BioCentury
REPRINT FROM FEBRUARY 27, 2017

PRODUCT DEVELOPMENT

GETTING THE MESSAGE

BY EMILY CUKIER-MEISNER, SENIOR WRITER

Hailed as a faster, cheaper way of producing therapeutic proteins than recombinant technologies, mRNA technologies have attracted some $2.5 billion from partnerships and investments from pharmas and VCs hoping to get in on the ground floor of the next big new therapeutic modality. But myriad scientific and clinical challenges remain.

Within the past five years, two of the field's nuts-and-bolts problems — stabilizing and manufacturing the oligonucleotides — have been sufficiently resolved to make pilot projects feasible for pharmas.

However, the issues that remain still leave question marks about the extent to which mRNA will be able to live up to its therapeutic promise. First is mRNA's tendency to be immunogenic, which is a clear impediment to indications that may need systemic or repeat administration.

Another is uptake of the mRNA into the right cells, with routes of administration beyond local injection posing additional delivery and safety challenges.

Once inside cells, the proteins encoded by the mRNA must be expressed for long enough and at high enough concentration to have the desired biologic effect.

About half the companies working on mRNA technology have identified vaccines and therapeutic immunomodulators as the nearest-term opportunities, because immunogenicity is a benefit for these products, and local injection of mRNA might yield enough protein to train the immune system to mount a durable response.

At least 10 of these candidates are in the clinic (see "mRNA Pipeline").

The levels of antibodies, T cells and/or cytokines these products stimulate will be early indicators of how successfully companies are delivering the mRNAs to cells, fine-tuning their immunogenicity and getting proteins expressed.

"The next two to four years should be critical for the field to get an understanding of the true potential of the technology," said Jeffrey Ulmer, head of preclinical R&D at GlaxoSmithKline plc's GSK Vaccines unit.

GSK is one of the few pharmas with an in-house mRNA platform, which it is using to complement its other infectious disease vaccine technologies.

But the ongoing studies won't reveal much about application of mRNA to replace proteins, which could be the greater opportunity because it opens up a broader set of therapeutic indications and may be given chronically.

At this stage, mRNA developers are unlikely to bother quantifying the amount of protein needed to trigger an immune response, because they are focused on measuring the response itself. And only a sliver of mRNA's potential applications are likely to use local injection. Most will need a vehicle designed to target other tissues, enable systemic delivery, or both — and the need for repeat administration sets a higher safety bar.

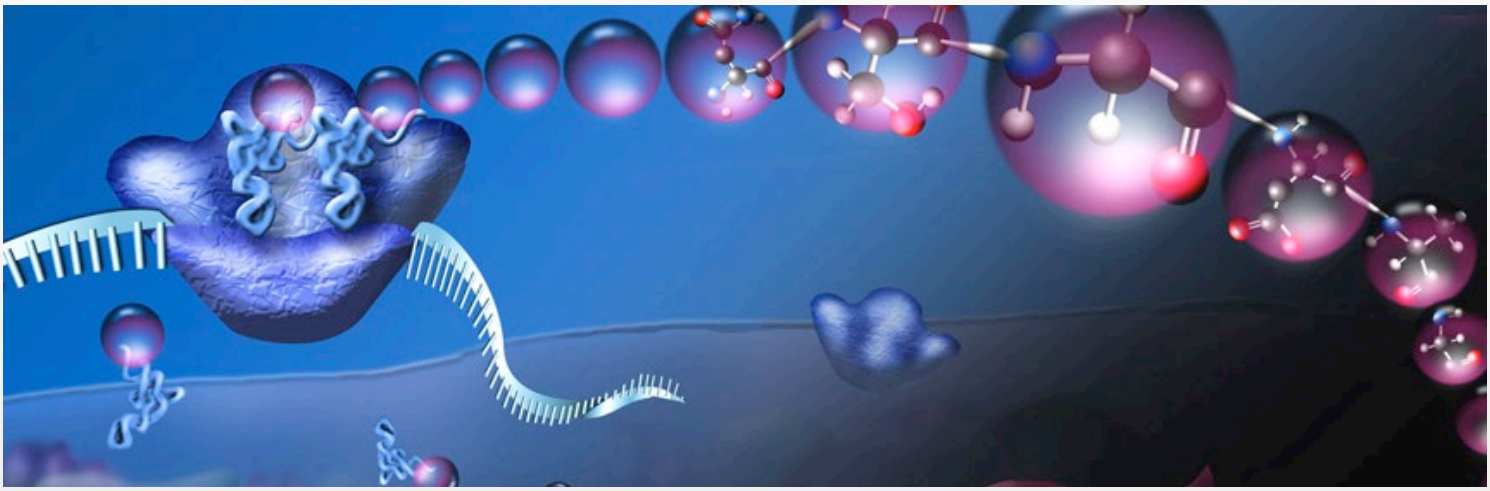
The first protein replacement to enter the clinic won't provide a road map because it comprises unformulated naked mRNA given locally. Moderna Therapeutics Inc.'s AZD-8601 is an mRNA-based therapy that encodes for vascular endothelial growth factor A (VEGF-A) that is in Phase I testing in patients with Type II diabetes.

Moderna and partner AstraZeneca plc plan to develop the compound, one of five clinical programs unveiled by Moderna in January, to treat cardiovascular and other ischemic vascular diseases.

Moderna declined to say what readouts it is measuring in its trial. But a few companies who also plan to advance protein replacement candidates in the next year or two may be able to get an early idea of efficacy in their studies using activity readouts of the new protein as a biomarker.

HOW IT WORKS

Endogenous mRNAs hold protein sequences copied from DNA and serve as the instruction templates for the protein synthesis machinery in cells. By administering a synthetic mRNA, vaccines and therapies give the body instructions for how to make a new protein using a person's own cells.



While mRNAs can be delivered to cells *ex vivo*, the hope is that they can be given *in vivo* as an alternative to recombinant products to deliver almost any protein that doesn't require synthetic modification. mRNAs can also produce intracellular and transmembrane proteins that can be difficult or impossible to deliver when produced recombinantly.

For some applications, the encoded protein itself carries out the desired biological function. But when using mRNA as a vaccine platform, the goal is to deliver antigens that will train the immune system to attack a pathogen or malignancy.

As a method of delivering antigens, mRNA offers several advantages over administering recombinant proteins directly. It can encode more complex antigens, be produced much more quickly and be made more stable than conventional vaccines.

"You can store it at room temperature for two years — it's a great need in terms of prophylactic vaccines," said CureVac AG Chairman and CEO Ingmar Hoerr.

For both cancer and infectious vaccines, mRNA encoding antigens of interest is either delivered directly to dendritic cells or is taken up and translated by other cells that pass the antigens to antigen-presenting cells by unknown mechanisms.

CHALLENGES MET

The past five years have seen the resolution of issues surrounding the mechanics of making mRNA suitable for clinical use as well as a profusion of ideas for how to resolve outstanding challenges in biology.

Companies now know multiple ways to improve the durability of mRNA within and outside of cells. Better purification and protective formulations can help prevent its degradation by extracellular elements. The mRNA itself can be engineered for further stability via codon sequence optimization, incorporation of modified bases, secondary structure control or additional motifs that enhance translation or further increase stability.

"You can say for RNA, one of the problems has been solved: stability," said Gary Nabel, CSO and head of the North America R&D hub at Sanofi. In 2015, the pharma partnered with BioNTech AG to develop up to five mRNA-based cancer immunotherapies.

Manufacturing capabilities also have improved, removing some uncertainty for potential partners and easing the barriers to entry for smaller players. Several mRNA companies have already built factories to produce GMP-grade material, and have demonstrated they can manufacture the products on a clinically relevant timescale (see "mRNA Test Kitchens").

Gita Dittmar, head of corporate development at ethris GmbH, noted that third-party GMP manufacturing is now more widely available, so small companies don't have to develop in-house capacity.

"Three years ago we would have had to; now CROs can do that," she said.

CHALLENGES YET

The main unresolved challenges to widespread use of mRNA as a therapeutic modality are controlling immunogenicity, expressing sufficient amounts of protein and doing so in the correct cells. Moreover, a solution to one challenge — such as a tissue-specific formulation — is inextricably linked to effects on the other two.

> **"YOU CAN SAY FOR RNA, ONE OF THE PROBLEMS HAS BEEN SOLVED: STABILITY."**
>
> GARY NABEL, SANOFI

Though there are no clear winning strategies for how mRNA companies should regulate the immunogenicity of their therapies or deliver them, the players have assembled a variety of technologies for doing so.

Some of the techniques that have improved RNA stability — such as better purification, modified bases, codon optimization and addition of motifs outside of the portion that encodes proteins — also have been used to reduce immunogenicity and increase protein expression.

One major advance was Drew Weissman and Katalin Karikó's finding that replacing uridine with modified oligonucleotides such as pseudouridine and 5-methyl cytidine greatly decreases the immune stimulatory properties of RNA. Weissman is a University of Pennsylvania professor of medicine. Karikó is a VP at BioNTech.

In 2015, CureVac scientists published a paper in *Molecular Therapy* that showed sequence changes alone were sufficient to tamp down

immunogenicity without relying on modified bases. Indeed, the authors concluded that changing the chemistry could be self-defeating.

"What we found out is that if you took chemical modifications and mixed them with sequence modifications, you destroy many of the benefits of sequence modifications," said Hoerr.

Companies using naturally occurring oligonucleotide bases can employ another tactic to boost protein production: self-amplifying mRNA systems that encode replication machinery in addition to the protein of interest (see "Message Repeat").

Another challenge is getting mRNA to the right cells using a formulation that is safe enough to give as often as needed for efficacy.

Even unformulated, or naked, mRNA can get into cells. But formulating it with lipids, polymers or other chemistries can protect the mRNAs from degradation, increase uptake and in some cases assist with tissue targeting.

Most companies delivering antigens by mRNA are doing so via local injections — often intradermally or intramuscularly for infectious applications, and intranodally or intratumorally for cancer indications.

So far, said GSK's Ulmer, it doesn't seem that vaccines need more precise tissue targeting than the choice of injection site.

"Targeting of RNA to a particular cell type may not be critical, at least in our hands with our RNA vaccines," he said. "We've observed that production of the antigen in muscle cells appears to be sufficient to prime a potent immune response."

By contrast, BioNTech CEO Ugur Sahin said the biotech chose to develop a dendritic-cell targeting platform for its mRNA cancer immunotherapies to recreate the "extreme immune response" provoked by a blood-borne pathogen.

The company did so by altering the charge and other properties of cationic liposomal nanoparticles that are given systemically and target the tissues where dendritic cells congregate.

In addition to lipid nanoparticle technology and polymer encapsulation or conjugation, companies are also investigating other delivery platforms that include delivering naked mRNAs themselves and protamine condensation.

VACCINE PROGRESS

A handful of clinical trials so far suggest mRNA vaccines express their intended antigens, and may be on track to do so safely.

According to Hoerr, CureVac has not seen any major safety signals in the more than 380 patients who have received the company's mRNA products, including some in cancer trials who have received the product monthly for over a year. Instead, the side effects have been "vaccine-like" — redness at the injection site, or flu-like symptoms — and transient.

The trials also show proof of principle that mRNA-based vaccines can produce antigen-specific T cell responses.

At the JPMorgan Healthcare Conference in January, Hoerr presented interim results from a Phase I study in healthy volunteers of CV7201, an mRNA vaccine that encodes for the G protein of the rabies virus. Virus-neutralizing antibodies were detected in all 21 subjects in the lowest dose cohort, and 17 (81%) of them had titers over 0.5 IU/mL, which Hoerr said is the World Health Organization's threshold for protectiveness.

CureVac plans to publish data on the immune responses, including virus-neutralizing titers, this half.

In June last year, BioNTech published data showing that its systemic FixVAC induced antigen-specific T cell responses in the first three patients treated in a Phase I melanoma study. The mRNA vaccine used in the study targets four shared tumor antigens.

These results aren't quite enough to say whether the antigen-specific immune response is strong enough for efficacy.

At the JPMorgan presentation, Hoerr reported that a second vaccine, CV9104, did not meet the primary endpoint of improving overall survival in a Phase IIb trial to treat castration-resistant prostate cancer

mRNA VS. RNAI

The first mRNA products potentially can be developed slightly faster than RNA interference products because mRNA and RNAi act by opposite mechanistic principles — adding vs. knocking down a protein — that could lead to inherently lower hurdles for mRNA developers.

GlaxoSmithKline plc's Jeffrey Ulmer said mRNA might not need to be distributed among cells as widely as siRNA to be effective. And for immunomodulatory applications, the mRNA might not need consistent or high expression — just enough to train the immune system. Ulmer is head of preclinical R&D at the pharma's GSK Vaccines unit.

Gita Dittmar, head of corporate development at ethris GmbH, said there are several diseases where generating even a small amount of target protein could have a substantial therapeutic effect, whereas siRNA "frequently requires very potent knock-down of endogenous mRNA."

Moreover, mRNA may act more durably than siRNA because each molecule can be translated more than once into multiple proteins, and the duration of effectiveness is determined not only by the persistence of the mRNA, but by the lifetime of the encoded protein.

Despite their differences, a few lessons gleaned from RNAi could help speed the timeline for mRNA.

The siRNA concept of using lipid nanoparticles as delivery vehicles is one of them. Most mRNA companies include the technology among delivery forms they are investigating, although the particles are often only a starting point.

Joseph Payne, chairman, president and CEO of Arcturus Therapeutics Inc., said the difference in size between siRNA and mRNA molecules — a single nanoparticle can contain hundreds of siRNA molecules, but potentially only one to three mRNAs — requires tailoring the approach.

"Messenger RNA requires a different formulation process and additional excipients to stabilize the particle," he said.

— EMILY CUKIER-MEISNER

(CRPC). CV9104 is a modified mRNA vaccine targeting six antigens overexpressed in prostate cancer given intradermally.

Hoerr said CureVac is considering developing CV9104 in combination with checkpoint inhibitors, saying the company has preclinical data showing that adding anti-checkpoint antibodies increases the immune response.

Other mRNA developers did not count the result as a strike against the concept, and weighed in with other possible explanations for the miss, including the choices of antigen encoded and administration by intradermal injection vs. other routes.

Art Krieg said adding a checkpoint inhibitor is logical because even a strong T cell response would not necessarily overcome PD-1 expressed on the tumor cell surface. Krieg is CEO of Checkmate Pharmaceuticals Inc. and has held positions at other oligonucleotide companies including as founding CEO at RaNA Therapeutics Inc.

He also said the CV9104 study and other trials don't resolve a biological paradox that could limit mRNA vaccines' effectiveness in general: that innate immunity triggered when mRNA activates toll-like receptors (TLRs) can inhibit mRNA translation, thereby limiting antigen expression.

"The early data and clinical failures just announced don't shed enough light on this," he said.

NEXT FOR VACCINES

More preclinical and clinical data will help determine the extent to which CV9104's failure is attributable to product-specific or class effects. Particularly helpful will be clinical data that measures the strength of an induced immune response, which is likely to be revealed in Phase I studies of other products.

Moderna CFO Lorence Kim said the vaccine field now has benchmarks for how strong an immune response is sufficiently protective for well-investigated infections like influenza, which means even early clinical trials can give credible indications of efficacy.

"People know how to interpret the antibody titers that one gets out of these studies," he said.

Moderna plans to publish top-line data from a Phase I study of mRNA-1440 this year. The mRNA vaccine is designed to prevent infection with influenza A virus subtype H10N8.

Even if the current crop of vaccines do not meet efficacy standards, mRNA developers are already working on next-generation approaches.

To solve the self-limiting challenge, Hoerr said companies may need to experiment with formulation options to titrate the degree of immune stimulation so that antigen-specific immune cells are produced, but not so much as to antagonize antigen production.

In the next few years, Ulmer suggested, the field will get a better understanding of when mRNA activates TLRs and intracellular RNA sensors, which will help companies devise strategies to tailor immune activation towards adjuvanting their products rather than compromising efficacy.

MOVING TO REPLACE

The next two years also will begin to position the space for data on the bigger therapeutic opportunities.

Several companies plan to bring mRNAs expressing therapeutic proteins into the clinic that will test the safety of their tissue-targeted delivery technologies and ability to make clinically relevant amounts of protein.

Readouts from ongoing mRNA trials won't shed much light on how much protein therapeutic the products can express: There's little reason for companies to quantify the amount of protein made because doing so uses different tools than measuring the subsequent immune response, which is more indicative of efficacy.

Even if the trials suggest enough protein is being made to train the immune system, Sanofi's Nabel said therapeutic uses will likely need higher expression levels than vaccines to achieve the desired biologic effect.

For non-vaccine cancer immunotherapies like the ones Sanofi is developing with BioNTech, he said biopsies can show whether the levels of cytokines induced by a therapy are high enough to predict clinical efficacy.

Nabel declined to say when the partners plan to advance a candidate to the clinic.

It's unclear how much Moderna's Phase I study of the first mRNA expressing a therapeutic protein will reveal about expression levels, as Kim declined to say what early efficacy measures the company might be looking for in the trial.

It is known the study won't shed much light on the safety of repeat administration or new formulations, as AZD-8601 is being given as a single injection of naked mRNA.

eTheRNA N.V. plans to begin clinical testing this year of an *in vivo* formulation of its TriMix immunotherapy to treat melanoma and breast cancer. TriMix is a therapeutic mRNA that encodes CD70 (CD27L); CD40 ligand (CD40L; CD40LG; CD154) and constitutively active TLR4.

MESSAGE REPEAT

At least two companies are using self-amplifying mRNA technology to increase the potency of their infectious disease vaccines.

Both GlaxoSmithKline plc and BioNTech AG are using the alphavirus genome to underpin their preclinical platforms.

The self-amplifying mRNA vaccines encode antigens of interest and proteins from the alphavirus replicase complex. When transcribed, the replicase allows the mRNA to copy itself, but does not become infectious because it lacks viral structural proteins.

GSK's Jeffrey Ulmer said the resulting boost means not as many mRNAs need to get into cells -- easing some of the pressure on delivery systems.

"It converts a few copies of mRNA into several orders of magnitude more," said Ulmer, who is head of preclinical R&D at the pharma's GSK Vaccines unit.

One drawback is that the mRNAs must be formed from RNA bases to be able to replicate -- which could make them less immunogenic -- but GSK thinks that will be outweighed by the potency boost from amplifying the RNA.

GSK's self-amplifying mRNA platform originated at Novartis AG in 2009. GSK acquired it along with Novartis' vaccines division in 2015.

BioNTech CEO Ugur Sahin said the biotech is developing self-amplifying mRNAs internally and for veterinary use under a 2016 collaboration with Bayer AG's Bayer Animal Health unit.

— EMILY CUKIER-MEISNER

The company has carried out a Phase Ib melanoma study using an *ex vivo* formulation of TriMix given in combination with mRNAs encoding melanoma antigens. In that trial, eTheRNA assessed both antigen-specific T cells and serum cytokine and chemokine levels before and after administration.

CEO Dirk Reyn said the company plans to measure both antigen-specific T cells and infiltration of immune cells into tumors in its upcoming Phase I studies of TriMix.

In the next few years, he expects new technologies will help trace mRNAs and the proteins they express in human trials.

"Technologies are emerging from universities and academic centers that are identifying where the mRNA ends up," Reyn said, "using particular labeling techniques that go almost to the level of individual mRNA molecules."

Products expected to enter the clinic by 2018 may tell more of the story.

For example, ethris' early studies of ETH-CFTR, an mRNA therapeutic that encodes the wild-type cystic fibrosis transmembrane conductance regulator (CFTR) channel, will include a functional measure of the new protein expressed.

Dittmar said ethris can do so by measuring ion conductance across the nasal epithelium — an established method of diagnosing CF — after administration of its nebulized formulation. This would indicate expression of functional CFTR.

Elsewhere, PhaseRx Inc. plans to measure blood ammonia level to gauge how well its intracellular enzyme replacement therapy PRX-OTC makes up for the missing enzyme in ornithine transcarbamylase deficiency (OTCD). PRX-OTC is slated to enter the clinic in 1H18.

The next few years will also yield safety data for tissue-targeted delivery modes not represented in the vaccine trials. The most advanced include inhaled lung-targeting formulations, and systemic formulations that target lung or liver.

Krieg said inhaled mRNA formulations may pose less of a challenge than systemic ones.

mRNA PIPELINE

At least three companies — **CureVac AG**, **Moderna Therapeutics Inc.** and **BioNTech AG** — have brought at least eleven mRNA therapeutics into the clinic alone or with partners. Selected products are shown below and exclude mRNAs given *ex vivo* and non-mRNA oligonucleotides.

The pipeline below shows cancer immunotherapies in blue, infectious disease vaccines in green and functional protein therapies in gold. (A) In January, CureVac announced CV9104 failed a Phase IIb monotherapy trial; the company is considering studying the vaccine in combination with checkpoint inhibitors; (B) In Phase I/II; *Source: BCIQ: BioCentury Online Intelligence, ClinicalTrials.gov, CureVac, Moderna, BioNTech and company websites*

COMPANY	PRODUCT	PH I	PH II
CureVac AG	CV9104	Prostate cancer (A)	
Moderna Therapeutics Inc.	mRNA-1325	Zika virus (B)	
BioNTech AG	FixVAC MERIT	Melanoma	
BioNTech AG	IVAC Mutanome	Melanoma	
		Breast cancer	
CureVac AG / Boehringer Ingelheim GmbH	CV9202 (BI 1361849)	NSCLC	
BioNTech AG	FixVAC Lipo-MERIT	Melanoma	
CureVac AG	CV7201	Rabies	
Moderna Therapeutics Inc.	mRNA-1440	Influenza	
Moderna Therapeutics Inc.	mRNA-1851	Influenza	
Moderna Therapeutics Inc. / AstraZeneca plc	AZD-8601	Cardiovascular	
Moderna Therapeutics Inc. / Merck & Co. Inc.	MRK-1777	Infectious	

"Going after pulmonary delivery is an intermediate between the vaccine — which can be local and injected anywhere — and IV therapy that's going to deliver to the liver and other organs," he said.

Companies whose inhaled mRNA products are nearing the clinic will have to show they can reach enough of the surface of the lungs and modify enough airway cells for effectiveness, which can be made more challenging by disturbances in ventilation and mucus that accompany conditions like CF.

RaNA CEO Ronald Renaud said the company thinks the "physiochemical properties" of its inhaled formulation of an mRNA encoding CFTR will allow it to diffuse through mucus and reach the lung surface, though he declined to give details. RaNA plans to begin testing in CF patients by year end.

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JEFFREY ULMER, GSK

ethris is developing an inhaled as well as systemic version of ETH-CFTR. President and CEO Carsten Rudolph said the biotech has extensively screened potential carriers for their ability to protect mRNA during nebulization and efficient translation once reaching the target cells. According to Dittmar, the company's preclinical data show broad distribution in airways as well as expression of the encoded protein in the deep lung.

ethris and other companies giving mRNAs systemically also will have to show their formulations do not provoke an intolerable non-specific immune response.

"Giving IV lipid nanoparticles has historically caused significant immune toxicity," Krieg noted.

Some companies are working to resolve that problem by changing the composition of their lipid nanoparticles.

For example, Arcturus Therapeutics Inc. CSO and COO Padmanabh Chivukula said the company's Lipid-enabled and Unlocked Nucleomonomer Agent modified RNA (LUNAR) platform uses biodegradable and non-permanently charged lipids that are expected to yield degradation products with lower toxicity than previous lipid nanoparticles.

In 2018 Arcturus plans to begin clinical trials of LUNAR-OTC, a LUNAR-formulated mRNA that encodes ornithine transcarbamylase. LUNAR-OTC particles passively target the liver by targeting ApoE.

Chairman, President and CEO Joseph Payne said OTCD is a popular indication among mRNA companies because it has a larger patient population than many other rare liver diseases, established animal models and an easy to measure biomarker — blood ammonia — that might be acceptable for accelerated approval. bc

COMPANIES AND INSTITUTIONS MENTIONED

Arcturus Therapeutics Inc., San Diego, Calif.
AstraZeneca plc (LSE:AZN; NYSE:AZN), London, U.K.
Bayer AG (Xetra:BAYN), Leverkusen, Germany
BioNTech AG, Mainz, Germany
Checkmate Pharmaceuticals Inc., Cambridge, Mass.
CureVac AG, Tuebingen, Germany
eTheRNA N.V., Brussels, Belgium
ethris GmbH, Martinsried, Germany
GlaxoSmithKline plc (LSE:GSK; NYSE:GSK), London, U.K.
Moderna Therapeutics Inc., Cambridge, Mass.
PhaseRx Inc. (NASDAQ:PZRX), Seattle, Wash.
RaNA Therapeutics Inc., Cambridge, Mass.
Sanofi (Euronext:SAN; NYSE:SNY), Paris, France
University of Pennsylvania, Philadelphia, Pa.
World Health Organization, Geneva, Switzerland

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