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An Overview OF MRNA Vaccine Technology

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AN OVERVIEW OF MRNA VACCINE TECHNOLOGY

by

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Abstract

This project will serve to analyze the mechanism, efficacy, and adverse effects of mRNA technology in detail while also comparing it with a previous recombinant Influenza vaccine trial. In this systematic literature review, three library data bases were searched including Pub Med, Clinical Key, and Ebsco Megafile. Search criteria included the key terms: mRNA vaccines, vaccines, and COVID-19. Additional phrases used included: mechanism, history, and efficacy. Each key word was entered as it appears in the databases with additional phrases used to narrow down the research. The search was limited to the last five years to ensure up to date information. The literature review yielded a total of 1,100 results which were further refined with the additional terminology used above. Articles were reviewed for bias, pertinence to the topic, and credibility. Exclusion criteria included: surveys, online newspapers, magazine articles, periodicals and systematic reviews or peer reviewed journal articles published before 2016. For this review 21 sources were chosen. Overall, research suggests that mRNA vaccine technology has a variety of benefits including ease of development, ability to code for multiple viral strains, increased efficacy rates compared to previous technology, and decreased rates of adverse reactions in vaccine recipients. It should be noted, that while this research does show many benefits of mRNA vaccine technology, more research should be conducted to evaluate long term efficacy and side effects of mRNA vaccines.

Keywords: vaccine, mRNA, COVID-19, efficacy, side effects

Introduction

Messenger ribonucleic acid (mRNA) vaccine technology is an area of research that has been ongoing since the 90's. Scientists see a potential for its implementation, and for the first time in the era of the COVID 19 pandemic, it has been put into emergency use. With this new technology as with many new steps in medicine and science, widespread apprehension as to the safety and efficacy of these vaccines has been apparent. This review aims to look at the history of mRNA vaccine research and to highlight the potential benefits of mRNA vaccines over the conventional attenuated vaccines that we have been implementing over the last century. Addressing the question of benefits of mRNA vaccines over conventional vaccination technology is the main goal of this research. Historical mRNA vaccine trials and systemic reviews will be thoroughly examined in hopes to expose potential benefits and potential harms over older vaccine technology.

Review of Literature

A comprehensive literature review was performed using the following search databases: Pub Med, Clinical Key, and Ebsco Megafile. Search criteria included the key terms: mRNA vaccines, vaccines, and COVID-19. Additional phrases used included: mechanism, history, and efficacy. Each key word was entered as it appears in the databases with additional phrases used to narrow down the research. The search was limited to the last five years to ensure up to date information. The literature review yielded a total of 1,100 results which were further refined with the additional terminology used above. Articles were reviewed for bias, pertinence to the topic, and credibility. Exclusion criteria included: surveys, online newspapers, magazine articles, periodicals and systematic reviews or peer reviewed journal articles published before 2016. In total 328 systematic reviews, peer-reviewed articles, and clinical trials remained.

Current Vaccine Technology & Development

Overall, vaccines prevent over 2 million human deaths annually. Two known diseases were completely eradicated due to widespread delivery of vaccinations. One of these diseases being human smallpox and the other being a disease of livestock known as rinderpest. Vaccines depend on the adequate activation of our immune systems to function well. As many health care professionals know, we have two different types of immunity, innate and adaptive. Innate immunity is composed of different 'front line' cells including neutrophils, dendritic cells, monocytes, macrophages, and eosinophils. These are the first cells that antigens encounter upon entering the human body. Once exposed, these cells either phagocytize the antigen, secrete cytokines to recruit more immune cells to the exposure cite, or activate other immune cells to start up our adaptive immune system (Karch et al., 2016).

The best vaccines rely on our humoral and cellular immunity, both of which are part of our adaptive immune responses. Humoral immunity alludes to our B cells and cellular immunity alludes to our T cell activation. B cells must be activated by antigen presenting cells to recognize an antigen and begin making antibodies to it. Helper T cells (Th) help to facilitate this process by presenting antigens to B cells. Th and B cells then go on to initiate cytotoxic T cells (Tc) to start the process of apoptosis, or programmed cell death, in cells that have been infected with virus. Vaccines are engineered with the hope of inducing immunological memory. Immunological memory requires survival of some of the B cells, Th, and Tc cells after they recognize and attack a specific antigen inducing cell apoptosis (2016).

History of Vaccines

The first vaccines recorded in history were developed in China in the 15th century where pustules of patients infected with smallpox were excised, dried, and then inhaled by other citizens. Since then, vaccines have come a long way. Edward Jenner, a scientist of the 17th century, started our first vaccine trial by inoculating an 8-year-old boy with cow pox in hopes to give him immunity to the much more deadly smallpox. Sure enough, when the child was inoculated with smallpox later on, he did not become symptomatic, as his immune system had created immunity to the disease. Louis Pasteur was another important character in vaccine history, as he was the first scientist to attenuate viruses. He discovered that treating the pathogen with chemicals to decrease its virulence helped to mount a similar immune response while not infecting the recipient (Karch et al., 2016). He started these experiments with trials on dogs and other mammals in hopes to protect them from the rabies virus and subsequent infections from similar pathogens. This was successful and helped to implement a different kind of vaccine into the market.

Along with inactivated vaccines, toxoid vaccines were also developed to fight diphtheria in the late 19th century. It was discovered that if the bacteria were not used to inoculate patients, but rather the toxin that the bacteria produced, a sufficient immune response was mounted. Lastly, polio vaccines were approved in the 1950's being both inactivated and attenuated-live vaccines. Research suggests that the live attenuated polio vaccine was favored over the inactivated due to its efficacy; however, it did have some setbacks including the fact that some patients inoculated with the live vaccine did get Polio or were 'carriers' once vaccinated (Karch et al., 2016).

Vaccine Development in the United States

The first vaccines within the United States were developed in the early 1900s. These vaccines were effective; however, they were being developed without observing safety measures to ensure patients who received them would not suffer adverse effects (Sathyanarayana et al., 2020). During the development of the Diphtheria vaccine in the United States, more than 14 deaths in children occurred. After tragedies like this occurred during vaccine development, laws were established to ensure their safety. These laws included the Biologics Control Act, the Virus-Toxin Law, the Pure Food and Drug Act, and the Federal Food Drug and Cosmetic Act (Sathyanarayana et al., 2020). With these new laws came new agencies that ensured vaccine safety. The Center of Biologics Evaluation and Research company also known as CBER is such an agency that ensures vaccine safety and follows a single criterion for approving vaccines for clinical use. This criterion ensures: that vaccine manufacturers produce their product under Good Manufacturing Practice regulations (cGMP) and the Code of Federal Regulations (CFR), both of which require transparency from vaccine manufacturers (Sathyanarayana et al., 2020). Many more acts with the intent of monitoring vaccine safety came shortly after this, all of which are followed today whenever a new vaccine is developed.

Development of vaccines requires a sequential process. When vaccines are first being produced, a sponsor of the vaccine manufacturing company must submit an investigational New Drug application to the FDA before clinical trials can begin. Then, vaccines will go through phase I clinical trials, where a small population is tested with the product. Next, a phase II study will occur with the sole purpose of developing the effective dose of the vaccine. Lastly, in phase III, a larger population will try the vaccine to ensure its safety and efficacy. After a vaccine has made it through these three trials, the sponsor must apply for a biologics license (Sathyanarayana

et al., 2020). For a vaccine to be approved for this application, the benefits to patients must outweigh the risks.

Some vaccines can get accelerated approval, such as the COVID 19 vaccines that have been given emergency authorized use in the recent year. This approval is granted when three criteria are met: adequate and controlled clinical trials are performed, the vaccine sponsor has agreed to conduct more clinical studies to ensure clinical benefit, and the vaccine manufacturers in question will have post marketing studies in place to monitor for long-term adverse effects (Sathyanarayana et al., 2020). A common example of a post marketing study is the Vaccine Adverse Event Reporting System. This system allows constant surveillance of any new adverse events from vaccines in use today. Patients and their loved ones, as well as clinicians, can report events that they observe. These events will later be investigated and deemed either vaccine-related or unrelated.

Today there are quite a few hurdles to vaccine development in the United States. Some vaccines for complex human diseases have not been developed yet due to their lack of effectiveness. HIV is one such disease that researchers have not been able to create a vaccine to. Another challenge for vaccine manufacturers is cost. Often, vaccines are needed around the world, especially in third world countries. Because these countries are not as profitable to distribute vaccines to, it can put them at an unfair advantage in terms of having access to vaccines for their populations. Lastly, human trials mark a big challenge for manufacturers as there are a lot of ethical boundaries and restrictions in place (Sathyanarayana et al., 2020). Such boundaries and restrictions are necessary for vaccine development; however, these elongate vaccine development.

Recombinant Influenza Vaccines

Prior to the era of mRNA vaccines, conventional recombinant influenza vaccines have been widely used across the globe. It is important for comparison purposes to examine influenza vaccines in depth. A clinical trial performed by Dunkle and colleagues (2017) examined the effectiveness of a quadrivalent, recombinant Influenza vaccine RIV4 with a standard dose quadrivalent inactivated Influenza vaccine IIV4. It examined a total of 8,963 participants with 4,474 taking the 45-microgram dose of the RIV4 vaccine and 4,489 taking the 15-microgram dose of the IIV4 vaccine. The majority of participants was white, and all participants were fifty years or older. Of the total 4,474 participants who took the RIV4 vaccine, approximately 96 of them contracted Influenza. Of the 4,489 participants who were administered the IIV4 vaccine, 138 contracted Influenza. Both vaccines had similar contraction rates of influenza after receiving either immunization. This yielded a vaccine efficacy rate of 27-36% (95% CI) for either vaccine when compared with the relative risk factors of either population of encountering influenza naturally (Dunkle et al., 2017).

Similar side effects were noted in either vaccine with the following being reported within the first 28 days after injection with the standard Influenza IIV4 vaccine: cough (5.8%), Influenza-like illness (4.6%), oropharyngeal pain (4.1%), headache (3.3%), upper respiratory tract infection (3.6%), fatigue (2.3%), and myalgia (1.8%). Local injection site reactions were reported in both groups. Within the IIV4 Influenza vaccine these included: pain (22.0%%), tenderness (37.1%), redness (2%), or swelling (2.7%) (Dunkle et al., 2017). Of note, fever was not a statistically significant local side effect of either influenza vaccine.

Attenuated Vaccine Development

Development of attenuated vaccines is a complex process. Live attenuated (deactivated) vaccines intend to grant the recipient a single immunity dose against a particular virus. With live virus vaccines comes the risk of viral mutagenesis and spread from people who have received it, even though they themselves are immune. Live vaccines, as well as inactivated vaccines, also require something that is called cold chain distribution. This keeps them viable for longer periods of time, but also increases the cost of storage and limits the time available from production to administration of the dose. Inactivated vaccines, much like most of our current vaccines utilized today, are inactivated pathogens or fractions of the pathogens themselves. These are attractive options for attacking different diseases; however, when used in the older immunocompromised populations, they require additives. This adds an additional step for vaccine manufactures and challenges the standardization of vaccine production (Shin et al., 2020). Viral vectors are also a type of vaccine in use today. Essentially these are a different virus that is similar to the one developers are trying to initiate an immune response against. Some advantages of these include widespread delivery to tissues, inherent adjuvant qualities, and ease of production.

Vaccine Standardization

All vaccines need effective standardization processes to be used at efficient and safe doses. Two processes were developed with this purpose in mind. The first being a single radial immunodiffusion (SRID) assay and the other being a rocket immuno-electrophoresis (IEP) assay. Of the two different assays, the SRID assay became the more widely accepted one (Wood et al., 2018). This measures the concentration of hemagglutinin in influenza vaccines. SRID basically outdid IEP technology in terms of standardizing and comparing vaccines because it was later found that IEP technology was inconsistent in measuring different vaccines within different

facilities. SRID is also low tech, meaning it can be used in laboratories around the world at cheaper costs, which is likely another reason why it was found to be more widely accepted.

SRID agents are produced for worldwide use by WHO regulatory laboratories. They are in Japan, NIBSC, and the UK. Without this type of standardization, it would be more difficult to match vaccines to the viral strain they are trying to eradicate or prevent the spread of and would be much harder to determine the immunogenicity of said vaccines (Wood et al., 2018). While this process is essential, it adds about 6-8 weeks into the development of vaccines.

With mRNA vaccines, hemagglutinins in combination with other agents will need to be measured to test efficacy via a SRID assay. This is concerning because in times of a pandemic this process could lead to decreased accessibility to vaccines when they are needed most. The trouble with mRNA vaccine technology and the use of SRIDS to standardize them is that researchers need to determine the properties of the vaccines that will determine their immunogenicity, much of which is largely understood at this time. This may lead to the need for development of an entirely new assay to test the effectiveness of mRNA vaccines (Wood et al., 2018). Aside from SRID assays, there are a few other tests that can measure agglutinin responses in a similar fashion. These include: SDS-PAGE testing, reverse-phase high-performance liquid chromatography, mass spectromy, surface plasmon resonance, antibody-based assays, and receptor binding assays. Any one of which may be employed in the future to monitor mRNA vaccine efficacy. Only time will tell if one of these technologies will be capable of standardizing mRNA vaccines for their future use.

Mechanism and Production of mRNA vaccines

At this time, we are currently using our first ever mRNA vaccine technologies to fight the COVID-19 pandemic. Research into mRNA vaccines has been ongoing since the 1990's. The largest challenges to their development have been problems with stability and later translational ability within the host's cells (Cagigi, Loré 2021). For this to occur reliably, researchers have found that not only do the nucleotides in the mRNA sequence need to be modified, but also the transport molecules that help to deliver the mRNA into the host cells. Once this process is streamlined, it is likely that future mRNA vaccines will take a fraction of the time to develop because the only new step for each viral disease will be that of coding the new sequence for each specific antigen (2021). Not only will this decrease time of production, but also it will decrease cost and increase ease of production. Overall, mRNA technology is better suited for mass produced vaccines in comparison to DNA because it does not need to be delivered to the cellular nucleus via separate, complex mechanisms.

Types of mRNA vaccines

There are three different types of mRNA vaccine platforms. These are conventional chemically modified and unmodified mRNA as well as self-amplifying mRNA vaccines. The difference lies in that self-amplifying mRNA not only contains the genome sequence for the antigen, but also contains the genome sequence that enables its continued replication (Cagigi et al., 2021). Currently, Pfizer and Moderna both utilize conventional chemically modified mRNA in their vaccines. This is paired with a lipid carrying drug delivery system composed of lipid nanoparticles. These transport molecules are positively charged so that they can bind to the negatively charged mRNA to protect it from degradation second to the host cells' RNAse enzymes. At this time, the biggest concern for side effects related to mRNA vaccines is that the

immune response initiated second to the mRNA may later result in a weakened adaptive immune response due to decreased protein translation.

mRNA vaccine production

When developing an mRNA vaccine, there are steps that researchers and developers must sequentially follow. The first step is sequencing a gene of choice from the antigen that is the target. Once this is done, an mRNA primer and the mRNA sequence of the antigen itself need to be made using DNA. This DNA is then used as a template for *in vitro* (within a cell) transcription. After this transcription occurs, a cap is added to the 5' end of the sequence to protect the mRNA from being destroyed. Lastly the mRNA sequence is purified, and impurities are removed through a variety of processes to increase potency of the vaccine and reduce degradation of the vaccine (Maruggi et al., 2019).

Currently, mRNA vaccines are produced via a 1 or 2 step *in vitro* reaction. They are then purified with DNase digestion, precipitation, chromatography, or tangential flow filtration (Rosa et al., 2021). These vaccines are advantageous because they only contain components that trigger our bodies' immune responses to a particular coded antigen rather than needing injection of the whole organism. It has taken many years to get to this point, starting from the development of virus-like particle vaccines, leading to recombinant viral-vectored vaccines and toxoids, and finally ending with polysaccharide or protein-based vaccines.

To produce mRNA vaccines, many different essential techniques are required. One technique that needs to be performed is the capping technique. All mRNA have a 5' cap; this is the first sequence of mRNA and is extremely important to the initiation of translation. Rosa and colleagues' site two different ways that caps can be added, one of which is by using a synthetic cap analog and the other is by using a capping approach (Rosa et al., 2021). The capping

approach seems to be in favor as it protects the manufactured mRNA from degradation and avoids an overactivation of body cell immune responses. A poly A tail must also be added during this process. As it sounds, the poly A tail is at the end of the mRNA manufactured sequence. This piece must also be added to increase stability and expression of the mRNA. Altered nucleotides are used in these processes as they improve translation efficiency (2021).

Based on current clinical trials and emergency authorized use (EAU) of mRNA vaccines, the two highlighting features over conventional technology are their increased safety and efficacy in comparison to conventional vaccines. While the process of producing our two EAU vaccines was relatively quick, most of the time spent on their production was used developing a particle with the capability of transporting and allowing the mRNA to enter host cells (in-vitro delivery). Mapping the sequence for the spike protein of SARS-COV2 was a relatively quick process, as researchers and scientists around the world alike worked on this task (Kim et al., 2021). Self-replicating mRNA vaccines (ones used in Moderna and Pfizer) rely on this in-vitro transcription process. This allows the mRNA itself to make many more copies of the mRNA coding for the specific spike protein from the SARS COV-2 antigen.

Production of mRNA vaccines requires no animal materials and has a reduced time of manufacturing which makes it less susceptible to contamination during storage. The price of mRNA production makes it a challenge to mass produce. Purification processes of mRNA have been scrutinized to find the best one. The best, most cost effective, purification process would be that of continuous manufacturing (Rosa et al., 2021). This allows compartmentalization of multiple reactions, all of which need to occur to properly purify mRNA. These reactions would go on to include in situ product removal, substrate feed and product recovery (SDPR), and multimodal chromatography, all occurring simultaneously (2021). This process may be just the

answer to reducing the costs and time for production of mRNA vaccine technology, allowing for it to be more readily mass produced and distributed.

mRNA Delivery

Something important to note is that there are many different delivery mechanisms of mRNA vaccines. They are injected via three ways: injection of naked mRNA, conjugation with lipid-based carriers, or injection via trans infection of dendritic cells. Along with these injections, there are many different transport molecules that are being studied to help aid in the efficacy of these vaccines.

When vaccines are made, the mRNA itself needs to be composed of a few components including an open reading frame (coding for the antigen), poly A tail, 5' cap, and anti-reverse cap analogs. The anti-reverse cap analogs are essential so that the open reading frame is not read backwards and therefore translated incorrectly. In terms of development with the intent to activate T cells, which are more necessary for tumor cell destruction or impairment, protein folding (coded by the mRNA) is very important.

There are many ways in which researchers have found to improve mRNA delivery to cells. One that was found was mediated by endocytosis. Dendritic cells can swallow the mRNA and work in their prime role as antigen presenting cells (activating T and B cells). Dendritic cells can also be targeted by a process called electroporation where the mRNA is directly and specifically delivered to the cytoplasm of dendritic cells (Heine et al., 2021). With the COVID vaccines in use today, delivery is done via codelivery with lipid nanoparticles (LNPS). These LNPs are nontoxic and mostly composed of cholesterol for stabilization. They also have a positive charge that allows for better penetration into cells.

LNPs function as transport and work by protecting the mRNA from degradation and allowing the mRNA to replicate numerous times to produce a more potent immune response. The LNPs in use with the EAU mRNA vaccines have also been studied to develop a co-transport for the mRNA; it protects the nucleic acids from nucleases, controls the release of nucleic acid, ensures cell and tissue selectivity, ensures high mRNA delivery yield, produces minimal toxicity, and is capable of stability in long term storage (Kim et al., 2021). LNP growth relies on pH and hydrophobic reactions that occur within the body. This growth ultimately affects the transport of mRNA. Researchers are unsure if the mRNA itself is transported within the LNPs or if it is transported within the membrane of LNPs. Regardless, LNPs have shown their capability of decreasing recognition of mRNA by RNAses, which in turn, allows for better longevity of the vaccine response (2021).

Reducing Immunogenicity of mRNA sequences

Immunogenicity of the mRNA itself has been a challenge for researchers over the years, as the mRNA itself is often detected by our own immune system or by RNAses prior to being translated into proteins coding for the antigen of interest. It has been found that by modifying the specific nucleotides much of this can be avoided. Some modifications that improve translation include adding a poly A tail to the mRNA sequence that has less uracil. Also, purification of the mRNA sequence prior to injection is vastly important as it eliminates additional “junk” that our body can easily recognize. A worrisome reaction based on the immunogenicity of the mRNA molecules is that of antibody dependent enhancement activity. Essentially, antibodies are made to the antibodies that are produced second to the injected mRNA’s translation (Wang et al., 2021). This effectively increases hypersensitivity reactions and would decrease the clinical utility of these types of vaccines if it occurred.

Another way in which the immunogenicity of the mRNA sequences themselves can be reduced is via transport molecules. Transport molecules are a huge player in terms of how mRNA vaccines work. Without them, many would be degraded prior to essential translation that is needed to generate an immune response. One trial looking at melanoma patients utilized a cationic nano emulsion delivery system using three different immune modifying molecules. In this trial, the mRNA was delivered into melanoma patients and showed increased immunity and clinically significant tumor growth impairment (Wang et al., 2021). A variety of different co-transport/carrier molecules are being studied today in clinical trials: lipid-based molecules, polymer-based carriers, peptide-based carriers, virus-like replicon particles, naked mRNA vaccines, and cationic nano emulsion.

mRNA Mechanism of Action

Adaptive immunity is activated by mRNA using different mechanisms including: infection of somatic (body) cells, infection of tissue-resident immune cells such as macrophages and dendritic cells, and infection of immune cells in secondary lymphoid tissues such as lymph nodes and the spleen. The importance of secondary lymphoid tissue infection deals with the activation of B cells, which promote longer lasting immunity and antibodies. Cytotoxic T cell, or MHC class 8 immunity, is established via antigen presentation of the membrane of host cells that have been injected with the mRNA. Injection of the mRNA occurs when the negatively charged mRNA binds to the positively charged lipid nanoparticle. Next, it fuses with targeted cell membranes found within the host. The host cell undergoes endocytosis and engulfs the mRNA and LNP. After this occurs, proton pumps within the endosome are triggered and reduce the pH inside of the endosome. This increases the positive charge and ultimately releases the mRNA into the cytoplasm (Wang et al., 2021).

Antigen presentation on the membranes of host cells happen relatively soon after administration of the vaccine because of the local immune responses that occur at injection sites. Helper T cells, or MHC class II (CD4) cells, are more reliably activated when antigens are presented extracellularly via macrophages or dendritic cells once they arrive at lymphoid organs (Kim et al., 2021). All these responses can be tailored for individual viral strains using the LNPs while only changing the mRNA sequence itself. Like CD4 cells, B cell activation is also best via extracellular antigen presentation. B cells are extremely important in producing long term immunity that is essential for a well-developed and effective vaccine.

Emergency Authorized Use mRNA Vaccines

The current pandemic that started the use of our first EAU mRNA vaccines is caused by a single positive sense RNA virus that is made of four proteins. These proteins include the N protein (membrane), M protein (envelope), E protein, and S protein (spike protein) (Kim et al., 2021). The spike protein is what Moderna, Pfizer, and many more clinical research trials are aiming to duplicate with their mRNA sequences (Kim et al., 2021). There has been a rate of 52% efficacy of Moderna and Pfizer vaccines based on the immune response initiated with the first dose. The most common side effects of both include the following: pain at the injection site, fatigue, headache, muscle pain and chills, joint pain, and fever (2021). All these side effects are much more frequently reported following the second, booster, dose of both. Moderna uses a 100-microliter dose while Pfizer uses a 30-microliter dose. One major downfall in terms of mass distribution is that Pfizer vaccines need to be stored at super cold temperatures, which is not possible at all storage facilities.

Benefits and Risks of mRNA vaccines

Rabies

Throughout the numerous trials of mRNA vaccine technology, few have been aimed at providing prophylaxis for the rabies virus. Alberer and colleagues conducted a trial aimed at evaluating the efficacy of an mRNA rabies vaccine. Their trial is a relatively small trial only comprising of 101 participants aged 18-40 years old. Participants were excluded if they showed any previous signs of autoimmune disorders, had received the conventional rabies vaccine or had plans to, or if they were of an immunosuppressed state (Alberer et al., 2017). The vaccine developed, CV7201, was that of an mRNA sequence encoding the rabies virus glycoprotein. Not only was it administered at different doses to patients, but it was also administered via different routes including intradermal injection, spring powered intradermal injection, and via an intramuscular injection.

Throughout the clinical trial process, participants who were vaccinated would have routine follow up visits where autoimmune markers such as ANA, TSH, and anti TPO antibodies were measured (Alberer et al., 2017). Participants' antibody titers and peripheral blood mononuclear cells were also measured at these follow up appointments. Lab abnormalities were seen in approximately 12% of the sample population following injection of the mRNA rabies vaccine; these abnormalities were not significant and resolved quickly.

Injection site reactions were common for participants that received the vaccine intradermally (94%) and intramuscularly (97%). Also, 5% of participants reported nasopharyngitis, headache, oropharyngeal pain, vertigo, and rhinitis within seven days after their first dose (Alberer et al., 2017). With this vaccine it should also be known that 78% of

participants in the trial at some point reported grade 3 events to include fever, headache, and chills (2017). These are determined by the WHO and include a systemic reaction such as a fever.

With regards to route of administration, it was found that IM injection via syringe yielded less of an immune response (no antibodies to rabies) than that of intradermal injection via a needle free injection device where 77% of participants had antibodies for the rabies virus (Alberer et al., 2017). While this was significant, at the one year follow up appointment, only 2 participants out of the fourteen who received the injection via intradermal needle free devices still had some antibodies against the rabies virus. This vaccine, regardless of route of administration, showed evidence of activation of the T-cell responses post vaccination. It also demonstrated induction of memory B and T cells, but overall, long lasting immunity was not successfully demonstrated.

COVID-19

At this time, the most common side effects encountered with the COVID-19 vaccines, Pfizer and Moderna, are the following: erythema, swelling, fever, fatigue, headache, and myalgias within the first 48 hours of vaccination (Castells et al., 2020). Research suggests that there is no increased rate of hypersensitivity reactions; however, there is a 10x increased rate of anaphylactic and anaphylactoid reactions in comparison to previous vaccine technologies (2020). Researchers are unsure as to why there is an increased rate of anaphylaxis like reactions, but they suspect that it may have to do with the polyethylene glycol component that is added to these vaccines to allow for increased stabilization of the mRNA. They also suspect that it may be caused by the antigen produced from the vaccine, residual mRNA protein products, or preservatives within the vaccines themselves (2020).

Pfizer and Moderna both use LNPs to increase delivery of mRNA to the cytoplasm of cells, so this may also play a role. Mast cell activation plays a role in these reactions and can be monitored via drawing serum Tryptase levels. There are three types of immediate vaccine reactions: IgE mediated, non-IgE mediated, and nonimmune, which are used to describe vasovagal syncope reactions related to the vaccines (Castells et al., 2020). There are also eight different types of delayed reactions that patients may have after administration of either of the COVID vaccines. These include site reactions, urticaria or benign exanthems, serum sickness, fever, skin reactions, organ damage, and neurological sequelae (2020).

The main differences between Pfizer and Moderna lie in their costs, storage requirements, authorized age demographics, side effects, and efficacy. Currently, Pfizer has been authorized for use in those ages 16+, has 95% efficacy for preventing COVID-19 for at least 119 days after the second dose, is the cheaper vaccine of the two, and has a lower rate of side effects compared with the Moderna Vaccine (Meo et al., 2021). The Moderna vaccine is authorized for use in those ages 18+, has a 94.5% efficacy rate for preventing COVID-19 for at least 119 days after the second dose, is roughly double the cost of the Pfizer vaccine, and has a higher side effect profile of the two (2021).

Cancer

This article discusses different mRNA technologies that are currently being investigated in the treatment of lung cancers. Different vaccines have been used with the intent of targeting a variety of tumor cancer genes including Kristen rat sarcoma (KRAS), epidermal growth factor receptor genes (EGFR) and echinoderm microtubule-associated protein-like 4-anaphlastic lymphoma kinase (EML4-ALK) (Khan et al., 2021). Essentially, these mRNA injections are used to silence oncogenes, in hopes of increasing efficacy of cancer treatments in patients who

are already undergoing radiation. There are also miRNA sequences that work by activating RNAse to cleave cancer DNA (2021). Both mRNA vaccine injections have the capability of targeting more than one antigen. This is especially useful in cancer treatment as there are many mutated genes and processes at play that allow for its progression.

Researchers have tried to target angiogenic oncogenes involved in lung cancer using mRNA technologies, but these were found to be associated with many adverse side effects (Khan et al., 2021). Regardless of this fork in the road, there have been several RNA sequences designed to target different lung cancer oncogenes. These oncogenes include metadherin, KRAS, STAT3, Bcl2, Survivin, AXL and Akt1. Metadherin is a cancer gene that decreases T cell efficacy and increases cancer cell signaling. mRNA used to target metadeherin has been found in trials to decrease lung cancer progression. Bcl2 and Akt1 targeting unfortunately showed limited improvement and efficacy in clinical trials because mRNAs used to target these were not adequately delivered to host cells' cytoplasm (2021). Trials targeting AXL showed increased tumor suppression and improved treatments for patients with non-squamous cell lung carcinomas. Patients who used mRNA that targeted Survivin in combination with their chemotherapy had increased efficacy of their cancer treatments. All in all, the best cancer trial from this article was that of a liposomal encapsulated mRNA-based vaccine that showed improved treatments for patients with melanoma (2021). This vaccine increased antigen responses as well as T cell immune responses against tumor cells.

The ongoing search for cancer treatments has been a large motivator into mRNA vaccine research and development, with the pandemic efforts shifted towards creating the first emergency authorized use vaccines for infectious disease processes. Interestingly, there have been trials that have shown tumor regression using RNA-pulsed dendritic cells against tumor

cells. Trials have also looked at using mRNA to treat HIV; however, at this time none have shown success (Rosa et al., 2021).

Diabetes Insipidus

One of the very first preclinical studies using mRNA vaccine technology was that of Jirikowski et al. who essentially found that mRNA injections that simulated oxytocin and vasopressin were able to temporarily reverse diabetes insipidus in mice. Since this initial trial, there have been many shown benefits of mRNA vaccines. These vaccines have shown immunogenicity, do not get integrated into the human genome, and are not subject to resistance (Wang et al., 2021).

Animal Trials

Several mRNA vaccine trials have been conducted. Influenza mRNA vaccines proved efficacious, and induced hemagglutinin inhibition titers that were comparable to inactivated virus vaccines (Maruggi et al., 2019). Immune responses second to the mRNA vaccine for rabies were significant but were found to decline in immunogenicity after just one year. The Zika virus mRNA vaccine trials proved to immunogenicity in mice and showed some conferred protection in the fetus when given to pregnant mice. There has also been a trial where mRNA coding for six different antigens was used and was successful in inducing protective immunity for all six viruses (2019). Lastly, trials within mice have shown promise of immune protection from different streptococcal species after injection with mRNA vaccines. Even parasitic, malarial trials within mice have shown immunogenicity of the host after vaccine injection.

Therapeutic utility of mRNA vaccines in the COVID-19 response

Gender Differences

This article emphasizes the need for women and pregnant women to have representation in vaccine trials. Women make up half of the population and are no doubt affected by pandemics and chronic diseases similarly to their male counterparts. Emphasis on female representation is especially important as females are especially impacted by COVID-19. It is important to note that women have a greater humoral immunity than men (Chang et al., 2020). Humoral immunity being B cells and their ability to produce antibodies against pathogens, something that is utilized greatly with the production of vaccines. While women do have a greater humoral immunity, they are also more likely to suffer from autoimmune diseases, which makes vaccines, especially mRNA vaccines, more challenging to create for their population.

Different vaccines activate B cells to initiate their maturity to later form memory B cells; this is often done when they migrate to the host's germinal centers (Chang et al., 2020). There are differences in the physiology of the germinal centers in men compared to women. This too affects the immunogenicity of different vaccines between the sexes. One example of differences between men and women and their responses to vaccines was that of a tuberculosis vaccine trial. It was found that TB vaccines inhibit anti-inflammatory proteins in women and enhance their effectiveness compared to men (2020). Estrogen plays a huge role in this process as it initiates antiviral reactions and can work at inhibiting influenzae virus replication. Another reason for this difference in women is that some genes located on the X chromosome have been found to detect viruses and initiate immune responses, perhaps why women are more likely to suffer from autoimmune disorders. There needs to be representation between the two sexes as they clearly have different responses to vaccines, and different immunogenic pathways.

Regarding the COVID-19 pandemic, there are some very viral specific differences between the sexes. One such being that men have a higher concentration of ACE 2 in their blood (Chang et al., 2020). The novel COVID-19 virus enters our own cells via this receptor which makes men at higher risk of contracting COVID. While this is true, numerous studies have been shown that suggest women lose their antibodies faster than men, which may indicate that vaccines do not last as long in women as they do in men (2020). With previous vaccines, such as the influenza vaccine, women have historically been able to get vaccinated. This immunity has been shown to be passed on to offspring. No mRNA vaccines have been intentionally tested in the pregnant population to date. This is something that should be done to better understand how women, especially pregnant women, react to the new mRNA vaccine technology that is in use today.

Pregnancy

Shimabukuro and colleagues conducted a preliminary study of 35,691 participants, aged 16-54, who were pregnant to see their most common reactions to the COVID-19 vaccine. This article also looks at whether maternal immunity granted from the COVID-19 vaccine is passed further down to the infants of these pregnant women. Injection site pain was a more common complaint in pregnant women; however, headache, chills, and fever were less frequently reported in pregnant women (Shimabukuro et al., 2021). Majority of the participants in this study were pregnant and were aged 25-34. Additionally, a large portion of participants were those that worked in the medical field (3,958). Most participants were ages 25-34. Those that received vaccine during pregnancy or in the periconception period were considered eligible.

The most common gestational adverse reactions that were reported related to COVID vaccination were spontaneous abortion and preterm birth (Shimabukuro et al., 2021). This study

was conducted using VSAFE software as well as the VAERS survey. Most participants received the vaccine in the second trimester of pregnancy. Of all the pregnant participants, 827 went on to full term pregnancy and of those, 712 had live births while 104 had spontaneous abortions and 1 participant had a still birth (2021). Majority of the spontaneous abortions occurred before 13 weeks of gestation suggesting that participants were either very late in their first trimester of pregnancy or early in their second. While the most common gestational side effects were the two previously mentioned, some participants also reported preterm birth 9.4% and small gestational size 3.2% (2021). Strong evidence suggests that maternal immunity is passed on to infants; however, at this time it cannot be quantified how many antibodies are passed on.

Mass Vaccination

Li and colleagues examined the precursor to what we now know as the Pfizer vaccine. Adverse effects were commonly reported after injection with the first and second doses of the BNT162b1vaccine. More people in the younger population reported side effects when compared with the more elderly population; however, both reported high occurrences of adverse effects from the vaccine (Li et al., 2021). In fact, greater than 80% reported adverse effects after the first dose and greater than 90% with the second dose. While these percentages are high, the only Grade 3, or severe systemic adverse reaction reported, was that of a fever (2021). Younger people were more likely to have headaches in relation to the vaccine than those that were older (2021). This along with a few other side effects like malaise and fatigue were much more likely to occur after the second dose rather than after the first. Another interesting finding with the BNT162b21 vaccine was that it decreased lymphocytes and platelets within patients' serum and increased their C-reactive protein levels (2021). This was because the mechanism of the vaccine was to send lymphocytes to the lymphoid organs to precent the antigen 'mRNA from the

vaccine' to host immune cells. As to be expected, younger participants in this trial elicited higher immunity than their elderly counterparts.

The current Pfizer vaccine in use today was found to have a milder systemic reactogenicity profile, therefore fewer side effects than the one analyzed within this trial (Li et al., 2021). This vaccine encodes the full-length S protein with mRNA and has been found to have broader immunogenicity than BNT162b21. This makes it a much better candidate for the emergency authorized use that it undergoes today.

Pfizer's current vaccine available on the market, BNT162b, has been found to induce strong TH-1 type CD4 and CD8 T cell responses, and was found to protect Rhesus monkeys' lungs from infection with the virus. It was also found to be 90% effective at preventing infection with COVID-19 when used in Rhesus monkeys (Li et al., 2021). Much like Pfizer's BNT162b vaccine, Moderna's mRNA-1273 also codes for the full length of the COVID-19 S protein. Both vaccines produced neutralizing serum in recipients that was equal to or greater than that of patients who had recovered from the virus itself (2021).

A human trial was conducted and executed by giving patients either a placebo or actual injection 21 days apart of the Pfizer vaccine. The trial included 43,448 participants, of whom 37,706 came back for a follow up an appointment two months after the second dose of the vaccine. The vaccine was eventually found to be reactive in 8,183 participants (Polack et al., 2020). This information was found using the Clopper-Pearson 95% confidence intervals to assess side effects of the vaccines. The most common side effects experienced were fatigue, headache, chills, myalgias, and joint pains (2020). Of note, pain and fever were both more common in younger people receiving the vaccine than their older counter parts. They also were found to be more likely to take fever lowering medications than older participants (2020). Overall, when

comparing patients who received the COVID vaccine to those who received the placebo, it was found that there were generally more side effects with the vaccine. Of these side effects some of the more serious ones included the following: shoulder injury, lymphadenopathy in the right axillary region, paroxysmal atrial fibrillation, and right leg paresthesia.

Through this trial it was found that protection from the Pfizer vaccine starts 12 days after the first dose (Polack et al., 2020). After the first injection there was found to be 52% protection conferred against COVID 19, and after the second dose there was a 91% coverage against COVID-19; this bumped up to 95% after 7 days post injection with the second dose (2020). While more immunity was conferred with this second dose, there was also more systemic reactions to it. The intent of researchers is to study the long-term safety of the Pfizer vaccine, but according to this trial they will not be able to do so against a placebo group as vaccines are becoming mandated.

Dagan and colleagues took a unique approach and conducted a study evaluating Pfizer efficacy where they matched Pfizer-vaccinated individuals to unvaccinated counter parts. These participants were in Israel and data was gathered from Clalit Health Services. Participants included those with zero to three pre-identified risk related conditions laid out by the CDC that puts patients at risk for severe COVID-19 infection. Each study group within this clinical trial included 596,618 participants. Eligibility criteria of this study included patients who were 16 or older, those who had no history of positive PCR COVID testing, and those who were members of the Clalit Health Services organization within the previous 12 months to this study.

This study, while not randomized controlled, included a much larger population of patients than that of the original phase 3 trial that Pfizer had initiated. Overall, 1,163,543 vaccinated participants were eligible for the study and were matched with 596,618 unvaccinated

controls (Dagan et al., 2021). Unvaccinated patients were on average younger than those who were vaccinated. Out of these vaccinated participants 10,561 infections were documented. Of these infections, 5,996 (57%) had symptomatic COVID, 369 required hospitalization, 229 participants had severe cases, and 41 cases resulted in death (2021).

During days 14-20 post initial dose, vaccine effectiveness against infection was found to be 46%. Effectiveness against symptomatic COVID-19 infection was found to be 57%. Protection from COVID hospitalization was found to be 74% (Dagan et al., 2021). Protection against severe COVID illness was found to be 62%. Overall, protection against COVID-19 death was found to be 72%. During days 21-27 post initial dose, vaccine effectiveness against infection was found to be 60%. Protection from symptomatic COVID-19 were found to be 66%. Hospitalization protection from COVID-19 was found to be 78%. Protection against severe COVID-19 was 80%. Lastly, there was found to be an 84% protection against COVID-19 death (2021). 7 days after the second dose of Pfizer there was ultimately found to be 92% protection against COVID-19 infection. 87% protection from COVID-19 related hospitalization and 92% protection against severe disease (2021).

Overall, of those who were vaccinated: 55 had severe COVID (compared to 174 in the unvaccinated group), ultimately finding 62% conferred protection in those vaccinated from days 14-20, 80% conferred protection days 21-27, and 92% for 7 or more days after the second dose (Dagan et al., 2021). While this study does not have specific information on effectiveness against variant strains of COVID-19, a plateau of active infections was observed in the later periods in vaccinated persons suggesting that there is some conferred protection against COVID variant strains.

Applicability to Clinical Practice

The information gathered in this literature review will allow providers to confidently educate patients on the risks and benefits of mRNA vaccine technology. It will encourage conversation with patients and allow providers to better explain the mechanisms by which mRNA vaccines work. Not only that, but also, this research will analyze the differences from previous vaccines and illuminate the major benefits and risks compared with older technologies. This will ultimately allow patients to make more informed decisions about their future vaccination status and will allow primary care providers to share an important part in this decision.

Discussion

After conducting this research, mRNA vaccines have the potential for not only preventing infectious diseases, but perhaps fighting chronic or cancer related illness as well. In making vaccines and conducting trials moving forward, it is especially imperative to include all different populations, including those of pregnant women. At this time based on the trial done by Shimbaruku and colleagues mentioned earlier, spontaneous abortions, preterm births, and low birth rates are consistent with rates seen in the general population even after vaccination. In fact, spontaneous abortion rates in this trial were less than that of the general population, only ranging at about 12%. While this is true, more research needs to explore the number of pregnancies and previous spontaneous abortions in these populations as these are factors that predispose women to spontaneous abortions in the first place.

Overall, the most common side effects of the COVID-19 vaccines and previous Influenza vaccines are relatively similar. Main complaints of either vaccine platform range from malaise, headaches, and chills with a more predominant emphasis in mRNA vaccine recipients having injection site pain. Of note, it has been found that there is a higher rate of anaphylaxis reactions in patients who receive Moderna or Pfizer vaccines, and at this point scientists are unsure why. More side effects are reported in those that receive Moderna over Pfizer, and Pfizer is relatively cheaper in comparison to Moderna. While this is the case, Pfizer has much more stringent storage requirements and is therefore not as easily accessible globally in times of a pandemic.

In terms of benefits of mRNA technology over conventional vaccine technology, a few have been highlighted in this research. Overall, mRNA vaccines are much quicker to produce than conventional vaccines as once the antigen sequence is identified the process has essentially been streamlined by adding a LNP to the antisequence. With this first round of EAU mRNA

vaccines production costs were relatively expensive; however, once more mRNA vaccines are produced in the future, the process should become less expensive. These vaccines also have much wider reach in terms of the populations they can be given to, because they can be given to immunocompromised patients unlike live vaccines without fear of infecting recipients. Another benefit of mRNA vaccine technology is that it has the potential to code for more than one antigen, and in the future may be easily manipulated to protect against more than one strain of virus at a time.

With these benefits being outlined there are still some setbacks to mRNA technology as some of the biggest challenges to vaccine development and progression through clinical trials has been their risk of failure as well as their risk for antibody dependent enhancement. Another consideration of mRNA vaccines is the need for supervision post vaccine injections. Particularly, when the virus was first erupting, hospitals were especially burdened, and this may not have always been possible. Fortunately, most of what is required of patients is to wait fifteen minutes after injection of their vaccine in the clinic to monitor for any severe adverse effects. Something, that under typical conditions can be accommodated for.

Currently, research is lacking in mRNA technology. While scientists have successfully created two vaccines that have been use in our current pandemic there are still a lot of questions as to the long-term side effects of these vaccines. There is also no proper way to standardize the doses of these vaccines and to compare efficacy across different labs. This is something that in the future will need to be done to properly assess effectiveness and clinical utility of these vaccines. Booster shots for these vaccinations have also begun development, with Pfizer's booster shot being FDA approved recently. Research into these boosters will need to be conducted as to evaluate their efficacy and side effect profiles.

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