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The 'anti-hype' vaccine

After years under the radar, RNA vaccines are finally emerging as immunotherapies to combat pandemics and cancer. But as Laura DeFrancesco reports, clinical validation remains elusive.

'Vaccines on demand' is an alluring, yet misleadingly simple, concept. Find an antigen of choice against a scourge of choice. Sequence and synthesize it. Formulate a product and deliver it to a site in the body where it elicits a clinically meaningful immune response. Such a vaccine would enable rapid-response agents against pandemic threats. Alternatively, it could provide personalized cancer immunotherapy, with each new batch of vaccine tailored to reflect the antigenic content of a patient's evolving drug-resistant tumors.

RNA—long overlooked as a starting point for immunotherapy—potentially ticks many of the boxes for a vaccine on demand, offering advantages in manufacture, flexibility, scalability and cost of goods. At the same time, clinical validation faces formidable barriers, such as antigen discovery, product formulation and delivery. To emphasize the challenge, vaccine developer CureVac reported in January that its prostate cancer product—the first RNA vaccine to be put into humans—had failed to improve survival over standard of care. Even so, a recent flux of funding and research interest promises to reinvigorate the field.

Rapid responses

For many years, RNA languished as the 'ugly stepchild' of nucleic acid vaccine development, with DNA reigning supreme. One reason for the slow development was that few in the research community considered RNA a good starting point (**Box 1**).

In recent years, a crucial impetus for advances in RNA vaccine technology—and their application to rapid response efforts—has been support from the US Defense Department's Defense Advanced Research Projects Agency (DARPA)—specifically its Biological Technology Office (BTO) in Washington, D.C. Since 2011, DARPA has provided substantial funding for several early-stage RNA vaccine programs (**Table 1**). "We like to think of ourselves as [funders of] early-breakthrough, high-risk, things that would not normally be funded. If you were going to do a safe biomedical research program, with incremental approaches to solving a problem, that would not be us," says Matt Hepburn, a program manager at DARPA's BTO. Their overriding interest is in having platforms in place that can respond quickly in the case of a pandemic. "Our mindset, day, night, 24/7, is what do we need to do to be ready for the next pandemic," he says.

The potential of RNA vaccines to perform in such a setting was demonstrated in 2013 during an outbreak of a deadly strain of (H7N9) influenza in China. The Chinese Center for Disease Control and Prevention deposited the sequence in GenBank, a web-based database, and folks at Novartis (with funding from DARPA and the Biomedical Advanced Research and Development Authority, BARDA) set out to see whether they could make an effective vaccine based on the electronic sequence. "We were lucky enough to be collaborating with Craig Venter's teams [Synthetic Genomics and Synthetic Genomics Vaccines, both based in La Jolla, California], so we made a vaccine in 8 days, put it in mice in 13 days and showed that, indeed, it works", says Andrew Geall, who was running Novartis's mRNA vaccine program until it was moved to GlaxoSmithKline (GSK) Vaccines in Rockville, Maryland, as part of an asset swap[1]. (Geall currently serves as VP of formulations at the RNAi company Avidity BioSciences in La Jolla, California). By "it works," Geall means that the vaccine raised neutralizing antibodies after one injection of 1 mg and all protect mice had HI titers considered protective after two doses.

DARPA could not be happier. "What you are seeing in the field now is that using a nucleic acid to get the body to do what you want it to do, whether it's a vaccine or other responses, is really exploding. We find that profoundly exciting," says Hepburn. Recently a DARPA-funded Moderna program in Zika was handed

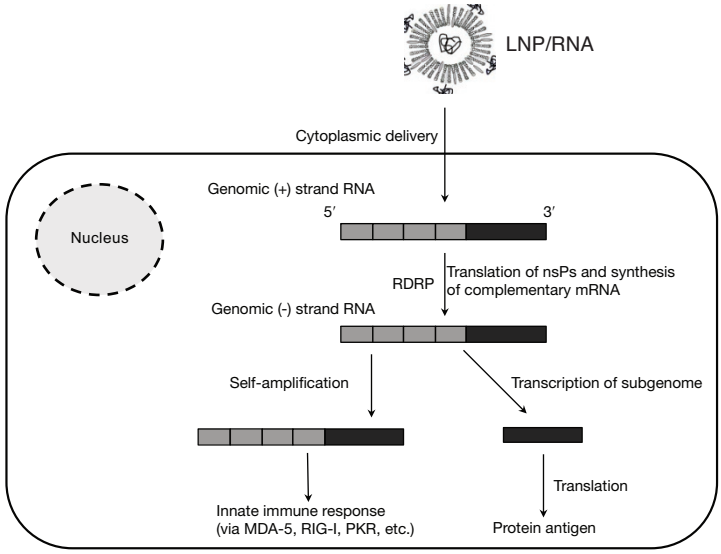

Figure 1 Cytoplasmic delivery of a self-amplifying mRNA using a LNP non-viral delivery system. LNPs are taken up by endocytosis and RNA is delivered to the cell cytoplasm. Once in the cytosol, the self-amplifying mRNA co-opts host cell translation machinery to produce the RNA dependent RNA polymerase (RDRP) encoded in the non-structural protein (nsP) domain (light grey boxes). This allows transcription of the positive strand genomic RNA into a negative strand template. Then, both full-length genomic RNA as well as the subgenomic RNA transcripts encoding the vaccine antigen (dark grey box) are generated. Self-amplification leads to innate immune stimulation which adjuvants the immune response to the vaccine antigen. Source: Andrew Geall, Avidity BioSciences.

off to BARDA—which serves the application and delivery side, as opposed to development side, of the US government's medical countermeasures program. DARPA is providing funding for preclinical development and a phase 1 trial of a chikungunya vaccine.

To infection and beyond

Although several commercial RNA vaccine programs are already moving forward in infectious disease, rapid response is not yet a theme (**Table 2**). Ingmar Hoerr, co-founder, chairman and CEO of CureVac in Boston–based CureVac, is focusing on rabies as a first proof of principle. According to Hoerr, infectious disease indications offer a wealth of traditional vaccines against which to benchmark RNA vaccine responses. "There is a vaccine and [World Health Organization] guidelines for titer level." This contrasts with cancer, where the lack of conventional vaccines makes it difficult for developers to assess effectiveness of RNA as an immunogen. In infectious disease, "you can see where you are," says Hoerr.

CureVac's rabies vaccine, which encodes the viral glycoprotein, stimulates both B-cell and T-cell responses in volunteers. "We have a safe product, you don't need eggs or cell culture. It's quite cheap and it could be a dollar a dose," says Hoerr. The company also recently showed that it is possible to immunize against more than one pathogen at a time, having combined several (up to six) flu hemagglutinin (HA) antigens in one vial. According to Hoerr, it's unusual to receive immune responses against all HA antigens at the same level in one vial.

Another company with an RNA vaccine in the clinic is Argos Therapeutics, based in Durham, North Carolina (and known as Merix until 2004). According to CEO Charles Nicolette, the company has been investigating the use of RNA encoding a modified form of the CD40 ligand protein to pretreat antigen-presenting dendritic cells (DCs). Nicolette notes that this protocol imitates the presence of $CD4^+$ helper T cells[2]. "The DC thinks it's in a healthy individual and is able to do its job and generate the immune response that we want", he says. Having a functioning DC that is independent of the requirement for $CD4^+$ T-cell help brought AIDS to mind. "The [human immunodeficiency] virus has elevated to an art form the destruction of $CD4^+$ T cells; it's what kills you in the end. So we asked the question: can we use our RNA technology to target autologous virus and be able to immunize the patients effectively?" According to Nicolette, the answer may be yes. The company has been able to induce antiviral immunity in HIV patients and, on the basis of this work, has attracted funding from the US National Institutes of Health (NIH) Division of AIDS. "We were awarded a contract for $40 million. Our HIV program is alive and kicking, and we're pretty excited about it."

Critical elements

Early signs that RNA might have some use in a therapeutic setting came in 1990, with the publication of pioneering work by John Wolff, Phil Felgner and colleagues at the University of Wisconsin–Madison demonstrating that native RNA can be injected directly into muscle in mice and expressed[3]. CureVac's Hoerr, at the time a PhD student at the University of Tübingen, was amazed that no one picked up on *in vivo* administration of RNA. Although still a student, he was able to directly administer RNA, but most other researchers steered clear of RNA, he says. "Nobody was trying our approach or taking account of what we were doing, although we published a lot of papers." In recent years, that has been changing, particularly as ways of making, delivering and expressing mRNA have improved and flagship companies such as CureVac and Moderna have sprung up, attracting both researchers and investors to the space.

The technology improvements that contribute to an effective RNA vaccine come in several forms. Modifications to the RNA molecule itself improve its stability and increase its expression upon delivery. These include adding a cap (m^7GpppG) to the 5′ end and tacking on a poly(A) tail to the 3′ end, optimizing codon usage and GC content, providing 5′ and 3′ untranslated elements that facilitate ribosome binding and boost expression[4].

But to get efficient translation, the RNA has to stick around, and cells have various mechanisms to dispose of incoming, presumably pathogenic nucleic acids. Upon engagement, these pathways—involving Toll-like receptors, the RNA helicase RIG-I and protein kinase R—induce an innate immune response, which, among other things, shuts down the cell's translational machinery. In 2005, Kaitlin Kariko, then at the University of Pennsylvania in Philadelphia, showed that it is possible to 'de-immunize' RNAs by incorporating naturally occurring modified bases[5], and that the translational efficiency of mRNA can be improved by replacing uridine with pseudouridine[6]. Following this work, Derrick Rossi, a co-founder of Moderna who is at Harvard Medical School in Boston, took it a step further and showed that substituting pseudouridine for uridine and 5-methylcytidine for cytidine works even better[7].

Another refinement to the RNA molecule that enhances its efficacy as a vaccine is the addition of the coding region of viral replicases, which render the molecule self-amplifying. First described for RNA in the 1990s by Peter Lijestrom, using Semliki Forest virus[8], self-amplifying replicons can reduce the amount of

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material required to raise an immune response. More recently, the Novartis vaccine group incorporated this feature into its RNA vaccine designs. Their influenza vaccine, for example, which uses the alphavirus replication machinery (**Fig. 1**), requires a single dose of less than 1 μg to get a response equivalent to two 80 μg doses with an unamplified RNA vaccine[1].

Finally, Moderna's new CSO Melissa Moore, who joined the company in October 2016, is applying her expertise on riboncleoprotein structure to enhance ribosome engagement. Moore has shown that secondary structure positively impacts translational efficiency[9].

Delivery, delivery, delivery

The development of delivery vehicles is another critical component to getting RNA to function *in vivo* (see 'Worth the risc?' doi:10.1038/nbt.3810). The vehicles primarily protect the RNA from degradation, but their composition can also influence uptake and localization *in vivo*, according to Philip Santangelo at The Wallace H. Coulter Department of Biomedical Engineering at Georgia Tech and Emory University both in Atlanta. Santangelo is developing imaging systems for live-animal-based visualization of RNA using ^{64}Cu-labeled molecules and positron emission tomography (PET) scanning[10]. With support from DARPA, Santangelo has been tracking RNA after delivery and, in unpublished work presented recently at a DARPA-sponsored conference, showed time series data of monkeys after intramuscular injection of *in vitro*–transcribed RNA. "The whole idea was to come up with a methodology that would allow you to come up with the whole picture," says Santangelo.

There are almost as many delivery vehicles as there are groups working in the space. Some encapsulate the RNA molecules in particles, whereas others use positively charged polymers to grab onto the RNA through charge interactions. Geall, who had worked on delivery systems for small interfering RNA (siRNA) at Novartis before heading up the vaccine program, borrowed from that work and used a cationic lipid nanoparticle (LNP) originally developed by Peter Cullis at the University of British Columbia for systemic delivery of siRNAs and commercialized by several RNA therapeutic companies. For a proof of concept, Geall's group used 1,2-dilinoleyosxy-3-dimethylaminopropane, which had been demonstrated to be effective at delivering siRNA intravenously[11]. In parallel, the team at Novartis vaccine developed a cationic nanoemulsion (CNE), based on the company's proprietary adjuvant MF59, which has an established clinical safety profile[12]. This adsorbs mRNA on the surface of an emulsion droplet, whereas the LNP encapsulates the mRNA in a lipid envelope. Both approaches provide protection to the mRNA from enzymatic degradation from RNases.

Mainz, Germany–based BioNTech, which was spun out of Johannes Gutenberg University in 2008, recently reported on a delivery platform that localizes in lymphoid tissue after intravenous injection. By systematic varying of lipid nanoparticle parameters (charge, size and lipid composition), they found that particles with a slightly negative charge home to lymphoid tissues. According BioNTech CEO Ugur Sahin, most people use positively charged vehicles, because they stick to cell membranes (regardless of cell type), giving good transfection efficiencies. Because they were interested in maximizing transfection efficiencies, many groups have stayed with positively charged particles, he says. But researchers at BioNTech found that with their negative particles, comprising two common lipids (DOTMA and DOPE), they can restrict uptake to DCs, capitalizing on the biological process of macropinocytosis, by which DCs and macrophages sweep up solutes from their environment. In addition to protecting the payload and mediating its uptake and eventual expression, the particle triggers interferon-γ (IFN–γ) release, leading to maturation of DCs *in situ* and a strong T-cell response. "At the end of the day, what we accomplished is that we have particles that behave like viruses," Sahin says[13].

Researchers at the Massachusetts Institute of Technology's Koch Institute in Cambridge have developed a 'tunable' dendrimer-based particle, comprising molecules of high amine density with organized branching structures that condense around mRNA molecules, folding them into monodisperse spheres. Omar Khan, a chemical engineer at the Koch Institute, points out the repurposing the delivery technology developed for siRNAs is not the right approach for mRNA that can be an order of magnitude larger. "If people are using old technology that was designed for siRNA, which is ~19 nucleotides long, and then we want these replicons that are at least 10,000 nucleotides long—so to us, making the molecular structure tailored for the replicon made sense," says Khan. Using their vaccine, Khan and his collaborators at MIT have shown that after a single intramuscular injection, mice are protected from a lethal exposure of influenza (using hemagglutinin protein antigen), Ebola (using Ebola glycoprotein antigen) and *Toxoplasma gondii* (with six *T. gondii*–specific antigens)[14]. Khan and Jasdave Chahal, a virologist who designed the replicon RNA molecules used in the vaccines, have formed a company with their faculty mentors, Tiba Biotech (*tiba* is a Swahili word for 'cure'). They also have partnerships with Charles Shoemaker at Tufts Veterinary School in North Grafton, Massachusetts, and the US Army Medical Research Institute of Infectious Diseases in Frederick, Maryland, to develop vaccines against the parasitic worm *Schistosoma mansoni* and Ebola, respectively. Ultimately, the duo would like to see their technology—which is self-assembling, fast (taking only 7 days to go from pathogen sequence to vaccine), single dose and capable of carrying multiple antigens—be applied in the global health arena.

Cancer in the crosshairs

There are two ways to administer an RNA vaccine. One is injection of complexed or encapsulated RNA under the skin or into muscle; the other is pre-loading of DCs (or a cell population enriched for such antigen-presenting cells) *ex vivo* with RNAs and using those cells as both delivery vehicle and RNA translator.

One of the earliest RNA vaccines to enter clinical trials was the latter type of platform, developed by researchers at Duke University in Durham, North Carolina[15]. The technology

Box 1 Making RNA

Attempts to vaccinate with nucleic acid date back decades. To date, a handful of nucleic acid vaccines have made it to registration, albeit for veterinary, not human, applications. Only one of those products is currently commercially available, a Novartis vaccine to protect farm-raised salmon from the infectious hematopoietic necrosis (IHN) virus. Some believe that an early focus on DNA rather than RNA sent the field down the wrong path.

Part of the problem is that RNA has been widely perceived in the research community as too fragile and difficult to work with. "There's a preconception that RNA is not a stable molecule, it's been designed to be unstable, hydrolyzed, and there are RNases everywhere. All of that is true, but it's controllable, and it's not going to be an issue pharmaceutically," says Avidity Biosciences' Geall.

Geall points out that people had not realized that RNA is actually easy to make; the only barrier was having enzymes sufficiently pure to make products that could be put into humans. He took this problem to several life-science suppliers and "gently twisted their arms" into considering making enzymes for a good manufacturing practice (GMP)-like production process. "They embraced it and began, and now those enzymes are available. And then everyone learned that making RNA is pretty simple," he says.

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Table 1 Funding for RNA vaccines

Company	Date of funding, source	Terms
BioNTech	November 2015, Sanofi	$60 million upfront and near-term milestones for co-development and co-commercialization of mRNA cancer immunotherapies
	September 2016, Genentech	$310 million upfront & near-term payments, 50:50 cost and profit share to develop novel mRNA-based, individualized cancer vaccines IVAC Mutanome
	May 2016, Bayer	mRNA vaccines and therapeutics specifically for animal health applications
CureVac	November 2011, Sanofi with DARPA funding	$33.1 million to co-develop RNA vaccines for infectious diseases using RNActive technology. Terms with Sanofi undisclosed
	November 2011, Sanofi	Option to exclusive license to mRNA vaccine to undisclosed pathogen, terms undisclosed
	October 2013, Johnson & Johnson	Partnership to develop influenza RNA vaccine, terms undisclosed
	March 2015, Gates Foundation	$52 million equity investment to construct GMP production facility with additional funding for prophylactic vaccines for infectious diseases
	September 2015, International AIDS Vaccine Initiative	Combine HIV trimer construct with RNAactive technology, terms not disclosed
	September 2014, Boehringer Ingelheim	€35 million ($45M) upfront payment and €430 million ($563M) milestone payments for exclusive global license for CV9202 in NSCLC
Infectious Disease Research Institute (Seattle)	October 2016, NIH	$500,000 for Zika mRNA vaccine
Moderna	October 2013, DARPA	$25 million, emerging infectious disease and engineered biological threats (including Chikungunya)
	January 2015, Merck	$50 million up front for partnership to discover, develop and commercialize five mRNA vaccines against four undisclosed viruses
	January 2016, Gates Foundation	Up to $100 million infectious diseases
	July 2016, BARDA	$125 million, Zika
Synthetic Genomics	January 2017, Johnson & Johnson	Partnership to develop RNA-based therapies for cancer and infectious diseases; terms not disclosed

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was licensed to Argos in 1997 (formerly Merix), which currently has two cancer immunotherapies in the clinic (**Table 2**). The company's platform involves extracting and amplifying mRNA from a tumor and transfecting a patient's DCs *ex vivo* with the entirety of the products. Before transfection, the DCs are preconditioned with a mixture of agonists to IFN-γ receptor and/or tumor necrosis factor-α receptor followed by secondary transient conditioning with a CD40 agonist. According to Argos CSO Charles Nicolette, "We do not sort through what is normal and what is mutated." He adds, "we amplify all the mRNA from the tumor sample, and we let nature figure out what it can attack and what it should leave alone." As a result, Argos departs from many cancer vaccine companies that attempt to sort through identified neoantigens and predict what ends up presented. "That's how we parted company [with the RNA vaccine field]. A lot of people don't think of us as a RNA company."

Argo's first product in human testing is for renal cell carcinoma (RCC) in combination with the tyrosine kinase inhibitor Sutent (sunitinib). The RCC patient population in the trial normally survive only 15 months from diagnosis. In a phase 2b trial of 21 patients, those immunized with tumor RNA had a median survival time of 30 months, and two patients remained in long-term remission for more than 7 years[16].

The vaccine, rocapuldencel-T (also called AGS-003), is now in a pivotal randomized phase 3 ADAPT (autologous dendritic cell immunotherapy plus standard treatment) trial, with 462 RCC patients stratified by risk factors for progression. Argos plans to expand clinical development to other tumor types, such as bladder cancer and non-small-cell lung cancer.

The alternative and more common direct injection route of vaccination was pioneered by German researchers at the University of Tübingen, using protamine-complexed RNA[17]. This program, which became the foundation of CureVac, which, after receiving regulatory clearance on RNA made following good manufacturing practice (GMP) standards, put its first RNA cancer vaccine product (CV9103) into patients in 2008, targeting four antigens: prostate-specific antigen (PSA), prostate-specific membrane antigen (PSMA), prostate stem cell antigen (PSCA) and six transmembrane epithelial antigen of the prostate 1 (STEAP1). "We did sequence modulation looking for natural elements that allow for high expression, ribosomes binding, RNA to be stable for a couple of days, not just a couple of hours," says Hoerr. With their optimized sequence, they observed strong T-cell responses in preclinical work.

Bolstered by the positive regulatory result for the DNA prostate cancer vaccine Provenge (sipuleucel-T, made by Dendreon, based in Seattle) and other positive data from vaccines in oncology trials, Hoerr focused initially on cancers where strong T-cell responses are needed. "When we planned our first trial, everybody thought monotherapy would be enough. You would have to encode a number of antigens make them immunogenic, inject them and it would be a beautiful T-cell response," he says. On the basis of promising phase 1/2 results—strong $CD4^+$ and $CD8^+$ responses, as well as responses to all four antigens[18]—the company initiated a phase 2b trial in castration-resistant prostate cancer with an updated vaccine (CV9104) carrying two additional antigens: prostatic acid phosphatase (PAP) and mucin 1 (Muc1).

Hoerr says that the CureVac team is still analyzing the data from the phase 2b trial—only the top-line results were reported in January. They are also looking toward combination trials with their lung cancer vaccine and checkpoint inhibitors. Since 2014, they have collaborated with Boehringer Ingelheim, based in Ingelheim am Rhein, Germany. They also have a recently completed trial in non-small-cell lung cancer, in which patients receive radiation, which has been shown to be synergistic, before RNA vaccination.

Elsewhere, BioNTech recently reported early results with a small cohort of melanoma patients treated with their RNA-lipoplexes delivery system, which contains RNA for four tumor-associated antigens: NY-ESO-1, MAGE-A3, tyrosinase and transmembrane phosphatase with tensin homology. The company detected α-interferon release as well as an immune response against the four antigens[13]. Sahin is encouraged by the

response to these antigens, which go against the current craze for neoantigens. "If you go to the literature, you will hardly find convincing tyrosinase immunogenicity in patients. We continue to see strong T-cell response, with these non-neoantigens… I am excited that this [liposomal RNA vaccines] might open the door to explore also non-neoantigens with different types of cancer," he says.

BioNTech is also working on two kinds of personalized cancer vaccines, which draw from a library of shared (off-the-shelf) antigens and from a library constructed from individually tailored approach constructed from the tumor neo-epitope of each patient[19]. Using a set of synthesized RNAs identified via algorithms that can identify neo-epitopes binding major histocompatibility (MHC) class II molecules, company researchers have shown that they can induce tumor regression in mice and have reported they can identify similar sequences in human tumors[20]. Both types of personalized vaccines are currently in phase 1 trials for melanoma and triple negative breast cancer.

Table 2 RNA vaccines in clinical trials

Company	Drug	Indication	Stage of development
BioNTech	Lipo-MERIT	Melanoma	Phase 1
BioNTech	IVAC mutanome	Melanoma	Phase 1
BioNTech	TNBC-MERIT	Triple-negative breast cancer	Phase 1
CureVac	CV9104	Prostate cancer	Phase 1, phase 2
CureVac	CV9202 plus radiation	NSCLC	Phase 1
CureVac	CV7201	Rabies	Phase 1
CureVac	CV8102	RSV, HIV, rabies	Phase 1 rabies
CureVac	CV9103	Prostate cancer	Complete
Moderna	mRNA 1851	Influenza H10	Phase 1
Moderna (DARPA funded)	mRNA1388	Chikungunya	Preclinical
Moderna (BARDA funded)	mRNA 1325	Zika	Phase 1/2
Moderna	mRNA1440	Influenza H7	Phase 1
Moderna and Merck (Kenilworth, New Jersey)	mRNA 4157	Cancer (personalized)	Preclinical
Moderna	mRNA-1647	CMV	Preclinical
Moderna	mRNA-1653	HMPV/PIV3	Preclinical
Argos	AGS-003	RCC	Phase 3
Argos	AGS-003	NSCLC	Phase 2
Argos	AGS-0004	HIV/AIDS	Phase 2
GSK and Vaccine Research Center at NIH	Self-amplifying RNA vaccine	Zika	Preclinical

PPD, Pharmaceutical Product Development; NSCLC non-small-cell lung cancer; RSV, respiratory syncytial virus; CMV, cytomegalovirus. HMPV/PIV3, metapneumovirus (hMPV) and parainfluenzavirus type 3 (PIV-3).

Early exuberance

It is still early days for RNA vaccines—probably too early to tell whether they will fulfill their promise in the clinic. "If you look at the history of all the new technologies, what happens initially is a huge amount of enthusiasm," says Margaret Liu, a consultant in the field of vaccines, who pioneered DNA vaccines at Merck in the early 1990s and has worked on vaccines and immunotherapies since. Fueling the recent wave of enthusiasm, in no small part, has been the amount of money that Moderna raised in short order. "People are afraid of missing out and would rather invest and be wrong than miss out. I don't think it's predictive of whether it will be a success, it certainly enables success," says Liu. By some measures, this has been borne out.

According to public disclosures, Moderna has advanced five programs into clinical trials and established four biopharma partnerships. It is building out a $110-million GMP clinical manufacturing facility.

But several key questions about this vaccine technology remain unanswered: how is RNA transcribed and then processed into epitopes on the cell surface? What is the best route of delivery, and how do responses differ depending on which tissues are targeted? What kind of immune response is protective, in the case of infectious diseases, or curative, in the case of cancer?

One issue that may be keeping pharma from entering the field is competition from existing vaccines. Geall says that at Novartis, he had to work against the notion that RNA vaccines would not measure up to commercial products. But, he says, his group was able to show that for both influenza and rabies, their product performed well in animal models, which he feels bodes well for human use. Not everyone agrees that the probability of success is high, based on animal results. Liu says, "Preclinical studies don't predict very well for vaccines. They can be negative, and that can be useful, but [positive results] don't mean that products are around the corner."

Despite the disappointment surrounding the results from CureVac's first RNA vaccine trial in humans, people working on these programs remain optimistic. "The failure was in a very difficult indication in oncology with an asset that they developed a long time ago," points out Geall. And encouraging preclinical successes continue to come. Just last month, a group of investigators reported results with a lipid nanoparticle mRNA vaccine for Zika; they were able to raise neutralizing antibodies as well as protect both mice and rhesus monkeys with a single dose[21].

"Ultimately, it will boil down to picking the right target—the right antigen being coded—and the right disease, whole pathophysiology of the disease," says Liu. But BioNTech's Sahin has no doubts. "I'm absolutely convinced, it's not hype. RNA is anti-hype. It remained under the radar for many years, and it's now mature enough to fulfill promises."

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1. Hekele, A. *et al. Emerg. Microbes Infect.* **2**, e52 (2013).
2. Tcherepanova, I.Y. *et al. BMC Mol. Biol.* **9**, 9 (2008).
3. Wolff, J.A. *et al. Science* **247**, 1465–1468 (1990).
4. Sahin, U., Karikó, K. & Türeci, Ö. *Nat. Rev. Drug Discov.* **13**, 759–780 (2014).
5. Karikó, K., Buckstein, M., Ni, H. & Weissman, D. *Immunity* **23**, 165–175 (2005).
6. Karikó, K. *et al. Mol. Ther.* **16**, 1833–1840 (2008).
7. Warren, L. *et al. Cell Stem Cell* **7**, 618–630 (2010).
8. Zhou, X. *et al. Vaccine* **12**, 1510–1514 (1994).
9. Ricci, E.P. *et al. Nat. Struct. Mol. Biol.* **21**, 26–35 (2014).
10. Santangelo, P.J. *et al. Nat. Methods* **12**, 427–432 (2015).
11. Geall, A.J. *et al. Proc. Natl. Acad. Sci. USA* **109**, 14604–14609 (2012).
12. Brito, L.A. *et al. Mol. Ther.* **22**, 2118–2129 (2014).
13. Kranz, L.M. *et al. Nature* **534**, 396–401 (2016).
14. Chahal, J.S. *et al. Proc. Natl. Acad. Sci. USA* **113**, E4133–E4142 (2016).
15. Heiser, A. *et al. J. Clin. Invest.* **109**, 409–417 (2002).
16. Motzer, R.J. *et al. Br. J. Cancer* **108**, 2470–2477 (2013).
17. Weide, B. *et al. J. Immunother.* **32**, 498–507 (2009).
18. Kübler, H. *et al. J. Immunother. Cancer* **3**, 26 (2015).
19. Diken, M. *et al. Methods Mol. Biol.* **1499**, 223–236 (2017).
20. Kreiter, S. *et al. Nature* **520**, 692–696 (2015).
21. Pardi, N. *et al. Nature* http://dx.doi.org/10.1038/nature21428 (2017).

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