Delivering the biorevolution: a BNext Workshop on cellular delivery technologies

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Dylan George[1], Tara O'Toole[2], BNext et al

Summary: This workshop was motivated by BNext's interest in technologies that facilitate timely response to infectious disease outbreaks through the rapid design and manufacture of vaccines against newly emergent pathogens.

A compelling technology for rapid response to an ongoing outbreak is nucleic acid-based vaccines. Nucleic acid-based vaccines are attractive for rapid response because, in theory, DNA or RNA antigens that provoke a protective immune response could be quickly and inexpensively designed, manufactured, and used speedily in the clinic. Big pharma and biotech companies are interested in advancing nucleic acid-based vaccines. Several candidates are in clinical trials, though no nucleic acid-based vaccines have achieved FDA approval. Among the hurdles associated with DNA or RNA-based vaccines are the following:

All Available Cellular Delivery Technologies Have Limitations - Major techniques to deliver the nucleic acid "payload" inside cells have been demonstrated - including electroporation, viral vectors and a variety of lipid nanocarriers – but all are problematic. Electroporation is suitable only for laboratory settings and not feasible in a mass casualty setting. Viral vectors carry the risk of unintentional immune reactions, and the virus carrier can only deliver certain types of payloads. Lipid nanocarriers are arguably the most advanced modality and are the delivery vehicle used in seven of eight ongoing RNA vaccine trials and in gene therapy trials. But they too are disadvantaged by the relatively "fragile" supply chain that is being used primarily for other products.

Manufacturing viruses and lipids is itself a hurdle to be overcome, especially if vaccine were needed in large quantities. For example, the supply chain capacity for GMP-grade lipids is limited, and currently being stretched by demand for the second-generation Shingles vaccine.

Similarly, manufacture of GMP-grade nucleic acid at scale is not currently possible at speed and would probably require 12 months. Making DNA in the U.S. Government's Advanced Development Manufacturing Facilities may make this possible in 6 months. Several biotech companies are working hard to improve de novo DNA synthesis, but we are not yet able to do this at the required scale and time frame. DARPA is starting a program to develop novel approaches for DNA manufacturing at scale too.

Regulatory approval of novel cellular delivery methods requires a time-consuming and costly investment of resources, a fact that creates a rational disincentive to innovate. Nonetheless, successful and safe cellular delivery is a central feature of many of the most promising new drugs, including gene therapies. The commercial stakes involved in these new approaches will likely advance the science of cellular delivery, hopefully to the benefit of nucleic acid-based vaccines.

Conclusions: Advances in delivery modalities other than the current mainstays – existing viral vectors, lipid nanocarriers - should be supported. Supporting alternative DNA synthesis technologies and nimble, efficient biomanufacturing capabilities should be a priority.

[1] Vice President, BNext, IQT
[2] Executive Vice President, BNext Director, IQT

Background: Advances in synthetic biology are driving the creation of innovative therapies and vaccines that could transform rapid response capabilities for pandemics. These technologies – gene therapies, cell therapies, oncological immunotherapies, nucleic acid vaccines - require delivery of modified RNA or DNA to targeted cells to program those cells in order to have the desired clinical effect has significant technical challenges. On August 21, 2019, BNext convened a workshop of subject matter experts from industry, academia, and U.S. government agencies (Amy Jenkins – Program Manager DARPA, Mark Feinberg – CEO IAVI, Keith Wells – biomanufacturing consultant) to explore potential approaches to successful intracellular delivery technologies for vaccines which could be rapidly designed and quickly manufactured at a large scale. This paper reports on the workshop findings. The workshop was convened by B.Next, a division of IQT Labs, the research venture of In-Q-Tel (IQT).

Vaccines are critical tools for countering infectious disease outbreaks: Outbreaks of infectious diseases are an increasingly common, devastating feature of modern-day life which threaten lives and livelihoods. Modern patterns of trade, travel, commercial development drive such outbreaks. These outbreaks are fought by brave front-line clinicians and public health professionals armed with outdated data technologies, insufficient resources, and typically without effective vaccines or drugs. More often than not they fight outbreaks with 20th century tools. We need 21st century solutions to confront these 21st century health security challenges. At IQT we are actively pursuing technologies that provide the capabilities needed to respond to novel emerging infectious disease outbreaks.

Vaccines are the single most effective medical capability for countering infectious diseases (1), but vaccine development typically requires 15-20 years and approximately a billion dollars (2). The current process and enabling tools to discover, design, manufacture, and test a new vaccine are not well suited for rapid response. As a result of this long, expensive development process, vaccines historically have been unavailable to counter outbreaks of newly emergent disease (e.g., SARS 2003; Ebola 2014; Zika 2016).

One workshop participant told the group about how the lack of a deployable vaccine allowed the Ebola outbreak of 2014-2016 in West Africa to rampage across Sierra Leone, Liberia and Guinea killing over 11,000 people and significantly destabilizing the region. At the time, no licensed vaccine or therapeutic was available, but several candidate Ebola vaccines had already gone through years of early stage development. Merck Vaccines was willing and able to step into the breach to advance a candidate through later stage development. With support from the USG and others, Merck, at considerable expense, licensed a candidate vaccine, contracted manufacturing capabilities, and began the process of testing the vaccine in hopes of providing life-saving vaccines to people in the region. Merck was able to shorten the development timeline from years to months. Fortunately the outbreak ended before this vaccine could be manufactured and deployed at scale. So, in the end, the vaccine did not significantly contribute to stopping that specific outbreak.

Despite the example of Merck Vaccines and other initiatives[3], participants agreed that we continue to battle novel pathogen outbreaks without effective vaccines (3). Because time is critical during an outbreak, current methods of developing vaccines are not sufficient and technologies that can be designed and manufactured quickly will have more impact. Technologies that enable the discovery, manufacture, development, and use of vaccines in timeframes that would significantly counter an ongoing outbreak remain critically important. Promoting and developing vaccine technologies that enable rapid design and scaled-up manufacture has been a focus of some DARPA programs (e.g., Adept, P3). B.Next also continues to seek technologies that would enable vaccine design and manufacture in timeframes that would be applicable to stopping an epidemic.

Nucleic acid-based vaccines are promising technologies: Nucleic acid vaccines, which deliver DNA or mRNA to generate an antigen, are particularly promising vaccine technologies for rapid outbreak response because, at least in principle, they can be rapidly developed and inexpensively manufactured (4).

mRNA is the intermediate molecule that enables the expression of a gene into a protein. It is the molecule that tells a cell what proteins to build. The idea behind mRNA vaccines is to design and use an mRNA that would tell the body's cells to generate a particular type of protein, an antigen, that will elicit a protective immune response for a specific disease (Figure 1). In short, nucleic acid vaccines biologically "program" a person at the cellular level to generate immunological protection. This programming should work as long as you are able to deliver the right information, that is the right mRNA, to the right cells in a body.

[3] See efforts by the Coalition for Epidemic Preparedness Innovation, https://cepi.net/

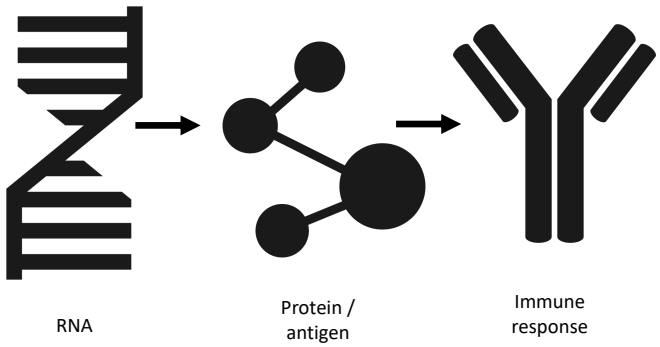

Figure 1. mRNA vaccines program cells to generate immune responses. mRNA vaccines accomplish immune responses by inserting an RNA molecule into cells to program the cellular production of a protective response in the body. The RNA molecule once in the cell is translated to a protein molecule. The protein, or rather antigen, elicits an immune response – generates antibodies or other mechanisms - that provides protection from the pathogen.

An advantage to mRNA vaccines is that RNA can be designed and, in theory, synthesized quickly using standardized processes. Traditional vaccine manufacturing is bespoke and typically requires a unique and expensive manufacturing facility for each vaccine, whereas with RNA production one manufacturing facility could be used for multiple vaccines because you are using a standardized system for RNA synthesis. Also, RNA-based vaccines can be manufactured cell-free, which reduces complications associated with maintaining GMP cell lines (5). Development of a mRNA vaccine can go from genetic sequence to mass production in three months, whereas traditional approaches would take many months to years to produce a new vaccine at scale. Despite such promise, however, no nucleic acid vaccines have yet been approved by the FDA, although several candidate vaccines have progressed to phase 1, 2 clinical trials (6).
Several participants were cautiously hopeful that mRNA vaccines could provide capabilities to address the challenges of rapid vaccine development, but the clinical trials still need to demonstrate candidate mRNA vaccines are safe and effective.

Intracellular delivery: a vital component for effective vaccines: A major challenge with nucleic acid vaccines is getting the genetic payload to the right place in the body so one's immune system can generate protection. The safe and effective delivery of genetic payloads within humans has been a focus for decades (7), (8).

Intracellular delivery includes not just the process of getting materials through cellular membranes, but also entails protecting payloads from degradation processes, and releasing payloads into a cell in a reliable way (3). Intracellular delivery is a linchpin for a range of therapeutic applications beyond vaccines, including gene-editing technologies. Participants noted that several Phase I trials of nucleic acid vaccines nusing novel delivery technologies are underway (4), (8), (9).

Participants discussed the three main delivery modalities for vaccines: electroporation, viral vectors, and lipid nanocarriers.

Electroporation is the process of applying an electrical field to a cell such that cellular membranes become transiently permeable, molecular cargo moves across the membrane, the cargo can be inserted into the cell, and the membrane is resealed (Figure 2). Electroporation has been used in microbiology since the 1970s and is widely used in basic and biomedical research. But there are limitations to its use outside a lab or in a mass administration situation. The process can be highly efficient, but it is expensive and can create cell death if the electrical fields cause a permanent destabilization of a cell membrane or components. Electroporation can cause pain and muscle contractions which makes it less than appealing for treatment adherence if more than one dose is required. Most importantly, electroporation requires equipment to establish the electrical field and the portability of this equipment limits how widely it could be used outside of a clinic or laboratory setting. The value of electroporation is most apparent for *in vitro* and *ex vivo* investigations and applications, and not necessarily *in vivo* delivery.

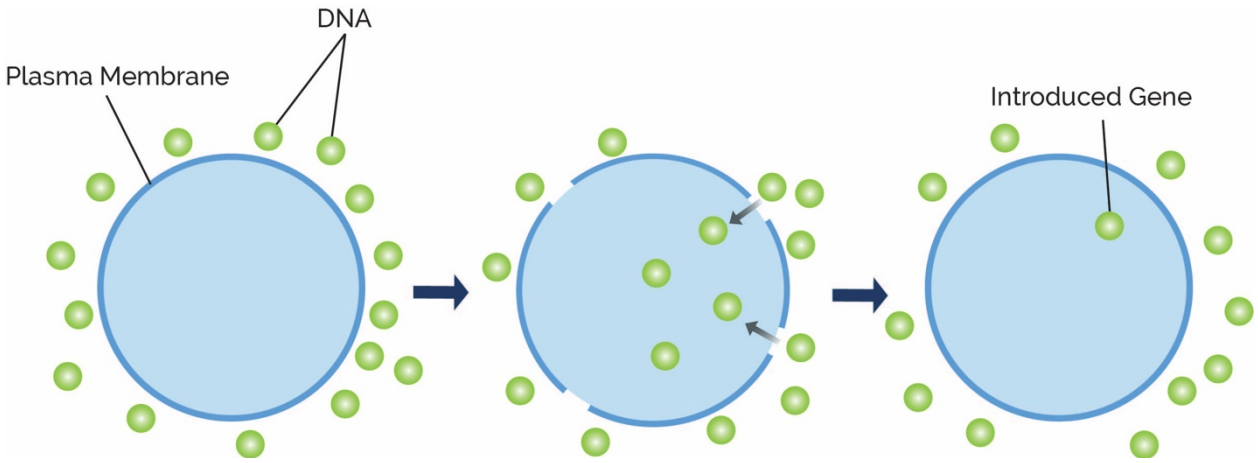

Figure 2. Electroporation is the process of applying a temporary electrical field to a cell. The electrical pulse causes transient pores to develop in the cell membrane. Material can be inserted into cells while pores remain open. [4]

Viral vectors are another intracellular delivery modality for therapeutics and vaccines. Viruses have been honed over evolutionary time to infect cells with genetic payloads. Simplistically, one can think of viruses as molecular machines with two functional components – the container and the cargo. Viral vectors use the natural infection machinery – the container - of a virus but with modified genetic material – the cargo - that is to be inserted into a target cell (Figure 3). Viral vectors have been used for decades and in many clinical trials (10), and have been used in gene therapies approved by the FDA[5]. Notably the recent recombinant vesicular stomatitis virus Ebola vaccine (11) and recent high profile gene therapies (12) use this approach.

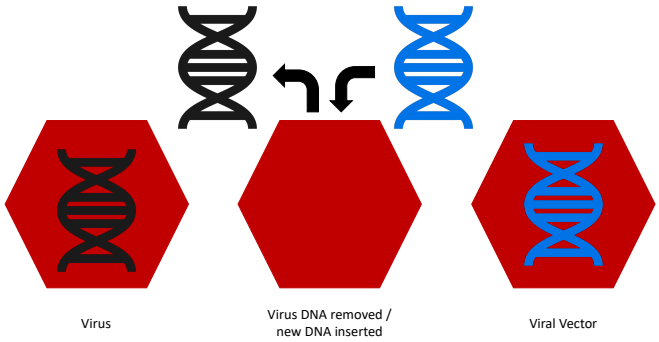

Figure 3. Viral vectors are made by using existing viruses, removing the virus DNA, and inserting new DNA that is to be used to program a cell for research or biomedical purposes.

However, this technology has limitations. The most well-known shortcoming of viral vectors has been unintentional immune reactions in patients. In 1999, a teenager suffering from a rare genetic disorder tragically died from an immune reaction to a viral vector used during a gene therapy trial. This tragedy set back the field of gene therapy for a decade. Even if we are able to avoid similar acute tragedies in the future, some people will produce

[4] Based on a figure from https://courses.lumenlearning.com/microbiology/chapter/microbes-and-the-tools-of-genetic-engineering/

[5] Zolegensma is a gene therapy for treating pediatric patients with spinal muscular atrophy, and was recently approved by the FDA in May 2019. Zolgensma uses an adeno-associated virus vector for intracellular delivery of the gene therapy.

immune responses to particular viral vectors. If this happens then the continued use of those vectors will not be possible in those individuals which will limit the therapeutics and vaccines that are in those vectors. Viral vectors are also limited by challenges in manufacturing large quantities of virus, the size of payloads, and by their ability to target many cell types. If the viral vector cannot infect certain types of cells, then we will not be able to program those cells with the genetic payloads. Finding viral vectors that can target specific cells will be an ongoing effort. So, researchers are actively searching for alternative viral vector systems to counter these limitations[6].

Lipid Nanocarriers - Delivering nucleic acids or proteins cargo into cells can be achieved by using chemical reagents to construct delivery vehicles that have different properties. Many alternative cell delivery approaches such as lipid nanocarriers, polymer nanocarriers, and other nanomaterials have been explored to bypass the limitations of viral vectors (13). Lipid nanocarriers are the most advanced of these technologies for nucleic acid delivery (8), are currently being used in the majority of current clinical trials on mRNA vaccines (6), and were used in the first RNAi drug ("Patisiran"), approved by the FDA in August 2018. A recent review of mRNA clinical trials and delivery modalities found that seven of the eight of the ongoing clinical trials on mRNA vaccines are using lipid nanoparticles as their intracellular delivery modality (6).

Carrier systems based on chemical reagents can be limited by the features of the cargo (e.g., size, chemical properties, unpackaging abilities) and the target cell types. As with viral vectors, getting into some cell types is easier than others depending on cell receptors, surface interactions, and internal cellular processing pathways. For example, immortalized cell lines can be easily transfected, whereas blood and neurological cells pose difficulties (8). Because lipid nanocarriers have been easier to make relative to viral vectors, generate adjuvant effects, and do not generate unintended immunogenic responses, they have been broadly used to deliver nucleic acids in drug development. But see below for the challenges associated with "fragile" supply chains associated with lipid nanocarriers.

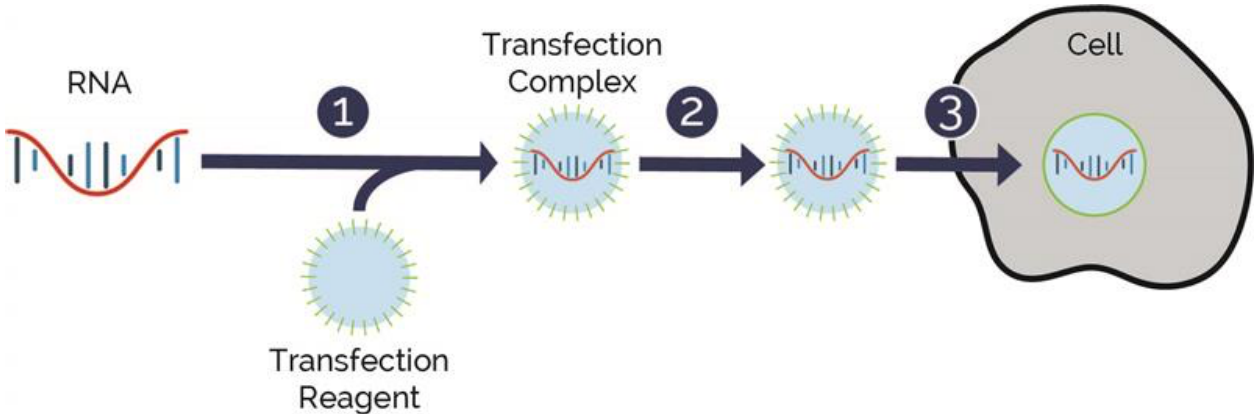

Figure 4: Getting programmed RNA into cells – the transfection process: **1)** A chemical reagent is combined with a nucleic acid making a chemical complex of the two entities. **2)** The combined reagent and nucleic acid interact with the cell surface. **3)** Cells internalize the complex and the nucleic acid is ultimately released to the cell cytoplasm. [7]

Challenges and opportunities:

Regulatory: A major regulatory issue is the innovation disincentive within big pharma. An effective regulatory environment for medical countermeasures is necessary but can slow innovation. For biological pharmaceuticals such as vaccines, the manufacturing process itself contains much of the valuable intellectual property. To meet regulatory standards, the production process must reliably produce the same product which requires considerable investment of time and resources. Once a process has been validated and approved by a regulatory agency there is a rational disincentive to modify the process because major changes would require further regulatory approval and cost to provide the needed data. (14). Regulatory disincentives slow the pace of innovation for intracellular delivery

[6] For example, see the company Ring Therapeutics.
[7] Based on figure from https://www.mirusbio.com.

technologies. Viral vectors and lipid nanocarriers are the delivery modalities that are furthest along in clinical trials for gene therapies (8) and mRNA vaccines (6); however, alternative delivery technologies – commensal viral vectors, polymer nanocarriers – need to be supported and tested as well.

Manufacturing at scale: Even when a vaccine that has been designed and tested in animal studies is available, manufacturing it at scale is challenging, especially for an ongoing outbreak that requires vaccine to be delivered quickly. Biotechnology companies typically lack resources to push vaccine development beyond preclinical work and early clinical trials. Late stage clinical trials and constructing unique manufacturing facilities drive the high costs associated with vaccine development. There are few major manufacturers[8] with the needed expertise working on vaccines (2), and they traditionally have developed bespoke manufacturing capabilities which constrain the speed and ability to pivot to novel threats.

The limits of current manufacturing have been a major motivation for developing nucleic acid vaccines which can be developed and produced at scale much more quickly than traditional approaches. One participant noted that while nucleic acid vaccines are promising, manufacturing quality nucleic acids at the scale needed for a mass outbreak have not been completely figured out (9) and likely will be deficient because manufacturing clinical GMP DNA can take anywhere from six to twelve months. Also, a participant highlighted that gene and cell therapies using delivery technologies will not require the same scale of manufacturing as would mRNA vaccines especially during surges of an ongoing outbreak. So, relying on commercial markets to develop the needed capacity may not yield the quantity of material demanded by a pandemic scenario. New synthetic biology approaches to manufacture DNA enzymatically instead of chemically potentially could be a means of addressing the manufacturing shortfall of clinical GMP DNA. Exploration of the potentials and deficiencies in nucleic acid synthesis are needed. Companies developing enzymatic approaches of DNA synthesis are exciting and warrant further attention as do companies developing novel, nimble, and efficient biomanufacturing capabilities.

Supply chain constraints: One participant reminded the group that as we think about new technologies for outbreak response, we need to think about manufacturing capability and the supply chain of constituent materials, particularly we need to consider "fragile" supply chains. Constraints in manufacturing supply chains may limit the development and use of nucleic acid vaccines and delivery technologies. Competing markets for component materials have caused shortages for manufacturing clinical GMP lipid products. A good example is the vaccine called Shingrix[9]. This vaccine prevents shingles (herpes zoster) and is made up of the antigen, glycoprotein E, and an adjuvant, AS01B. People generally lose capacity to generate an immune response as they age, and the vaccine was developed specifically to generate immune responses in older people. The adjuvant is critical to generating the immune response, and is liposome based. The market for Shingrix is large. So, GlaxoSmithKline, the manufacturer, has acquired a major portion of the lipid supply to maintain Shingrix production which has disrupted the lipid supply chain for other uses. It remains unclear if these lipid supply chain challenges will persist or manufacturing capabilities will eventually compensate. But, in the short run the lack of raw materials will delimit manufacturing capabilities for other lipid-based products such as delivery technologies.

Conclusions: The workshop found that there were persistent scientific, regulatory, manufacturing, and supply chain challenges for advancing nucleic acid vaccines and delivery technologies. Significant research is ongoing in novel delivery modalities and it will be exciting to see those results in the next few years. The interface of regulation and innovation will continue to provide safety assurances yet will disincentivize the adoption of innovation in biomanufacturing. Supply chains for component materials will be a fluid environment and should be monitored because they could significantly limit capacity during an outbreak scenario. Advances in delivery modalities other than the current mainstays – existing viral vectors, lipid nanocarriers - should be supported. Supporting alternative DNA synthesis technologies and nimble, efficient biomanufacturing capabilities should be a priority.

[8] For example, GlaxoSmithKline, Merck, Pfizer, Sanofi Pasteur
[9] https://www.shingrix.com/index.html

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