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Application of mRNA technology in vaccines against pandemic pathogens - present and futurey

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Abstract

MRNA vaccines have become an important resource in the fight against the COVID-19 pandemic, caused by the SARS-Cov2 virus. This article presents the history of vaccines against various pathogens, as well as the history of mRNA technology and its current application. It also points to the development of mRNA vaccines in the near future. This technology appears to be a milestone in global vaccinology.

Vaccine

Vaccination is one of the greatest achievements of civilisation in the fight against infectious diseases, including those causing epidemics and pandemics. The milestones in vaccinology were the invention of a smallpox vaccine by Edward Jenner (1796), rabies and cholera vaccines by Louis Pasteur (1885), and a vaccine against poliomyelitis virus (1952).[1,2,3,4,5]

The timetable for the introduction of new vaccines is shown in Table 1.[3,4]

Table 1. The history of vaccines[3, 4]

Year	Vaccine
1880	First vaccine for cholera by Louis Pasteur
1885	First vaccine for rabies by Louis Pasteur and Émile Roux
1890	First vaccine for tetanus (serum antitoxin) by Emil von Behring
1896	First vaccine for typhoid fever by Almroth Edward Wright, Richard Pfeiffer, and Wilhelm Kolle
1897	First vaccine for bubonic plague by Waldemar Haffkine
1921	First vaccine for tuberculosis by Albert Calmette
1923	First vaccine for diphtheria by Gaston Ramon, Emil von Behring and Kitasato Shibasaburō
1924	First vaccine for scarlet fever by George F. Dick and Gladys Dick
1924	First inactive vaccine for tetanus (tetanus toxoid, TT) by Gaston Ramon, C. Zoeller and P. Descombey
1926	First vaccine for pertussis (whooping cough) by Leila Denmark
1932	First vaccine for yellow fever by Max Theiler and Jean Laigret
1937	First vaccine for typhus by Rudolf Weigl, Ludwik Fleck and Hans Zinsser
1937	First vaccine for influenza by Anatol Smorodintsev
1941	First vaccine for tick-borne encephalitis
1952	First vaccine for polio (Salk vaccine)
1954	First vaccine for Japanese encephalitis
1954	First vaccine for anthrax
1957	First vaccine for adenovirus-4 and 7
1962	First oral polio vaccine (Sabin vaccine)
1963	First vaccine for measles
1967	First vaccine for mumps
1970	First vaccine for rubella
1977	First vaccine for pneumonia (Streptococcus pneumoniae)
1978	First vaccine for meningitis (Neisseria meningitidis)
1980	Smallpox declared eradicated worldwide due to vaccination efforts
1981	First vaccine for hepatitis B (first vaccine to target a cause of cancer)
1984	First vaccine for chickenpox
1985	First vaccine for Haemophilus influenzae type b (HiB)
1989	First vaccine for Q fever
1990	First vaccine for Hantavirus hemorrhagic fever with renal syndrome
1991	First vaccine for hepatitis A
1998	First vaccine for Lyme disease
1998	First vaccine for rotavirus
2003	First nasal influenza vaccine approved in U.S. (FluMist)
2003	First vaccine for Argentine hemorrhagic fever.
2006	First vaccine for human papillomavirus (which is a cause of cervical cancer)
2012	First vaccine for hepatitis E
2012	First quadrivalent (4-strain) influenza vaccine
2013	First vaccine for enterovirus 71, one cause of hand foot mouth disease
2015	First vaccine for malaria
2015	First vaccine for dengue fever
2019	First vaccine for Ebola approved
2020	First vaccine for COVID-19

Despite the many successes related to the effectiveness of vaccination, the world faces many problems due to the lack of control of many previously known pathogens, as well as the emergence of new ones that pose a threat to human health and life.[6,7,8,9,10]

The pathogens that currently pose the greatest risk to human health

To this date, the infectious diseases such as malaria, tuberculosis and influenza have not been controlled.[6,7]

Since the 1980s, HIV, HCV, viral haemorrhagic fever, prion diseases and infections caused by drug-resistant bacterial strains and, more recently, the COVID-19 pandemic caused by SARS-Cov2 have become public health problems.

The increase in the number of types of disease is influenced by the growth in the world's population, non-compliance with the principles of hygiene in the developing countries, population migration and the increase in the resistance of bacteria to antibiotics.

It should not be forgotten that some of the pathogens mentioned may, according to the current definition, be treated as biological weapons. Bioterrorism is defined as the unlawful, illegal use of biological agents against human beings with the intention of coercing some action or intimidating a government, the civilian population or any part of it in order to achieve personal, political, social or religious goals.[8]

WHO indicates health risks resulting from the possibility of spreading the following infections: Chikungunya, Cholera, Crimean-Congo hemorrhagic fever, Ebola virus disease, Hendra virus infection, Influenza (pandemic, seasonal, zoonotic), Lassa fever, Marburg virus disease, MERS-CoV, Monkeypox, Nipah virus infection, Novel coronavirus, Plague, Rift Valley fever, SARS, Smallpox, Tularaemia, Yellow fever, Zika virus disease.[9-18]

Among the threats identified, viral infections deserve special attention, including infections caused by haemorrhagic fever viruses (VHF), which represent a diverse group of animal and human diseases.

VHF can be caused by five different families of RNA viruses: the families Filoviridae, Flaviviridae, Rhabdoviridae and several families of members of the Bunyavirales order, such as Arenaviridae and Hantaviridae Some VHF agents cause relatively mild illness, such as Scandinavian nephropathy epidemia (hantavirus), while others, such as Ebola virus, can cause severe, life-threatening disease.

It is important to remember that after decline infections resulting from various pathogens health problems for a significant proportion of the population (malaria and tuberculosis are examples). Ministry of Health of the Democratic Republic of Congo declared an outbreak of Ebola Virus Disease (EVD) on 7 February 2021 after the laboratory confirmation of one case in Butembo, North Kivu Province.[15] Genetic sequencing analysis indicates that the outbreak is linked to the two-year long outbreak that took place in North Kivu and Ituri provinces from 2018 to 2020, but the infection source is yet to be determined.

The response was coordinated by the Provincial Health Department in collaboration with WHO and partners. WHO employed nearly 60 experts on the ground, and once the outbreak was declared, helped local staff to track contacts, provide treatment, engage communities and vaccinate nearly 2,000 high-risk people, including more than 500 frontline workers. Eleven confirmed cases and one probable case, six deaths and six recoveries were recorded in four health zones.

WHO continues to work with the Democratic Republic of Congo to combat other public health problems, such as measles and cholera outbreaks, the COVID-19 pandemic and an inefficient health system.

Coronavirus Diseases including SARS-Cov2 infection[19,20,21,22,23,24]

The first cases of coronavirus infection with severe respiratory distress (Severe Acute Respiratory Syndrome - SARS) were identified in Feb 2003 in China, followed by 26 other countries, infecting 8096 people, with 774 deaths.

It is thought that Severe Acute Respiratory Syndrome probably started with bats, spread to cats and then to humans.

SARS is characterised by breathing problems, dry cough, fever, head and body aches and is spread through respiratory droplets from coughing and sneezing.

Quarantine efforts proved successful and by July 2003, the SARS virus was contained and has not reappeared since.

SARS was seen by the global health professionals as a wake-up call to improve the outbreak response, and lessons from the pandemic were used to control diseases such as H1N1, Ebola and Zika.

On 11 March 2020, the World Health Organisation announced that the COVID-19 virus was officially a pandemic after it swept through 114 countries in three months and infected more than 118,000 people. And the spread was hardly over.

COVID-19 is caused by a new coronavirus - a new strain of coronavirus that has not previously been detected in humans. The symptoms include breathing problems, fever and cough and can lead to pneumonia and death. Like SARS, it is spread through droplets from sneezing.

The first reported case in China appeared on 17 Nov 2019 in Hubei province, but remained unrecognised. Eight more cases emerged in Dec 2019, with researchers pointing to an unknown virus.

Many learned about COVID-19 when ophthalmologist Dr Li Wenliang defied government orders and shared safety information with other doctors. Li died from COVID-19 just over a month later.

Without an available vaccine, the virus has spread beyond China to almost every country in the world. By December 2020, it had infected more than 75 million people and led to more than 1.6 million deaths worldwide. The number of new cases was growing faster than ever, with an average of more than 500 000 reported each day.

Worldwide, by 16:00 CEST, 29 December 2021, there were 281,808,270 confirmed cases of COVID-19, including 5,411,759 deaths reported to WHO. As of 28 December 2021, a total of 8,687,201,202 doses of vaccine have been administered (accessed at https://covid19.who.int/).

Patients who have recovered from acute SARS-CoV-2 infection often have persistent symptoms lasting several months.[25, 26] Long COVID is defined as the persistence or development of symptoms 4 weeks after illness onset, when testing for replication-competent SARS-CoV-2 has been negative for at least 1 week. Its epidemiology remains incompletely understood. Numbers vary from study to study, but several North American and European studies have reported a prevalence of long COVID of between 30 and 90% at 6 month. Given the scale of the COVID-19 pandemic, with currently over 150 million reported cases, long COVID may be emerging as a huge global medical and public health problem.[25, 26]

The world of science is currently looking for solutions to the following issues related to COVID-19[27]:

- Burden of disease
- New Variants of SARS-Cov2 (mutations in the SARS-CoV-2 genome)
- Modes of transmission
- Period of infectiousness
- Immune response and risk of reinfection
- Personal preventive measures
- Quarantine
- Vaccines
- Pre-exposure prophylaxis
- Post-exposure prophylaxis
- Public health guidance

Vaccines

Various technologies are now known that can be used to induce the desired immune response, including[28,29]:

- Live Attenuated Vaccines (e.g. influenza, MMR)
- Inactivated Vaccines (e.g. Hepatitis A, Polio)
- Viral Vectored Vaccines (e.g. Ebola vaccine)
- Recombinant-protein Vaccines (HPV)

Classical vaccination involves the delivery of ready-made antigens in the form of weakened (attenuated) microorganisms ("live"), inactivated (killed) pathogens or their isolated proteins (subunit vaccines). The mRNA of vaccines or genetically modified viral vectors is intended to deliver the genetic material encoding the desired antigen of a pathogenic microorganism ('vaccine' antigen) to the cells of the vaccinated person and to produce this antigen by the host cells. In this way it provides a precise 'recipe' for the antigen, but does not contain any ready-made protein or microorganism.

Recently, especially in COVID-19, the role of vaccination, including vaccination leading to herd immunity, has increased significantly. At the same time, the scientific world faced a huge challenge in 2020 in the form of a virus that emerged de novo humans.

While vaccine manufacturers were able to rapidly change the production lines during the influenza A (H1N1) pandemic, there are complex technological issues associated with COVID-19.

Vaccines to prevent SARS-CoV-2 infection are considered the most promising approach to contain the pandemic. By the end of 2020, several vaccines had become available for use in different parts of the world, more than 40 vaccine candidates were in human trials and more than 150 were in preclinical studies. The World Health Organization maintains an updated list of evaluated vaccine candidates. [30]

mRNA history

Years of intensive work by many scientists have led to the development of mRNA vaccines (https://cihr-irsc.gc.ca/e/52424.html).

Of the many scientists who have contributed to the development of COVID-19 vaccines, the most common are: Robert Malone, Pieter Cullis, Derrick Rossi, Kizzmekia Corbett, Drew Weissman and Katalin Karikó.

Messenger RNA (mRNA) is now considered a new category of therapeutic agents for the prevention and treatment of various diseases.[31,32,33] One of the most important factors associated with proper, safe, effective mRNA activity are stable delivery systems that protect the nucleic acid from degradation and enable cellular uptake and release of mRNA. Lipid nanoparticles have successfully entered the clinic for mRNA delivery.

Therefore, the milestones that led to the development of vaccines with mRNA against COVID-19 include both the discovery of the mRNA itself and the nanoparticle system (Table 2).

Table 2. Key milestones for mRNA and lipid nanoparticles discovery[31,32,33]

Year	Milestone
1961	mRNA discovery
1965	Development of liposomes
1978	Development of liposome-mRNA formulation
1989	Development of cationic LNP-mRNA formulation
1993	Development of liposome-mRNA formulations as influenza vaccine
2014-2017	Clinical trial of LNP-mRNA formulations for cancer immunotherapy, clinical trials of LNP -mRNA formulation as influenza vaccines
2018	Onpattro LNPs, encapsulations siRNA, approved by the FDA and EMA)
2020	Clinical trial of LNP formulation delivering gene-editing components for genetic disorders;
2020	mRNA-1273 and BNT 1626 (LNP-mRNA Formulations Covid-19 mRNA vaccine obtained authorisation from regulatory agencies in multiple countries

mRNA vaccines -technological processes

RNA vaccines were the first SARS-CoV-2 vaccines to be produced and represent a completely new approach to vaccines .[31] After administration, the RNA is translated into a target protein to induce an immune response.[31, 34, 35, 36]

These vaccines are produced completely in vitro, which makes the production easier. However, some vaccines must be maintained at very low temperatures, making storage difficult.

The mRNA vaccines encode the full length SARS-CoV-2 spearhead protein (S). The genetic information is transcribed in a tube from linear acidic DNA into RNA using the enzyme RNA polymerase. The resulting RNA is enzymatically modified to contain elements of natural mRNA molecules - a guanylyl cap at the 5' end of the strand and a polyA sequence at the 3' end. As a result, the preparation corresponds to fully mature cellular mRNA molecules that serve the cell as a matrix for protein synthesis. The mRNA is purified from other reaction components and then packaged in carrier lipid nanoparticles (NLP). The production process does not use cell cultures, is fast and very efficient.

The following technological developments have improved the properties of mRNA vaccines:

- Replacement of uridine-containing nucleoside with pseudouridines (improving the stability of the molecule)
- The cap of nucleosides G and A stabilizes the molecule and initiates translation UTR5 region - regulatory function (defines the target number of translations)
- Alpha globulin is produced quickly and in large amounts
- The amount of guanine and cytosine improves translation
- The embedded proline recreates the shape of the viral S proteinUTR-3 stabilizes mRNA
- Poly A - «tail» is responsible for the rate of degradation (detaching some adenosines after each translation).

The molecules mainly penetrate muscle cells and antigen presenting cells (APC, e.g. dendritic cells) at the injection site. Immediately after mRNA release into the cytoplasm, protein biosynthesis occurs on the delivered mRNA matrix (vaccine mRNA does not penetrate the cell nucleus). The proteins produced are post-translationally modified and transported according to their biochemical properties.

Shortly after translation, the mRNA molecule is degraded by a cytoplasmic ribonuclease naturally found in human cells.

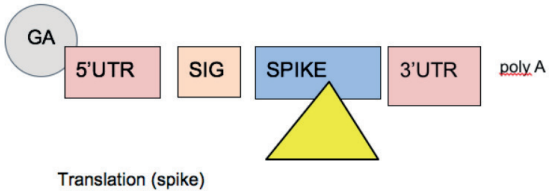

Fig. 1 The Covid-19 mRNA vaccine is constructed from a messenger RNA that contains instructions for synthesizing the coronavirus spike protein[31]

However, mRNA-based vaccine has many advantages but also some disadvantages.[34, Table 3]

The pros of mRNA include:

- the technology underlying mRNA vaccines is flexible, allowing for rapid updates as new virus mutations (variants) evolve or new viruses are discovered. Because mRNA vaccines are based on viral protein sequences, preparing a new vaccine can simply involve changing the mRNA sequence if you know what protein you want to make
- mRNA vaccines are also produced faster and more reliably than traditional vaccines. In the case of Moderna, the entire process - from vaccine design to production to shipping - took just 7 weeks. While mRNA vaccines may only take weeks to design and manufacture, the necessary clinical trials to assess safety and efficacy still require several months of testing.

The cons of mRNA vaccines include:

- despite the benefits of mRNA vaccines, there are still risks and unknowns
- they are not as stable at high temperatures, making packaging and distribution more difficult
- long-term storage and delivery of vaccines requires global investment in infrastructure, staff training and last mile coordination
- long-term effects are still unknown

Table 3. Advantages and disadvantages of viral vectored vaccines, DNA vaccines and RNA vaccines (according to Zhang et al., 34)

Vaccines	Advantages	Disadvantages
Viral vectored vaccines	Stimulation of innate immune response; induction of T and B cell immune response.	Induction of anti-vector immunity: cell based manufacturing
DNA vaccines	Non-infectious; stimulation of innate immune response; egg and cell free; stable, rapid and scalable production; induction of T and B cell immune response.	Potential integration into human genome; poor immunogenicity in humans.
RNA vaccines	Non-infectious, non-integrating, natural degradation, egg and cell free, rapid and scalable production; stimulation of innate immune response; induction of T and B cell immune response	Concerns with instability and low immunogenicity

mRNA vaccines in clinical practice

The following m RNA vaccines are currently available in Europe and the United States[37]:

1. the COVID-19 mRNA vaccine BNT162b2 (Pfizer-BioNTech COVID-19 vaccine) has been approved by the Food and Drug Administration (FDA). BNT162b2 (Pfizer-BioNTech COVID-19 vaccine), which is indicated for individuals aged 5 years or older
2. the COVID-19 mRNA vaccine mRNA-1273 (Moderna COVID-19 vaccine) mRNA-1273 (Moderna COVID-19 vaccine), which is indicated for individuals aged 18 years or older

As with other vaccines, particular attention should be paid to immunogenicity, efficacy and safety.

Due to the relatively short follow-up time, there are dilemmas in the use of mRNA vaccines in clinical practice, including:

- Booster doses
- Deviations from dosing recommendations
- Expected side effects
- Contraindications and precautions
- Impact of new variants on vaccine efficacy

It is important to stress that reluctance to vaccinate is a major obstacle to achieving vaccination coverage that is broad enough to result in herd immunity and slow transmission in the community.

According to WHO data from January 30, 2022, 52.4% of the population are fully vaccinated globally, including 56.96% in Poland (https://covid19.who.int/table).

mRNA vaccines beyond COVID-19

We appear to be witnessing the development of an exciting mRNA technology relevant to current and future vaccinology.[34,38,39]

Thanks to joint efforts of governments, funding agencies, academia, biotech and pharmaceutical companies, large-scale drug production is becoming a reality. The success of Moderna's and Pfizer/BioNTech's COVID-19 vaccines has helped to revitalise the ongoing mRNA research.

Both mRNA and self-amplifying RNA have shown potential as vaccines against a range of infectious diseases, including influenza, respiratory syncytial virus, rabies, Ebola virus, malaria and HIV-1. Combined with therapeutic applications, particularly as immunotherapy for cancer, mRNA technologies will continue to improve and expand as an integral part of future drug development.

Moderna is developing vaccines against viral diseases, mainly epidemic and pandemic, which are being worked on in collaboration with governments and non-profit organisations. The company is also researching vaccines against common infections such as those caused by cytomegalovirus (CMV: mRNA-1647), Epstein-Barr virus (EBV: mRNA-1189), Zika virus (mRNA-1893) and those that cause respiratory tract infections (COVID -19: mRNA-1273, respiratory syncytial virus (RSV: mRNA-1345), human metapneumovirus (hMPV), parainfluenza type 3 (PIV3: mRNA-1653) and pandemic / H7N9 influenza (mRNA-1851).

Conclusions

The COVID-19 pandemic has shown the importance of developing new antiviral vaccines. Therefore, this publication shows the history of vaccine development in the world, beginning with the first vaccines against cholera and rabies. Given the persistent pandemic and the mutations of the SARS-Cov2 virus, the progress made in the development of anti-COVID-19 vaccine should be constantly monitored. It is an important part of public health in every country, including Poland.

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