mRNAissance: To What Extent Could mRNA Technology Lead the Revolution in Vaccine Development?

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Abstract:

This paper explores the revolutionary significance of mRNA vaccine technology.

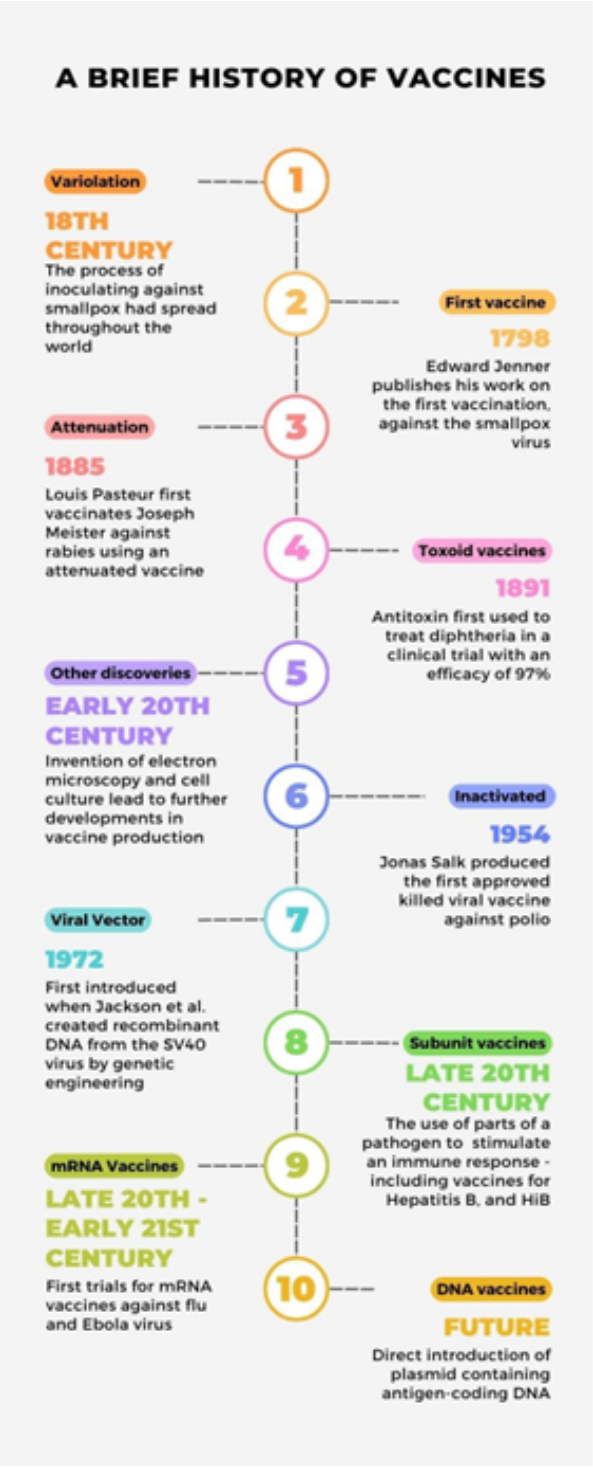
Beginning with the historical context of vaccine development, this paper considers the emergence of this innovative technology, exploring examples of mRNA vaccines in light of the COVID-19 pandemic. The mechanism and advantages of mRNA vaccines including their adaptability, speed of production and high immunogenicity are discussed. The Pfizer-BioNTech and Moderna vaccines also serve to highlight the importance of this technology in the pandemic, but also its limitations in the real-world setting such as reduced efficacy against new variants, storage challenges and concerns on immune exhaustion.

Next, the potential for mRNA technologies in challenging infectious diseases such as HIV (human immunodeficiency virus) and MDR-TB (multidrug-resistant tuberculosis) is also evaluated. mRNA technology can also have wider impacts in the field of healthcare, with implications in cancer therapy by targeting tumor associated antigens (TAAs), particularly in melanoma, and treatment for genetic diseases such as hemophilia and cystic fibrosis by encoding the relevant missing proteins. mRNA therapy can also induce pluripotency in somatic cells which could have profound impacts in regenerative medicine.

Finally, other developing vaccine technologies such as DNA vaccines, DIOSynVax and Caltech's EABR are explored within the context of mRNA vaccines, highlighting opportunities for combinations, and overcoming the limitations of mRNA technology. Ultimately, this research contributes to the understanding of mRNA vaccines and its profound implications in revolutionizing many aspects of global healthcare.

Introduction

Figure 1. Timeline of History of Vaccination. Adapted from History of Vaccines from College of Physicians Philadelphia [2] [See Reference List]



Vaccination, it is argued, has saved more lives than any other human intervention, apart from clean water and sanitation.[1] A timeline showing the history of vaccines is summarized in Figure 1.[2] The CDC estimates that over 4 million lives are saved per year due to childhood vaccination.[3] Despite their numerous successes, illustrated by the eradication of smallpox and two of three known types of polio, traditional vaccine technologies such as live attenuated vaccines or inactivated vaccines face challenges in cold-chain storage and scalability, stability of dosage, occurrence and severity of adverse effects.[4] The global pandemic has especially emphasized the need for innovative vaccine technologies - mRNA vaccines, which have revolutionized vaccine development since their introduction. This paper aims to explore how mRNA vaccines could transform vaccinology.

The significance of this topic is highlighted by how 30 years of research about mRNA vaccines fed into the development Pfizer and Moderna vaccines against the devastating SARS-CoV-2 virus, establishing the technology as a powerful alternative to traditional approaches because of its high potency, safety, efficacy and potential for rapid development and low-cost manufacturing [5,6]. This technology could hold the key to producing vaccines against many challenging pathogens.

The central question of this paper is: “To what extent could mRNA vaccine technology lead the revolution in vaccine development?” This report will investigate the rise of mRNA vaccines, the mechanism, advantages and disadvantages, current challenges and potential solutions of mRNA vaccines. Then, it will explore the applications of this technology in combating challenging viral diseases such as HIV, and the future implications of this technology. Finally, there will be an investigation into other emerging technologies such as DNA vaccines, EABR and DIOSynVax and their implications in advancing vaccine development.

Essential to any critical report is transparency about the scope and limitations of the project. This

is a broad topic, and the analysis will aim to give as much in-depth understanding as possible within the constraints of the word count. Also, since this is a rapidly evolving field, this investigation will represent mRNA technology at this time. There may be other progress that will be outside the scope of the paper. Furthermore, this paper will not delve into nuanced regional application of the mRNA technology, or the ethical aspects. Moreover, opportunities of primary research are severely constricted due to both lack of resources and time - yet there will be an interview with experts as a part of the primary research.

The Rise of mRNA Vaccines:

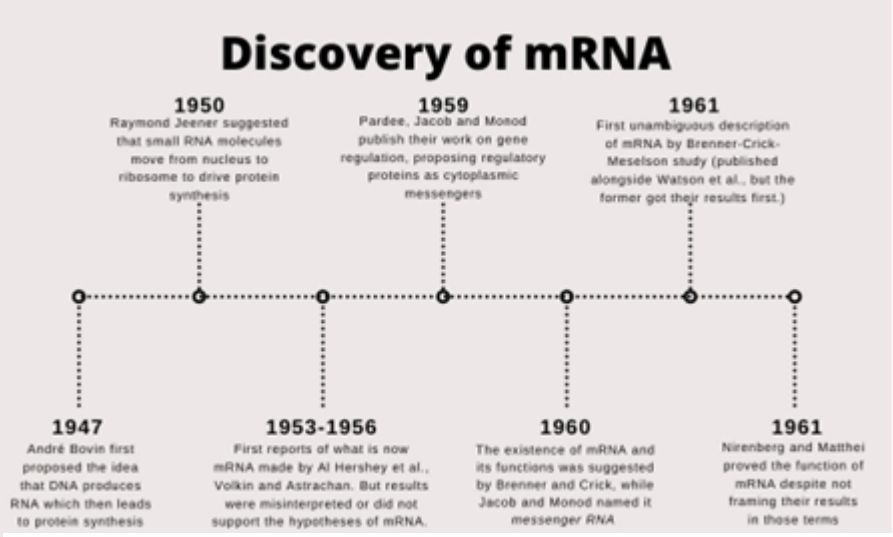


Figure 2. Timeline showing the Discovery of mRNA. Adapted from Cobb M. Who discovered messenger RNA? Current Biology. 2015

Despite the impression that the Pfizer's and Moderna's mRNA vaccines are new, there have been decades of research that formed the knowledge base of mRNA vaccines. The COVID-19 pandemic allowed a sudden influx of government funding, and the pooling of resources into creating a vaccine for the SARS-CoV-2 which catalyzed the production and emergency authorisation of mRNA vaccines in most countries.[7]

The first building block to the emergence of this innovative technology was the discovery of mRNA and its function - summarized in Figure 2., based on an article published in the *Current Biology* magazine.[8]
mRNA is a polynucleotide, transcripted from the antisense strand of DNA in the nucleus. It travels out of the nucleus via a nuclear pore in the nuclear membrane and attaches to a ribosome which translates the codons into amino acids, forming a protein.

Milestones in the Development of mRNA Vaccines:

In 1978, Giorgos J. Dimitriadis used fatty membrane structures, liposomes, to transport mRNA into mouse and human cells to induce protein expression.[9] In 1984, Melton et al. at Harvard University created the first biologically active mRNA in the lab.[10]

The idea of using mRNA as a therapeutic drug became prominent in 1989 when a start-up biotech company, Vical Inc, discovered that mRNA packaged in liposomal nanoparticles could successfully transfect mRNA into a variety of eukaryotic cells.[11] This discovery is controversial as scientist Robert Malone claims that this was his experiment in late 1987 which

was side-lined and not appropriately credited.[12] Most sources seem to agree that it was Malone's landmark experiment.[9] A few months later, Wolff et al. reported their experiment where 'naked' mRNA administered in muscle cells of mice led to the expression of the uncoded protein in the first couple of days.[12]

In 2005, after decades of resilient research, Katalin Karikó, and her colleague, Drew Weissman, made a breakthrough in mRNA vaccines that won them the 2023 Nobel Prize in Physiology and Medicine. They found a way to circumvent the inflammatory response produced in mice to their initial vaccine by replacing uridine (a nucleoside of uracil and ribose) in mRNA with pseudouridine, (see Figure 4) bypassing the Toll-like receptors and thus an undesired immune response.[13] The substitution also yielded an additional benefit of increased production of target protein in cells.[14]

After two years of research, Dr. Derrick Rossi succeeded in using mRNA to make adult cells function like embryonic stem cells in 2009. This discovery led to the founding of Moderna in 2010.[13] A detailed timeline of the discovery of the mRNA vaccine is accessible at *Nature Custom Review*.[14]

Another key technology used in mRNA vaccines was pioneered by Pieter Cullis' laboratory beginning late 1990s - lipid nanoparticles as a delivery system for nucleic acids. Initially used in gene-silencing therapy, two of Cullis' companies began working on using LNPs as a delivery mechanism for mRNA therapeutics in 2012.[12] Since the nucleic acid had a negative charge, the lipids needed to be positively charged to associate with the acid. However, this made them toxic. Cullis developed an ionisable cationic lipid that was positively charged at low pH, but neutral at physiological pH. That allowed researchers to load the DNA or RNA at low pH, and the nucleotides would remain associated with the nanoparticle at neutral pH.[14]
In 2014, Weissman successfully used nanoparticles produced by Acuitas Therapeutics, a company co-founded by Pieter Cullis, for Zika and flu vaccines in animal models. Work on these vaccines were ongoing when the pandemic hit, causing all efforts to be redirected.[14]

mRNA Vaccines Against COVID-19

Property	Pfizer-BioNTech	Moderna
Mechanism	mRNA vaccine	mRNA vaccine
Efficacy after two doses	95% in Phase III clinical trial	95% in Phase III clinical trial
Storage	-80ºC to -60ºC	-25ºC to -15ºC
Vaccinees	16 years and above	18 years and above
Dosage	2 shots of 30 µg each, 21 days apart; given intramuscularly in deltoid muscle	2 shots of 50 µg each, 28 days apart; given intramuscularly in deltoid muscle

Table 1. Comparing the Properties of the Pfizer-BioNTech and Moderna mRNA Vaccines. Adapted from sources [21-23] [see Reference List]

Becoming the first vaccine against SARS-CoV-2 to receive Emergency Use Listing by WHO on 31st December 2020 [16], Pfizer-BioNTech's collaborative Comirnaty mRNA vaccine was licensed less than a year

after the sharing of the virus' genome. [17]

Four months later, Moderna's mRNA vaccine, Spikevax, was listed by WHO for emergency use on April 30, 2021.[18] Table 1. acts as a summary of the key characteristics of both vaccines based on the Yale website and is cross-checked against most recent WHO data.[19] This data may be outdated as it is difficult to obtain more recent statistical information that is not regionally specific. It is important to note that both vaccines were updated for 2023-2024 years corresponding to COVID-19 variants, but data regarding those is currently unavailable. Tables from this study have been used to fill some information gaps.[20]

What is the mRNA Vaccine?

Having explored the emergence of the vaccine technology and discussed examples, this section will discuss the mechanisms of this technology, advantages and disadvantages along with the current challenges and potential solutions.

Mechanism of mRNA Vaccines

In principle, the mechanism of mRNA vaccines is straightforward. An mRNA molecule coding for the antigen is transcribed *in vitro*, then formulated with a synthetic delivery vehicle such as LNPs (Lipid Nano-Particles) and then delivered to the target cells in the host. Then, host cell ribosomes translate the antigen from the delivered RNA which then elicits innate and adaptive immune responses, offering protection against the target pathogen.

There are two classes of mRNA vaccines currently known: non-replicating mRNA, sometimes called modRNA standing for nucleoside-modified mRNA, which mimics endogenous RNA; and viral-derived self-amplifying RNA (saRNA).[21]

Non-replicating mRNA vaccines

Synthetic mRNAs are structurally modeled after cellular mRNAs. Thus, the minimal structural requirement is a 5' cap, open reading frame (ORF) and a 3' poly(A) tail for efficient translation of encoded protein. Untranslated Regions (UTRs) with regulatory function on either side of the ORF can improve mRNA properties. All these regions are modifiable, and are exploited to optimize translation efficiency, stability and immunogenicity of synthetic mRNA.[22]

5' cap

In eukaryotic mRNA, the function of 5' cap structure is to interact with cellular cap-binding proteins, like eukaryotic initiation factor 4e (eIF4E), which regulate mRNA processing, nuclear export and translation initiation. This structure also prevents mRNA decay by blocking decapping proteins and increases mRNA stability.

The first synthetic 5' cap, known as the mCap could be incorporated in forward and reverse directions. However, the reverse direction is not recognised by the cell's translation machinery leading to a large proportion of synthetic mRNA being unrecognizable, reducing translation efficiency. This led to the innovation of ARCA - Anti-Reverse Cap Analogue - which is chemically modified to improve translation efficiency by preventing the reverse orientation. There are also further modifications that increase translation efficiency.[22]

UTRs

Untranslated regions on either side of the ORF are regulatory elements which affect translation efficiency. In humans, 5' UTRs are associated with enhancing translation efficiency whilst 3' UTRs are longer than 5' UTRs regulate stability of the mRNA.
3' UTRs may induce repression of translation, deadenylation (shortening of the poly-A-tail), decapping, or cleavage and lead to mRNA decay. They could also be involved in the transport of mRNA closer to where it is needed. For synthetic mRNA, suitable 3' UTRs can prolong expression and protein level, increase stability and translation efficiency. In future mRNA designs, there is a high probability of increased UTR selection as these regions offer a lot of flexibility due to their significant regulatory role in protein synthesis.[22]

ORF

The open reading frame is a series of nucleotide triplets, known as codons, which code for specific amino acids. This is characterized by a 'start' codon (AUG) and a 'stop' codon (UAA, UGA, or UAG) at their respective ends. Codon bias (which is the use of the same codon to code for an amino acid more often than other codons coding for the same amino acid) may increase translation efficiency. Nucleotides need to be modified as natural nucleotides (A, T, G and C) in synthetic mRNA are excessively immunogenic. While it is necessary to induce both innate and adaptive immune responses for long-term immunity, excessive immunogenicity of mRNA vaccines result in reduced protein synthesis before an adaptive immune response.[22]

3' End and poly(A) tail

The poly(A) tail of the 3' end of mRNA is a sequence of adenine nucleotides of varying length; they are involved in regulating transcript half-life and initiation of translation. Longer tails have better stability at the cost of reduced translation efficiency. Sixty As seem to be an optimal number in most cells, except for some immune cells which require more As, however, these are harder to produce. Alternatively, the 3' end may have a histone stem loop which can be used to terminate the mRNA. However, these two structures are not mutually exclusive as few histone mRNAs are polyadenylated downstream of the stem loop in non-growing cells.[22]

Self-amplifying mRNA vaccines

saRNA can produce copies of themselves in host cells as they are derived from the genomes of positive-strand RNA viruses. This mimics a viral infection, resulting in sustained levels of the target protein with self-adjuvanting innate immune response. Non-virally derived self-amplifying mRNA vaccines have the potential to be highly versatile, potent, streamlined,

scalable and inexpensive. saRNA have all the structures listed in the above section in addition to a section of non-structural genes after the 5' UTRs.[22]

Advantages of mRNA vaccination

Unlike traditional viral vaccines, mRNA vaccines pose minimal safety risks as they have a low reactogenicity and a non-infectious nature. Given its high immunogenicity, its efficacy generates high titres of neutralizing antibodies, and T-cell activation across demographics, including vulnerable populations such as the elderly. Additionally, unlike a DNA vaccine, mRNA cannot integrate into the cell genome, reducing the risk of causing mutations. Moreover, mRNA is a minimal genetic vector, so it can avoid the risk of antivector immune response when using viral vector vaccines. Thus, mRNA can be used for multiple immunisations.

Furthermore, mRNA is subject to physiological destruction due to metabolic processes in the cell, hence does not have any lasting side-effects on the body. The ability to regulate mRNA's half-life through UTR modifications and delivery methods enhances the versatility of the technology. mRNA vaccines can also be lyophilised (frozen to remove water and subjected to a vacuum to remove ice through sublimation) to increase their thermostability too.[23]

Challenges in the Real World

Despite their vast advantages, the mRNA COVID-19 vaccines encountered challenges in real-world settings, including reduced efficacy against emerging variants and adverse effects. Over time, limitations of mRNA vaccines emerged in face of new COVID-19 variants. The most significant are the rare but serious adverse events specifically associated with these mRNA vaccines, partially caused by activation of Th17 immune responses (which can exacerbate inflammatory reactions). Other drawbacks include short-lived protection and reduced efficacy towards variants of concern.[24]

One source claims that due to the lack of historical data, mRNA vaccines' potential side-effects contribute to vaccine hesitancy in almost 50% of the US population.
Recent research indicates that both mRNA vaccines elicited similar and significant reactogenicity, when compared to placebo groups. In some instances, mRNA vaccines caused antibody-dependent enhancement resulting in the exclusion of immunocompromised patients from vaccine trials.[20]

Furthermore, the necessity for booster doses due to the loss of post-vaccination immunity raises concerns about immune exhaustion which correlates with frequent vaccination, causing further research.[24]

Regardless, mRNA vaccines have made significant contributions to the control of the pandemic. These challenges serve as areas for further research to optimize the full potential of this revolutionary technology.

Using mRNA Vaccines for Challenging Infectious Diseases

Overview:

Despite the challenges faced by mRNA vaccines, their advantages in rapid development, flexibility, safety and effectiveness prove the technology to be an avenue worth exploring - particularly for challenging infectious diseases, which do not have a vaccine that offers long-term immunity.

Furthermore, the wealth of data generated from the mRNA vaccines for COVID-19 will prove to be invaluable in developing mRNA vaccines for other technologies. There are many other pathogens, including dengue, influenza, zika viruses that do not have a satisfactory vaccine. A list of current clinical trials for mRNA vaccines against challenging pathogens is available at *Frontiers in Immunology*.[25]

Human Immunodeficiency Virus:

Overview of pathogen and disease:

Human immunodeficiency virus (HIV) categorized within the Lentivirus genus of the *Retroviridae* family [26], comprises types 1 and 2, with HIV-2 exhibiting geographical limitation and less aggression.[27] Untreated HIV may progress to AIDS, which WHO defines as the most Advanced HIV Disease based on a CD4 cell count less than 200 or clinical stages. In 2023, global HIV-related deaths reached 40.4 million. Antiretroviral therapy (ART), the current best treatment, effectively suppresses viral replication and reduces transmission however requires lifelong daily administration.[28] Despite ART's efficacy, viral replication persists due to drug resistance and causes various other side effects.[29]

Developing an effective vaccine proves to be difficult due to a multitude of reasons. Innate and specific immune responses fail to clear the virus as HIV integrates as a provirus into the genome of long-lived memory T-cells, persisting in a latent state.
Moreover, the virus' rapid replication, coupled with high mutation and recombination rates, results in diverse viral clones. Antigenic variation, particularly of the Env glycoprotein and hypervariability further complicate vaccine development, as the virus evades both humoral and cell-mediated responses. Additionally, downregulation of major histocompatibility complex class I antigen presentation reduces antiviral cytotoxic T-lymphocyte response. Lack of natural immunity and spontaneous recovery leads to a chronic infection. In addition, there is no ideal animal model resulting in lengthy and expensive human trials, further impeding progress toward an HIV vaccine.[27]

Promises of mRNA technology:

Due to the high variability of the virus, an optimal vaccine focuses on multiple epitopes from the Env glycoprotein to induce broad neutralizing antibodies that would be effective against viral variants.[25] mRNA technology could prove to be useful in this situation, as it can code for multiple different antigens at the same time. mRNA technology's flexibility and speed of development will be useful in combating the viral diversity of HIV, caused by its high replication and mutation rates.

There are many candidates for an mRNA HIV vaccine, all showing positive results in preclinical trials. Notably, the vaccine developed by International AIDS Vaccine Initiative (IAVI) and Moderna is said to target a subset of B cells in the germinal center known as naïve progenitor B cells. By targeting these cells, the vaccine intends to initiate the production of mature B cells that can generate a diverse range of antibodies.

Perhaps, a combination of ARTs to reduce viral load and an mRNA vaccine for multiple variants will prove most effective.

Multi-Drug Resistant Tuberculosis:

Overview of pathogen and diseases:

According to WHO, tuberculosis (TB) claimed 1.3 million lives in 2022, including 167,000 with HIV, making it the infectious disease with the second-highest mortality rate. The bacterial pathogen, *Mycobacterium tuberculosis*, primarily infects the lungs and spreads through airborne transmission and can have a latency period, making it highly transmissible. Fortunately, TB can be prevented and treated. Untreated TB can be fatal, however, fortunately, antibiotics are effective treatments. In certain regions, the Bacillus Calmette-Guérin (BCG) vaccine somewhat provides protection against extrapulmonary TB.[30]

Unfortunately, a rising concern with TB is drug resistance. Often caused by inappropriate medication use it results in the emergence of multidrug-resistant TB (MDR-TB). This poses challenges as this strain is resistant to first-line drugs forcing the need for costly, toxic second-line treatments.

Developing a vaccine poses challenges due to the complexity of the pathogen. Mycobacterium tuberculosis has a complex cell wall consisting of lipids and glycolipids that increases the difficulty of identifying suitable antigens for a vaccine. Furthermore, the pathogen can enter a latent (dormant) state, which can reactivate to cause an active infection. It is difficult to effectively target the pathogen in both forms. In TB endemic regions, individuals may have prior exposure to environmental mycobacteria resulting in pre-existing immunity. This complicates response to a new vaccine and the reliability of efficacy studies.

Promises of mRNA technology:

A WHO conference report in 2023 highlights recent research that has made the use of mRNA vaccines for TB more plausible and appealing. Through the selection of novel Mtb antigens mRNA vaccine targets, improved understanding of certain cell types contributing to infection control in non-human primates (NHP) models, and the evaluation of numerous experimental methods to identify correlates of protection (COP) for TB.[31] Compared to drug

resistance, vaccine resistance is unlikely, as they target the pathogen in multiple different ways.[32]

As with HIV, mRNA vaccines' numerous advantages make it an ideal vaccination candidate. There are many vaccines in various stages of trials, but perhaps the most promising is an investigational TB vaccine candidate (M72/AS01E), which was found to be significantly protective against TB disease in a Phase IIb trial conducted in Kenya, South Africa and Zambia, in individuals with evidence of latent tuberculosis infection.[33]

Some critics argue that, as suggested by the COVID-19 data, mRNA vaccines might not be as effective in inducing long-term immunity desirable for infections such as TB. However, more research and trial data are required before any conclusions can be made.[13]

Implications of mRNA Vaccines in Non-infectious Settings

mRNA technology itself has many implications in healthcare. Having talked about the potential for mRNA in infectious diseases, this section will delve into utilizing mRNA technology in other aspects of medicine.

Vaccines against cancer:

Cancer is a non-communicable disease characterized by the uncontrollable division of any cell, creating abnormal growth called neoplasm (tumors). These cells may grow past normal boundaries and invade other tissues in the body, known as metastasis, such tumors are malignant and more likely to be lethal.[34, 35]

Like using mRNA vaccines to code for a pathogenic antigen, it is also possible to code for a tumor antigen. In fact, this was the initial direction of exploration of mRNA vaccines.
There are two types of vaccines against cancer - prevention vaccines and therapeutic vaccines. It is estimated that around 15% of cancers are caused by viral infections from pathogens such as human papillomavirus, hepatitis B virus etc.[36] Vaccines against these pathogens act as prophylactic vaccines, preventing the onset of the disease. On the other hand, therapeutic mRNA vaccines apply immunotherapy to existing cancers by targeting tumor-associated antigens (TAA) which are expressed in cancerous cells

There are three ways mRNA vaccines can be involved in cancer therapy. The first, like vaccines against infectious diseases, involves the *in vivo* injection of mRNA into the body using delivery vehicles such as LNPs or protamine. The second is the *ex vivo* transfection of DCs with antigen-encoding mRNA to prepare a DC vaccine.[37] The third way is not strictly a tumor vaccine, but mRNA encoded immunostimulants can turn 'cold', low or no response tumors to 'hot' tumors with good immune responses. Thus, there are many clinical trials that have attempted to enhance antitumor efficacy.

However, many promising cancer vaccine candidates have failed in phase III trials, after much investment. These vaccines target only one TAA. With the development of mRNA vaccines, their ability to code for multiple TAAs or neoantigens simultaneously is more promising.[38]

Multiple TAAs mRNA vaccines

Effectiveness of antitumor response increases when simultaneously targeting multiple TAAs as this reduces the risk of tumor antigen escape (where tumor cells develop immune evasion strategies). Most ongoing clinical trials utilize this approach, including the promising candidate BioNTech's BNT111 for melanoma, which targets all four TAAs.

Tumors with high mutation rates tend to have stronger immune responses, making multitargeting mRNA vaccines promising for highly immunogenic cancers like melanoma, however, have not shown as much promise in other tumor types.

On the other hand, there are many challenges of producing cancer vaccines - including limited TAAs for certain tumors, immune tolerance to TAAs in normal tissues, and the risk of triggering autoimmune responses. mRNA vaccines encoding neoantigens offer a new avenue for cancer vaccine development.[39]

Personalized neoantigen-encoding mRNA vaccines

As mentioned in the previous paragraph, tumor cells proliferate quickly and avoid immune detection. mRNA vaccines offer an advantage by targeting neoantigens unique to only tumor cells. This also reduces the risk of triggering an autoimmune response. This neoantigen, also known as a mutanome, is unique to each tumor patient. Sequencing technology can be used to identify these neoantigens, allowing for personalized vaccine development. This approach has demonstrated great success in clinical trials, particularly against melanoma.

However, despite the rapid development of mRNA vaccines, the process can take time, making it unsuitable for patients with advanced tumors that require urgent personalized therapy. Nevertheless, mRNA technology may enable a new era of personalized medicine.[39]

mRNA transfected DC vaccines

Unlike the other two technologies discussed, dendritic cell (DC) vaccines are produced *ex vivo*, where mRNA coding for TAA is injected into DCs enhancing their ability to identify and attack tumors. Notably, in 2010, the FDA approved the world's first DC tumor vaccine against prostate cancer. Despite their potential, this technology also faces the same challenges as any personalized treatment - individualisation increases production time, and decreases cost-effectiveness. Thus, further research is required for wide clinical use.[39]

Genetic Disorders and Regenerative Medicine

Although not technically through vaccination, mRNA therapy can alleviate symptoms of genetic disorders such as hemophilia, cystic fibrosis, and muscular dystrophy that are caused by missing or faulty proteins due to mutations in the genes coding for them. By introducing mRNA coding for these specific proteins into cells, normal functioning proteins can be restored, supplementing existing proteins.

Briefly mentioned in a previous section of this article, mRNA therapy holds promise in regenerative medicine. It can induce cells to become pluripotent (capable of forming any body cell), differentiate into specialized cells for specific functions (cardiac cells or neurons), or undergo reprogramming. By delivering mRNA that codes for various proteins like transcription

factors, growth factors, or signaling molecules, mRNA therapy can generate different types of cells from somatic cells (fibroblasts or blood cells).

However, there is still much research to be done regarding epigenetic alterations, transcriptional activity and long-term safety data, as there is a risk that induced pluripotent cells can become cancerous.[38]

Other Technologies:

mRNA vaccines are not the only technology showing promise. This section will explore DNA vaccines, and other recent technology. Again, it is important to consider that in this section, information is as up to date as possible, thus only taken from reliable sources such as official university websites and scientific studies.

DNA Vaccines:

As a nucleic acid vaccine, DNA vaccines are similar in mechanism to mRNA vaccines, however, there is still no licensed vaccine to date. It involves the insertion of plasmid DNA encoding the target antigen into host cells allowing for *in situ* production of the antigen. Proof-of-concept studies[40] in animal models have displayed efficacy of DNA vaccines against a variety of pathogens including influenza, HIV, malaria and Hepatitis B.

Like mRNA vaccines, this approach offers numerous advantages, including the triggering of both B-cell and T-cell responses, absence of infectious agents, and scalability of manufacturing processes. In contrast, DNA vaccines pose more advantages than mRNA vaccines in a simpler production process, and increased thermostability facilitating longer storage, hence could be an alternate solution to mRNA vaccines' demanding storage requirements.[41]

However, DNA vaccines have limitations compared to mRNA vaccines. There is a chance of contamination during manufacture as DNA vaccines involve production in bacterial cells. The efficacy of DNA vaccines is impacted by cell division and there is a risk of genomic integration. Nevertheless, there has been no signs of genomic integration in studies so far, but the risk remains as some candidate vaccines move to clinical trials.[42]

DIOSynVax by Cambridge University:

DIOSynVax is a vaccine technology created to combat zoonotic spill-overs of coronaviruses (from animals to humans). The aim is to generate a cross-family and mutation-countering immune response. When combined with mRNA technology, it could address the drop in efficacy shown by the Pfizer-BioNTech and Moderna vaccines against newer variants.

It uses computational technology with viral genetic information to select target antigens for a vaccine by designing Vaccine Antigen Payload (VAP) specific to each virus. These VAPs are 'vector-agnostic', thus can be given in any vaccine technology - viral vector, DNA or mRNA. This technology is a potential solution to the challenge COVID-19 mRNA vaccines faced - reduced efficacy against novel strains.

DIOSynVax for COVID-19, using a DNA vector, is currently in clinical trials, after promising results in mice, guinea pigs and rabbits. This vaccine is delivered needle-free, by a high-powered jet of air that allows it to penetrate the layers of the skin. The manufacturing could also be scaled up to produce in more-stable powdered form, boosting global distribution, particularly useful in low- and middle-income countries.[43]

EABR Technology by Caltech:

This technology combines aspects of mRNA and protein-based vaccines to create a natural, infection-mimicking technology. Developed in response to the challenges of emerging COVID-19 variants such as Omicron, it utilizes the ESCRT and ALIX-binding region (EABR) technology, to engineer self-assembling enveloped virus-like particles (eVLPs) by incorporating an EABR into the SARS-CoV-2 spike protein's cytoplasmic tail (the part of the viral protein that extends into the cytoplasm of the host cell, having roles in viral replication and assembly). These VLPs mimic the structure of the virus, serving as a potent immune trigger, but remain non-infectious, so potentially still safe.

A study conducted by Caltech and Acuitas Therapeutics showed that EABR technology produced up to five times as many antibodies as other COVID vaccines in mice against the original and Delta variants.[44,45] Thus, this information was cross-checked in another article which highlights the promise of this technology.[46]

The EABR technology also shows promise in robust antibody production against HIV, whilst it struggles with influenza viruses due to the multiple glycoproteins involved in attachment and release of the pathogen. However, further research is needed regarding cross-reactivity (the phenomenon where a vaccine targeting a specific antigen may also provide some level of protection against related antigens) to optimize vaccine efficacy. Combining multivalent mRNA technology with EABR technology could prove to enhance efficacy beyond anticipated levels for challenging pathogens.

Conclusions:

In conclusion, mRNA technology represents a ground-breaking paradigm shift in vaccine development, offering incredible opportunities in combating infectious diseases and addressing long-standing medical challenges. This paper has explored the ascent of this innovative technology, driven by its flexibility, adaptability and its high immunogenicity. The rapid development of the world’s first mRNA vaccines during the COVID-19 pandemic underscores both the advantages and the future potential of this technology.

However, as highlighted by the COVID-19 pandemic, there are certain limitations to the current mRNA technology. These include storage challenges, limiting access in tropical countries; adverse effects such as exacerbated inflammatory reactions; reduced efficacy against variants such as Omicron, requiring multiple booster doses which raised concerns about immune exhaustion. Nevertheless, the versatility of mRNA vaccines poses potential solutions to challenging diseases such as HIV and MDR-TB, specifically the speed of production and the ability to code for multiple antigens simultaneously. A HIV mRNA vaccine developed by Moderna in collaboration with IAVI holds much promise.

Furthermore, the applications of mRNA technology extend beyond infectious diseases, contributing to therapeutic cancer vaccines by encoding TAAs such as the BNT111 vaccine against melanoma, genetic disorders to encode for missing proteins and regenerative medicine in inducing pluripotency of somatic cells.

Additionally, navigating the complexities of this technology needs a nuanced understanding of mRNA technology in the broader scope of vaccine development. It is crucial to recognise that each technology has its own advantages and limitations. For example, DNA vaccines are more thermostable, but pose a risk of genomic integration and contamination during the production process. Exploring combinations of technologies, as exemplified by the EABR technology and the DIOSynVax, has potential in optimizing vaccine efficacy and addressing multi-faceted healthcare challenges.

Essentially, mRNA technology holds transformative potential in all fields of healthcare, not just vaccine development. Its unique advantages have already demonstrated their potential in the light of the pandemic, whilst also inspiring increased innovation, paving the way for incredible advancements. Further research regarding specific uses is required to exploit the full potential of mRNA vaccines. To answer the initial question, it is evident that mRNA technology has and will continue to revolutionize vaccine development and other fields. mRNA technology is a testament to scientific ingenuity, playing a pivotal role in advancing global health.

References:

1. Greenwood B, Salisbury D, Hill AV. Vaccines and global health. Philosophical Transactions of the Royal Society B: Biological Sciences [Internet]. 2011 Oct 12 [cited 2024 Feb 24];366(1579):2733–42. doi:10.1098/rstb.2011.0076

2. College of Physicians Philadelphia. Vaccine timeline [Internet]. College of Physicians Philadelphia; [cited 2024 Feb 24]. Available from: https://historyofvaccines.org/history/vaccine-timeline/overview

3. Centers for Disease Control and Prevention. Fast facts on global immunization [Internet]. Centers for Disease Control and Prevention; 2023 [cited 2024 Feb 24]. Available from: https://www.cdc.gov/globalhealth/immunization/data/fast-facts.html

4. College of Physicians Philadelphia. Diseases [Internet]. College of Physicians Philadelphia; [cited 2024 Feb 24]. Available from: https://historyofvaccines.org/diseases

5. Anthony L. Komaroff M. Why are mrna vaccines so exciting? [Internet]. Harvard Medical School; 2021 [cited 2024 Feb 24]. Available from: https://www.health.harvard.edu/blog/why-are-mrna-vaccines-so-exciting-2020121021599

6. Gote V, Bolla PK, Kommineni N, Butreddy A, Nukala PK, Palakurthi SS, et al. A comprehensive review of mrna vaccines. International Journal of Molecular Sciences [Internet]. 2023 Jan 31[cited 2024 Feb 24];24(3):2700. doi:10.3390/ijms24032700

7. Sharma R, Mandepalli J. History and future of vaccination. Personal interview, Dec 27. Malaysia, 2023 (unpublished) [see Appendix: Interview Transcript]

8. Cobb M. Who discovered messenger RNA? Current Biology [Internet]. 2015 Jun [cited 2024 Feb 24]; 25(13). doi:10.1016/j.cub.2015.05.03

9. Li M, Li Y, Li S, Jia L, Wang H, Li M, et al. The Nano Delivery Systems and applications of mrna. European Journal of Medicinal Chemistry [Internet]. 2022 Jan [cited 2024 Feb 24]; 227:113910. doi:10.1016/j.ejmech.2021.113910

10. Melton DA, Krieg PA, Rebagliati MR, Maniatis T, Zinn K, Green MR. Efficient *in vitro* synthesis of biologically active RNA and RNA hybridization probes from plasmids containing a bacteriophage SP6 promoter. Nucleic Acids Research [Internet]. 1984 [cited 2024 Feb 24];12(18):7035–56. doi:10.1093/nar/12.18.7035

11. Verbeke R, Lentacker I, De Smedt SC, Dewitte H. Three decades of messenger RNA vaccine development. Nano Today [Internet]. 2019 Oct [cited 2024 Feb 24];28:100766. doi:10.1016/j.nantod.2019.100766

12. Dolgin E. The tangled history of mrna vaccines. Nature [Internet]. 2021 Sept 14 [cited 2024 Feb 24] ;597(7876):318–24. doi:10.1038/d41586-021-02483-w

13. Damian Garde JS— BG. The story of mrna: How a once-dismissed idea became a leading technology in the Covid vaccine race [Internet]. 2023 [cited 2024 Feb 24]. Available from: https://www.statnews.com/2020/11/10/the-story-of-mrna-how-a-once-dismissed-idea-became-a-leading-technology-in-the-covid-vaccine-race/

14. Nature Research Custom Media, Cytiva. How mRNA technology came to the rescue [Internet]. Nature Publishing Group; [cited 2024 Feb 24]. Available from: https://www.nature.com/articles/d42473-022-00159-1

15. Government of Canada CI of HR. The Long Road to mrna vaccines [Internet]. 2021 [cited 2024 Feb 24]. Available from: https://cihr-irsc.gc.ca/e/52424.html

16. WHO issues its first emergency use validation for a COVID-19 vaccine and emphasizes need for equitable global access. World Health Organization [Internet]. World Health Organization; 2020 Dec 31 [cited 2024 Feb 24]; Available from: https://www.who.int/news/item/31-12-2020-who-issues-its-first-emergency-use-validation-for-a-covid-19-vaccine-and-emphasizes-need-for-equitable-global-access

17. Burki T. First shared SARS-COV-2 genome: Gisaid vs Virological.org. The Lancet Microbe [Internet]. 2023 Jun [cited 2024 Feb 24];4(6). doi:10.1016/s2666-5247(23)00133-7

18. WHO. The moderna covid-19 (mRNA-1273) vaccine: What you need to know [Internet]. World Health Organization; 2022 [cited 2024 Feb 24]. Available from: https://www.who.int/news-room/feature-stories/detail/the-moderna-covid-19-mrna-1273-vaccine-what-you-need-to-know

19. Katella K. Comparing the COVID-19 vaccines: How are they different? [Internet]. Yale Medicine; 2023 [cited 2024 Feb 24]. Available from: https://www.yalemedicine.org/news/covid-19-vaccine-comparison

20. Chaudhury S, Ali T, Mujawar S, Sowmya A, Saldanha D. Dangers of mrna vaccines. Industrial Psychiatry Journal [Internet]. 2021 [cited 2024 Feb 24];30(3):291. doi:10.4103/0972-6748.328833

21. Pfizer. Harnessing the potential of mrna [Internet]. 2024 [cited 2024 Feb 24]. Available from: https://www.pfizer.com/science/innovation/mrna-technology

22. Yu D. (ed.) Petsch B. (ed.) *mRNA Vaccines;* Current Topics in Microbiology and Immunology. Switzerland: Springer Nature; 2022 [cited 2024 Feb 24].

23. Ilyichev AA, Orlova LA, Sharabrin SV, Karpenko LI. MRNA technology as one of the promising platforms for the SARS-COV-2 vaccine development. Vavilov Journal of Genetics and Breeding [Internet]. 2020 Dec 6 [cited 2024 Feb 24]; 24(7):802–7. doi:10.18699/vj20.676

24. Echaide M, Chocarro de Erauso L, Bocanegra A, Blanco E, Kochan G, Escors D. MRNA vaccines against SARS-COV-2: Advantages and caveats. International Journal of Molecular Sciences [Internet]. 2023 Mar 21[cited 2024 Feb 24]; 24(6):5944. doi:10.3390/ijms24065944

25. Matarazzo L, Bettencourt PJ. MRNA vaccines: A new opportunity for malaria, tuberculosis and HIV. Frontiers in Immunology [Internet]. 2023 Apr 24 [cited 2024 Feb 24]; 14. doi:10.3389/fimmu.2023.1172691

26. Seitz R. Human immunodeficiency virus (HIV). Transfusion Medicine and Hemotherapy [Internet]. 2016 [cited 2024 Feb 24]; 43(3):203–22. doi:10.1159/000445852

27. Plotkin S. (ed.) et al. Girard MP, Koffe WC. Human Immunodeficiency Virus Vaccines. In: Plotkin's Vaccines. 7th ed. Philadelphia, Pennsylvania: Elsevier; 2018. p. 400–29.

28. WHO. HIV and AIDS [Internet]. World Health Organization; 2023 [cited 2024 Feb 24]. Available from: https://www.who.int/news-room/fact-sheets/detail/hiv-aids

29. Dwivedi L, Shrivastava M. Anti-HIV therapy: Pipeline Approaches and Future Directions. Nature Precedings [Internet]. 2011 Jan 12 [cited 2024 Feb 24]: doi:10.1038/npre.2011.5540.1

30. World Health Organization. Tuberculosis. [Internet]. 2023 [cited 2024 Feb 24]. Available from: https://www.who.int/news-room/fact-sheets/detail/tuberculosis

31. Looney MM, Hatherill M, Musvosvi M, Flynn J, Kagina BM, Frick M, et al. Conference report: Who meeting summary on mrna-based tuberculosis vaccine development. Vaccine [Internet]. 2023 Nov [cited 2024 Feb 24] ;41(48):7060–6. doi:10.1016/j.vaccine.2023.10.026

32. Kennedy DA, Read AF. Why the evolution of vaccine resistance is less of a concern than the evolution of drug resistance. Proceedings of the National Academy of Sciences [Internet]. 2018 Dec 17 [cited 2024 Feb 24] ;115(51):12878–86. doi:10.1073/pnas.1717159115

33. WHO. New TB Vaccine Research [Internet]. World Health Organization; [cited 2024 Feb 24]. Available from: https://www.who.int/teams/global-tuberculosis-programme/research-innovation/vaccines

34. WHO. Cancer [Internet]. World Health Organization; [cited 2024 Feb 24]. Available from: https://www.who.int/health-topics/cancer#tab=tab_1

35. Neoplasm (tumor) [Internet]. Yale Medicine; 2023 [cited 2024 Feb 24]. Available from: https://www.yalemedicine.org/conditions/neoplasm

36. Benteyn D, Heirman C, Bonehill A, Thielemans K, Breckpot K. MRNA-based dendritic cell vaccines. Expert Review of Vaccines [Internet]. 2014 Sept 8 [cited 2024 Feb 24]; 14(2):161–76. doi:10.1586/14760584.2014.957684

37. Plummer M, de Martel C, Vignat J, Ferlay J, Bray F, Franceschi S. Global burden of cancers attributable to infections in 2012: a synthetic analysis. Lancet Glob Health. 2016 Sep;4(9). doi: 10.1016/S2214-109X(16)30143-7

38. Ye L, Swingen C, Zhang J. Induced pluripotent stem cells and their potential for basic and Clinical Sciences. Current Cardiology Reviews [Internet]. 2013 Feb 1 [cited 2024 Feb 24]; 9(1):63–72. doi:10.2174/157340313805076278

39. Donnelly JJ, Wahren B, Liu MA. DNA vaccines: progress and challenges. J Immunol. 2005 Oct 1;175(7):633-9. doi: 10.4049/jimmunol.175.7.633.

40. Sahin U, Türeci Ö. Personalized vaccines for cancer immunotherapy. Science. 2018 Apr 6;359(6382):1355-1360. doi: 10.1126/science.aar7112.

41. WHO. DNA [Internet]. World Health Organization; [cited 2024 Feb 24]. Available from: https://www.who.int/teams/health-product-policy-and-standards/standards-and-specifications/vaccines-quality/dna

42. Melo AR, de Macêdo LS, Invenção M da, de Moura IA, da Gama MA, de Melo CM, et al. Third-generation vaccines: Features of nucleic acid vaccines and strategies to improve their efficiency. Genes [Internet]. 2022 Dec 4 [cited 2024 Feb 24] ;13(12):2287. doi:10.3390/genes13122287

43. Vishwanath S, Carnell GW, et al. Self-amplifying RNA SARS-CoV-2 lipid nanoparticle vaccine candidate induces high neutralizing antibody titers in mice. Nat Nanotechnol. 2023 Mar;18(3):411-417. doi: 10.1038/s41551-023-01094-2.

44. Hoffmann MAG, Yang Z, Huey-Tubman KE, Cohen AA, Gnanapragasam PNP, Nakatomi LM, et al. ESCRT recruitment to SARS-COV-2 spike induces virus-like particles that improve

mrna vaccines. Cell [Internet]. 2023 May [cited 2024 Feb 24];186(11). doi:10.1016/j.cell.2023.04.024

45. Hoffmann M. Exploring hybrid mRNA vaccine technology for lasting immunity to COVID-19. Vaccine Insights [Internet]. 2024 Feb 02 [cited 2024 Feb 24]; doi: 10.18609/vac.2024.004.

46. Zost SJ, Carnahan RH. A budding new approach strengthens an important message. Cell [Internet]. 2023 May [cited 2024 Feb 24] ;186(11):2283–5. doi: 10.1016/j.cell.2023.04.034