WHITE PAPER

mRNA Vaccines:
Disruptive Innovation in Vaccination

May 2017
INTRODUCTION

Vaccines to prevent infectious diseases are the greatest medical innovation of all time. The CDC estimates that U.S. childhood vaccinations given in the past two decades will prevent Americans from 322 million illnesses, 21 million hospitalizations, 732,000 deaths, $295 billion of direct costs, and $1.3 trillion in social costs. For example, before the advent of the measles vaccine in 1963, the virus infected 500,000 Americans annually, causing 480,000 hospitalizations. Today, we see only 60 measles cases a year, primarily from foreign travelers. Smallpox, polio, diphtheria, pertussis, measles, mumps, and many other vaccines have also had an enormous impact on public health. (Figure 1)

However, despite these remarkable successes, there is significant room for innovation in vaccine research, development, manufacturing and delivery.

EXISTING VACCINE PARADIGM

Immunization against disease has been practiced for a thousand years, from variolation (deliberately exposing a healthy person to small amounts of infected material) to vaccination in all of its forms today. The goal of vaccination is to safely pre-expose our immune system to a small, harmless dose of all or a piece of a pathogen (called an antigen) so that, if and/or when we encounter the actual pathogen in the future, our immune system is already prepared to fight it and prevent disease.

Today we have vaccines against more than 25 different diseases1, using at least half a dozen different approaches. These include weakened or killed versions of pathogens, inactivated toxins, partial subunits of the pathogen, and conjugates (combinations of strong and weak antigens). All of these traditional approaches involve long, complex, and costly development and production.

Traditional vaccines face a number of challenges (Figure 2):

1. The target pathogens/antigens are grown in dedicated cell-culture and/or fermentation-based production before being extracted, killed, separated and purified. This involves a long, complex and costly process.

2. They often demonstrate efficacy empirically (i.e., without knowing why they work). The exact mechanism of protection may only be fully elucidated after the vaccine has been licensed and used and in some cases, such as pertussis (whooping cough), we still do not understand the mechanism of efficacy.

3. They require bespoke vaccine-specific production processes, production facilities and operators. Moreover, these capital investments must be made years in advance of vaccine approval, with all of the attendant risks that the vaccine could ultimately fail and waste this capital. This, in turn, limits the vaccine targets that developers are able or willing to substanably pursue.

4. Existing vaccines are only just learning to adjust the kind of immune response they induce, using adjuvants.

NUCLEIC ACID VACCINES

Nucleic acid vaccines, DNA and messenger RNA (mRNA), deliver the nucleotide sequence (e.g., “AAAGGCC…” that codes for the proteins that pathogens use to cause disease. The idea is that those proteins will act as antigens that the immune system will recognize. In other words, these vaccines enable the body to innately mimic a native infection to elicit an immune response, but without the ability to cause disease or spread.

1https://www.fda.gov/BiologicsBloodVaccines/Vaccines/ApprovedProducts/UCM093833
This approach has three main advantages over traditional vaccines (Figure 3):

1. The discovery stage can be exceedingly rapid because many of these antigens are already identified. Discovery also benefits from significant \textit{in silico} (computer-based) antigen design and rapid testing of vaccines in small animal models.

2. Production is standardized. It does not involve either pathogens or the development of target specific cell culture or fermentation. There is no need to grow the vaccine. As a result, a single facility can produce \textit{all} mRNA vaccines, with efficient utilization of a single set of processes, capital equipment, and labor.

3. The vaccine mimics natural viral infections in a way that the immune system recognizes. It's delivered to the muscle and immune cells, which process the nucleotide sequence just as they would do during an infection using viral DNA/mRNA inside the body's own cells (but safely).

Moreover, because the vaccine is DNA or mRNA, it can be sequenced and produced in a standardized process with fewer, more precisely controlled steps. This renders production faster, cheaper, and less vulnerable to unnecessary batch losses due to batch-to-batch variability. mRNA and DNA vaccines offer extraordinary improvements over traditional vaccines in both modularity and standardization.

In addition, nucleic acid vaccines offer the potential to adjust the balance between humoral protection and cellular protection based on the ability to precisely adjust the antigens being delivered. Because of this, nucleic acid vaccines can be designed to address pathogens that are exceptionally difficult to address using traditional vaccines approaches. (Figure 4)

**DNA VACCINES**

DNA vaccine work began thirty years ago, but as yet there are no licensed DNA vaccines and most remain in Phase 1 testing. (Figure 5)

The key challenge associated with DNA vaccines is that they must penetrate the cell nucleus (crossing two membranes; the cytoplasm and the nucleus). The DNA must then be transcribed in the nucleus into mRNA before moving to the cytoplasm to stimulate antigen production. This core complex pathway often requires both larger doses and special, often painful delivery devices using electric shocks or gold microspheres into person’s skin to deliver the DNA vaccine. Once inside the nucleus, DNA vaccines have a risk of permanently changing a person’s DNA.

**mRNA VACCINES**

There are now six prophylactic mRNA vaccines in clinical trials, four of which are being conducted by Moderna Therapeutics. These vaccines combine the advantages of DNA vaccines (natural antigen expression and production that is faster and standardized) while addressing many of the disadvantages. Unlike DNA vaccines, mRNA vaccines do not need to enter the nucleus, nor do they risk being integrated into our DNA, and they are directly translated into protein antigens. As a result, mRNA vaccines require only 1/1000 the dose of DNA vaccines and do not need special delivery devices. (Figure 6)

The first-ever published data demonstrating a prophylactic mRNA vaccine’s ability to elicit robust immunity in humans was published in \textit{Molecular Therapy} in April 2017. (Bahl \textit{et al.}, 2017)\textsuperscript{2} As with all new vaccines, time is needed to establish the level and duration of immunogenicity and the safety profile of mRNA vaccines in larger, more diverse populations. However, the innovation of mRNA vaccines offers the opportunity to improve upon DNA vaccines. These vaccines work seamlessly with the body to mimic the natural sequence of exposure and protection, without the dangers of a real infection. The precision and standardization of the antigen design and delivery offer public health and commercial advantages in terms of the speed and cost of discovery, the speed of development, the probability of success for many targets and the speed, cost and adaptability of production. mRNA offers us a new paradigm in vaccinations’ hundred-year history.

<table>
<thead>
<tr>
<th>Risk of DNA Integration</th>
<th>DNA Vaccine</th>
<th>mRNA Vaccine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Dose</td>
<td>mg</td>
<td>µg (1000x lower)</td>
</tr>
<tr>
<td>Special Delivery Device</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Factory Size</td>
<td>Large</td>
<td>Small</td>
</tr>
<tr>
<td>Membrane Penetration</td>
<td>Cytoplasm &amp; Nucleus</td>
<td>Cytoplasm</td>
</tr>
</tbody>
</table>

\textsuperscript{2}Bahl \textit{et al.}, Preclinical and Clinical Demonstration of Immunogenicity by mRNA Vaccines against H10N8 and H7N9 Influenza Viruses, \textit{Molecular Therapy} (2017), http://dx.doi.org/10.1016/j.ymthe.2017.03.035

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**Figure 1: Impact of Vaccines on Disease in U.S.**

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>Baseline U.S. 20th Century Morbidity (annual)</th>
<th>1998 Morbidity</th>
<th>% Decrease</th>
</tr>
</thead>
<tbody>
<tr>
<td>Measles</td>
<td>503,282</td>
<td>89</td>
<td>99.98%</td>
</tr>
<tr>
<td>Diphtheria</td>
<td>175,885</td>
<td>1</td>
<td>99.999%</td>
</tr>
<tr>
<td>Mumps</td>
<td>152,209</td>
<td>606</td>
<td>99.6%</td>
</tr>
<tr>
<td>Pertussis</td>
<td>147,271</td>
<td>6,279</td>
<td>95.7%</td>
</tr>
<tr>
<td>Smallpox</td>
<td>48,164</td>
<td>0</td>
<td>100.0%</td>
</tr>
<tr>
<td>Rubella</td>
<td>47,745</td>
<td>345</td>
<td>99.3%</td>
</tr>
<tr>
<td>Hemophilus influenzae type b</td>
<td>20,000</td>
<td>54</td>
<td>99.7%</td>
</tr>
<tr>
<td>Poliomyelitis</td>
<td>16,316</td>
<td>0</td>
<td>100.0%</td>
</tr>
<tr>
<td>Tetanus</td>
<td>1,314</td>
<td>34</td>
<td>97.4%</td>
</tr>
</tbody>
</table>

Average annual reported or estimated cases in years before vaccine licensure.

https://www.cdc.gov/mmwr/preview/mmwrhtml/00056803.htm#00003753.htm

**Figure 2: Comparison of Production Processes for Traditional, mRNA and DNA vaccines**
Nucleic Acid Vaccines (DNA and mRNA) can be Faster, Cheaper and More Potent Than Traditional Vaccines

<table>
<thead>
<tr>
<th>Traditional Vaccines (subunit/inactivated)</th>
<th>Nucleic Acid Vaccines (DNA/RNA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Slower (10-15 years)</td>
<td>Faster (4-7 years)</td>
</tr>
<tr>
<td>Customized for each vaccine</td>
<td>Process Development</td>
</tr>
<tr>
<td>Bespoke for each vaccine</td>
<td>Facility Scalability</td>
</tr>
<tr>
<td>$200mm-1bn</td>
<td>Costs</td>
</tr>
<tr>
<td>High volume of finished product</td>
<td>Stockpiling</td>
</tr>
<tr>
<td>May require adjuvant</td>
<td>Potency</td>
</tr>
<tr>
<td>Humoral response</td>
<td>Unlikely adjuvant</td>
</tr>
<tr>
<td></td>
<td>Ability to stockpile library of API (low volume and long shelf life expected) and formulate/fill &amp; finish as needed</td>
</tr>
</tbody>
</table>

America's Next Defense Against Zika and Other Foreign Invaders; Experimental DNA vaccines could shield against infectious-disease outbreaks that now spread around the world with alarming speed

By Betsy McKay and Peter Loftus

PHILADELPHIA—Dr. Keith Hamilton took his turn in the patient chair and braced for the sting of an experimental Zika vaccine.

The injection was the easy part. Next, a nurse jabbed three tiny needles in his upper arm with a device that delivered two electrical jolts strong enough to flex muscle. He said it felt like a needle piercing his arm, again and again.

Dr. Hamilton, an infectious-diseases doctor, was on a break from his rounds to volunteer in a landmark trial of a next-generation vaccine at the University of Pennsylvania’s medical school.

The Zika epidemic is accelerating work on this and other experimental DNA vaccines, which could turn out to be America’s best defense against infectious-disease outbreaks that now spread around the world with alarming speed, fueled by rising populations and global travel. These vaccines, made with synthetic DNA, can be developed and manufactured quickly.

Researchers in the U.S. and Canada have injected dozens of volunteers in the past few months with two competing DNA vaccines intended to provide immunity to the Zika virus. The mosquito-borne virus has caused hundreds of birth defects, including brain damage, and fetal deaths, mostly in Brazil.

Inovio Pharmaceuticals Inc., which makes the vaccine in the trial here, is in a race to market its vaccine and accompanying “electroporation device,” a tool the size of an electric toothbrush that uses a jolt of electricity to help usher the firm’s DNA vaccine into human cells.

One of the National Institutes of Health is pursuing its own DNA vaccine for Zika in a trial that began this summer.

While there are significant hurdles, some researchers believe DNA vaccines could provide faster, more effective ways to combat Zika, as well as Ebola, Middle East respiratory syndrome and other deadly viruses and bacteria that have sickened millions.

Scientists can develop DNA vaccines in weeks and begin human trials within months. DNA vaccines also may provide longer-lasting immunity compared with conventional vaccines and, in some cases, even cure the malady they are intended to protect against.

Conventional vaccines take years to develop and test. They often cost more than pharmaceutical companies are likely to recoup from sales in the mostly poor tropical countries where the diseases originate. The economics discourage many firms from producing them for new, emerging diseases.

Yet the stakes are high, concluded a report this year from health experts convened by the U.S. National Academy of Medicine: “A pandemic could kill as many people as a devastating war.”

Preparedness against most of the emerging infectious diseases that threaten the world is hobbled by a lack of vaccines and drugs, experts say.

More than 41 million people have died around the world over the past decade from AIDS, malaria, tuberculosis, Ebola and other tropical diseases, according to a tally by the Institute for Health Metrics and Evaluation at the University of Washington.

The World Health Organization declared last month that Zika was no longer a global public health emergency, but public-health leaders said it would remain a long-term crisis until a vaccine is developed to prevent its spread.

Zika has infected more than 170,000 people in the Americas, according to the Pan American Health Organization, with hundreds of thousands more suspected cases. Brazil is girding for a resurgence of the virus as summer arrives in the southern hemisphere.

DNA vaccines are made with so-called platform technologies, building blocks that shave years off development time. Ideally, they could deliver protection while an epidemic was still spreading instead of years later. Ebola, which struck West Africa in 2014, still has no licensed vaccine.

Traditional vaccines are developed by growing batches of viruses and bacteria, a slow, labor-intensive process. DNA vaccines are made by inserting a gene related to a particular virus or bacterium into pieces of synthetic DNA called DNA plasmids, an all-purpose platform.

Last fall, as it became apparent that Zika was taking a toll, researchers at the National Institute of Allergy and Infectious Diseases, or NIAID, retrieved an experimental DNA vaccine the agency had developed about a decade ago for West Nile virus. The vaccine had shown promise in human tests, but no company agreed to finish development and produce it.

The health agency retooled the shelved West Nile vaccine by substituting a gene for Zika in the platform DNA, providing “a big head start on Zika,” said Anthony Fauci, the NIAID director. “That’s where the field of vaccinology is going—having a series of readily interchangeable platforms.”
NIAID took less than four months from the time it settled on a vaccine design to begin a human trial, said Barney Graham, deputy director of the agency's Vaccine Research Center. The trial to assess its safety, and see if it generates an immune response, began in August. Initial results are expected by years-end. A second DNA vaccine is also being tested.

The DNA vaccines by Inovio and NIAID were the first two administered to human volunteers among nearly 30 Zika vaccines in development, according to the World Health Organization.

Even if Inovio's and NIAID's vaccines work in human trials, they aren't likely to be on the market for a couple of years, the approximate time needed to satisfy regulatory requirements for effectiveness and safety.

From a business perspective, the market may be small if public-health authorities determine that a Zika vaccine need only be stockpiled for emergencies rather than administered routinely to the general population.

Companies pursuing Zika vaccines are hoping public demand for widespread immunization will create a commercial market similar to the vaccine for rubella, another disease that causes birth defects. Any Zika vaccine wouldn't likely be aimed at pregnant women because of potential risks, but instead administered more broadly to young people.

Hundreds of millions of people are at risk, said Thomas Monath, chief operations officer of the infectious-disease division at NewLink Genetics Corp., which is developing two Zika vaccines.

Zika, he said, "is the biggest opportunity for a new vaccine that's come along in my career, and I've been in vaccines for 40 years."

Electric shocks

Scientists have been working on DNA vaccines for a quarter-century, including ones for the flu and severe acute respiratory syndrome, known as SARS, an infectious virus that sprang from China in 2002 and killed 774 people out of more than 8,000 infected on several continents. None of the DNA vaccines have made it to market for human use. The main problem has been that human cells don't easily absorb them.

Inovio's electroporation device is intended to be a solution. After the vaccine shot, the device delivers a mild electrical current to the same spot on the arm, temporarily opening cell membranes to allow the DNA inside. "That's the fire that cooks the meal," said J. Joseph Kim, Inovio's chief executive.

In late summer 2015, Dr. Kim read about the spread of Zika in South America and began work on a DNA vaccine to fight the virus. It only took about two weeks to design the DNA sequences on a computer and make a small batch of the vaccine. By December 2015, it was being tested on mice.

In June, the Food and Drug Administration approved a human study, based on animal tests that showed the vaccine triggered immunity to the Zika virus.

Inovio and GeneOne Life Science Inc., which are codeveloping the vaccine with academic collaborators at the University of Pennsylvania and the nearby Wistar Institute, a medical-research center, began their trial in July, around the same time as NIAID.

Dr. Hamilton, one of about 40 people who have volunteered to receive the experimental vaccine, said electroporation "felt more like putting a needle in multiple times rather than an electrical shock."

Helping science was worth the discomfort, he said: "This is a small piece in a larger puzzle to come up with an effective vaccine for the people who need it the most."

The Inovio trial is expected to be completed this month. Researchers are monitoring the volunteers for any side effects, as well as taking blood tests to see if the vaccine induces the expected immune response.

Inovio, based in Plymouth Meeting, Pa., also began a 10-month trial this summer with 160 participants in Puerto Rico, where Zika spread rapidly.

Dr. Kim, the company's 47-year-old chief executive, got his Ph.D. in biochemical engineering at the University of Pennsylvania, where he met David Weiner, a biologist and now a director at Inovio.

After working in vaccine manufacturing and research at Merck & Co., Dr. Kim co-founded a company called VirRx Genetics in 2000. The company later acquired an San Diego-based company called Inovio and took its name.

Inovio explored experimental DNA vaccines for HIV, among other technologies. At the time, the vaccines showed promise in animal studies, but failed in humans when given via standard vaccine injections.

Drs. Kim and Weiner turned to electroporation—first tried in the 1980s to boost the effectiveness of chemotherapy. In 2006, they tested an electroporation device, developed by a Texas company called Advisys, to immunize monkeys against a form of HIV.

One evening, close to midnight, Dr. Kim's home phone rang. It was Dr. Weiner calling to say the HIV vaccine, assisted by the electroporation device, had kick-started an immune response in the monkeys.

"That really triggered the path we're on now," Dr. Kim said. His company acquired Advisys in 2007.

The company reported positive results for its DNA vaccine against Ebola; 64 of 69 subjects mounted a strong antibody response after three doses. Inovio's vaccine for Middle East respiratory syndrome—a virus that can cause fatal respiratory illness—was developed with GeneOne in partnership with the Walter Reed Army Institute of Research. It is in an early clinical trial.

Inovio has had to delay a new trial of its vaccine against human papillomavirus vaccine until next year after the FDA in October asked the company for more information about the stability and sterility of the disposable parts in its electroporation device, which is used to both inject vaccine and deliver an electrical jolt. Its device in the Zika trial delivers only the shock.

Not everyone has embraced DNA vaccines. Merck & Co. explored a DNA vaccine for HIV several years ago, but dropped it after a study in 2007 found it didn't work.

Merck is now exploring synthetic RNA-based vaccines, a spokeswoman said. In theory, the approach is easier because RNA needs only to get into a cell's cytoplasm—the material between its outer surface and the core nucleus. DNA vaccines must penetrate the nucleus to work, said W. Ripley Ballou, head of Glaxo's U.S. vaccine research center in Rockville, Md.

GlaxoSmithKline also is exploring an RNA-based vaccine for
Zika, in collaboration with NIAID. It is being tested in animals and could move to human studies next year, Dr. Ballou said.

Using the method for different vaccines is a relatively simple process, he said, "once you figure out how to do it."

Health frontier

NIAID has pioneered DNA vaccines over the past several years for HIV, Ebola and other diseases.

Dr. Graham at the agency's Vaccine Research Center began thinking about a Zika vaccine after learning about the spread of the virus from a Brazilian researcher who approached him at a conference in July 2015.

NIAID researchers started work on the vaccine in November and accelerated their efforts after the World Health Organization declared in February that the complications of Zika posed a global public health emergency. By June, the agency had an experimental vaccine for the human trial.

NIAID's Zika vaccine doesn't use an electroporation device and it may not be necessary, according to Julie Ledgerwood, chief of the clinical trials program for the Vaccine Research Center.

Data from NIAID's trials of the DNA Zika vaccine in monkeys, and evidence from its progenitor West Nile vaccine in humans, "makes us think this vaccine is highly immunogenic and it should work well even delivered by traditional needle," Dr. Ledgerwood said.

A few studies conducted by other scientists have found little difference in the immune response generated by experimental DNA vaccines for HIV and the H5N1 flu that were given with and without electroporation.

NIAID has used electroporation in animal tests. "In our opinion, the device development probably still has a way to go," Dr. Graham said. "But the technology itself, I think, is promising."

Whether with or without an electrical shock, a successful DNA vaccine would accelerate the exploration of new technologies against Zika and other outbreaks, said Kayvon Modjarrad, associate director for emerging infectious disease threats at the Walter Reed Army Institute of Research.

Dr. Modjarrad is the principal investigator of the DNA vaccine trial for Middle East respiratory syndrome that uses electroporation.

"The way we make vaccines now and what's on the horizon," he said, "is very, very different from the way we've been making vaccines for the past 100 years."

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Figure 5: History of Vaccine Development

http://www.immunize.org/timeline/
https://www.vaccines.gov/basics/types/index.html#conjugate
http://www.who.int/biologicals/vaccines/pertussis/en/
http://www.inovio.com/company/revolutionizing-vaccines/history/
Figure 6: Differences between Traditional, MRNA and DNA vaccines (Delivery)

Traditional vaccine

Exogenously produced antigen

mRNA vaccine

Endogenously produced antigen

µg dose

DNA vaccine

Endogenously produced antigen

mg dose*

DNA vaccine requires 1000x larger doses

DNA vaccine requires an extra delivery device

Immunologic memory

*Assuming electroporation. Gold particles allow for smaller doses but are more expensive.

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Dr. Rachlin leads Strategy across Moderna’s portfolio of mRNA medicines. Prior to joining Moderna, Dr. Rachlin was a principal at Bain Capital Ventures, an engagement manager at McKinsey & Company, and an associate in the Portfolio and Decision Analysis Group at Pfizer. He received his M.D./M.B.A. from Harvard University.

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President, Valera, a Moderna venture

Dr. Watson is a UK-trained physician in internal medicine and infectious disease, bringing his twenty-year career in vaccine work to his role as President of Valera, Moderna’s infectious disease-focused venture. Previously, Dr. Watson was Global Head of Vaccination Policy and Advocacy at Sanofi Pasteur. He has also held positions including Head of R&D for Acambis, UK Medical Director of Aventis Pasteur MSD, as well as Head of Clinical and Epidemiology for SPMSD in France.