



New Generation Vaccines for COVID-19 Based on Peptide, Viral Vector, Artificial Antigen Presenting Cell, DNA or mRNA

Marzieh Rezaei¹ and Mahboobeh Nazari^{2*}

1. Department of Cell & Molecular Biology and Microbiology, Faculty of Science and Biotechnology, University of Isfahan, Isfahan, Iran

2. Monoclonal Antibody Research Center, Avicenna Research Institute, ACECR, Tehran, Iran

Abstract

At present, effective vaccines have been developed as the most successful approaches for preventing widespread infectious disease. The global efforts are focusing with the aim of eliminating and overcoming the Coronavirus Disease 2019 (COVID-19) and are developing vaccines from the date it was announced as a pandemic disease. In this study, PubMed, Embase, Cochrane Library, Clinicaltrial.gov, WHO reports, Science Direct, Scopus, Google Scholar, and Springer databases were searched for finding the relevant studies about the COVID-19 vaccines. This article provides an overview of multiple vaccines that have been manufactured from December 2020 up to April 2021 and also offers a perspective on their efficacy, safety, advantages, and limitations. Currently, there are several categories of COVID-19 vaccines based on Protein Subunit (PS), Inactivated Virus (IV), Virus Like Particle (VLP), Live Attenuated Virus (LAV), Viral Vector (replicating) (VVR) and Viral Vector (non-replicating) (VVnr) in progress or finalized as indicated by the WHO reporting of April 1, 2020.

Avicenna J Med Biotech 2022; 14(1): 30-36

Keywords: COVID-19, Pandemics, SARS-CoV-2, Vaccines

* **Corresponding author:**
Mahboobeh Nazari, Ph.D.,
Monoclonal Antibody Research
Center, Avicenna Research
Institute, ACECR, Tehran, Iran
Tel: +98 21 22432020
Fax: +98 21 22432021
E-mail:
Ma.nazari@avicenna.ac.ir,
nazari1980azar@yahoo.com
Received: 17 Jul 2021
Accepted: 20 Sept 2021

Introduction

SARS-CoV-2, as the third zoonotic coronavirus after SARS-CoV and MERS-CoV causes an acute respiratory disease^{1,2}. Because of similar structure and gene homology (75-80% to SARS-Cov, 50% to MERS-Cov) this new emergent virus was named SARS CoV-2³. In addition, the receptor of SARS-CoV-2 spike protein to bind to a host cell is the same as the SARS-CoV spike one and is known as the Angiotensin-Converting Enzyme 2 (ACE2)⁴. Virus attachment to the host cell, subsequent infection, antigen presenting and prevention of infection of the SARS CoV-2 is described in a simple schematic figure (Figure 1).

Since the SARS-Cov-2 emerged and caused a novel disease in 2019, scientists from all over the world have been on the research to overcome this pandemic. Many efforts in regard to COVID-19 vaccines started with the development of vaccines from various platforms against SARSCoV-2 infection. Different categories of the developing vaccine candidates are shown in figure 2. Most of them are based on the protein subunit specially the S-protein of SARSCoV-2⁵.

These current vaccine candidates developed based on various platforms, including Inactivated Viral vaccines (IV), Live Attenuated Viral vaccines (LAV), peptide or subunit based vaccines, Virus-Like Particles

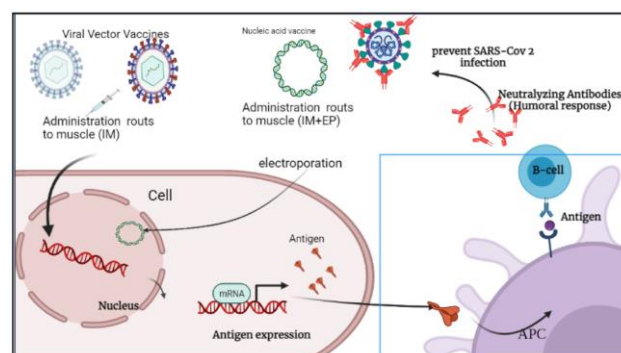


Figure 1. Schematic illustration of viral vector vaccines against SARS CoV-2 and prevention of infection. Intramuscular (IM), Electroporation (EP).

(VLP), replicating viral vector vaccines, on-replicating viral vector vaccines, and DNA or mRNA-based vaccines. Two classical vaccines such as LAV and IV vaccines have high potential to elicit a protective immune response, while some limitations are associated with an infection and LAV vaccines can revert to wild type⁶.

The aim of this study was to focus on the new plat-

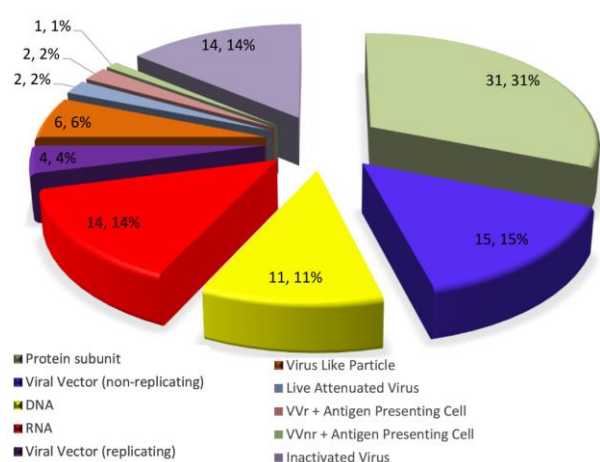


Figure 2. Different categories of SARS-CoV-2 Vaccines. Source: ClinicalTrials.gov website; WHO.

forms of the current vaccine candidates. New vaccine strategies based on recombinant DNA and peptide technology would have more safety and stability than the current vaccine strategies and would promise the manufacture and development of new generation of vaccines in a more predictable approach ^{6,7}. Some of these new generation platforms of vaccines are introduced in this study and include peptide based, nucleic acid based (DNA or RNA), viral vector and artificial Antigen Presenting Cells (aAPC) based vaccines ⁶.

Peptide based vaccines

Peptide vaccines are designed based on a single or highly immunogenic small epitopes. Most of COVID-19 candidate vaccines were designed based on protein subunit specially using the SARS-CoV-2 spike protein (S protein) or part of it as the immunogenic antigen. The glycoprotein S consists of S1 and S2 subunits. The Receptor Binding Domain (RBD) of the S protein potentially attaches to the receptor (ACE2) of the host cell ⁸.

The following vaccines based on peptide subunit are conducted in clinical phase III for the prevention of COVID-19 (the registration numbers are included in table 1):

- The Full length recombinant SARS CoV-2 glycoprotein S vaccine adjuvanted with Matrix M
- The Recombinant SARS-CoV-2 vaccine (CHO Cell) in which the RBD chemically conjugates to tetanus toxoid plus adjuvant)
- The CIGB-66 vaccine based on RBD plus aluminium hydroxide adjuvant
- EpiVacCorona vaccine in which short fragments of a viral spike protein are conjugated to a large carrier protein (has just been approved and no published article could be found).

Thus, they might be advantageous due to targeting immunodominant epitopes which induce more immune responses based on neutralizing antibodies instead of

larger proteins or whole inactivated virus ⁹; safety during production, and safely administered to immunosuppressed people ¹⁰. In addition, these vaccine candidates are not potentially infectious compared to the whole virus ⁸. Nonetheless, the T cell mediated immune response elicited by peptide based vaccine might be weaker in comparison to inactivated or live attenuated virus ¹¹. Actually, adjuvants are required to enhance antigen presenting to the host immune cells and improve the vaccine efficacy especially long term protection. Other disadvantages of the peptide based vaccines include: diminished uptake by Antigen Presenting Cells (APC) because of the small size of antigens, requiring more booster doses and adjuvants causing low immunogenicity, non-eliciting cellular responses, requiring confirmation of antigen integrity and restricting production due to low scalability ¹⁰.

Viral vector vaccine

Several viral vector based vaccines are available and divided as replicating and non-replicating viral vectors including adenoviruses and poxviruses. In the non-replicating type of viral vector based vaccines, a safe virus with an attenuated state can deliver the desired antigen and trigger a protective immune response (both cell- and antibody-mediated) without any replication in host cell ¹².

Furthermore, viral vectored vaccines present the desired antigens and mimic a natural infection. The current COVID-19 vaccine based on the non-replicating and replicating viral vectors are shown in tables 2 and 3, respectively. Thus, these types of vaccines can provide a potent immune response due to inducing cytokines and co-stimulatory molecules. Finally, viral vectored vaccines provide long lasting expression of target genes *in vivo* compared to DNA vaccines ¹².

Adenoviruses as a non-replicating viral vectors are simple to produce (in 2 weeks it could be enough to treat a thousand mice and dozens of monkeys with a novel adenoviruses vaccine), easy to purify to high titer, genetically stable, easily stockpiled, relatively inexpensive, and can be delivered *via* aerosol, oral, intradermal, and intramuscular routes. The aerosol route is considerably applicable when targeting a respiratory virus due to eliciting protective immune responses. It is also worth noting that viral vector vaccines tend to induce a robust response in both B cells and T cells ¹³ with a single dose and a good safety profile ¹⁰.

Common to some vaccines, adverse effects of viral vector vaccines include fever, pneumonia, diarrhea, transient neutropenia and lymphopenia, fatigue, labored breathing, headaches, liver damage, and fasting hyperglycemia. It should be noted that rare but grave adverse side effects include Bell's palsy, Guillain-Barre syndrome, gait disturbance, transverse myelitis, and an inflammatory condition in the spinal cord ¹³.

The most successful type of COVID-19 vaccine based on non-replicating viral vector is ChAdOx1-S (AZD1222) under the brand names Covishield as men

New Generation Vaccines for COVID-19

Table 1. Protein subunit vaccines, current clinical phases

Vaccines types	Strategy of design	Clinical phase
SARS-CoV-2 rS/Matrix M1-Adjuvant	(Full length recombinant SARS CoV-2 glycoprotein nanoparticle vaccine adjuvant with Matrix M)	Phase 2 and 3
Recombinant SARS-CoV-2 vaccine	(CHO Cell)	Phase 2 and 3
SCB-2019 + AS03 or CpG 1018 adjuvant	(Native like Trimeric subunit Spike Protein vaccine) plus Alum adjuvant	phase 3
COVAX-19®	Recombinant spike protein + adjuvant	Phase 1/2
MF59	Adjuvanted SARS-CoV-2 Sclamp vaccine	Phase 2
MVC-COV1901	(Spike-2P protein + adjuvant CpG 1018)	Phase 1/2 and Phase 2
FINLAY-FR1 anti-SARS-CoV-2 Vaccine	(RBD + adjuvant)	Phase 1/2 and Phase 2
FINLAY-FR-2 anti-SARS-CoV-2 Vaccine	(RBD chemically conjugated to tetanus toxoid plus adjuvant)	Phase 2 and 3
EpiVacCorona	(EpiVacCorona vaccine based on peptide antigens for the prevention of COVID-19)	Phase 2 and 3
Recombinant SARS-CoV-2 vaccine (Sf9 Cell)	RBD (baculovirus production expressed in Sf9 cells) Recombinant SARS-CoV-2 vaccine (Sf9 Cell)	Phase 2
IMP CoVac-1	(SARS-CoV-2 HLA-DR peptides)	Phase 1
UB-612	(Multipeptide based S1-RBD-protein based vaccine)	Phase 2 and 2/3
AdimrSC-2f	(Recombinant RBD +/- Aluminium)	Phase 1
CIGB-669	(RBD+AgHB)	Phase 1/2
CIGB-66	(RBD+aluminium hydroxide)	Phase 1/2 and phase 3
Recombinant Sars-CoV-2 vaccine	Spike protein, Aluminum adjuvant	Phase 1/2
Recombinant protein vaccine S-268019	(Using Baculovirus expression vector system)	Phase 1/2
SARS-CoV-2-RBD-Fc fusion protein		Phase 1/2
COVAC-1 and COVAC-2 sub-unit vaccine	(Spike protein) + SWE adjuvant	Phase 1/2
GBP510	A recombinant surface protein vaccine with adjuvant AS03 (Aluminium hydroxide)	Phase 1/2
Razi Cov Pars	Recombinant spike protein	Phase 1
MF59	Adjuvanted SARS-CoV-2 Sclamp vaccine	Phase 1
SpFN (spike ferritin nanoparticle)	Uses spike proteins with a liposomal formulation QS21 (ALFQ) adjuvant	Phase 1
EuCorVac-19	A spike protein using the recombinant protein technology and with an adjuvant	Phase 1/2
ReCOV	Recombinant two-component spike and RBD protein COVID-19 vaccine (CHO cell)	Phase 1
Recombinant SARS-CoV-2 Fusion Protein Vaccine (V-01)	Fusion Protein Vaccine	Phase 2

Source: ClinicalTrials.gov website; WHO.

tioned in table 2 with high efficacy (76.0% following the first dose and 81.3% at the second dose) ¹⁴.

Artificial antigen-presenting cells (aAPC) vaccine

The Dendritic Cells (DCs) are the most professional and specialized APCs that can be very efficient in activating T cells ¹⁵. *In situ* delivery of cargo to DCs could be beneficial owing to a new standardized generation of vaccines with efficient scalability, stability, and more ability to induce humoral immune response vs. cell mediated immunity and high safety with low risk of adverse reactions through reducing the administered dose ¹⁶. However, usage of the aAPC based vaccines have been limited because the process of isolation of specific DCs in this approach is labor-intensive and expensive ¹⁷. In addition, the widespread use of aAPCs could be restricted by the fact that for definition and

selection of MHC/peptide complexes identification of immunogenic antigens is required ^{18,19}.

LV-SMENP-DC vaccine

LV-SMENP-DC vaccine is in phase 1/2 clinical trials with registration ID NCT04276896 as clinical trials.gov reported (Table 2). Lent viral synthetic minigene vaccine is based on the selected, conserved and critical antigenic protein domain of SARS-CoV-2. In this approach, the DC is modified with LVs expressing SARS-CoV-2 minigene SMENP. Finally, the antigen specific CTLs will be stimulated by LV-DC expressing the desired antigens.

Nucleic acid-based vaccines

Nucleic acid-based vaccines can be classified as DNA or mRNA based vaccines that are capable to induce both humoral and cellular immune responses, but

Table 2. Viral vector (non-replicating) vaccines, clinical trials. Source: ClinicalTrials.gov website; WHO

Vaccines types	Strategy of Design	Clinical Phase
ChAdOx1-S - (AZD1222) (Covishield)	Non-replicating ChAdOx1 Vector Vaccine make Spike glycoprotein (S)	Phase 2/3, 3
Recombinant novel coronavirus vaccine	Adenovirus type 5 vector	Phase 2, 3
Gam-COVID-Vac	Adeno-based (rAd26-S+rAd5-S)	Phase 2/3, 3
GRAd-COV2	Replication defective Simian Adenovirus (GRAd) encoding S	Phase 2/3
VXA-CoV2-1	Ad5 adjuvanted Oral Vaccine platform	Phase 1
Human Adenovirus Type 5	hAd5 S+N vaccine (S-Fusion + N-ETSD). E2b- Deleted Adeno	Phase 1, 1/2
COH04S1 (MVA-SARS-2-S)	Modified vaccinia ankara (smVA) platform + synthetic SARS-CoV-2	Phase 1
Chimpanzee Adenovirus serotype 68 (ChAd)	Self-amplifying mRNA (SAM) vectors expressing spike alone, or spike plus additional SARS-CoV-2 T cell epitopes	Phase 1
COVIVAC	Newcastle Disease Virus (NDV) expressing membrane-anchored pre-fusion-stabilized trimeric SARS-CoV-2 S protein +/- adjuvant CpG 1018	Phase 1/2
SC-Ad6-1	Adenoviral vector vaccine	Phase 1

Table 3. Viral vector (replicating) vaccines, clinical trial

Vaccines types	Strategy of Design	Clinical Phase
DeINS1-2019-nCoV-RBD-OPT1	Intranasal flu-based-RBD	Phase 1 and 2
Covid-19/aAPC vaccine	The Covid-19/aAPC vaccine is prepared by applying lentivirus modification with immune modulatory genes and the viral minigenes to the artificial antigen presenting cells (aAPCs)	Phase 1
Dendritic cell vaccine AV-COVID-19	A vaccine consisting of autologous dendritic cells loaded with antigens from SARS-CoV-2, with or without GM-CSF	Phase 1, 1/2
AdCLD-CoV19	Adenovirus vector	Phase 1/2
BBV154	Adenoviral vector COVID-19 vaccine	Phase 1
NDV-HXP-S	Newcastle disease virus (NDV) vector expressing the spike protein of SARS-CoV-2, with or without the adjuvant CpG 1018	Phase 1/2

Source: ClinicalTrials.gov website; WHO.

several doses are required ⁶. Nucleic acid-based vaccines are likely to be the first priority of COVID-19 vaccines for entering clinical trials owing to their rapid adaptation to new emergence of virus ⁶. The list of current vaccines based on the nucleic acid is shown in table 4.

DNA based vaccines

DNA vaccines contain a DNA construct that codes for specific antigenic protein from a pathogen. The genetically engineered plasmid containing the DNA sequence is injected into the body and taken up by cells. Then, the antigenic protein is synthesized from the DNA construct based on the genetic code.

Like live or attenuated viruses, DNA vaccines effectively elicit both MHC-I and MHC-II pathways allowing the stimulation of the CD8⁺ and CD4⁺ T cells ²⁰ and inducing both humoral and cell-mediated immune responses ²¹. Some advantages of the DNA based vaccines are efficient scalability, fast design and development, and being extremely safe ¹⁰. Additional advantages of the DNA based vaccines are introduction of a vector encoding different antigens in a single vaccine; low-cost production, and high storage stability ^{22,23}. In addition, no infectious agent handling is required

and it can also induce humoral and cellular responses ¹⁰.

In comparison to protein vaccines, DNA vaccines are considered to be low-cost vaccines. DNA vaccines can enhance stability for transportation and can be administered to immunocompromised patients ²⁴. DNA based vaccine is known as a new successful approach, but the widespread use of DNA vaccination is limited due to its poor efficacy ^{25,26}, and low immunogenicity and may require multiple booster doses ²⁴. Currently, there is no nucleic acid vaccine approved. DNA vaccines require a special delivery platform ¹⁰. Despite the fact that clinical trials using DNA vaccines in humans elicit both cellular and humoral responses, these responses are often not sufficient to prompt significant clinical benefits. Consequently, DNA vaccines have only been licensed for use in veterinary medicine ^{21,23,27}.

Nevertheless, it would be important to focus on DNA vaccine optimization and delivery, including promoter design, codon optimization, adjuvants for use in humans, use of electroporation, and prime/boost immunization for refined vaccine design ²⁸. Regarding DNA vaccine administration, studies have shown that

New Generation Vaccines for COVID-19

Table 4. DNA or RNA based vaccines, clinical trial

Vaccines types	Strategy of Design	Clinical Phase
mRNA -1273	A lipid nanoparticle–encapsulated, nucleoside-modified messenger RNA that encodes the spike glycoprotein	Phases1/2, 2, 2/3 and 4
BNT162b2	Nucleoside-modified RNA, contained within a lipid nanoparticle, which encodes the full-length spike	Phases1/2, 2, 2/3 and 4
CVnCoV Vaccine	An optimized, non-chemically modified mRNA, encoding the prefusion stabilized full-length spike protein, formulated within Lipid Nanoparticles (LNPs)	Phases 2, 2/3, 3
ARCT-021	self-transcribing and replicating mRNA (STARR™) with LUNAR® lipid-mediated delivery technology	Phases1/2, 2
LNP-nCoVsaRNA	A purified, synthetic mRNA vaccine candidate encoding the S glycoprotein	Phase 1
SARS-CoV-2 mRNA vaccine (ARCoV)	A nucleoside-modified mRNA vaccine in lipid-encapsulated form that encoded the SARS-CoV-2 RBD (mRNA-RBD)	Phases 1, 2, 3
ChulaCov19 mRNA vaccine	SARS-Cov2 Wild-type S-spike mRNA/ lipid nanoparticle (LNP) vaccine	Phase 1
CoV2 SAM (LNP) vaccine.	A self-amplifying mRNA (SAM) lipid nanoparticle (LNP) platform + Spike antigen	Phase 1
mRNA-1273.351.	A lipid nanoparticle (LNP)-encapsulated mRNA-based vaccine that encodes for a full-length, prefusion stabilized S protein of the SARS-CoV-2 B.1.351 variant	Phase 1, 2, 4
MRT5500,	An mRNA vaccine candidate	Phase 1/2
HDT-301	Self-replicating mRNA vaccine formulated as a lipid nanoparticle	Phase 1
Covigenix VAX-001	DNA vaccines + proteo-lipid vehicle (PLV) formulation	Phase 1
CORVax	Spike (S) Protein Plasmid DNA Vaccine	Phase 1
bacTRL	Spike oral DNA vaccine	Phase 1
COVID-eVax	A candidate plasmid DNA vaccine of the Spike protein	Phase 1/2

Source: ClinicalTrials.gov website; Covid19.trackvaccines.org, WHO.

in vivo immune responses can be improved due to increased antigen delivery by electroporation (up to 1000-fold greater than delivery of naked DNA alone)²⁹. New advances have been reported in the immunization routes³⁰, for example the intradermal electroporation DNA delivery showed well tolerated, dose sparing and improving humoral immune responses in comparison to conventional intramuscular electroporation^{31,32}.

nCov vaccine as DNA based vaccine against COVID-19 is in phase 3 of clinical trials with registration ID nCov vaccine CTRI/2020/07/026352 (Table 4) and two others (CTRI/2020/07/026352; CTRI/2021/03/032051) are in phase 1/2 (Table 4).

mRNA based vaccine

mRNA-based vaccines are similar to DNA vaccines, except that the transcriptional stage is eliminated⁶. mRNA based vaccines are beneficial in comparison with conventional vaccines (e.g. subunit, killed and live attenuated virus, as well as DNA-based vaccines) because of its high potential for low cost production with high yields of *in vitro* transcription, fast development and safe and repeated administration. Easy and uncomplicated formulation of mRNA into carrier molecules such as lipid nanoparticles, causes fast uptake

and expression and finally enables entry of the mRNA into cells³³.

mRNA-based vaccines represent naturally immunostimulatory activity which is recognized by all cell surfaces, endosomal and cytosolic immune receptors. It also could provide adjuvant activity and thus elicit strong T and B cell immune responses³⁴. mRNA vaccines can be considered as highly safe vaccines because there is extremely low risk of infection or mutagenesis. mRNA based vaccines could be restricted due to difficult condition of storage and transporting and may need extremely low temperatures. mRNA vaccines exhibit instability and require storage at $<-20^{\circ}\text{C}$ ³⁴.

High efficacy of mRNA based vaccines would be achieved owing to more stability of mRNA and high translatability through various modifications in mRNA structure. Additionally, degradation of mRNA has been normally done by cellular processes, and *in vivo* half-life of mRNA can be increased through these modifications³⁵.

It is crucial to consider that unmodified mRNA itself is considerably sensitive, rapidly degradable, and highly immunogenic with high probability of activating a variety of pathogen-associated molecular pattern sen-

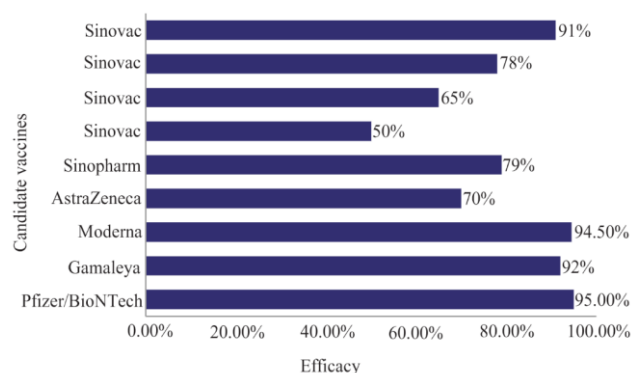


Figure 3. Sinovac efficacy trials have announced efficacies (for the same product) of 50%, 65%, 78% and 91%^{12,26}.

sors³⁴. Researchers showed that some modifications to nucleosides in mRNA molecules could improve the translatability and safety of mRNA vaccines as well as half-life³⁶. They reported that the incorporation of N1-methyl-pseudouridine (m1Ψ) in place of uridine increased the translatability (10-fold) over unmodified mRNA. This m1Ψ mRNA modification was included in the vaccine designing of two recently licensed mRNA vaccines mRNA-1273 and BNT162b2³⁷⁻³⁹.

As shown in figure 3, new research by Lombardi *et al* showed that the BNT162b2 (Pfizer) and mRNA-1273 (MODERNA/NIH) vaccines displayed an excellent efficacy (94%)⁴⁰. Safety data of mRNA BNT-162b2 and mRNA-1273 vaccines indicated that these vaccines are safe without any adverse reactions or health complications^{38,39}. Self-replicating RNA vaccines have high potency to express more antigens per cell and stimulate protective immune response with lower dose in comparison with classic RNA vaccines⁴¹.

Conclusion

Appearance of various subtypes of SARS-COV-2 virus due to different mutations, antigenic shift and antigenic drift may allow the virus to deceive the immune system even after the administration of the vaccine and offer insights into virus evolution. Dorp *et al* detected 198 mutations of SARS-COV-2 from the 7500 positive samples which caused the emergence of this novel virus with genomic diversity. Therefore, it can be concluded that these 198 mutations, which originated separately may indicate the evolution of the virus within the human host⁴².

Another point to consider is that, the benefits of an effective vaccine must outweigh the attributed known risks before its administration. Moreover, both safety and efficacy of the vaccine can be considered at least one year after administration. It is impossible to predict which types of candidate vaccines will be successful without running large-scale clinical trials.

In addition, the effectiveness of every vaccine, regardless of its targeting abilities, is also characterized by the dose, size, surface charge, cargo, using adju-

vants, route of administration, and the species to which it is delivered.

This review has some limitations which are presented as following future directions

Future directions of the COVID-19 vaccine must consider the optimizations of the COVID-19 vaccine which are known as the durability, dose, schedule and boosters; the effectiveness of the vaccine (Real word evidence or Herd immunity); safety (long term) and also the surveillance of the new subtypes (mutants) of virus.

Acknowledgement

We would like to appreciate the Monoclonal Antibody Research Center, Avicenna Research Institute, ACECR. This work was supported by a grant number of 990204-009 from Avicenna Research Institute.

Conflict of Interest

The authors declare no competing interests regarding this article.

References

1. Drosten C, Günther S, Preiser W, Van Der Werf S, Brodt HR, Becker S, et al. Identification of a novel coronavirus in patients with severe acute respiratory syndrome. *New Eng J Med* 2003;348(20):1967-76.
2. Zaki AM, Van Boheemen S, Bestebroer TM, Osterhaus AD, Fouchier RA. Isolation of a novel coronavirus from a man with pneumonia in Saudi Arabia. *New Eng J Med* 2012;367(19):1814-20.
3. Zhu N, Zhang D, Wang W, Li X, Yang B, Song J, et al. A novel coronavirus from patients with pneumonia in China, 2019. *N Engl J Med* 2020 Feb 20;382(8):727-733.
4. Walls AC, Park YJ, Tortorici MA, Wall A, McGuire AT, Veesler D. Structure, function, and antigenicity of the SARS-CoV-2 spike glycoprotein. *Cell* 2020;181(2):281-92. e6.
5. Dhama K, Sharun K, Tiwari R, Dadar M, Malik YS, Singh KP, et al. COVID-19, an emerging coronavirus infection: advances and prospects in designing and developing vaccines, immunotherapeutics, and therapeutics. *Hum Vaccin Immunother* 2020;16(6):1232-8.
6. van Riel D, de Wit E. Next-generation vaccine platforms for COVID-19. *Nat Mater* 2020;19(8):810-2.
7. Domínguez-Andrés J, van Crevel R, Divangahi M, Netea MG. Designing the next generation of vaccines: relevance for future pandemics. *mBio* 2020;11(6):e02616-20.
8. Li Y, Tenchov R, Smoot J, Liu C, Watkins S, Zhou Q. A comprehensive review of the global efforts on COVID-19 vaccine development. *ACS Cent Sci* 2021;7(4):512-33.
9. Ghaffari-Nazari H, Tavakkol-Afshari J, Jaafari MR, Tahaghoghi-Hajghorbani S, Masoumi E, Jalali SA. Improving multi-epitope long peptide vaccine potency by using a strategy that enhances CD4+ T help in BALB/c mice. *PloS One* 2015;10(11):e0142563.
10. Kyriakidis NC, López-Cortés A, González EV, Grimaldos AB, Prado EO. SARS-CoV-2 vaccines strategies: a

- comprehensive review of phase 3 candidates. *NPJ Vaccines* 2021;6(1):28.
11. Malonis RJ, Lai JR, Vergnolle O. Peptide-based vaccines: current progress and future challenges. *Chem Rev* 2019;120(6):3210-29.
12. Robert-Guroff M. Replicating and non-replicating viral vectors for vaccine development. *Curr Opin Biotechnol* 2007;18(6):546-56.
13. Kremer EJ. Pros and cons of adenovirus-based SARS-CoV-2 vaccines. *Mol Ther* 2020;28(11):2303-4.
14. Voysey M, Clemens SAC, Madhi SA, Weckx LY, Folegatti PM, Aley PK, et al. Single-dose administration and the influence of the timing of the booster dose on immunogenicity and efficacy of ChAdOx1 nCoV-19 (AZD1222) vaccine: a pooled analysis of four randomised trials. *Lancet* 2021;397(10277):881-91.
15. Bhardwaj N, Young JW, Nisanian AJ, Baggers J, Steinman R. Small amounts of superantigen, when presented on dendritic cells, are sufficient to initiate T cell responses. *J Exp Med* 1993;178(2):633-42.
16. Goyvaerts C, Breckpot K. Pros and cons of antigen-presenting cell targeted tumor vaccines. *J Immunol Res* 2015;2015.
17. Cohn L, Delamarre L. Dendritic cell-targeted vaccines. *Front Immunol* 2014;5:255.
18. Su Q, Igyártó BZ. One-step artificial antigen presenting cell-based vaccines induce potent effector CD8 T cell responses. *Sci Rep* 2019;9(1):18949.
19. Mitchell MS, Darrah D, Yeung D, Halpern S, Wallace A, Volland J, et al. Phase I trial of adoptive immunotherapy with cytolytic T lymphocytes immunized against a tyrosinase epitope. *J Clin Oncol* 2002;20(4):1075-86.
20. Wang B, Godillot A, Madaio MP, Weiner D, Williams W. Vaccination against pathogenic cells by DNA inoculation. *Curr Top Microbiol Immunol* 1998;226:21-35.
21. Hobernik D, Bros M. DNA vaccines—how far from clinical use? *Int J Mol Sci* 2018;19(11):3605.
22. Xu Y, Yuen PW, Lam JKW. Intranasal DNA vaccine for protection against respiratory infectious diseases: the delivery perspectives. *Pharmaceutics* 2014;6(3):378-415.
23. Silveira MM, Oliveira TL, Schuch RA, McBride AJA, Dellagostin OA, Hartwig DD. DNA vaccines against leptospirosis: A literature review. *Vaccine* 2017;35(42):5559-67.
24. Lee J, Kumar SA, Jhan YY, Bishop CJ. Engineering DNA vaccines against infectious diseases. *Acta biomater* 2018;80:31-47.
25. Leitner WW, Ying H, Restifo NP. DNA and RNA-based vaccines: principles, progress and prospects. *Vaccine* 1999;18(9-10):765-77.
26. Leclerc C, Ronco J. New approaches in vaccine development. *Immunol Today* 1998;19(7):300-2.
27. Coban C, Kobiyama K, Jounai N, Tozuka M, Ishii KJ. DNA vaccines: a simple DNA sensing matter? *Hum Vaccin Immunother* 2013;9(10):2216-21.
28. Li L, Saade F, Petrovsky N. The future of human DNA vaccines. *J Biotechnol* 2012;162(2-3):171-82.
29. Sardesai NY, Weiner DB. Electroporation delivery of DNA vaccines: prospects for success. *Curr Opin Immunol* 2011;23(3):421-9.
30. Smith TR, Schultheis K, Kiosses WB, Amante DH, Mendoza JM, Stone JC, et al. DNA vaccination strategy targets epidermal dendritic cells, initiating their migration and induction of a host immune response. *Mol Ther Methods Clin Dev* 2014;1:14054.
31. Jiang J, Banglore P, Cashman KA, Schmaljohn CS, Schultheis K, Pugh H, et al. Immunogenicity of a protective intradermal DNA vaccine against lassa virus in cynomolgus macaques. *Hum Vaccin Immunother* 2019;15(9):2066-74.
32. Tebas P, Kraynyak KA, Patel A, Maslow JN, Morrow MP, Sylvester AJ, et al. Intradermal SynCon® Ebola GP DNA vaccine is temperature stable and safely demonstrates cellular and humoral immunogenicity advantages in healthy volunteers. *J Infect Dis* 2019;220 (3):400-10.
33. Kauffman KJ, Webber MJ, Anderson DG. Materials for non-viral intracellular delivery of messenger RNA therapeutics. *J Control Release* 2016;240:227-34.
34. Pardi N, Hogan MJ, Porter FW, Weissman D. mRNA vaccines—a new era in vaccinology. *Nat Rev Drug Discov* 2018;17(4):261-79.
35. Thess A, Grund S, Mui BL, Hope MJ, Baumhof P, Fotin-Mleczek M, et al. Sequence-engineered mRNA without chemical nucleoside modifications enables an effective protein therapy in large animals. *Mol Ther* 2015;23(9):1456-64.
36. Karikó K, Muramatsu H, Welsh FA, Ludwig J, Kato H, Akira S, et al. Incorporation of pseudouridine into mRNA yields superior nonimmunogenic vector with increased translational capacity and biological stability. *Mol ther* 2008;16(11):1833-40.
37. Bettini E, Locci M. SARS-CoV-2 mRNA vaccines: immunological mechanism and beyond. *Vaccines (Basel)* 2021;9(2):147.
38. Callaway E. Why Oxford's positive COVID vaccine results are puzzling scientists. *Nature* 2020;588(7836):16-8.
39. Polack FP, Thomas SJ, Kitchin N, Absalon J, Gurtman A, Lockhart S, et al. Safety and efficacy of the BNT162b2 mRNA Covid-19 vaccine. *N Engl J Med* 2020;383(27):2603-15.
40. Lombardi A, Bozzi G, Ungaro R, Villa S, Castelli V, Mangioni D, et al. MINI REVIEW Immunological consequences of immunization with COVID-19 mRNA vaccines: Preliminary results. *Front Immunol* 2021;12:657711.
41. Vogel AB, Lambert L, Kinnear E, Busse D, Erbar S, Reuter KC, et al. Self-amplifying RNA vaccines give equivalent protection against influenza to mRNA vaccines but at much lower doses. *Mol Ther* 2018;26(2):446-55.
42. van Dorp L, Acman M, Richard D, Shaw LP, Ford CE, Ormond L, et al. Emergence of genomic diversity and recurrent mutations in SARS-CoV-2. *Infect Genet Evol* 2020;83:104351.