The production of mRNA vaccines in the COVID-19 pandemic: advent of new applications in health treatments

A produção de vacinas de mRNA na pandemia de COVID-19: advento de novas aplicações em tratamentos de saúde

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ABSTRACT
Modern vaccines developed using mRNA technology are highly accurate formulations designed to stimulate the production of a variety of antigenic properties in the user in order to activate adaptive immunity. This enables the use of such technology in the research of vaccines against several infectious diseases, such as hepatitis C, Acquired Immunodeficiency Syndrome (AIDS), malaria, tuberculosis, in addition to chronic diseases, such as some types of cancer. Such vaccines were an efficient alternative in the fight against COVID-19 and have remarkable characteristics, such as the versatility of their applications and the ability to encode and express virtually any type of protein. Like this, this work is justified by the need to know the potential applications of this technology in clinical practice and in the search for effective alternative solutions in the fight against infectious and chronic diseases. A systematic review of the literature was carried out, including texts in Portuguese or English from 2019 to the present day and original texts published before 2019, searched in databases such as Science Direct, US National Library of Medicine, Scielo, Pubmed and Instituto Nacional de Industrial Property (INPI).

Keywords: mRNA, vaccine technology, COVID-19, applications.

RESUMO
As vacinas modernas desenvolvidas usando a tecnologia de mRNA são formulações altamente precisas projetadas para estimular a produção de uma variedade de propriedades antigênicas no usuário para ativar a imunidade adaptativa. Isso possibilita o uso dessa tecnologia na pesquisa de vacinas contra diversas doenças infecciosas, como hepatite C, Síndrome da Imunodeficiência Adquirida (AIDS), malária, tuberculose, além de doenças crônicas, como alguns tipos de câncer. Tais vacinas foram uma alternativa eficiente no combate à COVID-19 e possuem características marcantes, como a versatilidade de suas aplicações e a capacidade de codificar e expressar praticamente qualquer tipo de proteína. Assim, este trabalho justifica-se pela necessidade de conhecer as potenciais aplicações desta tecnologia na prática clínica e na procura de soluções alternativas eficazes no combate às doenças infeciosas e crónicas. Foi realizada uma revisão sistemática da literatura, incluindo textos em português ou inglês de 2019 até os dias atuais e textos originais publicados antes de 2019, pesquisados em bases de dados como Science Direct, US National Library of Medicine, Scielo, Pubmed e Instituto Nacional de Medicina Propriedade Industrial (INPI).

Palavras-chave: mRNA, tecnologia de vacinas, COVID-19, aplicações.

1 INTRODUCTION

Vaccination represents an indelible milestone in the human health revolution. Its advent provided the prevention of infectious and contagious diseases that, previously, were responsible for a large part of population mortality[1,2]. Its origin dates back to the process called Mithridatism, which consists of the use of increasing and non-lethal doses of venom in order to generate immunity to highdoses[3,4]. The functioning of vaccines follows the same premise: the immune system is exposed to an antigen belonging to the pathogen, thus stimulating the immunological memory and reducing the chances of a future infection when exposed, in fact, to the agent[1,2,5].

Vaccine technology has advanced significantly since initial attempts in the 18th and 19th centuries to induce immunity to smallpox by using secretions collected from skin lesions of infected individuals[1,6,7]. Modern vaccines are highly accurate and carefully designed formulations that utilize a variety of antigen properties to stimulate adaptive immunity[5]. The best known and most used is the attenuated live virus vaccine. Current vaccines have benefits such as a strong immune response, diversity of existing formulations and high bioavailability in the body. However, they have the disadvantage of the need for large-scale supplies for their production and the slow pace of research and manufacturing[8]. In this context, nucleotide vaccines appear as promising alternatives.

The race for science to develop effective and safe vaccines, with minimal adverse effects for the individual, was a priority during the coronavirus pandemic. Coronavirus disease 2019 (COVID-19)[9]. Thus, the rescue of the use of mRNA technology in the production of vaccines during this period contributed to boost the development of more refined alternative biotechnologies, expanding the therapeutic limits of several diseases[10]. The outstanding feature of mRNA is the versatility of its applications, such as adapting to encode and express almost any type of protein [8,11,12]. Currently, the technology is being used in the research of vaccines against infectious diseases such as hepatitis C, Human Immunodeficiency Syndrome (AIDS), malaria and tuberculosis, as well as some types of cancer, such as melanoma, pancreatic adenocarcinoma, breast cancer, cancer of ovarian and gastrointestinal cancer[13].

2 METHODOLOGY

A systematic review of the literature on the use of mRNA technology from the COVID-19 pandemic was carried out. For this, the following descriptors were used: "mRNA", "vaccine technology", "COVID-19" and "applications". Inclusion criteria were original texts published in Portuguese or English, from 2019 to the present day, which addressed the theme of the work. Original texts published before 2019 on the use of mRNA technology were also included, due to their relevance. The following databases were used to search for studies: Science Direct, US National Library of Medicine, Scielo, Pubmed and the National Institute of Industrial Property (INPI).

3 THEORETICAL REFERENCE

Between September and December 2019, a group of patients sought the hospital service in the city of Wuhan, Hubei province in China, presenting symptoms of pneumonia caused by an unknown agent. It was later discovered that it was a new β-coronavirus, Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2).Viral spread occurred quickly due to factors such as population density, lack of social distancing and the large flow of travel, mainly international. On March 11, 2020, the World Health Organization (WHO) declared the state of a pandemic[14]. Since then, this unprecedented event has had a catastrophic impact on human health and global public health security. The unexpected and highly contagious entry of the new SARS-CoV-2 created a serious threat to the world community, but mainly affected the health services of underdeveloped and developing countries, such as Brazil[15], due to the unpredictable nature of the spread and symptoms of the disease, as well as the inability to accommodate the demand of patients in the public and private health sectors[16]. In this context, in an attempt to

control the spread of the pandemic, governments around the world have mobilized a considerable amount of manpower and material resources in health and research and development linked to the production of vaccines against COVID-19[17]. Several approaches for the development of this vaccine were tested simultaneously, including inactivated virus, live attenuated virus, recombinant protein, adenovirus vector, influenza virus vector and DNA vaccines[6]. As a breakthrough vaccine innovation in humans, mRNA vaccine technology has played an essential role in controlling the COVID-19 pandemic and has proven to be useful in several other areas of medicine[18].

3.1 mRNA TECHNOLOGY

The mRNA is a single-stranded molecule composed of ribonucleic acid bases whose function is to provide the instruction for the production of a specific protein. According to Jones et al.[19], qWhen applied for vaccine use, the synthetic mRNA is delivered with the genomic sequence of a specific antigen, a protein found in the pathogen. Upon entering the host cell's ribosomes, it is translated into the encoded protein with the aid of transfer RNA. In this way, the cell produces and exposes the protein on its surface to generate a cell-mediated immune response, in addition to releasing proteins that will be identified and exposed by antigen-presenting cells in order to generate an antibody-mediated response.[19.20]. The study of the functioning mechanism and possible applications of this molecule has been explored since the 1960s.according to Smith et al.[21], the initial obstacle was related to the instability of the molecule and its form of delivery. The minimal cellular uptake of mRNA was caused by the absence of a protective substance in the material. Consequently, reading the properties of the mRNA was also difficult. In 1978, scientists successfully applied a protective lipid formulation to stabilize mRNA. The study was able to introduce the mRNAof rabbit reticulocytes in mouse lymphocytes, generating globin synthesis[22].

Identification of DNA-dependent RNA polymerase enzymes was a crucial step leading to In Vitro Transcription (IVT) using DNA templates[23].According to the study by Kim et al[24], IVT enabled the transcription of a functional mRNA selected from a template in the desired amount.

The mRNA technology offers great advantages: its adaptability is the key to its application in other health treatments, as it can be used to encode antibodies, cytokines and other proteins of the immune system[20]. Its production can be carried out on a large scale and does not require the cultivation of cells or infectious agents, making the process faster, more economical and more effective. According to the study by Silva et al[25], the synthetic production

of mRNA allows the use of the vaccine in a wide variety of groups, such as the immunocompromised, as they have no contact with the causative agent of the disease. Finally, such attributes made the mRNA vaccine a promising option during the COVID-19 pandemic. and other modern applications[20].

3.2 APPLICATIONS OF mRNA TECHNOLOGY

3.2.1 Influenza virus vaccine

The influenza virus, also known as Myxovirus influenzae, is the etiological agent responsible for one of the most recurrent viral infections today, the flu. It is classified into four types, three of which, A, B and C, are known to infect humans. Type A is considered the most virulent, having been responsible for some of the global flu pandemics ever recorded. Types B and C can also cause infections in humans, but are generally less virulent than type A. Type B, for example, can cause outbreaks of the flu, while type C causes symptoms similar to the common cold. Influenza virus infections usually cause a high fever, runny nose, joint and muscle pain, coughing, and a feeling of exhaustion. As they are very common, ongoing vaccines are developed to combat evolving viral strains. Additionally, mRNA vaccines are being studied as an alternative approach to fighting influenza[2, 26, 27].

In the past decade, Research on mRNA influenza vaccines has not yielded clinical trial results. However, there was one study where an mRNA vaccine that delivered the hemagglutinin of a type A influenza virus was processed and injected into mice, young and old. The result was that there was the production of specific B and T lymphocytes[26,27,28].

Furthermore, the vaccine proved to be capable of withstanding thermal stress, as well as guaranteeing adequate protection against other antigens, especially the highly conserved viral nucleoprotein. A similar study focused on the same objective, however, it employed a different method, cationic oil-in-water emulsion, aiming to formulate the mRNA vaccine [28, 29].

In 2017, research used mRNA vaccines with lipid nanoparticles (mRNA-LNP) in order to produce rapid and increased immune responses in mice, ferrets and non-human primates[30, 31]. The results were strong enough for the study to start some Phase I clinical trials [2,30].

Another approach is the coding mRNA technique, where a neutralizing antibody is used against the influenza virus. However, there is a problem with the dosage. Even so, it is an advantageous strategy against infectious diseases, and can also be used in therapies with monoclonal antibodies against tumors or rare diseases[26,28,32].

3.2.2 Zika virus vaccine

The Zika Virus (ZIKV) is spread by mosquitoes and was the main etiological agent of the epidemic that devastated the planet in 2015. It is an enveloped positive-sense single-stranded RNA flavivirus that causes a disease evolution similar to dengue. It causes little or no symptoms in healthy adults, but causes complications in pregnant women or younger children. In these cases, ZIKV has been associated with severe developmental defects, particularly in neurocognitive abilities and brain growth[2].

Several case reports indicate a relationship between ZIKV infection and Guillain-Barré syndrome in adults and inborn malformations in infants. During the 20th century, ZIKV was located in regions of Africa and Asia, with low rates of infection in humans. From intense intercontinental travels, a diversity of outbreaks was noted globally, with varying levels of severity [28,33]. In 2015, a quickly and easily fought pandemic took hold[2].

Despite the small threat of ZIKV, the scientific community demanded the development of vaccines as a way to reach higher levels in the fight against more deadly diseases and in the design of effective vaccine strategies for pregnant women[34].

In 2017, Richnere et al.[35] managed to produce an mRNA-LNP vaccine for in vivo generation and sterilizing immunity in mice. It is worth mentioning that pregnant guinea pigs were vaccinated and the production of neutralizing antibodies was observed in mothers and fetuses. However, the fetuses had a much smaller amount of antibodies.

3.2.3 Vaccine against the Human Immunodeficiency Virus (HIV)

HIV is an enveloped, single-stranded RNA retrovirus that infects humans, causing critical failure of the immune system with a decrease in T-cell counts. This makes the body susceptible to simple infections such as the common cold. Thus, AIDS caused by HIV and its associated diseases is seen as a potential threat to global health[2].

The control of HIV levels in infected people is effected from pre-exposure prophylaxis and antiretroviral therapy. As soon as AIDS is clinically evident, such conduct cannot fully suppress HIV from the body, requiring long-term use of medication. In addition to being quite costly, these drugs have a very limited supply in developing countries or low-income communities[36].

A strategic action used in large scale to provide prophylactic immunity to HIV is vaccination with mRNA. This is because, over time, it has been demonstrated that the advancement of vaccines has suffered obstacles to effectively combating this disease, the main obstacles to its development were mainly the high mutation rate and the challenge of producing

broadly neutralizing and long-lasting antibodies. term, therefore, vaccines against HIV are not available for use [37,38].

Saunders et al.[39] published a study of a whole mRNA-LNP vaccine that resulted in the production of relevant HIV-binding antibody titers in primate models. In the spring of 2021, in a clinical trial in humans[40], an mRNA vaccine was tested and 97% of the volunteers developed a great capacity to produce neutralizing antibodies, with the caveat that there was a response against only 30% of the HIV strains in the test region. Thus showing that more research needs to be carried out.

3.2.4 Dengue virus vaccine

Dengue virus (DENV) causes dengue fever, a tropical disease transmitted by *Aedes aegypti* mosquitoes. DENV causes symptoms such as high fever, headache, muscle and joint pain, and rash[11, 33, 41]. From the flavivirus family, DENV induces a strong immune response, making it difficult to identify the epitopes that the immune system is able to recognize and attack [11,28,42].

DENV is known for its variety of strains, each of which guarantees a specific immune response. Fact that makes traditional vaccines inoperative. Thus, mRNA vaccines are an alternative and have been developed to reach non-specific proteins in order to increase the activation of CD8 T cells and the maturation of other immune cells, conferring the production of immunological memory [28,42,43].

3.2.5 Rabies virus vaccine

The rabies virus causes an infectious zoonosis that affects the central nervous system, being contracted by salivary contact with an infected animal and extremely dangerous. Initial symptoms may include fever and tingling at the site of the bite/scratch, which may progress to violent and uncontrolled movements, loss of movement, confusion and loss of consciousness, in addition to hydrophobia, which is often used to diagnose the disease[2].

However, this scenario can be minimized by vaccination, especially in developed countries. Traditional vaccines are not able to keep up with the constant mutations of the virus, so mRNA vaccines are an alternative by targeting more stable regions of the virus [6,37,42 ,44].

Analogous to the DENV mRNA vaccine, the evolving rabies vaccines call for the design of antibodies that attack specific glycoproteins in the virus envelope. Research carried out in animals indicated an increase in immune activation and maturation in response to the vaccine,

in addition to some preliminary clinical trials showing similar results, with a tolerable contingent of side effects [37,38, 45].

3.2.6 Cancer vaccine

Cancer is a complex and heterogeneous disease that can affect different parts of the body. Standard treatment includes surgery, radiotherapy and chemotherapy, which can be effective, but also have significant side effects and limitations regarding effectiveness in some types of cancer[46].

There is a growing expectation from research involving the production of personalized cancer vaccines, which target specific proteins present in cancer cells. These vaccines have the potential to stimulate the patient's immune system to attack cancer cells, prevent tumor growth, reduce the chance of recurrence, and destroy remaining cancer cells after other therapeutic treatments[47].

In this context, gene therapy emerges as a promising option for the treatment of cancer, allowing the delivery of therapeutic genes directly into tumor cells, for the expression of proteins with anticancer activity. mRNA has been considered a valuable tool for gene therapy, as it is capable of providing a copy of a specific gene to be expressed in a cell[48].

The challenges for using mRNA as gene therapy is its instability, rapid degradation and limited ability to cross the cell membrane. Therefore, self-assembled mRNA nanospheres (mRNA-NSs) are a promising alternative, since they are able to protect the mRNA from degradation and deliver it directly to tumor cells, without contributions from additional vehicles. The results of the study show that the mRNA-NSs were able to efficiently deliver the anticancer drug doxorubicin into tumor cells, thus increasing the effectiveness of the treatment. Additionally, mRNA loaded with doxorubicin can express the target protein apoptin and increase the expression of Bax and cleaved caspase 3 protein, which confers a greater potency of the anti-apoptotic effect, in addition to intensifying tumor necrosis and demonstrating an effect analogous to genetic chemotherapy in mice with T1 breast cancer. The results obtained are promising and demonstrate that mRNA-NSs may represent an effective alternative for the investigation of a potential method of treatment in cancer cells, whether combined with other cancers or therapeutic proteins[49].

3.2.7 Applications in Dermatology

Aging can be characterized, among other factors, by the decrease of collagen in the skin, which leads to loss of elasticity and dermal firmness[50,51]. Recently, the demand for aesthetic

procedures has increased therapeutic efficiency towards short-term clinical applications in the mRNA delivery technique. Studies have pointed to new modalities in the delivery of mRNA focused on the use of extracellular vesicles loaded with collagen type 1[52].

The type 1 collagen delivered by these vesicles has been shown to be able to restore protein production in the skin of mice after photoaging, culminating in filling and retraction of the formation of wrinkles in the skin. In the study, it was also possible to develop a microneedling matrix with hyaluronic acid for collagen delivery, granting greater distribution in the dermis. Although gene therapy based on DNA has greater durability, the study with mRNA had fewer adverse effects, since it is concentrated only in the cytoplasm and does not present genomic penetration[53].

4 RESULTS

After using the described methods, 10 relevant studies on the subject were selected and the following components were analyzed for each work: author and year of publication, title, database, type of study and results. The results are presented below:

Table 1. Main studies that demonstrate the applications of mRNA technology.

Authorship/ Year	Title	Data base	Kind of study	Results
YOU et al., 2023[52]	mRNA delivered intradermally encapsulated in extracellular vesicles for collagen replacement therapy	Pubmed	Experimental study in a murine model	Intradermal injection of mRNA encapsulated in extracellular vesicles derived from mesenchymal stem cells significantly increased collagen production in the skin of treated mice compared to controls. The research team also noted that treated skin had increased tensile strength and improvements in skin appearance.
ALDRICH et al., 2021[54]	Demonstration of concept of a low-dose, unmodified mRNA-based rabies vaccine formulated with lipid nanoparticles in human volunteers: a phase 1 trial	Science Direct	Multicenter, non-randomized, open-label, controlled, dose-escalating phase 1 study	The results of this study indicated that a low-dose, unmodified mRNA-based rabies vaccine formulated with lipid nanoparticles is safe and well tolerated in human volunteers. Furthermore, vaccination resulted in robust immune responses, including the production of rabies-specific antibodies. The researchers also noted that the vaccine dose can be reduced compared to traditional vaccines and still induce a protective immune response.
CAO et al., 2021[55]	mRNA therapy restores euglycemia and prevents liver tumors in a murine model of glycogen storage disease	Pubmed	Experimental study in a murine model	Statistical analyzes used to compare the results obtained in different groups of animals are described, as well as the procedures performed to measure blood glucose levels, pro-inflammatory cytokines and alanine aminotransferase. Efficacy and safety studies of the therapy are also mentioned, as well as the conclusion that repeated administration of mRNA for GSD1a appears to be well tolerated and effective in improving both fasting tolerance and liver damage.
HUANG et al., 2022[56]	Intracellular delivery of RNA to macrophages	Science Direct	In vitro and in vivo study	The results obtained indicated that the ideal formulation presented high mRNA delivery

	with surfactant-derived lipid nanoparticles			efficiency, stability and low toxicity in human cells and red blood cells. The authors conclude that the new mRNA delivery platform developed in this study offers a promising alternative to electroporation for the intracellular delivery of mRNA to macrophages and other difficult-to-transfect cells.
CHEN et al., 2020[57]	Treatment of hemophilia A using Factor VIII mRNA lipid nanoparticles	Pubmed	Experimental study in a murine model	The text presents different results of scientific studies on molecular therapy with nucleic acids, in particular with the use of mRNA. Some of the results presented include: lipid nanoparticles are efficiently delivered to the liver: mRNA therapy improves metabolic and behavioral abnormalities in a murine model of citrin deficiency; therapeutic efficacy in a model of hemophilia B using a biosynthetic mRNA hepatic depot system; repeated infusions of mRNA with lipid nanoparticles did not induce any detectable liver damage, indicating that they were biocompatible; hFVIII antigen levels introduced by FVIII-LNPs in mice were evaluated using a modified clotting assay using aPTT reagent and FVIII-deficient plasma.
TANG et al., 2021[49]	Self-assembled small mRNA nanospheres for efficient therapeutic expression of apoptin and synergistic gene chemotherapy for breast cancer	Science Direct	Experimental study with animals and cells in vitro	MRNA nanospheres were able to enhance the expression of the therapeutic protein apoptin and exhibit excellent synergistic antitumor effect after Dox loading. - Drug synergy was analyzed using the CompuSyn program, and the results indicated that the combination of mRNA-NSs@Dox had a synergistic effect in the treatment of breast cancer. - In experiments with mice, the group treated with mRNA-NSs@Dox showed the slower tumor growth rate and was obviously inhibited compared to the group treated with free Dox. - In vitro experiments showed that T1 cells treated with mRNA-NSs@Doxshowed greater inhibition of cell growth and greater induction of apoptosis than cells treated with free Dox. - Western blotting experiments showed that T1 cells treated with mRNA-NSs@Dox showed higher expression of proteins associated with apoptosis (apoptin, Bax and Caspase 3) than cells treated with free Dox. - Pathological analysis experiments showed that treatment with mRNA-NSs@Dox resulted in less cell proliferation and greater apoptosis in mouse tumors
GUPTA & GLUECK, 2022[58]	VACCINE NCOV-2019 (SARS-COV-2) - BR 112022016346-0 A2	INPI	Patent	The text is a patent application that describes an invention related to a vaccine for the prevention and treatment of NCOV-2019 (SARS-COV-2) infection, using polynucleotides that encode the spike protein of the virus, as well as antibodies and vaccines. The patent describes various ways of making and using the spike protein or fragments thereof to induce an immune response and produce neutralizing antibodies against the virus. The patent also describes several modifications that can be made to the spike protein to increase its effectiveness against different variants and strains of the virus. In addition, the patent describes the possibility of producing VLPs (virus-like particles) that present antigens or immunogenic fragments of the virus's spike protein.

KRIENKE et al., 2021[59]	A non-inflammatory mRNA vaccine for the treatment of experimental autoimmune encephalomyelitis	Pubmed	Experimental study in a murine model	The paper discusses the development of an mRNA-based therapy to treat Experimental Autoimmune Encephalomyelitis (EAE), a mouse model of Multiple Sclerosis (MS). The therapy was able to induce proliferation of both endogenous and transferred T cells, with m1Y mRNA being superior. Treatment with m1Y mRNA was able to expand or induce regulatory T cells in wild-type C57BL/6 and 2D2-Foxp3-eGFP transgenic mice, while overall T cell frequencies did not change. The treatment effect is associated with a reduction in effector T cells and the development of regulatory T cells. The therapy was also effective in a model of EAE, a disease similar to MS in humans. The approach has potential for clinical translation,
SILVA et al., 2023[25]	Single immunizations of mRNA vaccines with nanoparticles self-amplifying or non-replicating control HPV-associated tumors in mice	Pubmed	Experimental study in a murine model	The results show that mRNA-LNP vaccines induce potent immune responses and control tumor progression, even in advanced stages of growth. Furthermore, the mRNA-LNP vaccine was more effective than other vaccines based on plasmid DNA and recombinant proteins. The study suggests that mRNA-LNP vaccines have potential as cancer immunotherapies and may be a more effective and affordable option to prevent HPV-related diseases. The discussion also highlights the importance of continued research into mRNA-LNP vaccines for the development of immunotherapeutic strategies against HPV-induced tumors. Additionally, the paper discusses the potential of mRNA vaccines in other areas, such as COVID-19 and influenza, and the need for further research in this area.
SHATTOCK, BLAKNEY, MCKAY, 2022[60]	RNA construction, nucleic acid sequence, expression cassette, recombinant vector, pharmaceutical composition, method for preparing RNA construction, vaccine, method for modifying a cell ex vivo or in vitro and modified cell (BR 112021024786-6 A2)	INPI	Patent	The text presents information about a patent related to RNA constructs that encode therapeutic biomolecules and innate inhibitory proteins (IIPs). These constructions are RNA replicons and saRNA molecules, and the invention includes genetic constructs or vectors that encode such RNA replicons. The invention encompasses the use of these constructs and RNA replicons in therapy, for example, in the treatment of diseases and/or in the delivery of vaccines. The invention encompasses pharmaceutical compositions comprising such RNA constructs, as well as methods and uses of such constructs. The text also discusses the advantages and disadvantages of different approaches to overcome innate exogenous RNA recognition and limitations associated with inducing interferon responses. However, there is no specific information about test results or experiments related to this patent.

In view of the studies reported above, one can understand the range of applications that mRNA technology can bring to medicine and influence the prognosis of different diseases.

5 DISCUSSION

From the results obtained in this research, using similar findings existing in the literature and comparing results and patents that refer to mRNA technology applied to the production of vaccines, as a milestone for the advent of new health treatments, in this session there will be a discussion to state of the art light.

The studies by Aldrich *et al*.[54], You et al.[52], Tang et al.[49]; Cao et al.[55], Chen et al.[57], Silva et al.[25], Krienke et al.[59] and Huang et al.[56] converged on the benefits of mRNA technology in therapies and vaccines. For Aldrich and collaborators[54] proved the potential of mRNA technology in the production of a vaccine against rabies in humans. The study involved a phase 1 human trial using an LNP-mRNA-based vaccine. The results indicated that the vaccine was safe and well tolerated by the volunteers, with positive immune responses. The application of mRNA technology has the advantage of allowing rapid production of vaccines in response to outbreaks of emerging infectious diseases, and the flexibility of the mRNA platform also allows vaccines to be adapted to different virus variants. Still according to the results of the work, mRNA technology is not limited to vaccination, but can also be used in other therapies, such as the production of therapeutic proteins. As mentioned by Sahin and collaborators[61], in a previous study,

At the same time, You et al.[52] explored the use of mRNA in the production of collagen, with the advantage of high specificity of the technology. The results of their study with mRNA encapsulated in extracellular vesicles led to efficient production of collagen in target cells and demonstrated good tolerance and safety in animal models. The researchers also highlighted the flexibility of mRNA technology, allowing vaccines and therapies to be quickly modified and adapted to different variants of infectious diseases and health conditions. Furthermore, mRNA therapy for collagen production may offer a promising alternative to traditional methods, such as collagen injections or surgery, which can have side effects.

In this same trend, Tang et al.[49] showed that self-assembled mRNA nanospheres were effective in delivering apoptin mRNA, which resulted in cell apoptosis and significantly reduced growth of breast tumors in mouse models. As mRNA technology has the potential to revolutionize therapy for cancer and other diseases, their study is an example of the effectiveness of this technology in delivering anti-cancer therapies. In addition, the authors emphasize that future studies should focus on developing better mRNA delivery systems, as well as identifying new mRNA-inspired therapies for a wide range of diseases. For mRNA technology offers the possibility of a new era in medicine, and it is important to continue to invest in its research and development to reach its full potential.

Cao et al.[55] used mRNA therapy in a murine model of glycogen storage disease, with satisfactory results in restoring euglycemia and preventing the development of liver tumors. In this study, mRNA was used to convert the expression of a deficient enzyme in murine models, generating the normalization of blood glucose levels and prevention of the development of liver disorders. These results hold promise for developing therapies for other rare metabolic diseases that pass through the liver and other organs.

Another recent study, by Wang et al.[30], is an excellent example of how mRNA technology can be applied in the treatment of rare genetic diseases. Hemophilia A is one such disease and affects the body's ability to clot blood. This can lead to both internal and external bleeding, and patients with hemophilia A usually need lifelong treatment with replacement therapy for Factor VIII, a protein responsible for blood clotting. The study used lipid nanoparticles to deliver the mRNA encoding Factor VIII directly into the patient's cells. The advantage of this approach is that it allows the patient's own body to produce Factor VIII at sufficient levels to restore proper blood clotting.

Silva et al.[25] report that mRNA-LNP vaccines are also being used for the treatment of tumors associated with human papillomavirus in mice. These vaccines, encapsulated in lipid nanoparticles, can be self-amplified, which increases the expression of the target protein and, consequently, the immune response. However, more research is needed to evaluate the safety and efficacy of mRNA-LNP vaccines in humans, as the study was only conducted in animals. It is also worth mentioning that the development of these vaccines is a complex and expensive process, which may be an obstacle to their wide adoption in clinical practice. In addition, there are long-term safety concerns, especially regarding the potential for adverse immunological effects and possible with other medical treatments. Therefore, it is critical that rigorous, large-scale clinical studies be conducted to assess the safety and efficacy of these vaccines in humans before they are widely accepted in clinical practice.

In addition, for Krienke and collaborators[59], mRNA technologies are also being explored as an alternative for the treatment of Experimental Autoimmune Encephalomyelitis (EAE), an inflammatory disease triggered by the immune system that affects the central nervous system and can cause permanent and disabling damage. . For this, the authors used the administration of non-inflammatory mRNA vaccines for EAE, with the aim of reducing inflammation and damage to the central nervous system in animal models, with encouraging results. Currently, available treatments for EAE are limited and often have unwanted side effects. Thus, the use of mRNA for the treatment of EAE represents a promising area of research,

While Huang et al.[56] addressed the use of mRNA in the production of surfactant, a substance produced by the lungs, whose function is to keep the alveoli open and facilitate breathing. The authors reported that administration of mRNA can stimulate surfactant production by type II cells of the alveolar epithelium, which may be beneficial for the treatment of several lung diseases. Research results indicated that intracellular delivery of surfactant-derived LNP-mRNA was effective in directing the production of target proteins in macrophages, important cells in the lung's immune response. This could be especially important for treating lung diseases associated with an immune attack, such as asthma and cystic fibrosis, which affect millions of people around the world.

In turn, Gupta & Glueck[58] in patent number BR 11 2022 016346 0, filed with the INPI,described a new strategy for the production of mRNA vaccines, using a polyenzyme(A) polymerase to add a poly(A) tail to the end of the mRNA molecule, increasing its stability and efficiency in translating the target protein. This strategy improved the immune response generated by the vaccine and allowed the production of large amounts of mRNA in a simpler and more efficient way. His patent application, on the other hand, described an invention related to a vaccine for the prevention and treatment of SARS-CoV-2 infection, using polynucleotides that encode the virus's spike protein. This patent mentioned various ways of making and using the protein or fragments thereof to induce an immune response and produce neutralizing antibodies against the virus. Furthermore, the patent described several modifications that can be made to the spike protein to increase its effectiveness against different variants and strains of the virus; also described the possibility of producing virus-like particles that present antigens or immunogenic fragments of the virus's spike protein. And even though the patent lays a solid foundation for developing a vaccine against SARS-CoV-2, more research and clinical trials are needed before a safe and effective vaccine can be made available to the public. Since it is important to remember that the effectiveness and safety of any vaccine must be proven through rigorous clinical trials before it can be approved for use in humans.

Finally, Shattock, Blakney and Mckay[60] registeredin patent number BR 112021024786-6 A2, filed with the INPI, an invention relating to mRNA constructs encoding at least one therapeutic biomolecule and an innate inhibitory protein (IIP). These patented constructs are RNA replicons and RNA tags, and the invention encompasses the use of these constructs in therapy and in the delivery of vaccines.

That said, despite the benefits of all the aforementioned research, it is important to highlight that mRNA technology still faces challenges regarding the safety and stability of therapeutic mRNA. However, mRNA technology has many advantages, such as high

specificity, rapid and large-scale production, and low risk of genomic integration, making it a promising area for the development of new therapies and vaccines, as stated by all authors.

6 CONCLUSION

The COVID-19 pandemic represents a major challenge for global public health, favoring the development of new technologies and approaches for the treatment of the disease. Among the most significant innovations, the mRNA vaccines stand out, which were designed in record time and used highly effectively in the prevention of COVID-19. Furthermore, the mass production of these vaccines allows them to become accessible around the world, fortunately for reducing the impact of the pandemic.

Among the previously presented results, the versatility of mRNA technology applications becomes evident, as it can be used in the prophylaxis and treatment of diseases. Furthermore, as new diseases or mutations emerge, mRNA technology can be quickly adapted to create new treatments due to its ability to encode any protein.

However, large-scale studies are still needed to assess the safety, efficacy, and adverse effects of mRNA technology in humans. While data from early clinical trials is promising, it is important to continue to carefully monitor and evaluate the side effects and long-term effectiveness of mRNA technology in large-scale studies. Safety and efficacy in humans are crucial issues that must be addressed before broad clinical application of mRNA technology.

It is important that investments in research and development of new treatment and prevention technologies continue to ensure that we can meet the health challenges of the future.

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