technology to accelerate the process of scaling up the production of DNA required to produce milligram or gram-scale quantities of mRNA for clinical testing. The current way to do this is to build a "master cell bank" and prove that the DNA construct doesn't change over time as the bacteria amplify. Larger quantities of the bacteria can then be grown from cells from the master cell bank. But it often takes three months or more to establish a master cell bank. It can take six to nine months to produce a single gram of DNA under GMP conditions, and that's after you reach the top of the queue of a contract manufacturer.

"Part of what makes this approach hard is the work required to purify the DNA from the bacteria," Unger says. "And then there's a lot of quality control (QC) testing required to prove you did it right."

By synthesizing DNA in larger quantities without using cell-based cloning, Elegen anticipates enabling customers to bypass the bottleneck associated with master cell banks to save production costs and time. "We can get to a GMP product a lot faster with a lot less QC required because we're doing it with a purely cell-free, molecular-based method as opposed to using bacteria," Unger explains.

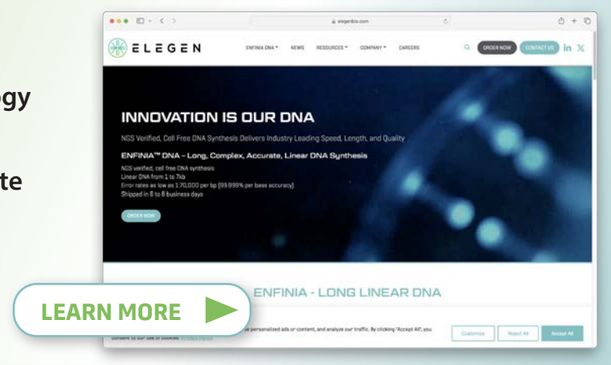
At the end of January, Elegen entered a multi-year collaboration and licensing agreement with GSK that allows the pharmaceutical giant to leverage Elegen's cell-free synthetic DNA production technology in the development of GSK's vaccines and medicines. The deal includes up to $35 million in upfront fees and purchase commitments of ENFINIA DNA, plus potential near-term milestone payments relating to the development of new product features and a potential equity investment in Elegen.



Elegen is disrupting the status quo for DNA manufacturing with their innovative technologies that bypass the decades-old processes like cell-based molecular cloning.

The deal is a marker of Elegen's strategy to partner with leading biopharma companies. "Given what we're doing and the value of it, we're talking to folks at the top, because they are the ones for whom we're making the most difference," Unger said. "We have the full stack of innovative technology here, and I'm going to say we have more copies of the innovation gene in our genome than some of the other companies that are making DNA—we replaced a technology that everyone has been using that was the standard and only way to do it for the last 50 years. That makes me sleep pretty well." ■

Learn More about how Elegen's cell-free DNA synthesis technology delivers fast turnaround, long, very complex, and highly accurate synthetic DNA at a competitive cost to streamline discovery and development.



Vaccine Developers Leverage mRNA and Other Powerful Technologies

To counter looming threats, innovative companies are developing safer and more effective mRNA vaccines, virus-like particles, and synthetic attenuated vaccines

Emergex Vaccines develops what it calls "synthetic, T-cell-priming immune set-point vaccine candidates" that are designed to evoke the body's natural T-cell immune response more safely than traditional live, attenuated vaccines. The candidates are meant to be delivered via microneedle patch technology. Indications that are being pursued by Emergex include viral infectious diseases and intracellular bacterial infectious diseases.

By JONATHAN D. GRINSTEIN, PHD and UDUAK THOMAS

Years from now, when we look back on today's vaccine development scene, we might say it was almost as consequential as the transition from variolation to vaccination. Variolation, an ancient method, involved the use of infectious material from a person with smallpox to inoculate others against the disease. Dried and powdered material from pus or scabs would be blown up the nose or jabbed beneath their skin. Although variolation had a fatality risk as high as 3%, as well the potential to contribute to the spread of smallpox and other diseases, no better protection was available, not until it was overtaken by vaccination in the 19th century.

Vaccination made inoculation far safer and far more effective. Also, it came to be used against a widening array of diseases. Analogous improvements are now promised by advanced vaccine technologies. Some of these technologies—such as mRNA-based technologies—are breaking new

ground. Others are updated versions of established technologies.

"Post-COVID-19, a huge portion of the technology focus has shifted to mRNA- based technologies," says Kate Broderick, PhD, a molecular geneticist and the chief innovation officer at Maravai LifeSciences. Broderick has worked for many years on vaccines for Ebola, Zika, Lassa fever, and Middle East respiratory syndrome.

"For an infectious disease vaccine, what you really want to see is what we call neutralizing antibodies," she continues. "The mRNA vaccines are really incredibly good at generating those particular types of antibodies. And they're showing that they're able to generate T-cell responses, too."

Indeed, mRNA vaccines work so well that Broderick believes many future vaccines will likely rely on the technology. Efforts are already underway to design mRNA-based vaccines ahead of a potential flu pandemic, as well as efforts to advance the development of self-amplifying mRNA vaccines, which use small doses of synthetic RNA to generate large quantities of antigen for a robust immune response.

This technology is still in its infancy, but according to Broderick, its potential is already emerging. "Self-amplifying RNA was kind of being developed at about the same time as mRNA, but it didn't get the focus that mRNA did during COVID-19," she relates. "Now we're starting to see some really, really interesting results there."

However, mRNA vaccines are not a magic bullet. "Just because it worked so brilliantly for COVID-19 [does not mean] it's going to work brilliantly for everything else," Broderick cautions. "Honestly, some things have worked, and some things haven't worked."

That means there is plenty of room for other technologies to proliferate and thrive, including updated versions of established technologies. For example, several companies are updating live, attenuated vaccine technology by incorporating some modifications to boost safety and efficacy. Also, subunit vaccine technology, which uses purified protein antigens derived from the target pathogen, is being used to develop new vaccines for respiratory diseases by companies like Novavax.

Innovation extends to vaccine delivery technology. One such technology is the microarray or microneedle patch. It is covered with tiny needle-like projections that penetrate and deliver vaccines to the top layers of skin. Its thermostability, ease of administration, and needle-free nature makes it an attractive option, particularly in resource-constrained contexts. Also, lipid nanoparticle technology is becoming safer and more stable, helping it to become the technology of choice for delivering mRNA-based vaccines.

There are also opportunities to use artificial intelligence (AI) in vaccine design and development. For example, AI can be used to model vaccine-protein target interactions, predict immune responses, and improve manufacturing efficiency. "An area that AI is really advancing is the management of [vaccine] clinical trials," Broderick points out. "If you are running a global infectious disease vaccine clinical trial at 10 sites in Africa and 3 sites in the United Kingdom, AI has the ability to integrate [the data] and provide feedback to the clinicians or the developers that is much more real time."

Virus-like particle vaccines get an upgrade

Launched in 2017, Osivax has offices in both France and Belgium, two hubs of vaccine development in continental Europe. The company is using its virus-like particle (VLP) technology platform to develop a novel class of T-cell vaccines that have broad-spectrum efficacy against multiple strains of a single virus. "This technology could be applicable to quite a few respiratory viruses," says Alexandre Le Vert, Osivax's co-founder and CEO. The company's first program is for a universal influenza vaccine.

One of the biggest hurdles in vaccine development is creating vaccines that are highly efficient. Current influenza vaccines are only about 40% effective because viruses can mutate rapidly and escape immune surveillance. Osivax's data, which has been published in *Lancet Infectious Disease*, shows that the company's vaccine has a much higher efficacy than current vaccines—about 84%—against viral strains responsible for seasonal influenza.

Osivax spun off from Imaxio, another French firm. Imaxio developed OligoDOM, the VLP technology that Osivax uses to make its vaccines. Osivax describes it as a self-assembling nanoparticle platform that is designed to trigger T-cell and B-cell immune responses. VLP technology is a well-established technology, used in vaccines to protect against viruses such as human papillomavirus and hepatitis B virus. "The issue that we used to have with VLPs is that they were able to trigger

Alexandre Le Vert, Co-Founder and CEO, Osivax

a strong antibody response, but not a strong T-cell response," Le Vert explains. "We've improved [the] technology to make VLPs that also trigger a very strong T-cell response."

In the context of influenza, Osivax used OligoDOM to design and produce a recombinant version of nucleoprotein, a highly conserved internal antigen. The recombinant protein self-assembles into a nanoparticle that activates the immune system. "It's an improved way to create nanoparticle vaccines to trigger broad protection against all the strains of a given virus," Le Vert maintains.

To date, Osivax has tested its universal influenza vaccine in over 1,200 participants in five clinical trials in multiple countries in Europe as well as in Australia. However, the vaccine is likely still years away from commercialization. At the time of publication of this article, Osivax has an investigational new drug application for its OVX836 universal influenza vaccine with the U.S. Food and

Drug Administration and has plans to launch additional trials, starting with a Phase IIb trial in 2024 followed by a third phase of testing thereafter.

In terms of the delivery mechanism, Le Vert says that Osivax's vaccine will be given as a standard intramuscular injection to ensure that it's easy for healthcare providers to administer and to ensure compliance from patients. Producing the vaccine on a large scale should also be straightforward. "It's the benefit of the [VLP] technology that we're using," he asserts. "Manufacturing is always a very big challenge in vaccines because you have to produce millions of doses. But compared to other vaccines, we think it's one of the strengths."

In addition to influenza, Osivax is developing vaccines for Sarbecovirus and human papilloma-virus, and is cultivating an mRNA vaccine program.

Live, attenuated vaccine revival

Clinical virologist and biopharma executive and entrepreneur Paul Grint, MD, believes that live, attenuated vaccines could play a larger role against many infections, particularly respiratory infections. "Credit to the mRNA technology and the Pfizers and Modernas of this world because they really stepped in when we had a massive global need," he says. "Those vaccines have had a profound impact—but SARS-CoV-2 is not going away."

Osivax uses OligoDOM, a virus-like particle–based vaccine technology, to develop vaccines that can trigger strong T-cell and B-cell immune responses and provide broad protection against all the strains of a given virus. Osivax's lead candidate, a universal vaccine for influenza, is currently in Phase II testing. The company also has vaccine candidates in development for sarbecovirus and human papilloma virus.

Grint is entrepreneur in residence at the University of California, San Diego, and holds board-level positions in several companies, including Codagenix, which combines live, attenuated virus design with codon deoptimization to develop synthetic biology–based vaccine solutions. Using live, attenuated viruses can be advantageous against respiratory infections, facilitating intra-nasal delivery and promoting mucosal immune responses. Other benefits include immunity that is systemic and of long duration.

"It's clear with some of the mRNA vaccines that you get a good antibody response but one that is relatively short lived," Grint notes. "In the influenza setting, you're seeing most of the infections really limited to the winter. That doesn't seem to be the case with COVID-19. We've seen significant increases in late spring and early summer over the last couple of years. Promoting booster vaccines in the early fall if you've lost most of your immunity coverage by January or February doesn't help if you then get potentially exposed in the late spring and early summer."

Unlike traditional approaches to live, attenuated vaccines, the Codagenix approach leverages synthetic biology and machine learning. Whereas the traditional approaches give viruses just a few mutations, the Codagenix approach introduces hundreds of intentionally placed edits, making it probabilistically impossible for the virus to revert to the wild-type phenotype.

The other piece of the synthetic biology puzzle for Codagenix pertains to manufacturing. "Some flu vaccines are still manufactured in eggs," Grint says. "Basically, the virus grows in the chicken

Peter Hansen/Getty Images

embryos that are harvested, put in a blender, and then slightly purified—that's the vaccine. The reactions to that vaccine can be very problematic, and clearly, it's not the type of manufacturing process one would like. You can use synthetic biology to turn that into something where, based on the cell line, you can easily produce large amounts of a highly purified vaccine."

Codagenix's live, attenuated viruses can be effectively produced in cell culture, which does not possess adaptive immunity at commercial scale in existing manufacturing facilities, is stored in conventional cold chains, and is easily delivered to far reaches of the globe.

Another company pursuing the live, attenuated virus route, but with a different twist, is Emergex Vaccines. Founded in 2014, Emergex initially planned to develop a vaccine for the emerging Ebola epidemic in West Africa. Challenges with testing that vaccine led Emergex to shift to being prepared for, rather than reacting to, an outbreak. The company now develops vaccine constructs that can be used against some of the world's nastiest viruses, and that can be quickly rolled out

and used in future pandemics or epidemics.

The idea is to create vaccines that stop emerging viruses before they can establish themselves, explains Thomas Rademacher, MD, PhD, Emergex's co-founder and CEO and an emeritus professor of molecular medicine at University College London. "You have to be able to make a vaccine that you have fairly good confidence is going to work," he says, and it has to be made "cheaply enough and easily enough such that if governments want to store 50 million doses or 10 million doses … it's not going to cost them $200 or $1,000 a dose."

Emergex vaccines target RNA viruses, which are responsible for diseases such as Ebola, dengue, and Zika. RNA viruses exist as clouds of related sequences—sometimes called quasi-species clouds—that work cooperatively to cause infection. These clouds are highly heterogeneous with multiple viruses present, so targeting only one member may not be enough to stop the infection.

Historically, live, attenuated viruses have been the best weapon against RNA virus–based infections by recruiting armies of T cells to kill off infected cells. But these kinds of vaccines can be lethal to their host. "No regulator is going to allow you to make an attenuated Ebola vaccine and [give] it to somebody," Rademacher states. He adds that you can get around this prohibition if you make vaccines that are not alive but can still do what live, attenuated vaccines do.

Emergex does this by extracting and sequencing viral signatures from infected cell cultures and using them to create completely synthetic attenuated vaccines that can trick the body's immune system into responding as it would if the vaccine was alive. These synthetic vaccines prime the T cells so that they are able to mobilize and destroy infected cells within hours of the initial infection, well before the viruses have time to produce and disseminate protein throughout the body.

"Under a normal infection, it takes about six or seven days to build up a T-cell army to kill that cell," Rademacher notes. "If I can kill that cell within the first 20 hours of infection, you have an abortive infection. It doesn't produce any virions, and the infection doesn't spread."

To have the best chance at generating an army of T cells, Emergex's vaccines are delivered using microneedle technology. "For vaccines to be effective, you have to have tissue-resident cellular immunity," Rademacher explains. "If you vaccinate into the epidermal layer, the T cells that are generated there go on to become resident T cells all over the body, and they create systemic immunization. If you give our vaccines intramuscularly or subcutaneously, they won't work."

So far, Emergex has conducted multiple vaccine trials including two in Switzerland that tested a dengue vaccine and a universal coronavirus vaccine at the same time. The company is now planning a universal flu vaccine trial in the United States. Emergex is also testing vaccines in Phase II trials in the Philippines and has agreements to test four vaccines in Brazil and South America. Separately, the company has purchased a facility in Fremont, CA, where it will manufacture its own microneedles and vaccines. ■

mRNA Vaccines and Therapeutics Combine Power and Finesse

Platforms for developing mRNA vaccines and therapeutics offer potent payloads, targeted delivery, and durable expression, as well as smooth paths to manufacturing

Companies focused on the day-to-day development of RNA vaccines and therapeutics may lack the bandwidth to attend to cGMP readiness. This is where cGMP integration and delivery specialists such as cGMPnow can help. cGMPnow advises that true cGMP readiness ranges from concept design to supply chain planning. In between, there are tasks such as clean room commissioning, qualification, and validation.

BY KATHY LISZEWSKI

Medical applications of mRNA technology are in the spotlight—not just mRNA-based vaccines, but mRNA-based therapeutics, too. The mRNA-based vaccines are the most conspicuous successes. They include the vaccines approved for use against COVID-19, of course, as well as the more recent vaccines approved for use against respiratory syncytial virus. Although regulatory approval has yet to be secured by any mRNA-based therapeutics, dozens of these

Orbital Therapeutics, a developer of RNA-based medicines, has announced that it is building a platform "designed to sit at the intersection of RNA technology, delivery methods, data science, and automation," and that its initial areas of focus include "vaccines, immunomodulation, and protein replacement." With a toolbox that contains both linear and circular RNA technologies, Orbital is exploring new ways to enhance human health. Circular RNA, for example, could extend the duration of antigen exposure and thereby enhance the immune response. As this image shows, process development work at Orbital includes RNA purification.

drugs are in clinical trials for indications such as cancer and cardiovascular diseases. There are also mRNA-based therapeutics in development for regenerative medicine applications.

The technological and clinical advances are accompanied by optimistic predictions from market researchers. For example, according to Research and Markets, the global mRNA vaccines and therapeutics market will reach $66.2 billion by 2028, up from $56.1 billion in 2022, reflecting an annual growth rate of 2.8%.

To realize the potential for mRNA vaccines and therapeutics, industry players are enhancing their tools and technologies. Some of the most prominent players—vaccine and therapeutic developers, platform providers, and manufacturing consultants—shared their insights at the mRNA Conference 2023. Let's revisit (and expand on) these insights here.

This article will describe self-amplifying mRNA that encodes antigen cassettes (for improving immune responses) and replicases (for prolonging antigen exposure); circular RNA that bears an internal ribosomal entry sequence (for initiating translation and ensuring durable antigen expression); synthetic DNA templates (for intensifying mRNA production); "programmed" nanoparticles (for improving targeted delivery); and cGMP preparations (for easing the transition to mRNA manufacturing).

AI-identified antigens, self-amplified mRNAs

Effective antiviral vaccines ideally generate neutralizing antibodies to viral proteins and T-cell responses to the small viral fragments displayed on the surface of virally infected cells. To create such vaccines, Gritstone Bio is employing a technology for antigen identification (a proprietary artificial intelligence–driven platform called EDGE) and a technology for self-amplifying mRNA (samRNA).

"Coupling these technologies enables the design of vaccines that can deliver combinations of full-length antigens and select T-cell epitopes, leading to potent and broad humoral and cellular immune responses," says Amy Rappaport, PhD, the company's senior director. "While current mRNA vaccines have effectively reduced the severity and mortality for COVID-19, antibody titers wane within six months, and frequent boosting is required, particularly in the context of emerging variants of concern.

"As the name suggests, Gritstone Bio's samRNA vaccine is self-amplifying. It leads to longer antigen expression and increased durability and breadth of immune responses, and it permits lower doses than conventional or first-generation mRNA."

Rappaport explains that samRNA technology works by encoding an alphavirus replicase complex in addition to an antigen cassette selected for the target disease. "The samRNA is encapsulated in lipid nanoparticles, which deliver the samRNA into cells following immunization," she continues. "Once in the cell, the replicase is expressed and drives the amplification of the antigen cassette, leading to high and sustained expression. This allows for durable expression at low doses, in contrast to first-generation mRNA vaccines, where the RNA that is delivered is all you get."

For infectious disease targets, Gritstone Bio is applying its technology in vaccine programs against SARS-CoV-2, HIV, HPV, multiple respiratory viruses, and influenza. The company also has two cancer vaccine programs. Both are designed to drive strong T-cell responses to tumor-specific neoantigens to achieve tumor cell killing and efficacy in combination with checkpoint inhibitors.

Circular and linear approaches to durability

Going around in circles seldom leads anywhere. However, newly emerging circular RNA–based therapeutic platforms are offering potential applications for a range of diseases and could help address current shortcomings of mRNA-based vaccines.

"Although mRNA vaccines have been democratized by the success of COVID-19 vaccines, some challenges remain, like the durability of the immune responses or the potential lack of memory responses," says Gilles Besin, PhD, chief scientific officer, Orbital Therapeutics. "For now, we see mainly efforts to develop vaccines against viral or cancer targets, but mRNA vaccine technology has the potential to be applied against fungal and bacterial targets."

Orbital is developing both linear and circular

Gilles Besin, PhD, Orbital Therapeutics

Heikki Lanckriet, PhD, 4basebio

RNA technologies with the goal of enhancing the immune response by extending the duration of antigen exposure. "We have a platform based on a closed circle RNA molecule," Besin relates. "It contains several important structural elements, like an internal ribosomal entry sequence (IRES), required to initiate translation.

"Because it resists exonucleases, closed circle RNA, or circular RNA, can extend durability of expression in a cell-dependent manner. Early studies suggest that circular RNA could elicit better T-cell responses." Moreover, early studies indicate that circular RNA-based vaccines can be administered more safely and manufactured more simply.

Although Orbital's closed circle RNA is currently at the preclinical stage, Besin believes that circular RNA will lead to strong candidates for next-generation vaccines and other applications. "We are an RNA medicines company," he declares. "We are also developing RNA-based treatments for rare diseases, cell therapies for cancer and immune diseases, and regenerative medicines. We will remain agnostic and develop RNA medicines for diseases where RNA can help."

Synthetic templates, nanoparticle vectors

To accelerate the development of mRNA vaccines, 4basebio is targeting novel synthesis and delivery platforms. "We provide a cell-free, enzymatic DNA amplification process called Trueprime," says Heikki Lanckriet, PhD, the company's CEO and CSO. "It delivers customized linear DNA templates for a range of applications.

"For mRNA, we recommend our opDNA template, which is ready to use for *in vitro* transcription to manufacture mRNA. Other formats cater to gene therapy vector manufacture, gene editing, and DNA vaccines." The main difference between the distinct proprietary linear DNA formats the company produces is the way the ends are finished.

The "opDNA" refers to a linear DNA template that has a hairpin closure on one of the ends. "For *in vitro* transcription, we recommend keeping opDNA open at the 3¢ end," Lanckriet advises. "[This format has been] used extensively and successfully by us and our clients. For smaller mRNA products, such as the products used in mRNA-based vaccines, this means nearly double the amount of mRNA can be produced with the same amount of DNA."

Lanckriet notes that using opDNA can also overcome other drawbacks of the traditionally used plasmid DNA such as long lead times and supply bottlenecks. "Because the enzymatic manufacturing process is cell free, it does not produce any cellular contaminants such as endotoxin," he explains. "Our unique template generation process eliminates all nonessential sequences present in a plasmid backbone. However, essential sequence context such as inverted terminal repeats or poly A tails for viral vector and mRNA production, respectively, is readily produced in high quality."

In addition, 4basebio has developed a nonviral targeted delivery platform called Hermes. "The nonviral nanoparticle comprises an interchangeable targeting moiety that allows for preferential uptake in a specific cell or target tissue of interest and mitigates off target effects," Lanckriet details. "In addition, this improves packaging of the payload and increases the thermal stability of the particles. The particles have low immunogenicity,

Stuart Milstein, PhD, Senda Biosciences

enabling repeat dosing strategies, and are suitable for various routes of administration. Manufacturing is scalable, cost-effective, and driven by self-assembly processes."

Programmable medicines

The typical delivery vehicle for mRNA vaccines is a lipid nanoparticle that consists primarily of synthetic components. A more natural approach is being explored by Senda Biosciences. The company is mining information from natural nanoparticles that evolved to precisely shuttle biomolecules between species and into human cells. Natural nanoparticles represent a rich source of biological targeting information.

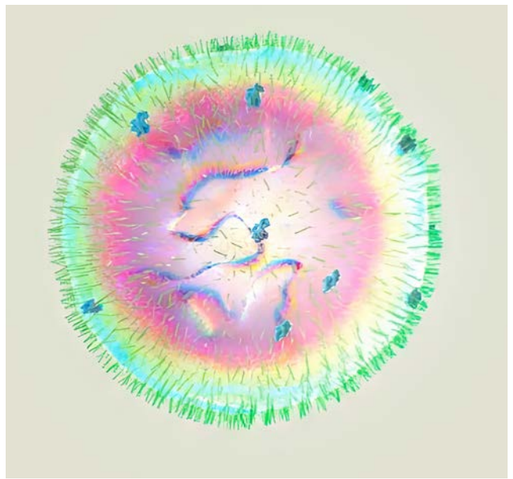
Senda Biosciences has developed the Senda Atlas. According to Stuart Milstein, PhD, the

company's chief platform officer, the Senda Atlas is designed to support the "programming" of nanoparticles. Basically, nanoparticles are endowed with properties to enhance therapeutic delivery.

"Meeting the challenge of 'programmable delivery' means that we can arrange for vaccines and therapies to be delivered to a specific tissue or cell," Milstein says. "It also means that we can de-target the liver, with the expectation that doing so will increase therapeutic efficacy and potency and potentially decrease reactogenicity."

According to Milstein, the company's breakthrough for developing the Atlas "followed systematically surveying myriad species across all accessible kingdoms of life to unpack the molecular composition of constituents that would be present in natural nanoparticles involved in 'intra- and interspecies communication'—moving biomolecules between different cells or organisms. It is a library of more than 75,000 molecular features that Senda has characterized and can use to program our own proprietary nanoparticles for potential therapeutic applications."

By applying deep molecular profiling and advanced analytics to natural nanoparticles and information molecules, Senda Biosciences is developing programmable medicines. For example, the company combines programmed mRNA and nanoparticles to create RNA medicines—SendRNA medicines—that can target a range of tissues and cells, and that can tune therapeutic function across multiple dimensions, including duration of effect, immune response, and route of administration.



Senda's lead preclinical studies are initially focused on COVID-19 vaccines. "Our first program focused on demonstrating superior performance to the COVID-19 commercial vaccines, which we believe we have achieved with strong preclinical results," Milstein reports. "As a next step, as well as seeking to move this program forward into the clinic, potentially in partnership, we are exploring additional applications for this vaccine technology, as well as potential indications in lung and hematopoietic stem cells, and we are advancing in vivo immune cell programing for applications such as in vivo chimeric antigen receptor T-cell therapies."

Milstein envisions being able to fill the gaps that prevent the right therapies from getting to the specific cells of interest. "Fully programmable medicines are the future," he declares. "It's not a question of 'if' but of 'when,' and we expect to play a key role in bringing them to the patients who need them."

cGMP readiness planning

Companies focused on the day-to-day development of mRNA vaccines may lack the bandwidth to attend to current good manufacturing practice (cGMP) readiness. These companies should consult with experts sooner rather than later, suggests Jeff Gilmore, the CEO of cGMPnow.

Jeff Gilmore, cGMPnow

"Start with a robust concept design by a company that knows the mRNA space," Gilmore says. "The primary consideration should be planning for cGMP readiness versus just facility readiness. Typical life sciences architecture/engineering and general contractor firms can support facility readiness (that is, facility construction punch-list completion). But clients need mRNA/cGMP manufacturing expertise to help them achieve true cGMP readiness, which involves matters such as cGMP equipment, computerized systems, CQV (commissioning, qualification, validation), staffing/training, and supply chain planning."

Unfortunately, failing to plan may mean falling behind or worse. "We see more delays in product-to-patient timelines driven by poorly planned cGMP manufacturing facility buildout projects," Gilmore warns. "These projects are riddled with scope creep, delays, cost increases, and subpar quality."

This is where engaging a cGMP specialist can help. According to Gilmore, a cGMP specialist should offer expertise in mRNA manufacturing processes and equipment, cGMP regulations, and project management. A cGMP specialist should be able to help clients develop (and comply with) realistic schedules and budgets for bringing facilities to cGMP readiness.

Gilmore asserts that cGMPnow specializes in full cGMP system integration and delivery. "We help clients assemble a concept design that provides very accurate scope, schedule, and budget estimates for the cGMP manufacturing facility buildout project," he details. "We also help clients navigate the path to cGMP readiness via cGMP manufacturing/QC equipment specification/procurement; cGMP computerized systems design/installation; and full facility, equipment, and computerized systems CQV. To further drive cGMP readiness, we provide support for facility staffing/training and supply chain planning (including raw materials, consumables, and associated electronic management systems). ■

Accelerating mRNA Synthesis With Linear "IVT-Ready" Template DNA

kirstypargeter/Getty Images

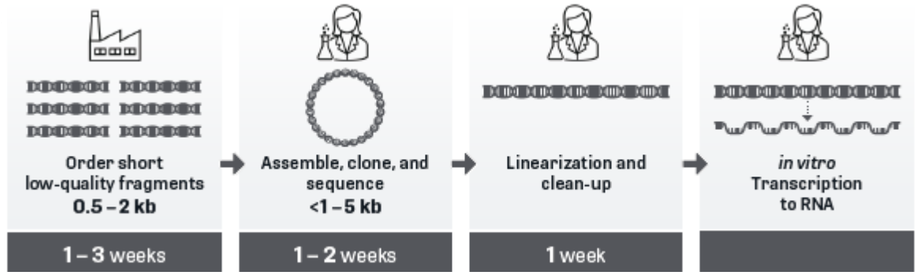
Introduction

By streamlining the mRNA vaccine development process with a fast and reliable supply of high-fidelity, high-purity DNA, scientists can more efficiently screen through antigen and recognition sequence variations to cast a wider net in their search for therapeutic candidates. Conventional mRNA synthesis relies on *in vitro* transcription (IVT) of a synthetic DNA template comprised of an antigen sequence and several ribosomal recognition sequences including a capping region, untranslated region (UTRs), and a poly-A tail. The quality of the final mRNA vaccine delivered to patients is directly impacted by the quality of the DNA template used in the IVT reaction. A sustainable and cost-effective supply of high-quality DNA for mRNA synthesis minimizes the risk of re-synthesizing mRNA, accelerating downstream development.

Conventional DNA template production

Using the conventional approach to build template DNA creates a bottleneck in the design-build-test-learn (DBTL) cycle at the core of mRNA discovery. To produce DNA for each design iteration, conventional suppliers follow a decades-old time-consuming cloning workflow that relies on the propagation of DNA through host cells, often bacteria, to produce a population

Using the conventional approach, a DNA build takes 3–6 weeks.



of sequence-perfect plasmid DNA. Plasmid DNA is isolated from the cells linearized and purified before IVT. The cell-based cloning process can easily extend the DNA build process to as long as 6 weeks.

ENFINIA™ DNA – Cell-free DNA production

To eliminate the DNA supply bottleneck and accelerate the DBTL cycle, scientists at Elegen have developed a scalable, turnkey cell-free DNA production platform that delivers a faster, reliable supply of high-quality DNA. Elegen's ENFINIA DNA offers very complex, linear, double-stranded DNA up to 7 kb in length with NGS-verified high per-base accuracy in 6 to 8 business days.

This unparalleled combination of speed, length, and accuracy provides a reliable supply of synthetic DNA to streamline and accelerate the development of mRNA vaccines and therapies.

Cell-free Synthesis of Template DNA for IVT

In a recent experiment, scientists at Elegen demonstrated the enzymatic addition of a poly-A tail to ENFINIA DNA, producing high-fidelity template DNA. To demonstrate the efficacy of the templates, Elegen scientists synthesized two genes with their standard ENFINIA DNA production process and enzymatically added poly-A tails (80 or 120mer) that were separately synthesized to each gene creating a ready-to-use template for IVT. Each tailed gene was analyzed by gel electrophoresis and compared to the untailed gene to measure the shift in molecular weight resulting from a successful addition of the poly-A tail.

Three replicates of two sample genes (Gene 01 and Gene 33) were prepared using the standard production process for ENFINIA DNA. After production, an 80 base pair poly-A tail was added

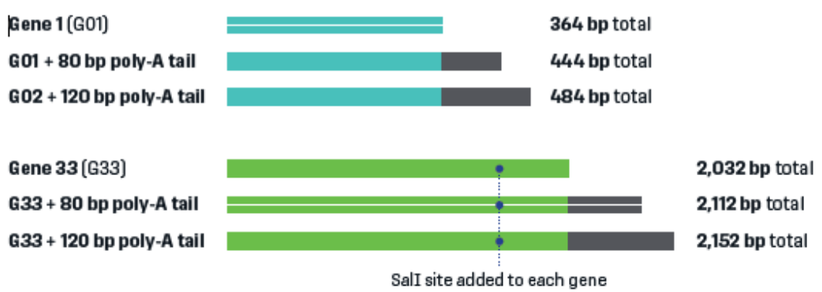


Figure 1. Elegen scientists synthesized two genes with their standard ENFINIA DNA production process and added chemically synthesized and purified poly-A tails (80 or 120mer) to each gene post-production. A SalI cut site was added to Gene 33.

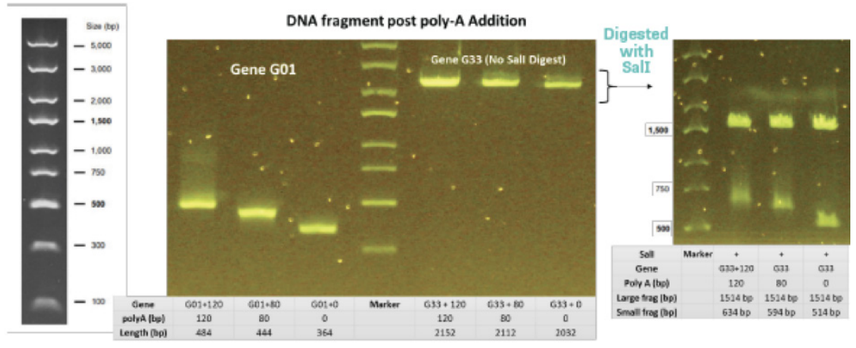


Figure 2. Gel analysis confirms that the addition of a 120 and 80 base pair poly-A tail synthesized to both Gene 1 and Gene 33.

to 1 replicate of each gene and a 120 base pair poly-A tail was added to another replicate of each gene. For Gene 33 a SalI cut site was added.

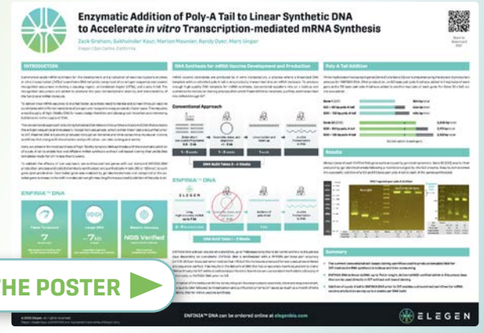
All replicates of each ENFINA DNA gene were analyzed by gel electrophoresis. Gene 33 (G33) was further analyzed by gel electrophoresis following a restriction digest by the SalI enzyme. Results demonstrate the successful addition of 120 and 80-base pair poly-A tails to each of the genes synthesized.

Conclusion

The popularity of mRNA-based vaccine development and increased market demand have highlighted the need for more efficient and cost-effective DNA build cycles. The decades-old cell-based cloning workflow used in the conventional DNA production process is a bottleneck that slows the pace of mRNA screening and lowers the overall efficiency of mRNA vaccine development.

The enzymatic addition of a poly-A tail to ENFINIA DNA bypasses cell-based cloning, enabling scientists to produce mRNA for screening and lead optimization more rapidly. ENFINIA DNA is linear, double-stranded DNA up to 7 kb long, delivered NGS-verified within 6-8 business days. As demonstrated, scientists using ENFINIA DNA to produce template DNA for mRNA vaccine development can save up to 4 weeks with each DNA build cycle. ■

Learn how Elegen produced the rapid synthesis of a high-fidelity template, enabling fast and efficient mRNA synthesis—without cell-based cloning, in less than 2 weeks.

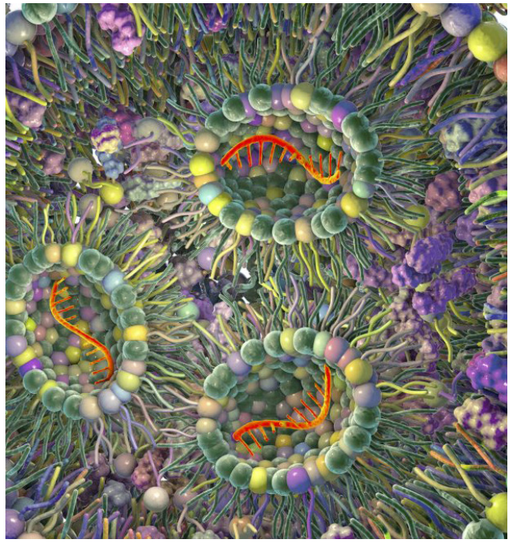


mRNA Therapeutics May Act Longer If Given Multiple Tails

Researchers from the Broad Institute and Massachusetts Institute of Technology (MIT) have engineered multi-tailed mRNAs that boost mRNA activity levels in cells by as much as 20x and last 2-3 times longer in animals compared to unmodified mRNA. When incorporated into a CRISPR-Cas9 system and used in mice, multi-tailed mRNAs also proved to be more efficient gene editors than their unmodified counterparts.

Details of the mRNAs are published in a new Nature Biotechnology paper titled, "Branched chemically modified poly(A) tails enhance the translation capacity of mRNA." Through this study, "we've shown that non-natural structures can function so much better than naturally occurring ones," said Xiao Wang, PhD, senior author of the paper, a Broad core institute member, and an assistant professor of chemistry at MIT. "This research has given us a lot of confidence in our ability to modify mRNA molecules chemically and topologically."

The results of testing in human cell lines and mice have also bolstered the researchers' confidence about the potential for using their engineered mRNAs in therapies that edit genes or replace faulty proteins. Excitement around the potential of mRNAs continues to grow driven by their successful application in COVID-19 vaccines, which only require a small dose of mRNAs to stimulate protein production. mRNA-based therapies have also been tested in clinical trials aimed at treating heart failure and to correct familial hypercholesterolemia, the researchers noted in the paper.



Kateryna Kon/Science Photo Library/Getty Images

Computer illustration of a lipid nanoparticle mRNA vaccine. A cross-section of a lipid nanoparticle carrying mRNA (orange) of a virus, for example, Covid-19 or influenza, is shown entering a human cell.

"However, mRNA therapies for applications such as enzyme replacement, antibody therapy, and gene editing require sustained high-level protein production, where the instability and low efficiency of traditional mRNA drugs necessitates high doses that may lead to cytotoxicity," they wrote. "To address these challenges, efforts to improve the translation duration and capacity (the overall protein production per RNA) of mRNA vectors are necessary."

Simply put, Wang's lab wanted to design an mRNA structure that could be stable, active, and

produce sustained therapeutic effects at low doses. This study "opens up many new opportunities for synthetically modifying mRNA to extend its therapeutic uses," said Hongyu Chen, first author on the paper and a graduate student from MIT Chemistry in the Wang lab. "I find mRNA very fascinating because as an informational molecule, its function is encoded by its sequence, while its stability is dictated by the chemical properties of its backbone. This feature gives chemists the versatility to extensively engineer the mRNA structure without worrying about changing the information it carries."

The poly(A) tail of mRNA plays a crucial role in protecting mRNA from degradation. In a 2022 paper published in *ACS Chemical Biology*, Wang and her collaborators showed that chemically modifying the poly(A) tail using deadenylase-resistant oligonucleotides slowed the natural decay of mRNA and improved protein production making them useful for various therapies. These so-called messenger-oligonucleotide conjugated RNAs or mocRNAs are made by ligating chemically synthesized oligos to the 3′ end mRNAs.

The research described in *Nature Biotechnology* builds on the work done developing these modified molecules. The researchers hypothesized that engineering a more complex mRNA structure with multiple modified poly(A) tails instead of a single tail would enhance the therapeutic effects of the mRNA. They tested mRNA structures with zero to three branches and three different kinds of chemical modifications. The modifications were phosphorothioate (PS), DNA, and 2′-O-methoxyethyl (2MOE). After testing multiple branched constructs, the researchers identified a three-branched construct with two types of chemical modifications (PS and 2MOE) on both the stem and branched poly(A) oligos had the best enhancement.

When the team tested the multi-tailed mRNAs in human cells, they sustained mRNA translation much longer than both natural mRNA and mocRNA, producing up to 20 times more proteins per dose over time. In experiments that delivered the mRNAs to mice, the researchers showed that protein production using the modified RNAs lasted as long as 14 days, nearly double the lifetime of unmodified ones.

Next, they used the multi-tailed mRNA to encode the Cas9 protein as part of a CRISPR gene-editing system and tested it in mice. They used it to edit two genes implicated in high cholesterol—Pcsk9 and Angptl3. Knocking down these genes is a possible therapeutic strategy for treating familial hypercholesterolemia. A single dose of multi-tailed Cas9 mRNA induced high levels of gene editing and resulted in decreased cholesterol in the mouse bloodstream compared to the control, according to the results reported in the paper.

For their next steps, the researchers are making their multi-tailed mRNA synthesis and purification process more scalable. They are also exploring how mRNA modifications affect its therapeutic stability and activity. "We want to see where else we can engineer mRNA's structure to increase efficiency," Chen said, adding that they are also interested in modifications that would improve the rate at which cells can scan and translate mRNA's instructions. ■

Next Steps for mRNA Vaccines and Therapeutics

According to etherna, mRNA platforms are overcoming technological and practical challenges to realize their vast clinical potential

Capabilities offered by mRNA technology platform companies typically include the design and optimization of RNA constructs. One such company is etherna. It produces all of its RNA via in vitro transcription. It also designs and manufactures customized lipid nanoparticle formulations tailored for the prevention and treatment of various pathological states.

By DAVID RICKETTS, DPHIL, BERNARD SAGAERT, STEFAAN DE KOKER, PHD and NEVIN WITMAN, PHD

In addition to COVID-19, viral diseases that are driving the development of mRNA-based vaccines range from influenza to genital herpes. These vaccines are forthcoming because mRNA development platforms are versatile. They help shorten the timelines and reduce the costs of vaccine development. Moreover, they are readily adapted to new targets—not just additional vaccine targets, but also therapeutic targets. Now that mRNA technology is of proven versatility, the industry is moving swiftly to exploit its potential in a vast range of therapeutic applications, ranging from autoimmune diseases to protein replacement therapies.

Long before COVID-19, mRNA vaccines were developed with oncology in mind, and it seems inevitable that the next major clinical breakthrough will come in the cancer sector. Companies such as Moderna, BioNTech, and CureVac are currently running clinical trials in diseases in advanced melanoma and other malignancies such as ovarian, colorectal, and pancreatic cancers. It is entirely conceivable that an mRNA cancer vaccine could reach the market by the end of the decade.

Cancer vaccines, however, will have to overcome stubborn challenges. For example, vaccine development for solid tumors must be personalized to target patient-specific neoantigens and thereby prevent or delay relapse following the surgical removal of cancerous tissue. Although manufacturers are developing increasingly innovative ways of manufacturing mRNA—for example, CureVac has struck a partnership with Tesla to explore whether bioprinters could automate the production process—the costs are likely to be high.

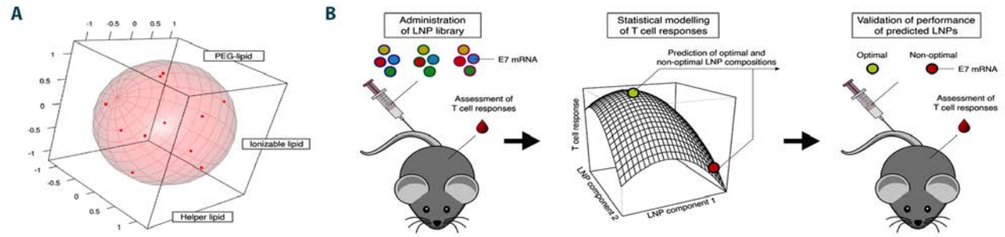
Nonetheless, mRNA technology could help overcome the manufacturing challenges encountered with CAR T-cell therapies such as Kymriah and Yescarta. These therapies have shown great promise against hematological cancers and lymphoma, but they rely on manufacturing processes that are time consuming, difficult to scale, and costly. Encouragingly, new evidence has emerged demonstrating that a vaccine-like injection of mRNA can induce CAR T cells in situ. Last year, as reported in *Science*, a team from the University of Pennsylvania together with Acuitas Therapeutics utilized mRNA to engineer regular T cells into functional CAR T cells inside the body. These cells reduced fibrosis and restored cardiac function in a mouse model of heart failure.

Another application that stands to benefit from mRNA technology is gene editing. For example, CRISPR-Cas9 mRNA and synthetic single guide RNA ribonucleoproteins make the nonviral delivery of gene editing components an attractive alternative to adeno-associated viruses. Although multiple clinical trials have shown that adeno-associated viruses have long-term efficacy, safety concerns remain. These include the potential for insertional mutagenesis and even carcinogenesis. The lower immunogenicity of nonviral approaches allows for redosing, while the greater control offered reduces off-target effects and toxicity.

Optimizing payload and delivery

All this serves to illustrate the many opportunities that lie ahead for RNA vaccines and therapeutics. But while we have seen many advances in the generation, purification, and cellular delivery of RNA, challenges remain, including optimizing the RNA payload, reducing toxicity, and improving targeting efficiency.

Many companies wish to explore the possibility of using an mRNA payload in a customized lipid nanoparticle (cLNP), a possibility that has already been realized in COVID-19 vaccines. etherna has dedicated a great deal of research to improving LNP technologies as well as optimizing the mRNA payload. Particularly, when it comes to mRNA construct design, there are many factors to consider beyond merely the coding sequence of the target. The capping of mRNA is crucial to



Scientists at etherna studied whether mRNA-cLNPs could be designed to induce strong CD8 T-cell responses upon intravenous immunization. (A) Distribution of 11 cLNP compositions (with variable lipid ratios). A cLNP library was prepared covering these 11 lipid ratios for three different PEG lipids. (B) LNP optimization approach. Using a design of experiments/Bayesian regression methodology, the scientists succeeded in tailoring mRNA-cLNP compositions to achieve high-magnitude tumor-specific CD8 T-cell responses within a single round of optimization.

minimize degradation and reduce the innate immune response, while well-designed and carefully paired UnTranslated Regions (UTRs) can significantly increase the expression of mRNA. Optimizing the final component of the construct, the poly A tail, is important to ensure the stability and integrity of the product.

mRNA and cLNP components also need to be carefully synergized for each individual application as both have biological activity and aggregate physiochemical properties. An integrated approach is required that involves not only the optimization of the mRNA payload, but also the screening of a library of ionizable lipids to identify the optimal formulation regarding both lipid chemistry and the mRNA-to-lipid ratio. For example, a more immune-stimulatory cLNP is applicable for vaccines where you want to build an antibody titer; alternatively, immune-silent LNPs might be more applicable for gene editing or treating an autoimmune disease.

Another key issue relates to establishing methods of improving the targeting efficiency of mRNA cLNPs, as cLNP biodistribution is generally broad and mainly directed to the liver. Therapeutics need to be targeted at the organ or even the cell type of interest while delivering enough of the payload deep into the tissue to elicit the desired response.

Broadly speaking, two different approaches try to address this problem. The first is called "active targeting." Here, a chaperone molecule is chemically conjugated to the cLNP to help guide it toward the desired location. The second is known as "passive targeting." Here, the cLNP can be designed or manufactured to have a predisposition for certain tissues by modifying the lipid composition and particle size. At etherna, we have shown that by carefully fine-tuning these two parameters, we can preferentially target spleen or liver following intravenous administration of cLNPs/mRNAs.

Researchers working in the gene editing field are particularly interested in finding better ways of targeting mRNA-LNPs toward the heart, lung, brain, kidney, and other tissues. Improved targeting would make it easier to design therapeutics for diseases that afflict these organs, including rare diseases such as enzyme replacement deficiencies and metabolic disorders.

The Holy Grail is to find a way of engineering mRNA-cLNPs capable of crossing the blood-brain barrier and unlocking enormous treatment opportunities ranging from brain tumors to neurodegenerative diseases such as multiple sclerosis.

Eliminating the cold chain

Although the rollout of COVID-19 vaccines has been a remarkable success, it was estimated in early 2023 that 2.3 billion people remain unvaccinated against the SARS-CoV-2 virus, with 89% of those living in the developing world. These discouraging figures are due, in part, to the cold chain requirements for mRNA-LNP COVID-19 vaccines. The need to store these vaccines between –50°C and –15°C hinders vaccination distribution, particularly in poorer nations with limited infrastructure. As a result, there has been considerable research over the last three years into lyophilization or freeze-drying. This commonly used pharmaceutical industry technique removes water from drug formulations to increase the stability and shelf-life of products. If technologies can be developed to lyophilize mRNA-cLNP vaccines, it would be possible to ship the vaccines worldwide without the need for cooling or freezing.

However, this process is far from straightforward and requires careful selection of lyophilization buffers, cycle process parameters, and temperatures. When the product is recovered, it is vital to demonstrate that the characteristics and traits of the mRNA and cLNP components have not been altered to a point where they are either unsafe or inefficient. Key physicochemical parameters such as particle size, proper payload encapsulation, and stability of the lipid components are critical to biological performance and must be retained during both lyophilization and subsequent storage.

Several research groups have successfully demonstrated the ability to lyophilize cLNPs containing either small interfering RNAs (siRNAs) or mRNA. However, retaining efficacy after reconstitution with water and during weeks or months of storage has proven challenging. Although siRNA-LNPs were successfully lyophilized by researchers at Carnegie Mellon University, they showed much lower efficacy *in vitro* following recovery. Another relevant lyophilization study was conducted by scientists at EyeGene, a Korean company. They successfully lyophilized mRNA-cLNP COVID-19 vaccines and showed that the reconstituted products could still induce strong immune responses in mice, but they did not examine whether the products remained stable after time in storage.

Almost every RNA company is attempting to develop new techniques to overcome these hurdles, and there are promising signs. Tekmira Pharmaceuticals has lyophilized an siRNA-cLNP for treating Zaire Ebola virus infection and shown that the reformulated version has equivalent efficacy in a Phase I trial.

The goal is to produce lyophilized vaccines that are thermostable and have a shelf life of more than six months. Such vaccines would be in huge demand. More important, they would be suitable for transport to nations around the world where the cold chain has traditionally

David Ricketts is director, business development, Bernard Sagaert works as interim CEO, Stefaan De Koker, serves as vice president, discovery, and Nevin Witman acts as a consultant at etherna.

proven cumbersome. No one has achieved this goal yet, but it is attracting significant resources.

The patent landscape

Previously undruggable pathways can be targeted by mRNA therapeutics. Accordingly, mRNA therapeutics represent a disruptive technology, one that is expected to change the standard of care for many diseases. However, innovations in RNA therapeutics may face nontechnological challenges. One such challenge concerns third-party intellectual property (IP), an important factor from the mRNA and cLNP perspective.

For example, several companies hold potentially restrictive patents pertaining to core components of mRNA technology. There are patents held on the most prevalent capping technologies, and much IP has locked up the use of modified nucleoside triphosphates, the building blocks of RNA. Other companies are in control of IP relating to lipid formulations and specific lipids used in cLNPs. The IP landscape is complex and dynamic. A number of summary articles provide an overview, including one written by partners at Neal Gerber Eisenberg.

This complex IP landscape could prove to be rate-limiting for the field because RNA startups will have to determine if they can afford the commercial licenses for these different technologies. When companies select modified nucleoside triphosphates, they already find that they must consider licensing issues in addition to what might be biologically optimal.

These are all hurdles that this nascent field will have to overcome. However, given the potential of mRNA platforms to tackle so many unmet clinical needs and the enormous amount of investment being directed toward mRNA, we are certain that the future is bright. ■

Synthetic DNA Advances Will Catalyze the Next Wave of Biotherapeutic Innovation

Recent innovations in DNA manufacturing are helping eliminate critical bottlenecks to bring new therapies to market faster than ever

istock

By DAN LACAZE

With ever-increasing competition, biopharmaceutical companies face significant challenges in swiftly bringing new therapeutics to market while effectively managing their capital and resources. In 2022, top biopharmaceutical companies allocated an average of over $2 billion to launch a single therapy from R&D through clinical trials to the market, highlighting the immense investment involved.[1] 2022 also saw FDA approvals for biopharmaceuticals pushed ahead of small molecules for the first time.[2] In comparison to traditional small-molecule drug production, biopharmaceuticals are more sophisticated and difficult to scale, as their manufacturing processes necessitate specialized facilities operated by highly skilled personnel, with annual operational expenses reaching between $37.3 to $55.5 million annually.[3] For some advanced therapeutic medicines, the cost to manufacture each therapy can be as high as $500,000.[4]

To maintain their leadership position and ensure a favorable return on investment, industry leaders must continually reassess their operational strategies and embrace innovative technologies to streamline processes, eliminate bottlenecks, reduce variability and enhance the speed and reliability of their development workflows. One ubiquitous and indispensable biomanufacturing input ripe for improvement is a scalable, turnkey solution for high-quality synthetic DNA. DNA serves as the programmatic code for every living cell and plays a crucial role in the development of all biopharmaceuticals, including mRNA vaccines, cell/gene therapies and precision medicines.

A fast and reliable supply of DNA is imperative for biopharmaceutical companies to ensure the overall efficiency and success of their operations. However, manufacturing DNA that meets the necessary length, accuracy, complexity, purity and scale requirements to advance therapeutic candidates from discovery to the market has proven to be anything but turnkey.

Recent innovations in DNA manufacturing are helping eliminate critical bottlenecks to bring new therapies to market faster than ever.

The cell-based DNA synthesis bottleneck

In the early stages of development, biopharmaceutical candidates heavily depend on an iterative design-build-test-learn (DBTL) cycle. This cycle involves testing and re-designing synthetic DNA constructs until potential therapeutic candidates are identified. However, conventional manufacturing methods often result in lengthy, weeks-long production times, especially for longer and more complex DNA constructs. Consequently, researchers spend a significant amount of time building DNA or waiting for suppliers to do so, delaying the crucial task of testing hypotheses and optimizing candidate therapies.

A closer look at mRNA therapy

A clear example of the DNA supply bottleneck can be found in the development of mRNA therapeutics produced by *in vitro* transcription (IVT), a process whereby a linearized DNA sequence template is transcribed into mRNA. The conventional production of long, double-stranded DNA templates starts by assembling short, single-stranded oligonucleotides of 60–100 bases into short, double-stranded DNA fragments, typically up to 2,000 base pairs (bp) long.[5] Due to errors inherent in the synthesis methods used, the short fragments produced are not pure, often containing molecules with errors in the DNA sequence. Manufacturers use a weeks-long process to correct the errors in the fragments and assemble them into a cloning vector, which is then propagated in bacterial cells that are ultimately plated on agar plates to grow into colonies. Bacterial colonies are sampled and sequenced to identify a sequence-perfect clone. Once a colony carrying a sequence-perfect plasmid is selected, the plasmid DNA is isolated from the cells, linearized and purified to serve as a template for mRNA synthesis by IVT.

The task of linearizing and purifying plasmid DNA prior to IVT should not be regarded as trivial by any means. Inefficient linearization of the plasmid can result in the expression of backbone DNA

wacomka/Getty Images

containing antibiotic-resistant markers. This, along with any remnants of host DNA or other contaminants from cell-based cloning, must be removed prior to IVT, adding time and cost to the process.

The entire process is then repeated to test each new DNA template design, taking months to identify candidates for preclinical studies. For therapeutics using self-amplifying RNA and trans-amplifying RNA formats, the frequency of design iteration can be higher than traditional mRNA designs, taking even more time. Speed is critical in the biopharmaceutical industry, and waiting weeks for DNA for each iterative development round is not an optimal solution.

The challenge of scaling mRNA vaccine production

After a therapeutic candidate has been identified and the decision is made to move into clinical testing, large quantities of plasmid DNA, manufactured under GMP compliance, are required to synthesize enough mRNA to perform clinical studies and treat patients. For this, conventional GMP workflows rely on the same weeks-long, tedious and cumbersome molecular cloning process, as well as two to three months of initial studies to select, characterize and establish a master cell bank (MCB), a large population of cells that serve as a seed stock. GMP DNA is then isolated, purified, linearized and transcribed from the MCB into large quantities of mRNA using IVT.

While cell-based processes are arguably cost-effective for high-scale amplification, they require months for processing and are plagued with many problems, batch-to-batch variability, extensive purification and testing, and potential downstream toxicity resulting from residual cell debris post-cellular lysing. Not to mention that many desired therapeutic constructs are toxic to bacterial hosts, preventing them from being

manufactured in the first place, despite their potentially superior therapeutic performance. Realizing that they cannot continue to rely on antiquated processes, forward-thinking organizations are exploring DNA manufacturing innovations that eliminate conventional cloning, large fermentation tanks and the rising costs of associated infrastructure and labor.

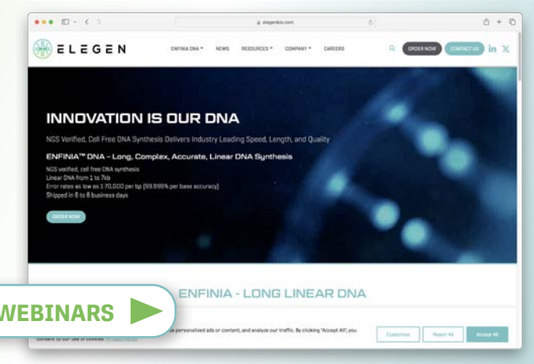
New innovations enable cell-free DNA synthesis

Surprisingly, there has been relatively little innovation in conventional DNA synthesis over the past several decades. Recent years have seen some incremental innovations that address singular steps of the build workflow. However, although promising, these singular improvements fail to deliver a meaningful impact on the performance of the overall DNA manufacturing process.

Synthetic DNA is produced by a continuous build process to transform single, low-quality oligonucleotides and fragments into longer DNA and genes. Therefore, innovating a single step, such as oligonucleotide synthesis, only to then use conventional cloning to build full-length DNA or scale the production of DNA is not an effective solution. No singular innovation can solve the DNA supply bottleneck in genetic medicine development. Radical changes are needed throughout the DNA production process, not least of which includes the cell-based cloning step, used by synthetic DNA manufacturers for the past several decades.

Recently, new suppliers have emerged with a focus on producing long, double-stranded DNA in novel ways. These companies use innovative cell-free synthesis, assembly and cloning strategies to accelerate and scale full-length DNA production. The result is sequence-perfect, multi-kilobase, double-stranded linear DNA that can be manufactured in days and used in therapeutic workflows with minimal or no additional cloning. Companies have received NGS-verified, high-complexity DNA as long as 7,000 bp in less than a week, ready for immediate use downstream without further processing. With this length and accuracy, even long gene constructs, e.g., 20,000 bp, can be assembled within two weeks. With accuracy nearly equivalent to

Learn More about how Elegen's cell-free DNA synthesis technology delivers fast turnaround, long, very complex, and highly accurate synthetic DNA at a competitive cost to streamline discovery and development.



plasmid DNA, the high-quality linear DNA can be used directly in IVT workflows, eliminating the weeks of time needed to clone, purify and linearize plasmid DNA.

With an agnostic approach to oligonucleotide synthesis chemistry and cell-free cloning technology, this groundbreaking innovation in DNA manufacturing offers unparalleled scalability and cost-efficiency compared to traditional methods. It is revolutionizing the discovery and development of mRNA therapies and will enable rapid scale-up of therapeutic candidates for preclinical and clinical studies. By eliminating bottlenecks in genetic medicine workflows, this technology not only accelerates the development process but also ensures the delivery of full-length, high-quality DNA free from cellular contaminants. Looking beyond mRNA biotherapies, this capability is particularly crucial for individualized therapy, where the timely production of safe and effective treatments, devoid of cellular contaminants, can significantly extend patients' lives.

Conclusion

The COVID-19 pandemic and the development of its mRNA-based vaccines brought attention to the need for faster healthcare innovations to meet medical challenges. Indeed, companies are racing to apply this technology to prevent and treat many illnesses, including influenza, shingles, HIV and cancer. Biopharmaceutical organizations seeking to remain competitive must adopt innovations, like Next-Gen Gene Synthesis using Cell-Free Cloning, to unleash the potential of synthetic biology and accelerate therapeutic development across all facets of genetic medicine. ■

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