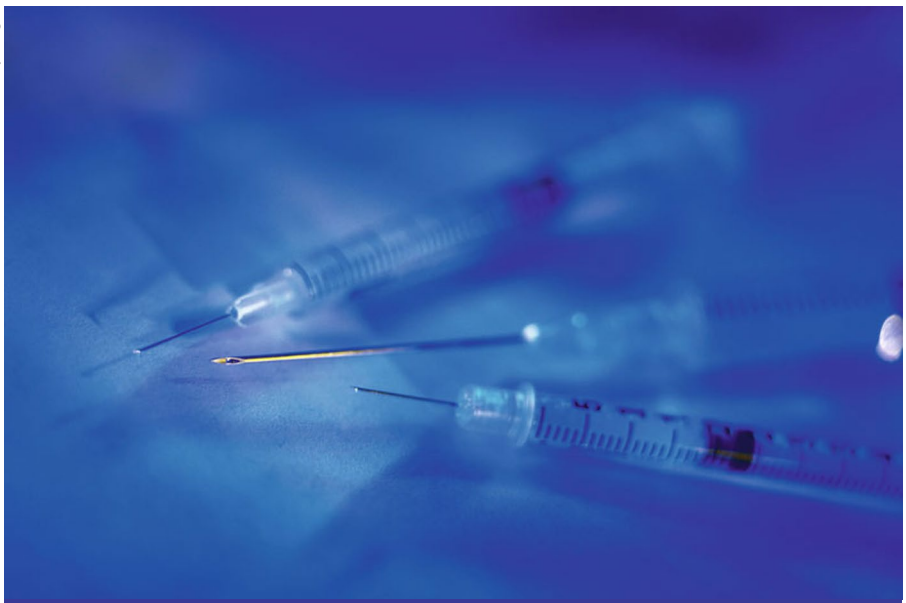

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mRNA flu shots move into trials

COVID-19 provided an opportunity to show that mRNA vaccines can work. Now, drug companies are racing to apply the technology platform for influenza.

Elie Dolgin

Hot off the successful deployment of mRNA vaccines for the prevention of COVID-19, three leading drugmakers have moved seasonal flu vaccine candidates built with the technology into early clinical testing. More companies intend to follow their leads next year (TABLE 1).

These candidates, if successful, could dramatically bolster the efficacy of a vaccine class that often delivers lacklustre protection. For mRNA's proponents — led by Moderna, Pfizer and Sanofi, all of which initiated phase I trials in recent months — new flu jabs could prove lucrative or help maintain standing in a global market projected to exceed US$10 billion by decade's end.

But flu shots could also prove a more challenging test for mRNA than did COVID-19. That's because unlike with SARS-CoV-2 — for which there were no established medical interventions — nine flu jabs from four different vaccine manufacturers are already available in the United States alone.

Those vaccines are safe, but their efficacy leaves room for improvement. Existing flu shots, whether built around inactivated viruses or recombinant proteins, typically offer only 40–60% protection from infection. In theory, mRNA might make for a better product: elicited immune responses may be broader, expressed proteins should have better sequence fidelity, strain selection may be more accurate and the technology makes it easy to incorporate large numbers of antigens. All of these features could translate into greater immune protection.

But mRNA, at least when formulated in lipid nanoparticles (LNPs), is prone to tolerability issues. Moderna and Pfizer/BioNTech's authorized mRNA jabs for COVID-19 often cause sore arms, headaches, low-grade fevers and fatigue. These same symptoms can occur with approved flu shots, but typically are much milder in degree.

mRNA "is a tool that does offer some upside potential," says Gary Nabel, former CSO of Sanofi, the founding director of the NIH's Vaccine Research Center (VRC), and CEO of the stealth-stage immunotherapeutics company ModeX Therapeutics. "The big stumbling block is safety."

To impact the established market, the platform would need to find a more tolerogenic sweet spot, as well as offer major efficacy advantages.

And yet, it's no sure thing that mRNA will prove capable of effectively delivering haemagglutinin glycoproteins, the main antigen found in flu vaccines. "Did we just get really incredibly lucky with COVID vaccines because of the antigen design and the immunodominancy of that protein?" asks Anna Blakney, an RNA bioengineer at the University of British Columbia. "Or have we stumbled on something that's functional for other viral glycoproteins as well?"

Making it

Many of the leading mRNA-based flu contenders were working on flu vaccine candidates before the pandemic. Then came the novel coronavirus, and "we literally swapped out flu coding sequences and swapped in SARS-CoV-2 sequences," says Philip Dormitzer, head of viral vaccines research at Pfizer.

The whole field has benefited. Seqirus stayed out of the COVID-19 vaccine race, despite having a self-amplifying mRNA platform that delivers both the antigen-coding sequence and the replication machinery needed for the construct to copy itself inside the host cell. (Seqirus acquired that platform from Novartis in 2015.) "Seeing an mRNA vaccine functional and generating such a robust response has somewhat derisked the outside concerns that people may have had around the general technology," says Ethan Settembre, vice president of R&D at Seqirus.

And the potential benefits of mRNA for flu prophylaxis are many.

Some of these boil down to how the vaccines get made. Because mRNA vaccines are manufactured synthetically, by encoding a target antigen sequence into a plasmid template, they offer high fidelity: encoded antigens exactly match the flu strains selected for each year's vaccine. By contrast, inactivated virus vaccines that are made in egg- and cell-based systems often suffer from sequence mutations that weaken their effectiveness.

Recombinant protein vaccines offer that same fidelity advantage, but the manufacturing process for these is comparatively cumbersome. The flexibility and speed of mRNA vaccine production

> "The big stumbling block is safety"

Table 1 | **Select mRNA vaccines for seasonal influenza in and approaching the clinic**

Sponsor	Product name	Technology	Type	Flu antigen(s)
In the clinic				
Moderna	mRNA-1010	mRNA	Quadrivalent	Haemagglutinin
Sanofi/ Translate Bio	MRT-5400, MRT-5401	mRNA [a]	Monovalent (H3N2)	Haemagglutinin
Pfizer	PF-07252220	mRNA	Monovalent (H1N1) and monovalent (B/Yamagata), to be combined into bivalent and quadrivalent	Haemagglutinin
Preclinical				
Moderna	mRNA-1020, mRNA-1030	mRNA	Multivalent	Haemagglutinin + neuraminidase
Moderna	mRNA-1073	mRNA	Quadrivalent + COVID-19	Haemagglutinin
Innorna		mRNA	Quadrivalent and pentavalent (two H3N2 strains)	Haemagglutinin
Sanofi		mRNA	Quadrivalent	Haemagglutinin
NIAID		mRNA	Monovalent (H1N1)	Haemagglutinin
NIAID		mRNA	Universal	Haemagglutinin stem
Seqirus		saRNA	Quadrivalent	Haemagglutinin
Pfizer		saRNA	Undisclosed valency	Haemagglutinin
Arcturus		Undecided	Quadrivalent	Haemagglutinin
GSK/CureVac		Undecided	Undisclosed valency	Undisclosed

[a]MRT-5400 and MRT-5401 rely on non-modified RNA. NIAID, National Institute of Allergy and Infectious Diseases; saRNA, self-amplifying RNA.

mean that vaccine makers could wait longer to begin manufacturing — starting production in May, say, instead of February, for the northern hemisphere. This would enable them to make more informed decisions about what strains to include.

"Every bit of time helps," says Sally Mossman, head of GlaxoSmithKline's vaccines R&D centre in the United States.

Enter the multiplex

There may be other efficacy benefits. The US relies on quadrivalent vaccines, containing haemagglutinin antigens (either purified from inactivated viruses or manufactured recombinantly) or live attenuated viruses that confer protection against four strains of influenza. (Other jurisdictions still use trivalent vaccines.) Some researchers have argued in favour of adding protection against additional strains, but doing so is logistically challenging with existing platforms.

> If you want to show that the technology works, the easiest place to start is with an influenza vaccine

Not necessarily so with mRNA.

A team led by Norbert Pardi, from the University of Pennsylvania Perelman School of Medicine, and Raffael Nachbagauer, then at the Icahn School of Medicine at Mount Sinai, showed as much last year. They immunized mice against one subtype of influenza with an mRNA vaccine encoding four different proteins: haemagglutinin stalk, neuraminidase, matrix-2 ion channel and nucleoprotein. Together with Mount Sinai's Meagan McMahon, Pardi and his colleagues have since repeated the exercise for two other flu subtypes. They plan to test a combined vaccine containing 10–12 antigens in mice and ferrets. Ideally, this will induce such broad immune protection that it doesn't need to be taken every year.

Many researchers also hope that mRNA will spur stronger or more diverse immune responses than traditional platforms. If true, this could prove especially beneficial for older adults, who often have weak responses to flu vaccines, notes Jenna Bartley, who studies immunological aging at the University of Connecticut School of Medicine.

It could even aid in the development of a universal flu vaccine that protects against all strains of the virus, says Duke University's Tony Moody. With NIH backing, he is now developing an mRNA-based haemagglutinin stabilized-stem vaccine, modelled after a protein-based one that is already in the clinic. "It's an informative experiment," says VRC director John Mascola. "We don't know how to induce a high level of durable antibodies to stem, and mRNA may be different in the way that it induces that response."

Current vaccine technologies mostly elicit only humoural responses that block viral invasion. mRNA vaccines, because they somewhat mimic natural infections, may bring about better T-cell responses.

Barney Graham also sees a broader societal opportunity: by working on seasonal mRNA candidates today, companies should be better prepared to tackle flu pandemics in the future. "That is the place mRNA could really change the deal," says Graham, who retired as deputy director of the VRC at the end of August. "If we really did have a pandemic, you could design [a new vaccine] within 10 minutes after you got the sequence, and you could have mRNA going into arms within a few weeks."

Show me the data

The only human data reported to date on an mRNA-based flu vaccine come from Moderna's first foray into the clinic. Beginning in late 2015, the company evaluated a pair of two-dose vaccines, each designed against a different strain of avian influenza.

According to former Moderna CMO Tal Zaks, the main goal of those trials was to give the company's mRNA a test run. "If you want to show that the technology works, the easiest place to start is with an influenza vaccine," he says. With influenza, he explains, the correlates of protection — measurements of immune parameters that allow prediction of vaccine efficacy — are well defined. There are also established vaccines to reference clinical results to.

To many onlookers, Moderna's first trials were a disappointment. Although most study participants who received higher doses of the experimental vaccines developed influenza-specific antibody titres above the threshold thought to be protective — as measured by the well-validated haemagglutination inhibition (HAI) assay — those antibody levels tapered off within just months of immunization. This suggests that the protection these offer might

be short-lived. T-cell immunity was not detectable at all.

Former company executives involved in the trials see things differently. Both Giuseppe Ciaramella, ex-head of infectious diseases research, and Michael Watson, former president of Moderna's vaccines unit, argue that the vaccines induced a robust pool of memory B cells, which remember antigens and can trigger rapid production of antibodies when people are re-exposed to the virus.

They highlight data from 5 participants who received their two shots 6 months apart, instead of 3 weeks apart like everyone else. Those individuals achieved much higher average HAI titres — "evidence," says Watson, "that the first dose had primed really well." Watson is now executive chairman of VaxEquity and chief executive of MEVOX, neither of which is working on influenza.

Watson is less sanguine about the tolerability findings, though. Although Moderna has since changed its LNP formulation and aspects of its mRNA design, its early trial data suggested that the therapeutic index for an mRNA-based flu vaccine might be narrow. This could prove problematic for a multi-component seasonal flu shot designed to train the immune system to recognize at least four antigens.

Moderna is nevertheless forging ahead clinically — and it has already begun adding additional antigens to its next generation of flu products. Neuraminidase, another surface glycoprotein, will be included in the company's follow-on clinical candidates. Other antigens that might better stimulate T-cell immunity are under consideration as well. Plus, the company is preparing combination vaccine candidates that could protect against multiple respiratory viruses. One of these, for COVID-19 and flu, is due to enter clinical testing next year.

But the addition of antigenic targets will likely necessitate higher total vaccine doses, points out Watson, potentially triggering more severe adverse reactions. "That's definitely the yin and the yang there," he says.

Open season

Nachbagauer, who joined Moderna last year to lead infectious disease R&D efforts, is mindful of the tolerability concerns, but he is taking a wait-and-see approach to the problem. The company's first seasonal flu vaccine candidate, mRNA-1010, consists of four mRNA sequences encoding haemagglutinin glycoproteins from the viral lineages recommended by global health authorities, formulated in the same LNPs used to deliver the company's COVID-19 vaccine. "We're really interested in what our reactogenicity looks like," he says.

The immunological contexts around pandemic and seasonal flu vaccines are quite different, adds Nachbagauer. Pandemic flu vaccines have to protect against a novel threat. Because people generally do not have pre-existing immunity to these, a two-dose, prime-boost immunization regimen is key. With the seasonal flu, most people have been exposed to the related strains or received earlier flu jabs. Just one shot can therefore be enough to protect against the latest adaptation of a continuously mutating adversary. If the COVID-19 vaccines are any guide, a one-dose candidate could prove more tolerable than a two-dose product.

Moderna launched a 180-person, dose-ranging trial of mRNA-1010 in July. It is expected to run through early 2022.

Sanofi and Pfizer have also moved their first mRNA-based flu shots into phase I testing. These companies are taking measured approaches to derisking mRNA platforms, starting with candidates containing just a single mRNA encoding the haemagglutination head domain from one type of influenza virus.

Sanofi is evaluating its monovalent H3N2 vaccine candidate in two different LNP formulations. "If results are positive, clinical development of a quadrivalent flu vaccine would be the next step," says Frank DeRosa, chief technology officer of Sanofi subsidiary Translate Bio. (Sanofi acquired Translate earlier this year for $3.2 billion.)

The company's flu vaccine, for now, uses a form of mRNA that lacks the chemical modifications found in most other mRNA products. Sanofi's COVID-19 vaccine, built around this unmodified mRNA platform, successfully elicited antibody responses in early clinical testing. But it remains unproven in an efficacy trial. CureVac's unmodified mRNA COVID-19 vaccine stumbled in phase III trials, however, raising the possibility that this strategy may prove inferior to modified mRNA candidates.

That perhaps explains why Sanofi is planning to also have a quadrivalent flu vaccine, based on modified mRNA, in the clinic by next year. CureVac, in partnership with GlaxoSmithKline, is considering both mRNA options for its flu jab.

Meanwhile, Pfizer is moving ahead with two parallel programmes: one involving modified mRNA, and the other with a self-amplifying construct. Its modified mRNA candidate, PF-07252220, is first up. (Pfizer has licensed BioNTech's technology, and has elected to advance its flu programme solo.)

> I think for people to really be convinced to switch to a new vaccine platform, they're going to want to see efficacy as well as all the immunological comparisons

In September, it began a phase I trial of this monovalent vaccine at four dose levels, each shot containing a single antigen from either a type A or B influenza strain. It plans to later combine those into a bivalent vaccine, administered at different dosages, before moving onto a quadrivalent product — all benchmarked against an approved flu shot.

Pfizer plans to begin trials of its self-amplifying mRNA candidate in the coming months, too.

A path well trodden

For any mRNA-based vaccine that advances into later-stage testing, the regulatory pathway to approval is well defined: companies will either need to demonstrate efficacy through classical clinical trials or show non-inferiority against a licensed comparator on a surrogate measure of protection, such as HAI titre. "That's the beauty about flu," says Pad Chivukula, chief scientific officer and chief operating officer of Arcturus Therapeutics. "The path for licensure is pretty straightforward."

The correlate approach is lower cost and involves less risk, especially for vaccines optimized to induce antibody production. But it won't capture protection accrued through induction of T-cell responses or other arms of the immune system, a potential downfall for mRNA contenders. The correlate approach also won't support claims about the superiority of the platform when it comes to the breadth or longevity of protection. So most companies with mRNA-based vaccines in the works anticipate eventually running large-scale trials over one or more flu seasons.

"Ultimately," says Dormitzer, "I think for people to really be convinced to switch to a new vaccine platform, they're going to want to see efficacy as well as all the immunological comparisons."

Although last year saw a sharp drop in flu cases around the world, likely the result of mask-wearing and social-distancing in response to COVID-19, most experts expect influenza to come storming back. When it does, companies hope their mRNA-based vaccine candidates will be ready.