Nucleic Acid Vaccines for COVID-19

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Nucleic acid vaccines employ genetic material from a pathogen, such as a virus or bacteria, to induce an immune response against it. Based on the vaccination, the genetic material might be DNA or RNA; as such, it offers instructions for producing a specific pathogen protein that the immune system will perceive as foreign and mount an immune response. Nucleic acid vaccines for multiple antigens might be made in the same facility, lowering costs even more. Most traditional vaccine regimens do not allow for this. Nucleic acid vaccines could also be applied to COVID-19.

Keywords: mRNA ; COVID-19 ; DNA ; nucleic acid ; SARS-CoV-2 ; vaccine ; coronavirus

1. Introduction

A vaccine is the best approach for infectious disease prevention [1]. The first effective vaccine developed was against the smallpox virus in the form of live attenuated, and, through worldwide mass vaccinations, smallpox was declared eradicated [2]. Pasteur and his team coined the idea of attenuation and defended its use with pasteurella multocida, responsible for diarrhea in chickens. Later, anthrax vaccine in sheep and rabies vaccine in animals and humans were developed using the attenuation approach [3]. The concept of inactivation of a pathogen without losing immunogenicity was devised at the end of the 19th century. Later, various inactivated (killed, dead) vaccines were formulated against typhoid, cholera and plague diseases. Over time, various novel technology-based vaccines have been developed, such as diphtheria toxoid and recombinant technology-based hepatitis B vaccine [4]. Despite the success, vaccine development is still a challenging task as scale-up productions and funding resources have commonly hindered much of their development. This process is usually challenging, complex and takes 15–25 years for the final approved product, and by then, the patent life would have ended. It often requires expenses of more than $500 million. Pre-COVID-19 era, only 7% of the vaccines developed progressed to human clinical trials from pre-clinical studies [5]. Additionally, of those in clinical trials, only 20% show safety and efficacy [6]. Other considerations required for vaccine development are stability, storage conditions, number of injections, route of immunization, optimal dose, scale-up, manufacturing and distribution of the vaccine globally.

The novel coronavirus strain, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) , emerged from Wuhan, China, in late December 2019 [7][8]. Rapidly, it spread around the globe and was declared a pandemic soon after [9].

In parallel, there was and is a worldwide race for vaccine discovery. A number of different modes of vaccine formulations have been developed at an unprecedented speed [4][10], such as live attenuated, inactivated (dead), subunit, viral vector and genetic (nucleic acid), against the SARS-CoV-2 virus [11][12][13]. Currently, seven vaccines using three different approaches have been approved by the WHO for emergency authority use. Generally, the vaccine development process requires 15–25 years for approval. However, COVID-19 vaccines have been approved within 6–9 months in an accelerated development process in response to the pandemic situation [14]. In fact, the speed has been possible due to the method used for vaccine development in the instance of nucleic acid-based vaccines. These vaccine formulations have been around for over 30 years and tested in humans for a number of diseases, such as cancer, HIV and other viruses, but none have been approved for human use. However, the pandemic has enabled their use to be fast tracked, a technology that instigates revolution in the vaccine development process and overcomes the limitations of previously available technologies [15]. It involves the administration of nucleic acid coding for antigens in the body. This nucleic acid coding acts as a set of instructions and guides the host cells to produce antigenic proteins, which, subsequently, stimulate specific immune responses against the gene-delivered antigen. Thus, the nucleic acid vaccine immunizes the host against the specific pathogen [10].

2. Advantages of Nucleic Acid-Based Vaccines

Upon immunization, nucleic acid-based vaccines imitate a viral infection, causing vaccine antigens to be expressed in situ, which tends to result in the initiation of both humoral and cytotoxic T-cell responses [16]. Nucleic acid-based anti-SARS-CoV-2 vaccines may have advantages over traditional vaccines for the following reasons: (i) The high potency of mRNA vaccines is capable of generating potent antiviral neutralizing antibodies by activating both CD4+ and CD8+ T-cells with only one or two low-dose immunizations [17][18]. (ii) The structural modification of mRNA results in higher immunogenicity by improving its stability and translation efficacy [17]. (iii) Because of its degradation process in cells, mRNA-based vaccines reduce the risk of infection and insertion-induced mutagenesis [19]. (iv) These vaccines are easier to design in roughly a day and are also easy to produce on a large scale [20]. (v) Nucleic acid vaccine candidates generate

strong protection via antibody generation and activating the cell-based immune pathways [21]. (vi) These vaccine candidates, particularly DNA vaccines, have a great amount of shelf-life stability and elicit a potent immune response [22][23]. (vii) Considering the pandemic situation and global vaccine demands to eradicate the same, these vaccines can be scaled up and mass-produced easily as compared to the conventional vaccine formulations [23]. These vaccine candidates have demonstrated sound immune response with potential efficacy against the novel coronavirus in clinical trials thus far.

3. Challenges for Nucleic Acid Vaccine Development

Vaccines comprising nucleic acids, such as DNA in the form of plasmids and RNA in the form of mRNA, have received significant attention in recent years in the generation of new, improved vaccines [24][25][26]. DNA vaccines contain genes that are delivered to the host either as 'naked' DNA or via a plasmid or vector. On the other hand, mRNA-based vaccines comprise mRNA that encodes a protein and are either non-replicating mRNA, in vivo self-replicating mRNA or, in vitro dendritic cell non-replicating mRNA [27][28]. Nucleic acid vaccines exhibit significant advantages over traditional vaccines in terms of efficacy, safety and induction of both arms of immunity, humoral and cell-mediated immune responses [29][22][30].

However, both DNA and mRNA-based vaccines are associated with several challenges that need to be addressed during vaccine development to ensure their quality and effectiveness.

○ DNA vaccines can change the genetic composition of the host. DNA vaccines are delivered into the nucleus of the cell and transcribed into mRNA, which enters the cytoplasm, and the cells make the antigen. As such, DNA vaccines are associated with the risk of altering the genetic makeup of the host cell permanently (insertional mutagenesis). mRNA-based vaccines do not pose this risk as they do not enter the nucleus [30][31].

○ Naked DNA has low immunogenicity, and it is essential to include vectors, adjuvants and appropriate delivery methods to increase its immunogenicity.

○ DNA vaccines are relatively cheap to produce compared to protein-based vaccines and are stable, making them viable for storage and worldwide distribution. The challenge for DNA-based vaccines is their poor immunogenicity, often requiring multiple booster injections.

○ mRNA needs to cross the cell membrane to enter the cytoplasm. This is challenging due to its extremely large size, the negative charge of the molecule and degradability. Manufacturing clinical-grade mRNA is also a challenging task.

○ With the emergence of the recent COVID-19 pandemic, a significant number of DNA (26 candidates) and mRNA (35 candidates) vaccines (**Table 1**) are in preclinical and clinical trials despite these limitations, and eventually, two vaccines have been approved from the mRNA platform for mass application [32][33], and one from DNA platform, i.e., ZycovD (Zydus Cadila, India), has been approved [34].

○ The Pfizer-BioNTech and Moderna vaccines have demonstrated very good efficacy and safety in human trials, despite the evidence of increased risk of blood clots in a small number of subjects. However, the long-term safety, vaccine stability and efficacy still need to be established for this platform and is a subject matter of future studies.

○ The efficacy of nucleic acid-based vaccines is hindered by viral mutations, and the approved mRNA vaccines have demonstrated variable reduced efficacy against these mutant strains as compared with the efficacy against original non-mutated strains [32][33]. As viruses are known to mutate, mutations will continue with the SARS-CoV-2 virus, and hence, constant modifications of the vaccine are required to be effective against the new variants.

○ Some studies have shown that mutations in the target proteins of the SARS-CoV-2 virus may lead to the development of drug and vaccine resistance and eventually lead to vaccine in-efficacy.

○ The mRNA-based vaccines have the advantage of being stable, cost-effective, easy to make, and there are no requirements of purification steps that are commonly used for protein-based vaccines. However, it requires ultra-cold storage limiting its worldwide distribution, and a few booster shots may be required to generate appropriate immunity [35][36][37].

○ The elevated immune response induced by mRNA in the cytoplasm might cause cells to secrete greater portions of type-I IFN and other interferons, which can inhibit mRNA translation and inevitably lead to translational stagnation, RNA degradation, reduced activation of CD8 (cluster of differentiation 8) + T-cells and ultimately immune response cessation [38][29][39].

Table 1. Nucleic acid-based vaccine candidates for SARS-CoV-2 in different stages of clinical development.

Vaccine Name	Innovator/Country	Vaccine Platform	Vaccine-Triggered Immune Response	Stage of Clinical Development	Clinical Trial ID Number (https://covid19.trackvaccines.org/vaccines/79/) — Accessed on 18 August 2021)
mRNA-1273	Moderna/USA	RNA-based	Post-administration of mRNA vaccine, there is a trigger of type-1 interferon production, which subsequently promotes the Th1 response that is the characteristic of actual viral infection [40][41][42]. The innate immune responses from helper T-cells prime both CD8+ and CD4+ T cells to differentiate into effector and memory subsets [43].	Emergency use approved (EUA) in 72 Countries. This vaccine is also manufactured by Takeda (TAK-919)	PHASE 1: NCT04813796, NCT04785144, NCT04839315, NCT04889209, NCT04283461
					PHASE 2: ISRCTN73765130 NCT04847050, NCT04889209, NCT04649151, NCT04748471, NCT04761822, and NCT04405076 NCT04894435 NCT04796896
					PHASE 3: NCT04860297, NCT04649151, NCT04811664, and NCT04470427 NCT04796896 NCT04805125 NCT04806113
BNT162b2 (Tozinameran, Comirnaty)	Pfizer & BioNTech/USA	RNA-based (Encodes a prefusion stabilized, membrane-anchored SARS-CoV-2 full-length Spike protein)	The mRNA serves as an antigen as well as an adjuvant that will stimulate both adaptive and innate immune responses, respectively. Toll-like receptor 7 (TLR7) and melanoma differentiation-associated 5 (MDA5) are triggered by mRNA, which stimulates S-protein-specific naive T-cells, which become activated and differentiated into effector cells to form cytotoxic T-lymphocytes or helper T-cells. Strong Th1 cell response helps in antibody-secreting plasma cells. Stimulation of the type-1 interferon also aids in T-cell memory [44][43][45][46]	EUA in 99 countries.	PHASE 1: EUCTR2020-001038-36, and NCT04380701 NCT04839315, NCT04889209, NCT04816643 NCT04588480
					PHASE 2: ISRCTN73765130 and ISRCTN69254139 EUCTR2020-001038-36, and NCT04380701 NCT04368728 NCT04894435 NCT04889209, NCT04761822 and NCT04754594 NCT04824638 NCT04860739 and EUCTR2021-001978-37 NCT04649021 NCT04588480
					PHASE 3: NCT04368728 NCT04805125 NCT04800133 NCT04816669, NCT04713553, and NCT04754594

Vaccine Name	Innovator/Country	Vaccine Platform	Vaccine-Triggered Immune Response	Stage of Clinical Development	Clinical Trial ID Number (https://covid19.trackvaccines.org/vaccines/79/) — Accessed on 18 August 2021)
TAK—919 (Moderna formulation)	Takeda/Japan	RNA-based	Post-administration of mRNA vaccine, there is a trigger of type-1 interferon production, which subsequently promotes the Th1 response that is the characteristic of actual viral infection [40][41][42]. The innate immune responses from helper T-cells prime both CD8+ and CD4+ T cells to differentiate into effector and memory subsets [43].	EUA in 1 country.	PHASE 1 and PHASE 2: NCT04677660

Vaccine Name	Innovator/Country	Vaccine Platform	Vaccine-Triggered Immune Response	Stage of Clinical Development	Clinical Trial ID Number (https://covid19.trackvaccines.org/vaccines/79/) — Accessed on 18 August 2021)
mRNA	Walvax/China	RNA-based	Post-vaccination, the mRNA binds with TLR7 and MDA5, which triggers IFN1 production along with a strong Th1 cell response that helps in antibody-secreting plasma cells [44][43].	Under trials in 4 countries. Phase 1: 2 trials	PHASE 1: ChiCTR2000034112, and ChiCTR2000039212
				Phase 2: 1 trial	PHASE 2: ChiCTR2100041855
				Phase 3: 1 trial	PHASE 3: NCT04847102
CVnCov	Curevac/Germany	RNA-based		Under trials in 12 countries. Phase 1: 1 trials	PHASE 1: NCT04449276
				Phase 2: 4 trials	PHASE 2: ISRCTN73765130 2020-003998-22 NCT04652102 NCT04515147, PER-054-20
				Phase 3: 6 trials	PHASE 3: NCT04838847 and NCT04848467 NCT04860258 EUCTR2020-004066-19, and NCT04674189 and NCT04652102 2020-003998-22
BNT162b1	Pfizer & BioNTech/USA	RNA-based (Nucleoside-modified mRNA vaccine that encodes the trimerized receptor-binding domain)		Under trials in 5 countries. Phase 1: 2 trials	PHASE 1: EUCTR2020-001038-36, and NCT04380701 ChiCTR2000034825, and NCT04523571
				Phase 2: 2 trials	PHASE 2: EUCTR2020-001038-36, and NCT04380701 NCT04368728
				Phase 3: 1 trials	PHASE 3: NCT04368728
MRT5500	Sanofi Pasteur/USA	RNA-based		Under trials in 1 country. Phase 1 and 2: 1 trial	PHASE 1 and PHASE 2: EUCTR2020-001038-36, NCT04380701
EXG 5003	Elixirgen Therapeutic Inc./USA	RNA-based		Under trials in 1 country. Phase 1: 1 trial Phase 2: 1 trial	PHASE 1 and PHASE 2: NCT04863131
BNT162a1	Pfizer & BioNTech/Germany	RNA-based (Encodes an optimized SARS-CoV-2 receptor-binding domain)		Under phase 1 and phase 2 trials in Germany	PHASE 1 and PHASE 2: EUCTR2020-001038-36, NCT04380701
BNT162c2	Pfizer & BioNTech/Germany	RNA-based (A candidate using self-amplifying mRNA		Under phase 1 and phase 2 trials in Germany	PHASE 1 and PHASE 2: EUCTR2020-001038-36, NCT04380701
BNT162b3	Pfizer & BioNTech/Germany	RNA-based (A candidate using self-amplifying mRNA		Under phase 1 and phase 2 trials in Germany	PHASE 1 and PHASE 2: NCT04537949, and EUCTR2020-003267-26-DE
DS-5670a	Daiichi Sankyo Co., Ltd./Japan	RNA-based		Under phase 1 and 2 trial in Japan	PHASE 1 and PHASE 2: NCT04821674

Vaccine Name	Innovator/Country	Vaccine Platform	Vaccine-Triggered Immune Response	Stage of Clinical Development	Clinical Trial ID Number (https://covid19.trackvaccines.org/vaccines/79/) — Accessed on 18 August 2021)
Chulacov19	Chulalongkorn University/Thailand	RNA-based		Under phase 1 and phase 2 trial in Thailand	PHASE 1 and PHASE 2: NCT04566276
LUNAR-cov19/ARCT-021	Arcturus Therapeutics inc/USA	RNA-based		Phase 1: 1 trials	PHASE 1: NCT04480957
				Phase 2: 3 trials	PHASE 2: NCT04668339 NCT04728347 and NCT04480957
RTX-COVID19-B	Providence therapeutics holding Inc/Canada	RNA-based		Under phase 1 trial in Canada	PHASE 1: PRO-CL-001, NCT04765436
HDT-301	Senai simatec/Brazil	RNA-based		Under phase 1 trial	PHASE 1: NCT04844268
mRNA-1283	Moderna/USA	RNA-based		Under phase 1 trial in America	PHASE 1: NCT04813796
mRNA COVID-19 vaccine	Stemirna Therapeutics Co., Ltd/China	RNA-based		Phase 1 trial in 0 country	PHASE 1: ChiCTR2100045984
LNP-nCoV-saRNA	Imperial/UK	RNA-based		This vaccine is on hold	PHASE 1: ISRCTN17072692
AG0302-COVID19	AnGes/Japan	DNA-based	Post-vaccination, dsDNA triggers TLR9 to induce type-I interferon, which stimulates S-protein-specific naive T-cells, which become activated and differentiated into effector cells to form cytotoxic T-lymphocytes or helper T-cells [44][43].	Under phase 1, 2 and 3 trials in Japan	PHASE 1: NCT04527081 PHASE 2: NCT04655625 and NCT04527081 PHASE 3: NCT04655625

4. Conclusions

Vaccines are sometimes referred to as one of the most important public health interventions in modern history, owing to their significant impact on global morbidity and mortality. Vaccine development typically takes 10–25 years, and research and analysis expenses can easily reach billions of dollars. So, in light of the COVID-19 pandemic, the inevitable inquiry is: How were the presently available vaccinations developed so quickly? In terms of development and production, it is obvious that nucleic acid-based vaccines represent a technology platform for novel urgent vaccines. There are over 60 such DNA and mRNA-based vaccine candidates under development. Both of these classes of vaccines have drawbacks, such as safety, the necessity for an adjuvant and the requirement for refrigerated storage. Nucleic acid vaccines based on mRNA and DNA are increasing in popularity and are beginning to occupy the pre-clinical and clinical pipelines to address such deficiencies. In addition, a number of delivery methods are being explored to protect mRNA or DNA-based vaccines against degradation, and as such, RNA nanocomposites are being developed that should, in theory, confer resistance against degradation, increasing its immunogenic potential. The delivery mechanism needs to be adequate and delivered to antigen-presenting cells in endosomes, then escape into the cytoplasm and protein production and the stimulation of adaptive immunity. In a very short time, nucleic acid vaccines have been produced and tested in human clinical trials with great outcomes thus far. Advancement in the formulation technology helps in the stabilization of the mRNA with a simple and robust manufacturing process. Nucleic acid vaccines provide similar protection to that of conventional vaccines without having to risk the serious consequences. As it is designed in the lab, the manufacturing process is comparatively easy and robust than conventional vaccines. Out of 22 EUA vaccine candidates, three are based on an mRNA platform, while one is based on a DNA platform that defines the ultimate success of this platform. To meet the global demand, a significant global collaboration between vaccine manufacturing and supply, funding bodies, legislators and federal governments is critical. In the next few years, it needs to be determined if these vaccines confer safety and protection long-term and whether this mode of vaccine formulation can be used for a number of other current and emerging diseases.

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Vaccine Name	Innovator/Country	Vaccine Platform	Vaccine Triggered Immune Responses	Stage of Clinical Development	Clinical Trial ID Number (https://covid19.trackvaccines.org/vaccines/79/) — Accessed on 18 August 2021)
ZyCoV-D	Zydus cadila/India	DNA-based	Post vaccination dsDNA triggers TLR9 to induce type I interferon which stimulates S-protein-specific naive T cells, which become activated and differentiated into effector cells to form cytotoxic T-lymphocytes or helper T cells. Strong Th1 cell response helps in antibody secreting plasma cells. Stimulation of Type 1 interferon also aids in T cell memory [44] [47]	EUA in India	PHASE 1: CTRI/2020/07/026352 and CTRI/2021/03/032051 PHASE 2: CTRI/2020/07/026352 and CTRI/2021/03/032051 PHASE 3: CTRI/2021/01/030415
INO-4800	Inovio/USA	DNA-based	Post vaccination, dsDNA triggers TLR9 to induce type I interferon, which stimulates S-protein-specific naive T-cells, which become activated and differentiated into effector cells to form cytotoxic T-lymphocytes or helper T-cells [41][43].	Under phase 1, 2 and 3 trials in 3 countries	PHASE 1: NCT04336410 NCT04447781 PHASE 2: NCT04642638 ChiCTR2000040146 NCT04447781 PHASE 3: NCT04642638
GX-19	Genexine/Korea	DNA-based		Under phase 1 and phase 2 trials in Korea	PHASE 1 and PHASE 2: NCT04715997 and NCT04445389
AG0301-COVID19	AnGes/Japan	DNA-based		Under phase 1 and 2 trials in Japan	PHASE 1 and PHASE 2: NCT04463472
GLS-5310	GeneOne Life Science Inc./Korea	DNA-based		Under phase 1 and 2 trials in Korea	PHASE 1 and PHASE 2: NCT04673149
COVID-eVax	Takis/Italy	DNA-based		Under phase 1 and 2 trial in Italy	PHASE 1 and PHASE 2: EUCTR2020-003734-20, and NCT04788459
COVIGEN	University of Sydney/Australia	DNA-based		Under phase 1 trial in Australia	PHASE 1: NCT04742842
bacTRL-Spike	Symvivo/Australia	DNA-based		Under phase 1 trial in Australia	PHASE 1: NCT04334980

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