The next generation of RNA vaccines: self-amplifying RNA

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The global COVID-19 pandemic has brought tremendous momentum to the field of messenger RNA (mRNA) vaccines. The advantages of this vaccine platform, such as rapid development and high efficacy, resulted in mRNA vaccines being the first approved vaccines against COVID-19. Looking forward to the development of future vaccines, how can we make RNA vaccines even better? While improvements in the stability of the formulation and cost of the vaccine are inevitable, one of the main challenges is lowering the dose of RNA in order to avoid side effects associated with high doses of RNA. One way to do this is by using self-amplifying RNA (saRNA), a type of mRNA that encodes a replicase that copies the original strand of RNA once it's in the cell. Here, we discuss the origins of saRNA, how it works in comparison to mRNA, current challenges in the field and the future of saRNA vaccines.

The RNAissance

The global COVID-19 pandemic has showcased the advantages and greatly accelerated the field of messenger RNA (mRNA) vaccines. Although the first paper to show that mRNA could be used as a vaccine was published in 1990, the efficacy of an mRNA vaccine in a phase III clinical trial was not determined until last year (2020). Strikingly, both the Pfizer/BioNTech and Moderna mRNA vaccines were shown to have >94% efficacy against COVID-19 with two doses of mRNA encoding the wild-type pre-fusion stabilized SARS-CoV-2 spike antigen. In addition, mRNA vaccines are more rapid to develop and move into clinical trials than other vaccine platforms such as viral vectors or proteins, with Moderna reporting that they completed the design and preclinical testing in just 66 days prior to starting their phase I clinical trial. Given the rapidity of development and high efficacy, we have begun the RNAissance, an age of mRNA transforming prevention of infectious diseases, as well as other applications such as oncology and rare or inherited disorders.

The recent success of mRNA vaccines has been enabled by advances in RNA stability, modulation of innate immunity against RNA, and in vivo delivery. The stability of mRNA is highly dependent on the sequence, especially the 5' and 3' untranslated regions (UTRs), the 5' cap and 3' polyA tail. Furthermore, all mRNA is known to trigger intracellular innate receptors, such as toll-like receptors (TLRs) which lead to production of type I interferon and inhibition of translation. In order to overcome this, Kariño and others have shown that using modified nucleotides, such as pseudouridine, partially suppresses this innate sensing. Perhaps the biggest hurdle in the field was efficient delivery of mRNA. While many delivery vehicles have been investigated, lipid nanoparticles (LNP) consisting of an ionizable lipid, phospholipid, cholesterol and PEGylated lipid are the most clinically advanced and are used in both the Pfizer/BioNTech and Moderna vaccines. The combination of these advances has enabled the approved COVID-19 vaccines and set the stage for future mRNA technologies.

How are RNA vaccines different?

How is an mRNA vaccine different from a traditional vaccine, such as an inactivated virus or protein vaccine? Let's consider the goal of vaccination. The goal of a vaccine is to train your immune system to recognize a pathogen, such that if you ever encounter it, you are protected from either infection or severe disease. For COVID-19, vaccines target a protein on the surface called the 'spike' protein. Thus, for any COVID-19 vaccine, the strategy is to introduce the spike protein into a patient. There are many ways to do this (Figure 1), such as using an inactivated virus, the protein itself, or a carrier to deliver a nucleic acid that encodes the spike protein. The viral vector COVID-19 vaccines that have been approved (J&J and AstraZeneca/Oxford) utilize adenoviruses to deliver DNA that encodes the spike protein, whereas the mRNA vaccines (Pfizer/BioNTech and Moderna) utilize mRNA that encodes the spike protein. This mRNA is taken up by cells and the vaccine antigen is then translated by the ribosome. One major difference between mRNA vaccines and viral vector or protein vaccines is the manufacturing process. For viral vector or protein vaccines, large-scale bioreactors are required to grow batches of cells that produce the virus or protein, which
is both costly and time consuming. For mRNA vaccines, the RNA is produced synthetically and encapsulated in LNPs. Thus, the patient’s own cells are employed as a bioreactor. Because mRNA can be produced quickly and synthetically, this greatly minimizes the time required to manufacture these vaccines.

How can we make RNA vaccines better?

If they’re rapid to develop, manufacture and highly efficacious, why do we need to improve mRNA vaccines? There are a number of obvious starting places for progressing this technology, including improving the stability of the formulations, minimizing the dose of RNA and lowering the cost of the vaccines. One of the main issues with the first-generation mRNA vaccines is that they require cold-chain storage at −80°C or −20°C, which makes the widespread distribution to warmer climates or rural destinations more challenging. Research is actively being performed to stabilize mRNA formulations in either a liquid or lyophilized state. Investments in the manufacturing infrastructure and raw materials required for mRNA vaccines will also lower the cost of these vaccines in due time. Thus, a final frontier of the future of RNA vaccines is minimizing the dose of RNA required. The data from the clinical trial shows that ~50% of participants had side effects even though the RNA was prepared with modified nucleotides. This is caused by innate sensing and detection of foreign RNA that humans have evolved to fight infections from RNA viruses. These side effects are directly proportional to the total administered dose of RNA. Therefore, minimizing the dose of RNA required for vaccines will not only lessen side effects, but also the cost and time required to produce doses.

Self-amplifying RNA: harnessing viral evolution to fight pathogens

Enter self-amplifying RNA (saRNA). What exactly is saRNA? saRNA is a type of RNA that has many structural similarities to mRNA: it is a linear, single-stranded RNA molecule that is synthesized with a 5′ cap, 3′ polyA tail and 5′ and 3′ UTRs. The main difference is that saRNA is a much larger molecule as it encodes four extra proteins in addition to the vaccine antigen or gene of interest. The four extra proteins are the non-structural proteins, usually derived from an alphavirus, that...
encode a replicase. This replicase enables amplification of the original strand of RNA upon delivery into the cell (Figure 2), which yields a much higher amount of protein expression and thus a minimal dose of RNA required. However, this also means that saRNA (~10,000 nt) is typically much larger than mRNA (~2,000 nt), and thus more difficult to deliver.

The saRNA constructs that have been used for gene delivery or vaccine applications have been historically derived from alphaviruses, such as the Venezuelan equine encephalitis virus (VEEV), Semliki Forest virus (SFV) or Sindbis virus. These saRNA constructs contain the four non-structural proteins, a subgenomic promoter, and the gene of interest (replacing the viral structural proteins). By deleting the viral structural proteins, the RNA is incapable of producing an infectious virus. After delivery to the cytoplasm, the non-structural proteins form an RNA-dependent RNA polymerase (RDRP) that replicates both the genomic RNA (entire RNA strand) and subgenomic RNA (gene of interest). Each of the four non-structural proteins plays a role in the formation of the RDRP, which is a complex and multistage process. This RNA replication is what leads to higher antigen expression than non-replicating mRNA.

Researchers have begun to innovate new saRNA constructs in order to improve upon this technology. Viruses have evolved over time to have their own proteins to inhibit the interferon response, which shuts down host cell translation through mechanisms such as phosphorylation of eukaryotic initiation factor 2 (eIF-2), in order to increase the viral replication. I’ve used a similar strategy to increase the antigen expression from saRNA. I designed constructs that incorporate interferon inhibiting proteins directly into the saRNA construct, whereas others have looked at incorporating these proteins into a separate mRNA molecule. While both have been shown to be effective strategies to increase the protein expression from saRNA, encoding the interferon inhibiting protein in the same molecule ensures that the protein is produced in the same cell where the RNA is sensed. I also observed that this increased the vaccine immunogenicity for an saRNA rabies vaccine and enabled us to use higher doses of RNA without the detrimental effects usually observed with increased dose, such as lower protein expression and cytokine secretion. Studies have also been done to investigate encoding the replicase and gene of interest in separate RNA molecules. These *trans*-amplifying systems have been shown to have similar replication efficiency and protein expression as the traditional *cis*-amplifying saRNA constructs. The main advantage would be that developing new vaccines may be easier if the replicate constructs do not vary, and only the new antigen construct would need to be developed and produced.

Historically, research was performed with virus replicon particles (VRP) which use the same replicase elements but as a virally launched replicon system as opposed to an RNA-launched replicon. These studies

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**Figure 2.** Schematic of the intracellular mechanisms of mRNA vs saRNA
optimized the structural elements and sequences, primarily for cancer applications but also some infectious disease vaccines, which are now being used in in RNA-launched systems, such as saRNA vaccines. The convergence of these two fields (VRPs and mRNA vaccines) has led to the modern research being done with saRNA vaccines.

Delivery and the future of saRNA

saRNA is a large, negatively charged molecule which is not efficiently taken up by cells without a delivery system. The paradigm in the field of saRNA delivery is to use a cationic or ionizable delivery vehicle to complex the saRNA, which neutralizes the charge and forms a particle of ~100 nm in order to promote cellular uptake and cytoplasmic release. There are a number of strategies that have been utilized for saRNA delivery to date, including LNPs (similar to those used in the approved Pfizer/BioNTech and Moderna vaccines), polymeric nanoparticles and cationic nanoemulsions. There are advantages and disadvantages to each of these systems, but overall, the field of delivery is an ongoing area of importance. Although the sensing mechanisms of RNA are well known, the sensing of delivery vehicles is not well understood although it likely highly influences vaccine immunogenicity. Ideally, delivery systems would be able to target certain tissue or cells, adjuvant the saRNA in a complementary manner and ensure release of a large proportion of the saRNA into the cytoplasm.

The first-in-human clinical trial for an saRNA vaccine was performed in 2020 for a COVID-19 vaccine by our team at Imperial College London, which concluded the combined phase I/II clinical trial in January 2021. The doses used in the trial, ranging from 0.1 to 10 µg, compared to 30 µg for the Pfizer/BioNTech vaccine and 100 µg for the Moderna vaccine, illustrate the benefits of this platform. In the context of a pandemic, these doses would enable 10–1,000 times as many doses from the same batch of RNA. However, COVID-19 is not the only pathogen that’s been explored for this platform. Other applications have included infectious diseases such as influenza, rabies, HIV-1, malaria, Chlamydia trachomatis, Ebola, RSV and Zika viruses, as well as oncology applications such as melanoma and colon carcinoma. Future research will likely include optimizing both the molecular design of saRNA (sequence and structural elements), the delivery vehicle, as well as the manufacturing and quality control processes used for these vaccines.

Further Reading


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