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Messenger RNA-based vaccines: Past, present, and future directions in the context of the COVID-19 pandemic

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ABSTRACT

mRNA vaccines have received major attention in the fight against COVID-19. Formulations from companies such as Moderna and BioNTech/Pfizer have allowed us to slowly ease the social distancing measures, mask requirements, and lockdowns that have been prevalent since early 2020. This past year's focused work on mRNA vaccines has catapulted this technology to the forefront of public awareness and additional research pursuits, thus leading to new potential for bionanotechnology principles to help drive further innovation using mRNA. In addition to alleviating the burden of COVID-19, mRNA vaccines could potentially provide long-term solutions all over the world for diseases ranging from influenza to AIDS. Herein, we provide a brief commentary based on the history and development of mRNA vaccines in the context of the COVID-19 pandemic. Furthermore, we address current research using the technology and future directions of mRNA vaccine research.

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Contents

1. Introduction . . . 2
2. General overview of vaccines . . . 2
3. Vaccine types . . . 3
   3.1. Live-attenuated vaccines . . . 3
   3.2. Inactivated vaccines . . . 4
   3.3. Subunit/recombinant vaccines . . . 4
   3.4. Other vaccines (DNA/Toxoid) . . . 4
   3.5. mRNA vaccines . . . 5
      3.5.1. Introduction . . . 5
      3.5.2. Unique Mechanism of action . . . 6
      3.5.3. Advantages over current standards of vaccination . . . 7
   3.6. mRNA vaccine applications in prophylactic immunity and disease treatment . . . 8
      3.6.1. SARS-CoV-2 . . . 8
      3.6.2. Influenza virus . . . 9
      3.6.3. Zika virus . . . 9
      3.6.4. HIV/AIDS . . . 10
      3.6.5. Dengue fever . . . 11
      3.6.6. Rabies virus . . . 11

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4. Future directions and perspectives . . . 11
Declaration of Competing Interest . . . 13
Acknowledgements . . . 13
References . . . 13

1. Introduction

When the ongoing COVID-19 pandemic brought the world to a standstill in the spring of 2020, the medical and scientific communities, alongside political leaders and national governments, rapidly arrived at a consensus that wide-scale vaccination against the SARS-CoV2 virus would be the most effective strategy to control the impact of the disease and allow for the quickest return to normalcy. This steadfast belief in the public health impact of vaccines highlights the transformative role that they have played in ensuring human health. After several difficult months, billions of dollars in investment, and the near single-minded focus of the scientific community, multiple novel vaccines were produced against the new disease. Today, these vaccines are being administered at an unprecedented rate and slowly allowing "normal" life to resume for societies around the world. This pandemic is not yet over - many countries, particularly developing ones with low vaccine access and/or poor population compliance, are still being ravaged by the virus, new variants are frequently emerging which continue to create new outbreaks and challenge the effectiveness of these vaccines, and individuals are still reeling from the economic and health impacts caused by the virus, but experts can confidently state that the worst of the pandemic is behind us, and this paradigm shift can be significantly attributed to newly developed mRNA vaccines.

Historically, live, killed, and subunit vaccines have been critical in controlling the spread of similar diseases such as smallpox and hepatitis, but these diseases have been limited in their international presence, infectivity and case fatalities. COVID-19 presented several novel challenges in this regard; the ease of spread of the disease and the high observed fatality rate made it impossible to ignore or downplay the impact of the virus for the general population. Scientists maintained that lockdowns, social distancing/physical isolation, mask-wearing, and other stopgap measures would continue to be necessary for public health until a vaccine for COVID-19 became widely available, but such conditions are unsustainable for extended periods of time, as evidenced by the disruption of economies and livelihoods around the world and the increase in suicide rates and mental health issues caused by isolation. For these reasons, scientists and pharmaceutical companies faced a unique pressure from citizens and governments to create, manufacture, and widely distribute a vaccine immediately, as opposed to after the several years of development, testing, and quality control this process normally requires.

In the spring and summer of 2020, academic research groups and pharmaceutical companies turned their attention to messenger RNA (mRNA) vaccination, a different vaccination strategy which utilizes the host's cellular machinery to synthesize a viral protein product and establish protective immunity within the host. mRNA therapeutic delivery previously had limited applications in humans due to the instability of mRNA *in vivo* and was primarily being explored as an experimental strategy for cancer therapy, but the urgency of the pandemic required rapid development of a vaccine with a high safety profile, ease of scale up/production, and strong therapeutic efficacy, which a mRNA vaccination could provide. Recent advances in nanotechnology, and particularly lipid-based nanoparticles, offered an avenue for researchers to deliver mRNA to the body's tissue and harness the technique to produce a next-generation subunit vaccine, and many leading pharmaceutical companies were eager to try and do so. As of today, companies such as Moderna and Pfizer/BioNTech have been successful in producing COVID-19 mRNA vaccines. Their formulations successfully passed in Phase 3 clinical trials in the fall of 2020 and received a rare emergency use authorization from the United States Food and Drug Administration (FDA). Both Moderna and Pfizer/BioNTech utilized an mRNA approach requiring 2 shots, while Johnson Johnson a viral vector approach requiring just a single shot. The latter approach utilized a different virus in order to genetically encode instructions intended to fight off COVID-19 infections. Pfizer/BioNTech was the first to get approval on December 11, 2020, with Moderna getting approval a week later. Johnson & Johson's vaccine was approved on February 27, 2021. All of the vaccines are currently approved for use in adults, while the Pfizer vaccine has been approved for adolescents as well. Additionally, all three of these vaccines were given emergency use authorization in various countries. On August 23, 2021, the US Food and Drug Administration (FDA) officially approved the Pfizer/BioNTech vaccine. Widespread administration of these vaccines began around the same time as their debut and continues today; though there was certainly initial hesitancy in the eyes of the general public (some of which still persists well into 2021), extensive trials and studies have confirmed the safety and efficacy of these vaccines. Over 44% of the global population [1] has now been partially vaccinated against COVID-19 as of September 2021, and world stability is contingent upon the sustained success of vaccination efforts.

The breakneck pace of the development of COVID-19 mRNA vaccines highlights the benefits and utility of mRNA delivery as a vaccination strategy, and it is worth exploring how this technology can be refined and improved upon to provide prophylactic and therapeutic treatment solutions for a wide range of diseases. Looking beyond COVID-19, several infectious and tropical diseases continue to harm populations in developing countries with limited avenues for prevention. The foundational principles behind mRNA vaccines could potentially be applied to create effective solutions for these diseases. We examine the progression and current state of mRNA vaccines and their modern utility, their benefits/limitations and applications in treating and generating immunity against different diseases, and how principles of bionanotechnology can be used to further improve mRNA vaccine applications in the future.

2. General overview of vaccines

Vaccines have played a pivotal role in revolutionizing human health and society over the past two centuries; their advent in the late 1800′s converged years of seminal research in germ theory and immunology to significantly reduce the disease burden of many deadly infectious diseases. Vaccines are based on the fundamental premise of protective immunity at both the individual and population levels. By exposing a recipient to a noninfectious element of a disease-causing pathogen, the vaccine can stimulate the host's adaptive immune system to generate immunological memory against the pathogen, significantly reducing the chance of infection when exposed to the pathogen in the future. Simulta-

neously, widespread vaccination can protect the most vulnerable members of a population through a process known as herd immunity -- with enough vaccinated members in a group, the virus will have little opportunity for community spread. To date, there are vaccines available in the United States for nearly 30 different diseases ranging from influenza to Ebola, and many more worldwide [2]. Additionally, hundreds of vaccines are currently being researched and developed for preventative applications in treating other diseases -- most notably SARS-CoV2 -- and vaccines and their associated delivery strategies are also being explored for prophylactic treatment against diseases such as cancer.

Vaccination has reduced the burden of infectious disease, second only to clean drinking water in reducing mortality worldwide [3]. However, infectious diseases remain the second leading cause of death worldwide, disproportionately affecting children under the age of 5 and people in low-income countries. In fact, five of the top ten leading causes of death in low income countries are caused by infectious agents: lower respiratory infections (*e.g.*, pneumonia), HIV/AIDS, diarrheal disease, malaria and tuberculosis [4]. While some of these killers lack a current vaccine for disease control, many deaths result from vaccine-preventable disease, indicating substantial room for improvement in vaccine technology and administration.

3. Vaccine types

The innate immune system, consisting of natural barriers such as skin, mucous membranes, nonspecific macrophages, and enzymes serves well as a first line of defense against many disease-causing antigens, but fails to respond robustly and specifically to antigens, possibly resulting in damage to the host through excessive inflammation and delaying the response against pathogens. The human body's adaptive immune system bridges these shortcomings by generating a pathogen-specific immune response and retaining memory of the pathogen's key features. By doing so, the system can direct cellular and humoral elements to rapidly neutralize a pathogen when it re-enters the body, and generating this immunological memory is the key focus of most vaccination strategies [5].

Vaccine technology has advanced significantly from the initial attempts in the 18th and 19th centuries to induce smallpox immunity through lesion transfusion - modern vaccines are highly precise and carefully engineered formulations that utilize a variety of antigen properties to stimulate adaptive immunity. A summary of the various common vaccine types and their properties are presented below in Table 1.

3.1. Live-attenuated vaccines

Live-attenuated vaccines are one of the most common and effective vaccine types, with several formulations existing for diseases such as influenza, polio, and typhus, to name a few. Such vaccines rely on a living strain of a pathogen which has been attenuated to be non-infectious via growth in non-human tissue; this harmless pathogen strain is then inserted into a human and is recognized/killed by the host immune system. Effector B and T cells and pathogen specific antibodies are created in response to the pathogen and will be present to neutralize future infectious strains. A notable example of a live attenuated vaccine is the yearly influenza (flu) vaccine, which is commonly developed by culturing infectious influenza strains in cell cultures or fertilized chicken embryos to produce a live attenuated vaccine administered via nasal spray. The CDC estimates that over 169 million influenza vaccines were administered in 2019 alone and that vaccination

Table 1
A summary of vaccine types and their properties used today.

Vaccine Type	Mechanism of Action/Key Features	Modern Uses	Seminal Research	Future Directions in Disease Treatment/Delivery
Live-attenuated	Weakened/non-virulent pathogen detected by host immune system and generates immunity against future exposure	Influenza, polio, typhus, measles, mumps, rubella (MMR)	Edward Jenner, smallpox vaccine (1796) - virtually eradicated in humans now	Preclinical exploration of vaccination strategies for herpes[6] and COVID[7] (intranasal)
Inactivated	Fully killed pathogen is exposed to host immune system, generating immunity while avoiding virility	Cholera, pertussis, COVID-19 (exploratory)	Typhoid, plague, cholera (1896), influenza (1940 s)	COVID[8], orotransmucal delivery using artificial intelligence[9]
Subunit/ Recombinant	Only the antigenic component of the pathogen is introduced into the host and presented to immune cells	Influenza, meningitis, hepatitis A, hepatitis B	Anthrax (1970 s) and hepatitis B (1980 s)	Hydrogel modulation for influenza subunit co-delivery,[10] oral delivery[11]
Other approaches (DNA/Toxoid)	DNA: Insertion of DNA into host cell to create antigenic productsToxoid: Introduction of antigenic product to host - similar to subunit but focuses on product, not pathogen	Diphtheria, influenza, *C. difficile*, tetanus	DNA: 1983 (smallpox vaccine experimentation), 2016 (Zika virus) - work in progressToxoid: Diphtheria (1923)	DNA: Zika virus,[12] Phase 1 clinical trial for COVID vaccineToxoid: Exploration of PLGA-chitosan as delivery vector[13]

reduces an individual's chances for influenza infection by 40–60% [14].

Live-attenuated vaccines have several benefits; they are relatively simple to design, generate a strong immune response, and retain their bioavailability for longer periods of time, ensuring consistent generation of immunity. However, they can be dangerous to manufacture, can cause strong adverse immune reactions in immunocompromised patients, and possess the potential for reverse mutations to an infectious strain, such as in the infamous case of the oral poliovirus vaccine inducing poliomyelitis in patients. These issues can largely be addressed and mitigated through modern genetic engineering approaches, and in fact, several ongoing clinical trials with live-attenuated COVID-19 vaccine candidates are proceeding to determine efficacy in producing antibodies against the virus' characteristic spike protein [15]. However, a significant challenge in utilizing live-attenuated vaccines to combat the COVID-19 pandemic lies in their stringent transport requirements. Vaccine manufacturers are required to ensure a "cold chain" from the point of manufacture to the point of use to ensure vaccine stability and effectiveness, and the high costs of transporting live vaccines in a temperature-controlled environment and low feasibility of cold chain maintenance over long distances and with a large number of doses make live attenuated vaccines an insufficient short-term solution to the pandemic. This issue is seen with certain formulations of mRNA vaccines as well, and those face the same issues in the form of limited reach [16].

3.2. Inactivated vaccines

Inactivated vaccines include the original Salk vaccine for poliomyelitis (polio) and the cholera vaccine [17,18]. Similar to live-attenuated vaccines, inactivated vaccines are generally grown in culture in non-human tissue. However, the pathogen itself is killed to reduce the virility and prevent infection from the vaccine. Common methods to kill the pathogen include heat and/or formaldehyde. More recently, hydrogen peroxide has been explored as a killing agent [19]. While the pathogen has lost virility, the guiding principle behind inactivated vaccines is that the body is still able to produce specific antibodies that bind to the various fragments of the killed pathogen. This action then gives the host immunity, as the body has antibodies that will be able to recognize part of the live pathogen.

While inactivated vaccines were incredibly common in the mid-20th century, they produce slightly weaker immune responses in comparison to live-attenuated vaccines [20]. This creates a major drawback in that often several doses of inactivated vaccines are needed to be effective as the body loses its immune memory over time in the absence of reproducing viruses. Thus, inactivated vaccines are often reserved for people with weaker immune systems, such as those with immunodeficiencies and the elderly, although utilizing an adjuvant such as aluminum hydroxide can increase immunogenicity. For the case of the Salk polio vaccine, it was replaced within a decade of its discovery by a Sabin oral live vaccine due to it requiring multiple doses to be effective and the slight increase in polio cases as a result of the need for multiple doses [17].

As development continued on mRNA vaccines in response to COVID-19, researchers explored more traditional strategies, such as inactivated vaccines, to provide a second type of defense against the new disease. Much of the belief in the efficacy of mRNA vaccines was predicated on the assumption that just the spike protein was enough to elicit a robust immune response; however, certain individuals and communities around the world exhibited immune senescence and would require more immunostimulatory material, such as a whole virus, to establish protective immunity. Though the development using inactivated vaccines took longer, there were several benefits to pursuing research in inactivated vaccines - namely, a stronger clinical response and more global accessibility due to existing supply chains [21]. The most promising advantage of inactivated vaccines, however, is the generation of a more general immune response. As new COVID-19 variants continue to be discovered, mutations in the spike protein structure may render mRNA vaccines ineffective (though this is a fear yet to be realized as current vaccines have demonstrated immunity against variants as well). Inactivated vaccines provide multiple antigenic features to the host immune system, resulting in more robust immunity against spike protein-mutated variants. Though mRNA vaccines were more readily adopted in the West, Chinese vaccine manufacturers Sinovac and Sinopharm, and India's Bharat Biotech have produced inactivated vaccines authorized by the WHO for emergency use. These vaccines - made with an aluminum hydroxide adjuvant - are currently being deployed in many countries around the world and have demonstrated strong production of neutralizing antibodies. Additionally, the French company Valneva hopes to bring inactivated vaccines that are "variant-proof" to the United Kingdom, but their formulation is still in early-stage clinical trials [22].

3.3. Subunit/recombinant vaccines

Subunit vaccines utilize the antigenic components of the targeted pathogen, with the purpose of using these sections to induce a protective immune response. Generally, the components are a specific set of proteins and/or glycoproteins. After the administration of the vaccine, adaptive immunity can help ensure that further exposure to that specific component will result in an immune response. In general, subunit vaccines are used when the inactivated vaccine proves to be ineffective in preventing complications or is too immunogenic for the patient's immune system. The first subunit vaccine was developed for inoculation against pertussis (whooping cough). Initially, inactivated *Bordetella pertussis* bacteria were used, but this caused developments of adverse reactions. This led to the development of vaccines based on components of the bacteria rather than the full inactivated bacteria. Currently, the most common use of subunit vaccines is with immunization for Hepatitis B. In addition, there are vaccines in development for tuberculosis and cholera [23,24].

Due to their higher safety profile, subunit vaccines are primarily developed for use with elderly patients for applications such as inoculation against shingles [25]. Also, many mRNA vaccines are being compared to their subunit counterparts, due to subunit vaccines' great ability in instilling lasting immunity [26,27]. In addition, subunit vaccines are being used in studies on oral delivery of vaccines. Since most subunit vaccines are protein-based, they provide an avenue to test the protection against the acidic conditions of the gastrointestinal tract [28]. Finally, in the early stages of the COVID-19 pandemic, one vaccine approach was a subunit vaccine. The researchers in that study focused on creating a vaccine that targeted multiple amino acid motifs, thereby increasing the effectiveness of the vaccine compared to the current mRNA vaccines. Using a computational approach, they were able to assess the effectiveness of their designed vaccine and concluded that this vaccine was viable for general public use [29].

3.4. Other vaccines (DNA/Toxoid)

The most common vaccines in use today utilize the techniques described above, but other vaccination strategies have demonstrated potential for human use and are being explored in research settings. For example, toxoid vaccines are centered on the delivery of only the immunostimulatory product of a disease-causing agent to generate protective immunity. Similar to subunit vaccines (but using an antigen product as opposed to the antigen itself), these

vaccines have shown high efficiency in protecting against diphtheria, tetanus, pertussis, and *C. difficile* infections. Of note, a promising 2012 study by Foglia *et al.* succeeded in generating an intramuscularly administered toxoid vaccine using the enterotoxins and cytotoxins produced by *C. difficile.* Phase I and II clinical trial data indicated promising immunogenicity and safety profile [30]. Though toxoid vaccine applications for *C. difficile* are still at the clinical trial stage, the popular DTaP vaccine (immunity against diphtheria, tetanus, and pertussis) utilizes toxoid proteins for the diphtheria and pertussis components.

In response to the low immunogenicity and high safety risks of certain modern vaccines, a novel approach to vaccination is being explored that centers on the delivery of a recombinant bacterial plasmid. DNA vaccines, which are highly similar to mRNA vaccines, aim to stimulate humoral and cellular immunity by synthesizing an antigen or antigen product *in vivo* from an injected DNA sequence which will be transfected into the host genome. In laboratory settings, these vaccines have demonstrated antigen production against several diseases such as influenza, but translation to primate and human applications remains unrealized [31]. Despite their intended goals, DNA vaccines remain unable to safely generate significant immune protection in most trials. With DNA vaccines, integration and disruption of the host genome is a significant concern, and researchers have yet to demonstrate a dose-limited administration strategy to avoid such issues. Additionally, the benefits of DNA vaccines over conventional vaccination methods is still a subject of consideration; as of 2020, no DNA vaccines have been approved for human use.

Of note, the Oxford-AstraZENECA vaccine emerged in late 2020 as an effective vaccine against COVID-19 utilizing the DNA of the SARS-CoV2 spike protein. This is not technically considered a DNA vaccine - rather than incorporating into the host genome, the genetic material is delivered to the cell via a chimpanzee adenovirus vector and migrates to the nucleus, where it is transcribed independently of the host genome and then follows a similar path as mRNA vaccines. This vaccine has a much lower efficacy than the mRNA vaccines and is further reduced against novel variants, but it remains a promising option for tropical and lower-income countries due to its low price per dose and less stringent storage requirements [32]. It is yet unclear whether there is a significant advantage of integrating into the host genome in this particular disease, but the potential drawbacks of off-target effects relegate this as a consideration for the distant future.

Another recent vaccine that utilizes an adenovirus vector with non-incorporating DNA strands is the Johnson and Johnson/Janssen COVID-19 vaccine. This vaccine is novel in that it claims to only require one administration, as opposed to the two required by other vaccines. It demonstrated about a 66% efficacy rate against disease prevention, which is lower than other frontrunner vaccines, but it is still being utilized as an important tool in the fight against COVID-19. However, a very small number of recipients developed rare and dangerous blood clots in the brain, in a condition known as cerebral venous sinus thrombosis (CVST) [33]. This was observed with the AstraZeneca vaccine in Europe as well, and administration of both vaccines was temporarily paused. Regulators eventually decided that the benefits of administration outweighed the risks and resumed administration.

3.5. mRNA vaccines

3.5.1. Introduction

Conventional vaccination strategies have been significantly impactful for human health, COVID-19 presented a new challenge for not only vaccine development, but also deployment and administration. The world has been hard-hit by the pandemic, with over 160 million cases and 3 million deaths in over 218 countries as of May 2021 [34] .International commerce and travel were ground to a halt as cases continued to spread exponentially and fatalities steadily rose around the world. Vaccines utilizing live or killed viruses, though capable of strong and broad immunogenic responses, required years to develop and a complex manufacturing process to properly scale, produce, package, and deliver, which crippled economies and weary populations around the world could not afford to wait for.

As research groups and pharmaceutical companies worked to identify a feasible vaccination strategy against the novel coronavirus in the spring of 2020, a unique approach unexpectedly emerged from the field of immuno-oncology. Cancer immunotherapy has long been an area of interest for researchers seeking to improve upon the current standard of chemotherapy; by altering the body's immune response to be able to more effectively detect and eliminate tumors. Immunotherapy holds the potential to offer an effective and safe form of cancer treatment. One specific approach to re-engineering the body's immune cells is the delivery of messenger RNA encoding tumor antigens to immune cells, allowing the immune system to recognize and create antibodies against tumors. The now-famous 2005 studies by Kariko and Weissman were the first to identify mRNA's immunostimulatory properties via activation of Toll-like receptors (TLRs), as well as demonstration of the fact that slight nucleotide modification could allow mRNA to be engineered as a human therapeutic by avoiding the innate immune system and producing a large amount of protein with a relatively high safety profile [35]. In 2007, Mockey *et al.* explored a strategy to halt melanoma progression in mice models through administration of mRNA encoding melanoma-associated antigen, MART1 [36]. This approach involved delivering the mRNA to the cytosol of dendritic cells *in vitro*, which would then produce the antigenic protein and present it to CD8 + cytotoxic T lymphocytes, activating them and enabling them to lyse tumor cells. The use of mRNA was favored for several reasons over other gene-editing approaches: mRNA's quick, universal, and transient translation allowed for easy and safe administration, and engineered mRNA avoided the challenge of a premature immune response before presentation to cytotoxic T lymphocytes (CTLs) as would be observed with protein/subunit delivery. Administration of the mRNA, delivered through polyethylene glycol PEGylated and histidylated liposomes, resulted in a significant and specific decrease in the rate of tumor growth, which was directly correlated with protective CTL activity [36].

Despite the promising trials with mRNA based therapeutics in laboratory settings, the facts remained that mRNA is highly unstable inside the body and prone to degradation by immune agents and nucleases, possesses a high potential for adverse immunogenicity, and initially generates weaker protective immunity than conventional vaccines [37]. With the modern advances in lipid nanoparticle technology, researchers saw an avenue to effectively deliver nucleic acid vaccines and transcribe antigenic components *in vivo* [38]. From 2010 onwards, pharmaceutical companies such as Moderna, Pfizer, and BioNTech began to explore the development of mRNA therapeutics/vaccines and raised millions of dollars in funding towards this research, though it was only until recently that this work came into the spotlight. With the advent of the COVID-19 pandemic in early 2020 and the unique challenges present in ensuring a fast, scalable, and effective international vaccination strategy for the novel virus, these companies were well positioned to pivot their experimental explorations with mRNA towards a vaccination solution [39]. Moderna's vaccine was manufactured with smaller independent contracts, and BioNTech partnered with the pharmaceutical giant Pfizer for logistical and manufacturing support [40]. By early summer, mRNA vaccines

had exhibited the fastest development timeline along with high generation of immunity, and the first COVID-19 vaccines to receive emergency FDA approval in December were mRNA-based [41].

In the fall and winter of 2020, mRNA vaccines began concluding their Phase 3 clinical trials; exhibiting very high efficacy against COVID-19, Moderna and Pfizer-BioNTech's formulations received emergency use authorization from regulatory agencies around the world in a historic move and began being distributed for large-scale vaccination. These vaccines built upon the simple principles demonstrated by previous work in the field - an exogenously engineered mRNA strand is introduced to the body and used to produce the antigenic component of the SARS-CoV2 spike glycoprotein. These peptide fragments are then localized to the cell membrane and presented to immune cells, resulting in immunostimulatory activity and generation of long-term immunity. Clinical trials pointed to over 94% transmission/infection prevention efficacy and 100% severe infection/death efficacy for Pfizer and Moderna's vaccine, and by December 2020, both had received authorization with more traditional formulations by manufacturers such as AstraZeneca and Johnson and Johnson following closely behind [42]. A third company, CureVac, has an mRNA vaccine candidate in late stage clinical trials with the promise of long-term stability at regular refrigerated temperatures; however, its Phase 3 efficacy results displayed disappointingly poor potential for clinical translation. Scientists point to the lower mRNA dose and inflammatory nucleotide uridine (as opposed to the substitution of pseudouridine in most other formulations) as potential reasons for the initial failure [41]. However, CureVac is continuing to explore formulations with unmodified mRNA sequences. The small US-based vaccine manufacturer Novavax has demonstrated a vaccine candidate with 90% efficacy in Phase 2–3 clinical trials that also promises many of the transportation/storage benefits of the CureVac vaccine, though it is yet to receive emergency approval [43].

The development of these vaccines resulted in the largest global vaccination campaign in human history [41]. As of September 2021, an estimated 6.1 billion doses have been administered worldwide with roughly 31 million doses continuing to be given per day [1]. These vaccines have single- handedly altered the trajectory of the COVID-19 pandemic, slowly reducing the need for masks and stringent social distancing measures and allowing society to return to its normal state of functioning. On May 13th, 2021, the United States Center for Disease Control (CDC) updated their guidelines to state that masks are not required in most indoor/outdoor settings for fully vaccinated individuals, pointing to ever-decreasing case numbers and fatalities in the country - stemming in a large part from increased vaccination rates [44]. However, as the Delta variant began to proliferate in the USA in August of 2021 and cases/hospitalizations began to increase - including a small number of breakthrough infections but the vast majority in unvaccinated individuals - these guidelines were revised to encourage masking in high density settings and in regions of high spread [45]. On August 13th, 2021, the CDC further recommended a third booster dose after eight months of the Moderna and Pfizer/BioNTech vaccines in immunocompromised individuals, with plans to recommend booster doses for the general population expected to follow soon [46]. mRNA vaccines are not the only vaccines available against COVID-19, but they were the first ones to be produced and authorized for human use, and they continue to be absolutely pivotal in the global effort to combat the disease. Fig. 1 highlights the drastic impact that mass vaccination has had on decreasing the incidence of global COVID-19 cases in 2021.

3.5.2. Unique Mechanism of action

mRNA vaccines, as the name suggests, are built around the principle of *in vitro* transcribed (IVT) mRNA. Using the unique developments from the past couple of decades, it has become recently possible to engineer mRNA strands with slightly modified nucleotides that are capable of activating humoral and cellular immune responses through the production and display of protein products but limited immunostimulatory behavior from the mRNA strand itself. To combat the issue of low *in vivo* stability and susceptibility to degradation, modern vaccine formulations can utilize lipid nanoparticles (LNPs). These ionizable formulations are usually comprised of a main amino lipid along with helper lipids (cholesterol, PEGylated lipid, phospholipid) to facilitate cellular uptake and release. The microfluidic mixing process to form the encapsulated lipoplexes is performed at a low pH to encourage electrostatic complexation of the positively charged LNP and the negatively charged mRNA strand [48]. Experimentation is underway to improve the efficacy of these carriers as well - current areas of interest include engineering these carriers for better organ specificity and targeting, drug release and endosomal escape, and cellular uptake [49]. Moderna Therapeutics was the first commercial organization to demonstrate *in vivo* stability of mRNA-LNP formulations against influenza in the past, and the findings from that work were central to the development of their COVID-19 vaccine, as well as Pfizer's formulation. Another critical step in the development of clinically applicable mRNA was discovering the ability to engineer mRNA constructs with modified 5′ methylguanosine triphosphate caps and 3′-poly-adenosine tails. These post-transcriptional modifications are critical for ensuring proper ribosome binding, stability, and protection from nuclease mediated degradation, and ligating custom sequences can allow IVT mRNA to avoid common degradation factors, promote longer bioavailability, and assist in more effective translation [50].

Additionally, it is important to make a distinction between replicating and non-replicating mRNA. Non-replicating mRNA is a much simpler construct and consists of a transcript encoding the antigen of interest flanked by untranslated regions and the previously mentioned post-transcriptional additions. Such vaccines do not contain any extraneous genes encoding replication factors and are meant to be degraded in a relatively short period of time after generating the antigen [51]. The current COVID-19 mRNA vaccines utilize this form of technology as the transcript fits more easily in the lipid nanoparticles and is more economical to manufacture. Replicating mRNA, in contrast, contains additional genes encoding self-replication factors such as RNA-dependent RNA polymerase. These constructs produce additional transcripts, allowing for sustained expression of the antigen and a longer immune response [52]. However, they are much bulkier and not yet feasible for clinical applications, though there have been many promising recent studies in animal models [53]. Fig. 2 illustrates the structural differences between non-replicating and self-replicating mRNA constructs.

Following administration of the LNP-mRNA therapeutic, the goal then becomes to transiently produce and express a translated protein product. Upon crossing the cell membrane and being released from the LNP into the cytoplasm, the mRNA strand, containing conventional post-translational modifications, such as an open reading frame and a poly-adenosine tail, is recognized by the host cell's ribosomes and is translated into a protein product in the typical RNA-to-protein pathway. Though current vaccine formulations are non-replicating, a second class of mRNA vaccines is also being explored; termed self-replicating, these mRNA strands also encode viral replication machinery which can allow for sustained expression of the mRNA [55].

The translated antigen is then either localized to the cell membrane or exported outside of the cell, where it can stimulate humoral and cellular immune responses. Typically, dendritic cells (antigen-presenting cells) will phagocytize the antigen and mature, migrating from around the body to lymph nodes and pre-

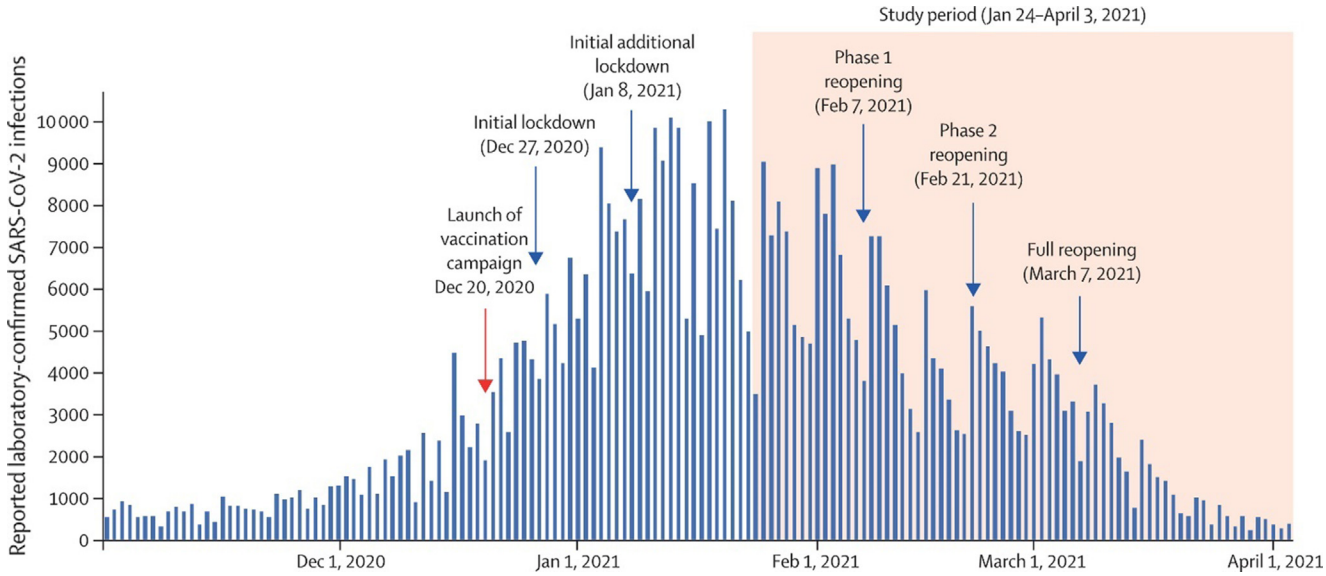

Fig. 1. Daily laboratory-confirmed SARS-CoV-2 infections in Israel (Nov 1, 2020, to April 3, 2021). Reproduced with permission from Haas *et al.* 2021 [47].

senting antigen fragments on their surfaces to developing T cells. This results in the mounting of an immediate immune response by CD8 T cells, as well as the generation of humoral immunity capable of neutralizing future infections from the same pathogen. B cells are activated either by direct antigen recognition at the B cell receptor (BCR) or CD4 T cell assistance, and these cells then produce antigen-specific antibodies. These antibodies can later recognize the same antigen present on the actual pathogen and rapidly mount an immune response before proliferation and infection. This humoral protection is the basis of long-term vaccine-mediated immunity [56]. A general overview of the intracellular process is shown in Fig. 3 and an expansion on intercellular immune generation is presented in Fig. 4.

3.5.3. Advantages over current standards of vaccination

Though other vaccination techniques have demonstrated high efficacy against many types of diseases, mRNA vaccination holds promise as a safe, controllable, and efficient alternative to pathogen-based viruses. The key safety advantage offered by mRNA vaccines is the non-integrating mechanism of action - with all activity localized to the cytosol, genomic disruption and off-target effects are not a concern as they are with DNA-based vaccines. Secondly, mRNA can be easily modified at the nucleic acid level to further reduce unwanted immunogenicity, increase effective half-life, and improve safety; modifications at the untranslated regions (UTRs) of the molecule can also promote ribosome binding and protein product translation. mRNA molecules are easily pro-

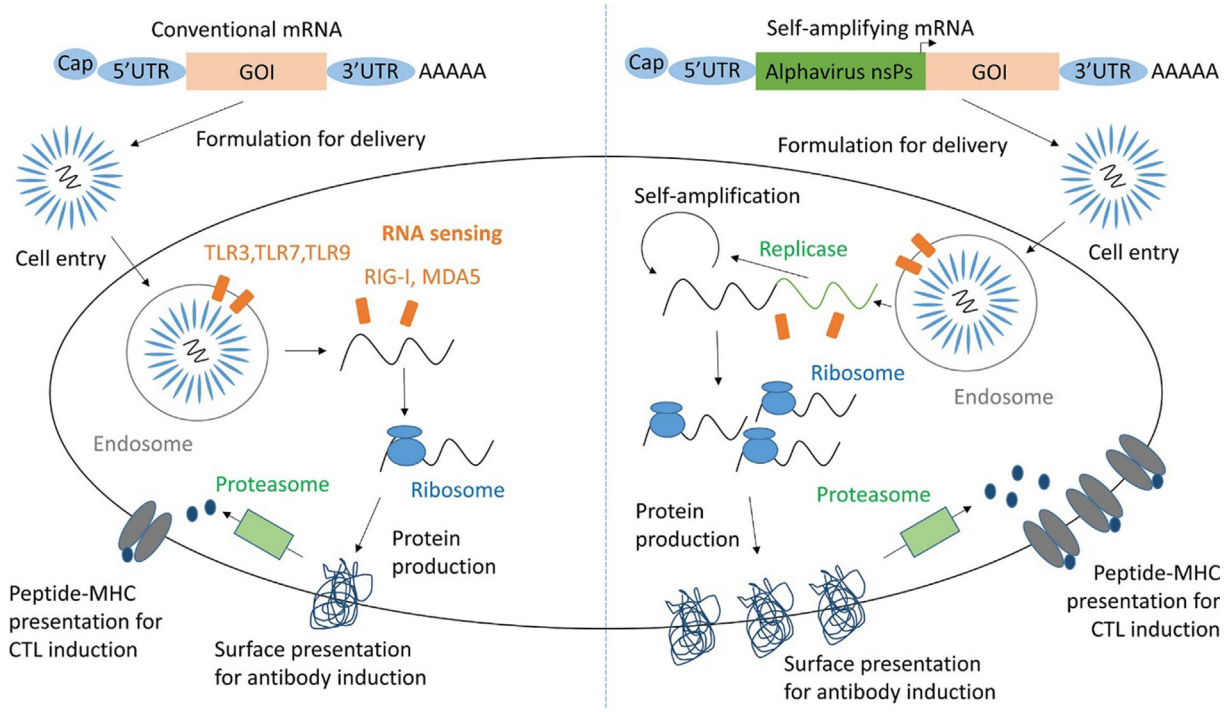

Fig. 2. mRNA and saRNA protein production in antigen presenting cells. Reproduced without changes from Sandbrink and Shattock, 2020 [54]. GOI: Gene of interest; UTR: Untranslated regions; nsPs: non-structural proteins; CTL: cytotoxic T lymphocyte.

duced in bioreactors, safety requirements in the manufacturing process are much less stringent than with live/inactivated vaccines due to the absence of live virulent agents, and nanoparticle technology has improved significantly in the last decade, enabling mRNA based therapeutics to have high *in vivo* viability, cellular uptake, and gene expression. Clinical and laboratory trials have shown that antigen presenting cells (APCs) are able to exhibit mRNA protein products to a similar degree as vaccination by more conventional methods [41].

Production of mRNA vaccines is relatively straightforward, with a plasmid containing the isolated gene of interest serving as a template for the mRNA strand. This process is easily scalable and does not require the use of dangerous live viruses or carefully monitored cell cultures, resulting in a high safety profile at the production stage. Subsequent purification and encapsulation into nanoparticles results in the final product. This manufacturing process is quite rapid - Moderna was able to isolate the COVID-19 spike protein genes within 4 days of receiving the genome and had their first formulations ready for *in vivo* animal trials in 2 months [58]. However, although the naked mRNA is easier to produce and store compared to other types of vaccine elements, the final therapeutic requires a stringent cold chain - in the case of the Pfizer/BioNTech vaccine, approximately −70 °C. This significantly affects the transportation and storage of these vaccines in tropical and developing countries, and these areas have been relying more on inactivated formulations as a result. However, this technology is still new, and if the more thermostable formulations of companies such as CureVac pass clinical trials, then the expansion of mRNA vaccines to these areas will become more feasible. Moderna's formulation is stable at commercial refrigeration temperatures but distribution was initially lagging due to limited manufacturing capabilities. The price per dose for mRNA vaccines currently ranges from $20-$40 depending on the manufacturer, which is significantly more expensive than more traditional formulations (which are closer to $2-$10 per dose) [59]. However, this cost is currently being subsidized by many governments around the world and is expected to decrease as manufacturing capabilities increase.

3.6. mRNA vaccine applications in prophylactic immunity and disease treatment

3.6.1. SARS-CoV-2

We have discussed much about the history and background of the COVID-19 pandemic elsewhere in this paper, but it is important to understand the biological underpinnings of this disease as well. Coronaviruses are a general family of enveloped ssRNA viruses characterized by the crown shaped proteins embedded on their outer surface. Other notable coronaviruses include SARS-CoV and MERS-CoV, which have caused smaller, more contained pandemics in recent history (Sudden Respiratory Arrest Syndrome and Middle Eastern Respiratory Syndrome, respectively). The novel coronavirus originated around the end of 2019 with initial outbreaks in various countries and rapidly spread around the world in the spring of 2020 [60]. SARS-CoV-2 infection can present radically differently in individuals, with most younger people with healthy immune systems exhibiting mild to no symptoms while older people/people with co-morbidities can develop symptoms such as a fever, pneumonia, and severe respiratory distress. Cases are characterized by a significant immune response that causes tissue and organ inflammation, and in some cases, the damage from this response can persist long after the infection [61]. Overall, the mortality rate of this virus has been estimated to be around 4%, with a significant skew towards older populations. Several variants have been identified in many countries around the world, including the United Kingdom, South Africa, and India. These variants tend to have mutations in the spike proteins that can result in an increased lethality rate and a decreased response from the immune system and current vaccines/therapeutics. However, more research is needed to confirm the unique properties of these variants as well as their response to current vaccines.

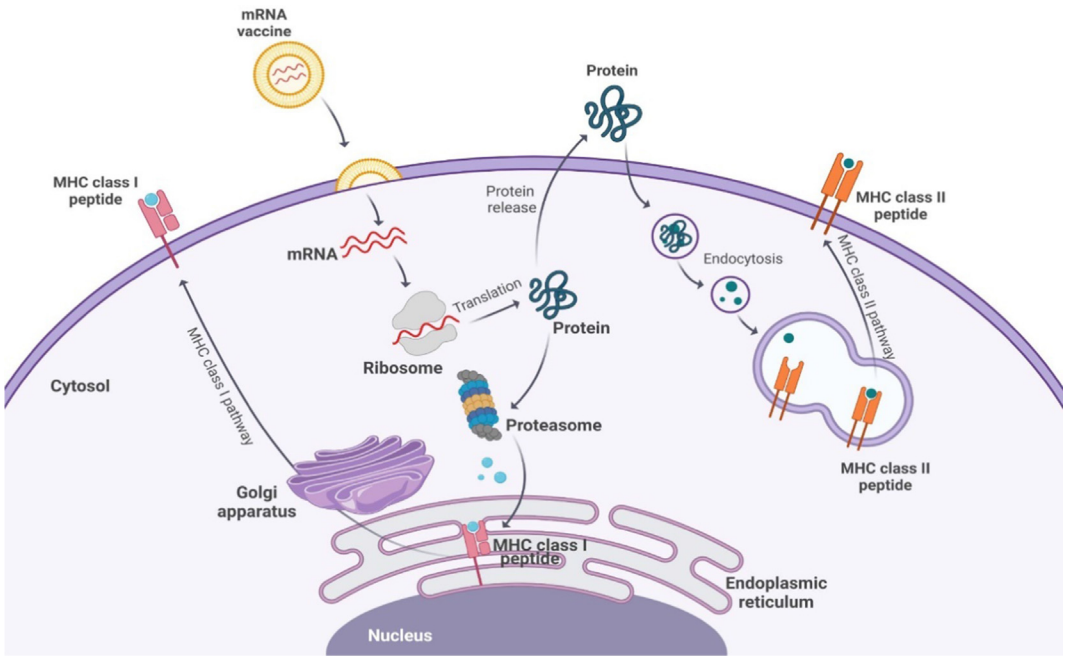

Fig. 3. Mechanism by which mRNA vaccines elicit immunity. The mRNA encoding the viral protein enters the cell where it is translated into protein by the ribosome. The resulting protein is broken down into peptides by the proteasome or transported by the Golgi apparatus to the outside of the cell. The remaining fragments in the cell are presented as a complex. Additionally, protein outside of the cell can be taken up by various immune cells and fragmented into smaller pieces by the endosome. Figure created using BioRender.com.

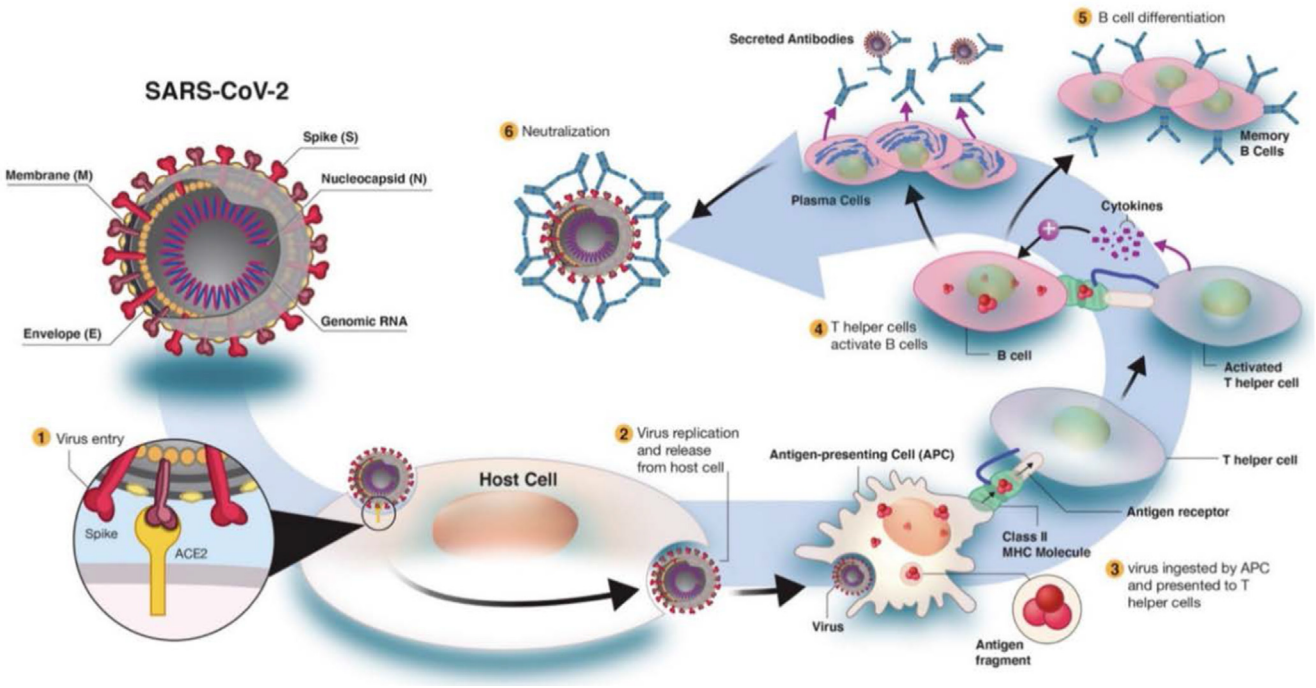

Fig. 4. Cellular mechanism of immune activation. Reproduced from Ghaffari *et al.*, 2020. [57] (1) The SARS-CoV-2 virus enters the host cell via interaction between viral spike and host angiotensin-converting enzyme 2 (ACE2) proteins. (2,3) Following replication and release from the host cells, a subset of viruses will be engulfed and digested by antigen-presenting cells (APCs) like macrophages or dendritic cells. (4) Fragmented SARS-CoV-2 antigen(s) will be presented to T helper cells, which in turn will interact and activate B cells. (5) Activated B cells will proliferate and differentiate into plasma or memory B cells with high-affinity binding receptors for the original SARS-CoV-2 antigen. Plasma cells secrete their SARS-CoV-2-specific receptors in the form of IgM, IgG, or IgA antibodies. (6) Antibody-mediated neutralization occurs when SARS-CoV-2-specific antibodies bind to viral antigen(s) and prevent virus interaction and entry into host cells.

A significant challenge with COVID-19 is its ability to spread latently - symptom presentation in a newly infected individual can take up to 14 days, if it occurs at all, and they are able to transmit it to others through close contact with respiratory fluids during that time [61]. Apart from prophylactic immunity through vaccinations, treatment options are limited; due to the rapid spread of the virus, longitudinal efficacy studies have been difficult. Several therapeutic options have been put forth, such as a combination of treatment with hydroxychloroquine and azithromycin, nucleotide analogs such as remdisivir, and convalescent plasma. However, studies utilizing these treatment options have produced conflicting results, and in most mild cases, isolated rest at home is recommended for the course of the disease. In more severe cases requiring hospitalization, supplemental oxygen, along with fluids and possibly the aforementioned treatment options. For patients exhibiting lung failure, a mechanical ventilator can also be used to assist in breathing until the body can mount a sufficient immune response [62]. The current standard of prophylactic treatment is vaccination; a summary of the various vaccine types is provided in Table 2.

3.6.2. Influenza virus

The influenza virus, commonly known as the "flu", is one of the most common viral infections today. There are four major types of influenza viruses, with three types - type A, B and C - known to infect humans. Type A is the most virulent of them, responsible for some of the global flu pandemics. In general, most influenza virus infections result in high fevers, runny nose, joint and muscle pain, coughing and feelings of exhaustion. Given that the infections are so common, there are yearly vaccines developed to combat the evolving viral strains.

Much like with other vaccines, mRNA vaccines are being explored as an alternative approach. The current research on mRNA influenza vaccines has not yet reached clinical trials, but there are promising results in animal models. In one study, an mRNA vaccine targeting the hemagglutinin of a type A influenza virus was developed and injected into mice, both young and old [63]. The vaccine was able to elicit both B- and T-cell protection. In addition, the vaccine was able to withstand thermal stress, as well as give some protection against other antigens, most notably the highly conserved viral nucleoprotein. The latter result indicates that this particular mRNA vaccine could have use for immunity beyond just influenza. A similar study focused on the same target but utilized a different method, oil-in-water cationic emulsion, to formulate the mRNA vaccine [64]. Finally, a study in 2017 used lipid nanoparticle-modified mRNA vaccines to generate rapid and increased immune responses in mice, ferrets and nonhuman primates [65]. The results were robust enough that the study elicited the start of some Phase I clinical trials.

3.6.3. Zika virus

The Zika virus (ZIKV) is a mosquito-borne virus which was responsible for a brief global epidemic in 2015. The virus, an enveloped, ssRNA flavivirus that has a disease progression similar to dengue fever, often presents little to no symptoms in healthy adult humans, but complications arise when pregnant women or young children are infected; in such cases, ZIKV has been linked to severe developmental defects, particularly in neurocognitive abilities and brain growth. Case reports have indicated linkages between Zika virus infection and Guillain–Barré syndrome in adults and congenital malformation in infants. Prior to the 21st century, the Zika virus demonstrated low infection rates in humans and was localized to regions in Asia and Africa. However, due to intercontinental travel, several outbreaks were observed around the world with varying levels of severity. The 2015 outbreak was severe enough to warrant worldwide travel advisories and several countries

Table 2
A summary of the various mRNA vaccine candidates for the prophylactic treatment of SARS-CoV-2

Manufacturing Company	Vaccine Type	Vaccine Mechanism of Action	Current Regulatory/Approval Status
Moderna	mRNA	LNP-encapsulated modified mRNA encoding for COVID-19 spike protein delivered intramuscularly over two doses with a potential third dose booster	Emergency use authorization in several countries around the world
Pfizer/BioNTech	mRNA	LNP-encapsulated modified mRNA encoding for COVID-19 spike protein delivered intramuscularly over two doses with a potential third dose booster	Emergency use authorization in several countries around the world
Johnson and Johnson/ Janssen	Viral vector	Single dose, antigen-encoding genes delivered in a single dose utilizing a chimpanzee adenovirus vector	Emergency use authorization in several countries around the world
University of Oxford/ AstraZeneca	Viral vector	Antigen-encoding genes delivered in a single dose utilizing a chimpanzee adenovirus vector	Lower-cost alternative to other formulations; worldwide emergency use authorization with focus in developing countries
Gamaleya (Sputnik-V)	Viral vector	Two-dose intramuscular injection of adenovirus-enclosed antigen genes	Limited emergency use authorization around the world, highest use in Eastern Hemisphere
Sinovac (CornoaVac)	Inactivated virus	Two-dose intramuscular injection of chemically inactivated COVID-19 virus	Limited emergency authorization use around the world, highest use in Asia and Africa
Sinopharm	Inactivated vaccine	Two-dose intramuscular injection of chemically inactivated COVID-19 virus	Full authorization in China with emergency use authorization in several developing countries
CanSino (Convidecia)	Viral vector	Single shot adenovirus vector vaccine delivered intramuscularly	Full authorization in China with very limited emergency use authorizations in developing countries
Bharat Biotech (Covaxin)	Inactivated virus	Vero cell-grown COVID-19 virus, chemically inactivated and	Very limited emergency use authorizations in various tropical and developing countries
Valneva	Inactivated virus	Single-dose chemically inactivated virus, promises strong performance against variants	Phase III clinical trials
Novavax	Subunit	Thermostable formulation of COVID-19 spike protein delivered using LNPs	Phase II/III clinical trials
CureVac	mRNA	Unmodified mRNA encoding COVID-19 spike protein encapsulated in LNPs	Preclinical/early clinical trials after poor Phase III results in initial formulation

advised couples to delay pregnancies until viral transmission was under control. However, this pandemic was short-lived and most cases either died out on their own or were easily combated with interferon treatment. Despite the relatively low threat of the Zika virus, there is scientific interest in vaccine development as a stepping-stone towards deadlier diseases and designing effective vaccine strategies for pregnant women. Several Zika virus vaccines are currently in clinical trials, and Moderna is currently in Phase 1 and 2 clinical trials with a potential mRNA vaccine candidate [66].

In a 2017 effort by Richner *et al.*, the authors succeeded in producing a lipid nanoparticle (LNP) encapsulated modified mRNA vaccine against ZIKV and observed *in vivo* generation of sterilizing immunity in mice models [67]. The delivered mRNA strand, which encoded for ZIKV structural proteins, was modified at the 5′ and 3′ untranslated regions and had base substitutions to ease translation. Lipid nanoparticles were selected as delivery vectors due to their strong performance in previous trials with siRNA and were delivered to mice via intramuscular inoculation. Mice receiving 2 μg or 10 μg of the vaccine demonstrated high levels of induced neutralizing antibodies against ZIKV and significantly improved performance over DNA plasmid/inactivated vaccines. The study also explored safety of administration to pregnant mice, and it was observed that while safety to mother and fetus with the mRNA vaccine was not a concern, the fetus had a significantly lower neutralizing antibody titer.

3.6.4. HIV/AIDS

Human immunodeficiency virus, or HIV, and its associated disease, acquired immunodeficiency syndrome (AIDS), are characterized as a significant global health threat. The virus is a type of human-infecting retrovirus (enveloped, ssRNA) that causes critical failure of the immune system - the decreased T cell count makes the body incredibly susceptible to even simple infections like the common cold. HIV is a sexually transmitted virus and can be passed perinatally as well, making it particularly troublesome for parts of the world with limited testing capabilities. As of 2020, around 38 million people globally are infected with HIV, with about 20% of that number unable to receive a confirmatory test (HIV.gov). AIDS was classified as an epidemic in the 20th century due to the high death counts and potential for uncontrolled transmission, but several therapeutic options have since become available for HIV-exposed individuals. Pre-exposure prophylaxis and antiretroviral therapy can control the HIV levels for these individuals, preventing the clinical presentation of AIDS, but these medications cannot fully rid the body of HIV and must be taken long-term. Additionally, they can be extremely expensive and are not widely available in developing countries or lower-income communities, resulting in limited access to care and an inequitable disease skew towards such groups [68].

mRNA vaccination has been explored as a strategy to provide prophylactic immunity to HIV. Vaccine development has not gone well historically for this disease; the high mutation rate and the challenge of generating long-term and broadly-neutralizing antibodies have made development difficult, and no HIV vaccines are currently available for use. However, with the advances in mRNA technology and its recent resurgence as a potential therapeutic, there may be potential avenues for the development of an effective mRNA vaccine. In 2018, Leal *et al.* demonstrated that naked mRNA could activate human dendritic cells in HIV positive patients in a phase 1 clinical trial; this was observed as good tolerability to the therapeutic and increased HIV-specific T-cell production [69]. However, in 2019 an error was published stating that the mRNA sequence used contained two start codons which may have impacted protein expression [70]. More recently, Saunders *et al.* developed a full mRNA-LNP vaccine and demonstrated its antibody generation capability in primate models. Vaccination with the formulation resulted in high titers of serum HIV-binding antibodies with good binding specificity. The authors pointed to the ease of production and scaleup, but also the challenges that the requirement of a cold chain could cause. The first in-human clinical trial

of an mRNA vaccine began in the spring of 2021 - led by Moderna in partnership with the International AIDS Vaccine Initiative (IAVI) and Scripps Research, the trial demonstrated that 97% of recipients developed the capability to produce broadly neutralizing antibodies, with the caveats that it was only responsive against 30% of HIV strains in the trial regions and that more research is needed to observe antibody maturation [71]. However, this trial was a promising sign for the eventual translation of mRNA vaccines to human therapeutics for HIV.

3.6.5. Dengue fever

The Dengue fever is a tropical disease brought about also by a mosquito-borne virus, the dengue virus (DENV). Symptoms include a high fever, headache, vomiting, muscle and joint pains and the characteristic skin rash. Early in the infection, the skin rashes blanch when pressed and later become a large red rash with smaller white areas. These symptoms generally take 3–14 days to develop [72]. Currently, dengue fever is common in more than 120 countries, including those in south/southeast Asia and South America [73]. After seeing an uptick in infections after World War II, increased effort was made to understand the nature of the virus, including the disease transmission and progression.

The virus is a part of the flavivirus family, small spherical viruses with a lipid envelope, just like the Zika virus [72]. The virus also induces a strong immune response, making it difficult to identify the specific components of the virus that the immune system is able to recognize and target. This ability to hide from the immune system is heightened by the virus targeting Langerhans cells, dendritic cells that present pathogens to the rest of the immune system. In addition, the dengue virus is notorious for having multiple strains, with each strain warranting its own developed immune response. Both of these factors have made conventional vaccines ineffective. Instead, mRNA vaccines for the dengue virus are in the works. These vaccines are designed to target some of the non-specific protein epitopes to increase CD8 T-cell activation and maturation of other immune cells to confer immunity memory [74].

3.6.6. Rabies virus

The Rabies virus is a zoonotic infectious disease that targets the central nervous system. It is contracted from salivary contact from an infected animal and is extremely dangerous. Early on, symptoms include fever and tingling at the site of the bite/scratch and can progress to violent, uncontrolled movements, loss of motion, confusion and loss of consciousness. Another key symptom is hydrophobia, fear of water, which is often used as the key symptom in the diagnosis of rabies. The progression time depends on how far the virus has to travel from the peripheral nervous system to the central nervous system. For many, once stronger symptoms start showing, it is too late for treatment and the virus is generally fatal in these situations. As of 2016, only 14 people have survived rabies once they started displaying symptoms [75].

However, vaccination can help mitigate this issue, particularly in developed countries. In developing countries, the current vaccines are unable to keep up with the rapidly-mutating virus. Thus, a new mRNA-based vaccine approach is currently being investigated that targets some of less rapidly-changing parts of the virus [76]. Much like the dengue virus mRNA vaccine, the developing mRNA Rabies vaccines look to create antibodies that target specific glycoproteins on the virus shell. In animal studies, research has demonstrated an increase in immune activation and maturation in response to the vaccine, as well as some preliminary clinical trials that indicate similar results, with a reasonably low amount of side effects [76,77].

4. Future directions and perspectives

It was not long ago that mRNA vaccines were on the fringes of the scientific community with a bleak outlook for translation to human therapeutics. The COVID-19 pandemic, as devastating and dangerous as it is, has managed to spur innovation in this novel type of vaccination, and the ongoing and future research in this area potentially holds huge implications for human health. Diseases that plague tropical populations, for example, could eventually see a reduction in mortality and case counts as work in mRNA vaccination against Zika, Ebola virus, and HIV comes to fruition. The influenza virus requires a yearly vaccine reformulation due to its constantly evolving nature; Freyn *et al.* demonstrated in 2020 that it was possible to design a universal mRNA vaccine against various serotypes of the influenza virus that may soon reduce the burden these viruses place on society every year [78]. A subsequent phase 1 clinical trial of the same formulation in humans demonstrated that vaccination was safe and an immune response was observed against the conserved viral hemagglutinin protein [79].

Though mRNA vaccines have come a long way in such a short time, their technology is not perfected yet, as evidenced by their slightly less-than-complete conferment of immunity against COVID-19 and the increasingly alarming number of COVID-19 Delta variant breakthrough infections post vaccination. The current formulations struggle with thermostability, potential for harsh side effects due to the impurity of the lipid nanoparticles, and may eventually become completely ineffective against new variants as the virus continues to evolve [80]. One observed issue was a small number of allergic reactions to polyethylene glycol used in stabilizing the lipid nanoparticles of the Pfizer-BioNTech vaccine [81]. Replacing PEG with other biocompatible stabilizing polymers, such as poly(N-vinylpyrrolidone) (PVP) and poly(N-(2-Hydroxypropyl) methacrylamide) (PHPMA) may decrease these adverse reactions, although much more preclinical and clinical testing is required to confirm the immunogenic profiles of these polymers [82]. Another potential strategy for improving the efficacy of mRNA vaccines would be to increase their targeting of dendritic cells via surface conjugation of specific ligands. Grafting mannose or hydrophobic-interaction-inducing lipids to the surface of lipid nanocarriers would enable them to more effectively target effector cells and generate immunity [83]. Several past and ongoing studies have demonstrated the improved targeting and immunogenic abilities of nanocarriers with targeting moieties attached to the surface [84]. Future strategies for improving the efficacy of intramuscularly administered mRNA vaccines could certainly benefit from incorporating these various aspects of nanotechnology.

One promising future direction for mRNA vaccines could be oral delivery applications. Many diseases in areas of poor hygiene in developing countries are caused by enterotoxins or enteropathogens such as Vibrio cholera, Escherichia coli, Salmonella, and Shigella. These pathogens cause infection upon crossing mucosal barriers, and inducing the formation of an immunologically strong mucosal barrier would prevent infection. The current standard of subcutaneous or intramuscular injection of vaccines elicits strong humoral and cellular immunity, but does not generate similar immunity at mucosal surfaces. However, orally administered vaccines must pass through the digestive tract and can confer immunity at these surfaces, allowing for prophylactic treatment against these particular toxins. Oral delivery has successfully been demonstrated and utilized for live-attenuated vaccines against the human rotavirus, and oral delivery of mRNA vaccines could utilize similar mechanisms [85]. There are several other advantages to oral delivery, including less stringent purification requirements, higher patient compliance due to the elimination of needles, and

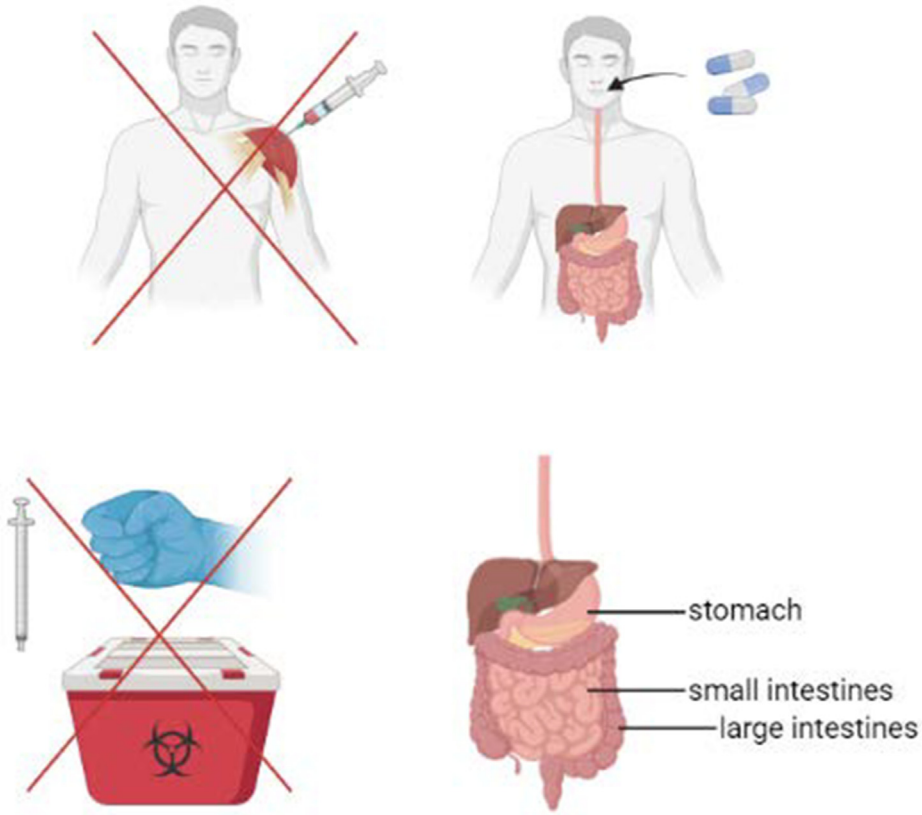

Fig. 5. Advantages of oral vaccine delivery. Advantages include the lack of a need for painful intramuscular injections and decreased generation of biohazardous plastic material by utilizing the body's existing gastroenteric mechanisms. Figure created using BioRender.com.

significantly decreased amount of biohazardous and plastic waste (Fig. 5) [86]. Additionally, mRNA vaccines could take advantage of developments in nanotechnology, such as the 2014 study by Duran-Lobato *et al.*, which demonstrated the *in vitro* targeting abilities of surface-modulated nanocarriers [87].

Oral delivery of mRNA vaccines, though promising, does come with several associated challenges. There are significant barriers to oral delivery in general, which are compounded by mRNA's low stability *in vivo*. The digestive system is a harsh physical and chemical environment with drastic changes in pH, and even at the mucosal layer, mucosa-associated lymphoid tissue can cause problems of immune avoidance and epithelial layer transport through tight junctions

and mucous layers may result in a lower bioavailability. mRNA and/or associated delivery proteins would rapidly become targets for proteases or exonucleases as well [88]. Previous studies in increasing stability and bioavailability have explored utilizing protective carriers derived from chitosan/alginate [89] and freeze-dried lettuce cells[90] due to their structural rigidity. Nanoparticles (notably polylactic acid and poly(lactic-co-glycolic) acid) in particular allow for targeting of immune cells, improved diffusion rate across tissue/mucosal layers, and sufficient protection against biochemical barriers [95]. Structures such as liposomes, synthetic and natural polymeric nanocarriers, and dendrimers have all been explored for oral vaccine delivery applications. Bioavailability and immune activation have been shown to depend on a variety of nanoparticle parameters, including nanoparticle size/shape, surface charge, and surface conjugation of targeting moieties [92].

Other strategies include utilizing specific entry points in the gastrointestinal system, including the thinner and more penetrable epithelium present in the oral cavity (sublingual and/or buccal routes) [91]. Though these approaches may provide for greater mucosal access and increased therapeutic adsorption than lower GI entry, fluid disturbance from saliva and enzymatic activity create new problems. Despite these challenges, the potential for expansion of mRNA vaccines into the parenteral delivery space remains huge and the renewed interest in mRNA research promises interesting developments in the near future. Technological devices, such as microneedle arrays [93] and high pressure liquid jets,[94] have successfully demonstrated mucosal penetration capabilities with vaccine formulations, and research in nanofiber-based mucosal patches for sustained vaccine release have also been explored [95].

An evident disparity in the COVID-19 pandemic is the difference in vaccination rates between developed and developing countries; according to the World Bank, of the 4 billion plus vaccines administered worldwide, low-income countries have received approximately 1.1% of them, with the balance being concentrated in wealthy countries [96]. These differences can largely be attributed to the ability of wealthy countries to pay for more doses, but also to the stringent transportation and cold chain requirements of the current formulations, which can make delivery of doses to warmer/tropical regions challenging. As discussed earlier, thermostable variations of these formulations are currently under development and local manufacturers in the Eastern Hemisphere have ramped up production of their domestic COVID-19 vaccines. Looking beyond COVID-19, many of the other potential applications of mRNA vaccines (HIV, Dengue fever, *etc.*) are most applicable to low and middle income tropical countries which are disproportionately ravaged by these diseases. Research is also being conducted on developing mRNA vaccinations against parasites, such as the malaria parasite, which may transform the way that malaria prevention is approached in several African countries

[97]. Future research in mRNA vaccines should certainly aim to consider these underserved regions of the world in their applications.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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