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Vaccine Development

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المرحلة الخامسة

اشراف

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Contents

1.0Abstract

The coronavirus disease 2019 (COVID-19) pandemic caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is the most formidable challenge to humanity in a century. It is widely believed that prepandemic normalcy will never return until a safe and effective vaccine strategy becomes available and a global vaccination programe is implemented successfully.

Here, we discuss the immunological principles that need to be taken into consideration in the development of COVID-19 vaccine strategies. On the basis of these principles, we examine the current COVID-19 vaccine candidates, their strengths and potential shortfalls, and make inferences about their chances of success. Finally, we discuss the scientific and practical challenges that will be faced in the process of developing a successful vaccine and the ways in which COVID-19 vaccine strategies may evolve over the next few years.

2.0 Introdution

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a positive sense, enveloped RNA beta coronavirus that emerged in Wuhan, China, in December of 2019 [1]. It is the cause of the clinical disease known as COVID-19 that has resulted in more than 50 M infections and more than 1.25 M deaths according to the World Health Organization [2].

COVID-19 is the third respiratory pandemic or epidemic caused by infection with a novel coronavirus. The first, SARS, developed in Hong Kong in the early-2000s, presented an average 6 days after infection with fever, chills, headache, myalgia, and cough. The principal organs involved were the lungs, which with computerized tomography (CT) imaging demonstrated consolidations that evolved within 7–10 days into pulmonary infiltrates.

A number of patients required mechanical ventilatory support, and by day 21 following initial onset of SARS-CoV, most patients had recovered, with mortality rate of approximately 9.6% [3,4].

The second clinical epidemic caused by a novel coronavirus was dubbed Middle East Respiratory Syndrome (MERS), and arose in 2012 in and near the Arabian Peninsula. This disease was associated primarily with fever, cough, and shortness of breath and it had a much higher 35% mortality rate [4,5].

Although SARS-CoV-2 shares sequence similarity with both SARS-CoV (79%) and MERS-CoV (50%), it has been most closely linked to two bat-derived SARS-like viruses (bat-SL-CoVZC45 and bat-SL-CoVZXC21, ~88% similarity) [1].

3.0Viral biology

SARS-CoV-2 likely has more than one mechanism of cellular entry [3, 4], the most closely examined is entry via the angiotensin-converting enzyme 2 (ACE2) protein [5] (Fig. 1).

Viral entry into the cell is also highly dependent on transmembrane protease serine 2 (TMPRSS2). Other cleaving proteases such as cathepsins B and L (CatB/L) have also been shown to induce viral cleavage and facilitate cellular entry, but to a lesser extent [6, 7]. Both ACE2 and TMPRSS2 have been detected in nasal and bronchial epithelium, while ACE2 gene expression has been identified in alveolar type II epithelial cells [6,8]. Additionally, ACE2 is found in the heart, cornea, esophagus, ileum, colon, liver, gallbladder, kidneys, and testis [6, 9]. This broad ACE2 tissue expression likely drives SARS-CoV-2 viral entry, inflammatory reaction, and subsequent widespread damage across a variety of organs and organ systems [9 ,10]. The envelope-anchored spike protein (S protein) of SARS-CoV-2 binds to ACE2 to facilitate viral entry [[11], [12], 13]]. Cryo-electron microscopy suggests ACE2 is a homodimer that binds up to two S proteins of SARS-CoV-2 [14,15]. S protein is translated within the cell as an S1-S2 complex, with S2 being cleaved off (via the aforementioned TMPRSS2 and cathepsins)

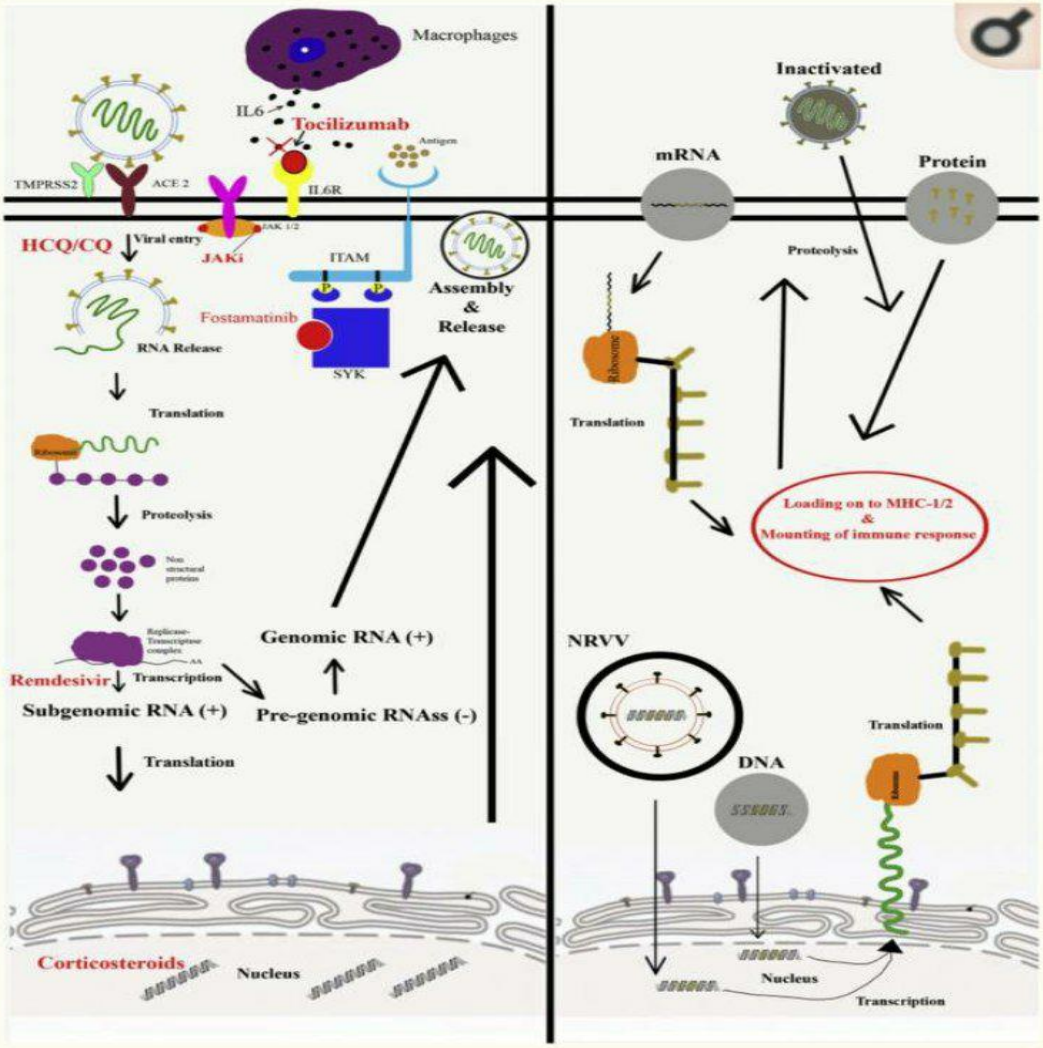

Fig. 1

(A) Mechanisms of action of various therapeutic agents being deployed against SARS-CoV-2 and COVID-19; (B) An overview of the various vaccine candidate types and their mechanism of action.

itis still not fully understood how SARS-CoV-2 viral infection drives the pathology seen in the COVID-19 clinical disease, but certain factors have been suggested. Among these is interruption of the renin-angiotensin-aldosterone (RAS)

pathway. ACE2 acts in the renin-angiotensin-aldosterone system (RAS) as an inhibitor of angiotensin II and its downstream effects. As a negative regulator of the pathway, ACE2 either converts angiotensin II into Ang-1-7, which acts on the MAS pathway, or angiotensin I into Ang-1-9, which works on the angiotensin II (ATII) receptor [16,17]. One of the functions of ACE2 in the lungs is to protect against ARDS and acute lung injury via reducing local inflammation [18]. A 2005 article by Kuba et al. demonstrated that SARS-CoV (the viral etiology of the first SARS pandemic) binds to the ACE2, elicits endocytosis, and reduces ACE2 protein levels on the cell membrane [19]. This type of viral entry ultimately leads to an increase in levels of angiotensin II and a decrease in ACE2, which could explain the pulmonary pro-inflammatory cytokine landscape and ARDS seen in COVID-19.

Although SARS-CoV-2 is very similar to SARS-CoV [1], key functional differences include receptor binding affinity and immunological evasion capacity. First, the receptor-binding domain (RBD) of SARS-CoV-2 Spike protein has a greater affinity to the ACE2 receptor than SARS-CoV [20,21]. Secondly, the SARS-CoV-2 S protein receptor binding domain has one “up” and two “down” conformations, equating to a less ‘exposed’ S protein and improved immune surveillance evasion [25]. This may ultimately prolong patient recovery time due to delays in the development of an appropriate antibody-mediated immune response in vivo [23,23,24,25].

4.0Vaccine development

4.1non-replicating viral vectors (NRVV)

The most widely utilized virally vectored candidates for non-replicating SARS-CoV-2 vaccines are adenoviral based (Table 1 , Fig. 1). Adenoviruses are double-stranded DNA viruses that are typically rendered replication ineffective via deletion of their E1 region 26]. Upon infection of target cells, there are high levels of transgene expression and upregulation of costimulatory molecules that elicit the cytokine and chemokine responses [27], improving immunogenicity. Considering that SARS-CoV-2 uses S protein to gain entry into cells, all the vaccines currently in trials express either full-length S protein or S protein subunits [[28], [29], [30], [31]].

One of the biggest disadvantages when using an adenoviral-vectored vaccine design is that most patients have pre-existing immunity against multiple adenovirus strains, which commonly circulate as upper respiratory infection pathogens, or they develop immunity soon after the first vaccine dose is administered [32]. A SARS-CoV-2 candidate made by CanSinoBiologics in collaboration with the Beijing Institute of Biotechnology uses adenovirus type 5

Type	Organization	Clinical phase
Inactivated	Sinovac	3
	Wuhan Institute of Biological Products/Sinopharm	3
	Bejing Institute of Biological Products/Sinopharm	3
	Institute of Medical Biology, Chinese Academy of Medical Sciences	1/2
	Research Institute for Biological safety Problems, Republic of Kazahstan	1/2
	Bharat Biotech (Whole Virion Inactivated)	1/2
Non-Replicating Viral Vector	University of Oxford/AstraZeneca	3
	Cansino Biological Inc./Bejing Institute of Biotechnology	3
	Gamaleya Research Institute	3
	Janssen Pharmaceutical Companies (Johnson&Johnson)	3
mRNA	Moderna/NIAID	3
	BioNTech/Fosun Pharma/Pfizer	3
	Curevac	1/2
saRNA	Arcturus/Duke-NUS	2
	Imperial College London	1

Table 1

(Ad5). This design has proven to be effective; however, certain groups' pre-existing immunity led to a slight decrease in efficacy [33,34]. There have been reports that intranasal inoculation in animals is more effective than the intramuscular injection of Ad5 [35], thereby increasing Ad5 vectored vaccine efficacy. To avoid pre-existing adenoviral immunity, The University of Oxford, in collaboration with AstraZeneca, has designed a chimpanzee adenovirus-vectored vaccine that expresses the full S protein (AZD-1222, previously known as ChAdOx1-nCoV). The initial inoculation is followed by a booster dose 28 days

later in order to ensure long-term immunity development. Overall, the vaccine has proven well tolerated and immunogenic.To overcome Ad5 immunity concerns, the Gamaleya Research Institute has developed a vaccine candidate using recombinant Ad5 (rAd5) as the initial dose, followed by a booster dose using recombinant Adenovirus type 26 (Gam-COVID-Vac). Despite ChAdOx1 causing higher neutralizing antibody titers, Gam-COVID-Vac nonetheless demonstrated titers equivalent to recovered COVID-19 patients [36].

Clinical trial preliminary reports of nonreplicating viral vector vaccine candidates for SARS-CoV-2 have shown all these vaccines to be safe and immunogenic, where immunogenicity has been defined as the detection (using enzyme-linked immunosorbent assays, ELISA) of antibodies induced against the spike protein. Additionally, some groups have used measurements of interferon-gamma (IFNγ), IL-2, and TNFα to infer cellular immunity development. All of the aforementioned NRVV candidates induced anti-spike antibodies and induced a measurable cellular immune response. NRVV vaccines caused small local reactions after injection, such as swelling and redness, and systemic symptoms including malaise, fatigue, fever, and headache, all of which generally resolve within 96 h after vaccination.

4.2 Messenger RNA vaccine candidates

Although there have been to date no FDA-approved mRNA vaccines available for use in humans, the unprecedented times brought on by the COVID-19 pandemic require unusual solutions. Production of nucleic acid vaccine candidates is generally faster and cheaper than protein subunit vaccines and, thus, there have been several mRNA-based SARS CoV-2 vaccine candidates developed and currently undergoing testing. Conventional mRNA vaccine design includes an

open reading frame of the targeted antigen (in this case, spike protein) with a 3′ polyadenylated tail, which generally produces both humoral and cellular

which generally produces both humoral and cellular immune responses [37] (Fig. 1).

A significant hurdle to mRNA vaccine development has been the propensity of mRNA to degrade; thus, stability and appropriate intracellular translation of mRNA are vital for the success of these vaccine candidates [35]. Various strategies have been developed to address these problems, including removal of double-stranded RNA and embedding of mRNA in lipid nanoparticles [38]. These lipid nanoparticle delivery vehicles have also been leveraged as adjuvants, leading to increased T follicular helper- and germinal center B-cell responses [39].

The leading mRNA SARS CoV-2 vaccine candidate is being developed as a collaboration between the National Institute of Allergy and Infectious Diseases (NIAID) and Moderna. Their vaccine, mRNA-1273, encodes the spike-2 protein antigen, made up of SARS-CoV-2 glycoprotein with the transmembrane anchor and an intact S1-S2 cleavage site [38]. It was initially evaluated in nonhuman primates and has successfully induced a robust anti-SARS-CoV-2 neutralizing antibody response and rapid protection against pulmonary injury [40,41].

BioNTech and Pfizer have created four RNA-based vaccine candidates explored in early-stage clinical trials (Table 1), two of which proceeded to further testing. Their vaccines were also embedded in LNP and encode perfusion-stabilized, membrane-anchored SARS-CoV-2 full-length spike (BNT162b2) and secreted trimerized SARS-CoV-2 receptor-binding-domain (BNT162b1). Based on recently published preliminary results, BNT162b2 reduces systemic adverse reaction to the vaccine in all participants, especially in older adults [41]. Both BNT and mRNA-1273 require booster doses in order to ensure high neutralizing antibody titer and

(presumably) long term immunogenicity. Despite the necessity of a second dose, the antibody response against the SARS-CoV-2 receptor-binding domain (RBD) of both vaccines showed significantly higher titers compared to patients who have recovered from COVID-19 [42,43,45]. Furthermore, the BNT162b1 vaccine demonstrated an anti-RBD IgG titer rising by nearly fifteen-fold on day 28 following first inoculation. Although these vaccines induced mild adverse symptoms following the initial dose, including mild fatigue, chills, headache, myalgia, and localized pain at the injection site, adverse reactions progressed with an increase in dosage, and booster doses in some patients caused moderate to severe local and systemic reactions

4.3Self-amplifying messenger RNA vaccine candidates

In addition to the above non-amplifying mRNA vaccines, self-amplifying RNA (saRNA) vaccine technology has been recently developed (Fig. 1). saRNA vaccines are synthesized using plasmids of Trinidad donkey Venezuelan equine encephalitis virus strains (VEEV). The VEEV structural coding regions are then replaced with pre-fusion Spike protein of SARS-CoV-2, while the self-amplifying coding region of VEEV alphavirus remains conserved [43,44].

These vaccines are promising because they have the potential to induce a more robust immunological response than a non-replicating mRNA vaccine. However, a major disadvantage of these vaccines is the length, given that they contain RNA sequence for replicon and the Spike protein. Some of the SARS CoV-2 saRNA vaccines currently under study include candidates by Imperial College in London [74], Arcturus/DUKE-NS, and the University of Washington (Table 1). All of these utilize self-amplifying RNA constructs embedded in various forms of nanoparticles with adjuvant properties [[44], [45], [46]]

4.4 DNA vaccine candidates

As early as 1990, investigators have shown that direct injection of intact nucleic acids into muscles of mice was a potentially useful vaccination strategy [47]. There are several advantages to a DNA-based vaccination approach, including the dramatically increased stability of the DNA molecule compared to RNA and the potential of DNA constructs to produce a large number of mRNA molecules, thereby increasing the target antigen's immunologic exposure.

aditionally, the thermal stability of DNA means that DNA-based vaccines have fewer refrigeration requirements than their mRNA-based counterparts.

DNA vaccines targeting both MERS and SARS-CoV-2 have both been developed. Inovio Pharmaceuticals had previously engineered MERS-CoV DNA vaccines, and now have designed a SARS-CoV-2 DNA-based vaccine candidate (INO-4800) (Table 1, Fig. 1). The vaccine was created based on a consensus SARS-CoV-2 spike glycoprotein sequence with an N-terminal IgE leader, added to enhance expression in target cells and increase immunogenicity [78]. In guinea pig testing, INO-4800 has shown humoral immunogenicity with anti-SARS-CoV-2 antibodies which inhibited viral binding to the ACE2 receptor. Furthermore, bronchoalveolar lavage fluid analysis revealed the presence of both cellular and humoral immune components after inoculation. There are three additional DNA vaccine candidates listed in the US clinicaltrials.gov database, but no additional preliminary data are available.

4.5Inactivated whole-virus vaccine candidates

Vaccines that use inactivated pathogens to induce immunity have a longstanding history in pandemic response. Although this type of vaccination represents the vast majority of historically effective vaccines, their long production time has put them

at a disadvantage in the current COVID-19 pandemic. The most promising inactivated SARS-CoV-2 vaccine candidates are currently in a phase 3 clinical trials in China (Table 1, Fig. 1).

This vaccine approach utilizes variants of the SARS-CoV-2 virion that are propagated via Vero (African Green Monkey) cell lines. Upon viral extraction, beta-propiolactone is used for inactivation with the viral particle then adsorbed onto an adjuvant (aluminum hydroxide) [48,49]. The present trials are investigating anti-viral immunity development at 14 and 28 days post-inoculation, with variations in timing and dose of booster vaccine, including an evaluation of two booster doses. When compared to other vaccine types, these inactivated viral vaccines appear to have reduced adverse effects. Most systemic adverse reactions were mild with no severe adverse reactions, while localized injection site redness and pain were common [44]. All adverse reactions have resolved 72 h after vaccine administration.

Comparing induced antiviral immunity of these inactivated viral vaccine candidates to the other vaccine types mentioned previously is difficult, as no comparisons were made between induced antiviral antibodies and those of recovered COVID-19 patient convalescent plasma. Nevertheless, the plaque reduction neutralization test (PRNT) analysis used in one of these studies mirrored that used in the mRNA-1273, BNT162b1, and ChAdOx1 studies. Inactivated vaccines showed similar titers to other approaches mentioned elsewhere in this article, and even higher titers than Ad5-vectored vaccines [50].

Despite this, there are several potential disadvantages of inactivated viral vaccines. Despite inactivated vaccines being considered one of the safer options in worldwide vaccination, the use of aluminum hydroxide as an adjuvant has been previously linked to vaccine-associated enhanced respiratory disease (VAERD), a driver of even more enhanced viral pulmonary pathology that has been reported

since the 1960s as the complication in vaccine trials and studies of measles and respiratory syncytial virus [51,52]. No indications of VAERD have been noted in phase 1 or 2 of these trials published to date. More concerningly, the previously-developed inactivated viral vaccines against SARS-CoV (the causative agent of the initial SARS epidemic) showed that anti-viral IgG levels rapidly decline 16 months after inoculation, rendering them practically undetectable three years after inoculation [53], raising concerns for durable immunity in what is expected to be a multi-year pandemic.

4.6 Protein subunit vaccine candidates

An alternative method of vaccine construction, the synthetic-protein subunit approach lies between nucleic acid-based techniques and inactivated whole virus vaccines. This group of SARS-CoV-2 vaccine candidates contain a recombinant spike protein expressed in various (typically insect) cell lines. Similar to RNA-based approaches, peptides are often unstable in vivo and are typically packaged into nanoparticles adsorbed onto specific adjuvants structured to increase the uptake of protein cargo into host antigen presenting cells. The leading SARS-CoV-2 protein vaccine candidate contender is NVX-CoV2373, produced by Novavax, currently in phase 3 clinical trial. This vaccine candidate consists of a nanoparticle containing the full-length wild-type Spike glycoprotein that was engineered to be resistant to proteolytic cleavage and capable of binding ACE2 receptors with high affinity. Protein production was optimized in the established baculovirus Spodoptera frugiperda (Sf9) insect cell expression system, while the adjuvant used to increase the vaccine's immunogenicity is Novavax's Matrix-M1 [84]. In addition to the Sf9 expression system, another commonly used cell line for vaccine production is Chinese hamster ovary (CHO) [[55], [56], [57]]. The CHO cell line

has been utilized in research settings for the production of MERS and SARS-CoV vaccines and has been used for COVID-19 serological testing [88].

Adverse effects from NVX-CoV2373 have been mild, including redness and swelling at the injection site, arthralgia, fatigue, headache, myalgia, nausea, and malaise dissipated after at least two days. No serious adverse effects have been noted. NVX-CoV2373 induces humoral and cellular immunity slightly stronger than that observed in convalescent plasma from recovered COVID19 patients

5.0Vaccinations

There are several safe and effective vaccines that prevent people from getting seriously ill or dying from COVID-19. This is one part of managing COVID-19, in addition to the main preventive measures. As of 3 June 2021, WHO has evaluated that the following vaccines against COVID-19 have met the necessary criteria for safety and efficacy:

1-AstraZeneca/Oxford vaccine

2-Johnson and Johnson

3- Moderna

4-Pfizer/BionTech

5-Sinopharm

6-Sinovac

Take whatever vaccine is made available to you first, even if you have already had COVID-19. It is important to be vaccinated as soon as possible once it’s your turn and not wait. Approved COVID-19 vaccines provide a high degree of protection against getting seriously ill and dying from the disease, although no vaccine is 100% protective. [58]

What's the recommended dosage?

1-AstraZeneca/Oxford vaccine

The recommended dosage is two doses given intramuscularly (0.5ml each) with an interval of 8 to 12 weeks. Additional research is needed to understand longer-term potential protection after a single dose. [58]

2-Johnson and Johnson

SAGE recommends the use of Janssen Ad26.CoV2.S as one dose (0.5 ml) given intramuscularly. There should be a minimum interval of 14 days between the administration of this vaccine and any other vaccine against other health conditions. This recommendation may be amended as data on co-administration with other vaccines become available.[58]

3-Moderna

SAGE recommends the use of the Moderna mRNA-1273 vaccine at a schedule of two doses (100 μg, 0.5 ml each) 28 days apart. If necessary, the interval between the doses may be extended to 42 days. Compliance with the full schedule is recommended and the same product should be used for both doses.[58]

4-Pfizer/BionTech

protective effect starts to develop 12 days after the first dose, but full protection requires two doses which WHO recommends be administered with a 21 to 28-day interval. Additional research is needed to understand longer-term potential protection after a single dose. [58]

5-Sinopharm

SAGE recommends the use of BIBP vaccine as 2 doses (0.5 ml) given intramuscularly. WHO recommends an interval of 3–4 weeks between the first and second dose. If the second dose is administered less than 3 weeks after the first, the dose does not need to be repeated. If administration of the second dose is delayed beyond 4 weeks, it should be given at the earliest possible opportunity. It is recommended that all vaccinated individuals receive two doses. [58]

6-Sinovac

SAGE recommends the use of Sinovac-CoronaVac vaccine as 2 doses (0.5 ml) given intramuscularly. WHO recommends an interval of 2–4 weeks between the first and second dose. It is recommended that all vaccinated individuals receive two doses. If the second dose is administered less than 2 weeks after the first, the dose does not need to be repeated. If administration of the second dose is delayed beyond 4 weeks, it should be given at the earliest possible opportunity.[58]

Common side effects after vaccination:

In most cases, minor side effects are normal, which indicate that a person's body is building protection to COVID-19 infection include:

1-Arm soreness

2-Mild fever

3-Tiredness

4-Headaches

5-Muscle or joint aches

Contact your care provider if there is redness or tenderness (pain) where you got the shot that increases after 24 hours, or if side effects do not go away after a few days. If you experience an immediate severe allergic reaction to a first dose of the COVID-19 vaccine, you should not receive additional doses of the vaccine.

It's extremely rare for severe health reactions to be directly caused by vaccines. Taking painkillers such as paracetamol before receiving the COVID-19 vaccine to prevent side effects is not recommended. This is because it is not known how painkillers may affect how well the vaccine works. However, you may take paracetamol or other painkillers if you do develop side effects such as pain, fever, headache or muscle aches after vaccination.[58]

6.0safty

Vaccine candidates must fulfil several requirements: safety, efficacy and quality. Because of the current escalation of the global COVID-19 pandemic, some aspects may change. The speed of vaccine development may push public health ministers, heads of states and the pharmaceutical industry to change their strategy for bulk budget investment for vaccine research. They must decide to prepare mass production events based on the limited data of promising vaccine candidates [44]. The need to protect billions of earth's inhabitants pushes governments and societies of the world to a 'great expectation' for the new vaccine. The overriding expectation, although with diverse interests, may influence the objective judgement typically required of candidate vaccine safety. Protecting human lives should be the priority.

mRNA- [59] and DNA-based vaccine technologies [54, 55] are being implemented in humans, especially as vaccine candidates. Several concerns about mRNA vaccine safety have been identified besides its promising potential advantages. The most important risks include the possibility that mRNA vaccines

may generate strong type I interferon responses that could lead to inflammation and autoimmune conditions [57]. The safety concerns of DNA-based vaccines involve the possibility that the targeting of DNA into the chromosomal DNA of the acceptor will trigger mutagenic effects in the functional gene located in the insertion loci [58]. At present, there are no mRNA- and DNA-based vaccines against any disease authorised to be marketed.

The strategy of DNA vaccines is similar to gene therapy in that a delivery system, such as plasmid, delivers targeted DNA into cells, where it is translated into proteins that induce the acceptors' immune response to generate targeted T-cell and antibody responses [59]. We have experience in using DNA for several gene therapies mostly related to inherited diseases or familial predispositions. Mainstream gene therapy scientists have stated that gene therapy is only suitable for terminally ill patients because the risks are very high [60].

Vaccine administration is completely different from interventions with gene therapy since the vaccine is for healthy human subjects, and the risk–benefit consideration would be completely different too. Both terminally ill and healthy persons have the same risk for the introduction of foreign DNA into their body, but terminally ill persons may benefit through having a chance to recover from their deadly disease, whereas healthy individuals may not have any benefit because they have never encountered the particular pathogen.

7.0Conclusion

Since the emergence of SARS-CoV-2, the scientific community has been working restlessly to find both short-term therapeutic approaches and a longer-term vaccine solution to reduce spread and curb COVID-19 morbidity and mortality. Despite significant progress and promising results from vaccine candidate studies, many obstacles remain, including the logistical difficulties surrounding mass production and delivery of millions or billions of doses to the worldwide population, which will likely represent the largest pipeline bottleneck.

Unfortunately, some vaccine types, such as mRNAs, are quite unstable at room temperatures and may require freezers not commonly found in rural clinics and hospitals away from academic research centers; non-refrigerated vaccine types may prove a more viable solution for these locations. Despite the challenges posed by this novel and rapidly spreading viral infection, the world has seen an unprecedented level of scientific engagement and cooperation which no doubt will serve as a model for future pandemic responses.

8.0reference

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