The Techniques Used on the Development of COVID-19 Vaccine

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Abstract. The COVID-19 pandemic, also known as the coronavirus pandemic, is caused by the severe acute respiratory syndrome coronavirus (SARS-CoV-2). It was first discovered in Wuhan, China, in December 2019 and continues until now, which becomes one of the deadliest pandemics in history and leads to a global recession. Thus, the production of the vaccine against SARS-CoV-2 become extremely urgent for all countries in order to control the epidemic. Therefore, the scientific community has made the rapid and significant progress in the development of vaccines against COVID-19, i.e., the entire vaccine manufacturing and production cycle has been greatly shortened. Up to now, more than 200 candidate vaccines have been created. In this paper, several vaccine technologies commonly used in the manufacture of COVID-19 vaccine are summarized. Besides, the different technologies that have been utilized for manufacturing are introduced. Furthermore, the corresponding clinical data are listed and discussed for the sake of indicating the success or failure of the vaccine.

1Introduction

1.1 Overview of COVID-19

Starting in 2020, all the people in the world face the crises and the challenge of COVID-19 pandemic, which is also known as the coronavirus pandemic. It has great infectiousness and causes an enormous number of infections. Figure 1 demonstrates the complete pathogenesis of COVID-19 representing all the facts related to its virulence inside the host body. On this occasion, each country is eager to find an effective treatment and pour plenty of funding on the research of the vaccines.

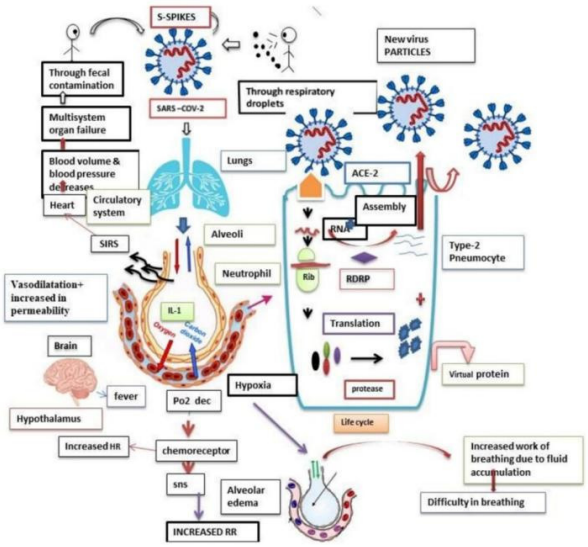

Figure1 Schematic representation of COVID-19 pathogenesis: A complete profile of entry, integration and replication of virus [1]

1.2 Structure of COVID-19

Compared with the SARS coronavirus (a kind of virus that spread in 2003 in China), COVID-19 and SARS have 80% sequence similarity in the genes. Like SARS, COVID-19 has a layer of single-stranded RNA coated on the outside of another single-stranded RNA covering by the surface of some spines. As a matter of fact, it is the typical structure of coronavirus, which is a linear single stranded RNA virus. Besides, novel coronavirus is usually spherical with irregular shape [2].

1.3 Transmission of COVID-19

COVID-19 spreads mainly through the air and when the infected person breathe, cough, sneeze, or speak. Moreover, the incubation period of COVID-19 is long (about 1 to 14 days even up to 24 days), which means people will spread the virus even without symptoms. In addition, COVID-19 has highly variable of symptoms showing up from none to severe illness. All these problems cause the highly infectious characteristics of COVID-19 and increase the difficulty of the work to limit the speed of the virus. Until now, more than 90 million people were diagnosed with more than 2 million cumulative deaths. To be more specific, the U.S.A has more than 350,000 people dead in that disaster, which is the country with the highest number of deaths; Brazil stayed at the second position, owned more than 200,000 people dead due to COVID-19. In order to restrain the disease, all the countries in the world have paid the great financial, material and human resources. For example, most of the governments block down the frontier, limit the

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transfer of citizens, looking for the cure to the COVID-19 as well as support the development of vaccines.

1.4 Treatment of COVID-19

Unfortunately, we still haven't found any effective treatment options so far. In this case, most of the methods applied in clinic treatment are comprehensive treatments. Currently, with the support of respiratory, only some antiviral drugs (e.g., Redecivir, Chloroquine phosphate, Fapilavir, etc) are effective for the treatments. As for the Redecivir, originally a drug used to treat Ebola, it is able to improve clinical symptoms (e.g., reduce the fever). With regard to Chloroquine phosphate, an commonly used antiviral or anti-malarial drug, it inhibited the Novel Coronavirus infection of cells. For Fapilavir, a drug used to treat influenza, it was also found to have significant inhibitory effect on novel coronavirus in clinical trials. Inspiringly, the development of the vaccine already has great success with plenty of novel vaccine technology including mRNA vaccine, inactivated vaccine, virus vector vaccine, and recombinant protein vaccine(as shown in table1).

Table1: Classification of COVID-19 vaccine technical route

DNA	RNA	Recombination Protein	Inactivated	Virus vector
Genexine(	Moderna	cdbio	sinovac	AstraZeneca
AnGes	BioNTech	Vaxine	Immunitor	cansinotech
Inovio	Arcturus	Kentucky BioProcessing	Bharat Biotech	Gamaleya
Cadila(CADILAHC NS)	CureVac(CVACO)			

2 mRNA Vaccine

2.1 The introduction of mRNA

The concept of mRNA as a therapeutic drug was formally proposed in the 1990s. Subsequently, mRNA technology gradually developed into a platform with great potential for vaccine products due to the simple structure. As the smallest genetic structure, it contains only the elements that are directly required for protein expression. Meanwhile, it has the good security with following reasons. First of all, the mRNA is unable to interact with the genome. In addition, recombination between single-stranded RNA molecules can occur in rare cases, i.e., potentially harmful genome integrations are ruled out. Moreover, mRNA is only a transient information carrier owing to the not-replicable characteristic and genomic integration gaps [3, 4].

In theory, as a therapeutic molecule, mRNA could provide breakthroughs in treatments and vaccines. Since mRNA can write and express all proteins, it suggests a principle possible way to develop vaccines for the prevention and treatment of various diseases, e.g., influenza, cancer and COVID-19. The sequence of RNA molecules changes the coding protein, i.e., proteins are not affected by the physical and chemical properties of different products [5]. Thus, the vaccine platform will be more timesaving and cost-saving. The manufacturing of mRNA avoids the lengthy cell-culture and purification process involved in traditional viral vaccine production, as well as the stringent requirements for biosecurity measures. Thereby, it is useful and suitable for the suppression of pandemic [6].

In terms of efficacy, mRNA does not need to cross the nuclear membrane to treat patients compared to DNA, which makes it even more effective [7]. mRNA vaccines lack MHC haplotype limitations compared to polypeptides, which is the requirement that APC or target cells express MHC molecules recognized and responded by the T cell. In addition, mRNA vaccines can be designed as self-adjuvants (an advantage over peptide- and protein-based vaccines) on account of the binding functions with pattern-recognition receptors.

2.2 Introduction of mRNA Vaccine

With the development of mRNA modification and delivery tools, the field of mRNA vaccines has progressed rapidly in recent years in terms of basic and clinical research. Clinical trials of mRNA vaccines against viral diseases (e.g., Zika, Ebola, influenza, rabies and cytomegalovirus infection) have been conducted in many countries [6]. Meanwhile, as long as the selected antigen of the pathogen target is found out, the gene sequence of the pathogen can be detected and cloned into the DNA template plasmid, which greatly increases the manufacturing efficiency of mRNA vaccine. After the vaccine is injected into a subject, the mRNA vaccine uses the host cell's mechanisms to convert the mRNA into his antigen in the body. The final cell location of antigen is determined by signal peptide and transmembrane domain. This can be inherent to the natural protein sequence or engineered to guide the protein into the desired cell compartment. Thus, antigens are able to be expressed as intracellular, secretory, or membrane-bound proteins [8]. Owing to the RNA replication mechanism (which contains the virus gene), the structural protein sequence will be replaced by other genes. Therefore, the self-replication of genes containing viruses will be guided by RNA and multiple antigen-encoding messenger RNAs will be produced. This mimics viral infection and causes the body to produce antibodies, leading to an effective humoral and cellular immune response [9]. The host's innate system is capable of sensing and responding to the RNA sequences from which the virus originated, i.e., an mRNA vaccine will induce a strong innate response. These include the production of chemokines and cytokines at the injection site, such as interleukin-12 (IL-12) and tumor necrosis factor (TNF). These are key factors for inducing an effective adaptive response to the coding antigen successfully [10]. In addition, mRNA is fully synthetic and almost any sequence can be designed and synthesized in silicon, delivered as an mRNA vaccine as well as quickly tested in vivo in animal models.

Although there are significant theoretical benefits to an mRNA vaccine, the fastest developing product is still in clinical trials. The biggest obstacle is the activation of innate immune response, which is a double-edged sword for mRNA vaccines. On the one hand, mRNA induces immune protection through immune activation. On the other hand, excessive activation of innate immunity will prevent mRNA translation and degrade mRNA. From a druggable point of view, a qualified mRNA vaccine product requires that the mRNA structure is stable enough to be delivered and expressed in target cells while inducing the desired adaptive immune response.

2.3 Mechanism of mRNA vaccine

So far, two phase III mRNA vaccines, BNT162B2 and MRNA-1273, have been shown to be at least 50 percent effective [11]. BNT162B1 is a lipid nanoparticle whose nucleoside modified mRNA encodes the S-receptor binding domain. It has another version, which is encoded stable, membrane-anchored full-length pre-fusion S BNT162B2 [12]. mRNA vaccine technology based on lipid nanoparticles (LNP) will deliver precise genetic information and adjuvant effects to antigen presenting cells S4. The efficacy of this technique has been proved against a variety of virus targets. RNA vaccines synthesized have been shown to be safe and well tolerated in clinical trials from LNP and liposomes for the prevention of infectious diseases or the treatment of cancer [13]. In addition, the BNT162B1 vaccine optimizes RNA for high stability and translation efficiency. Furthermore, it has a receptor binding domain (RBD) of the SARS-CoV-2 spike protein, which is a key target of neutralizing antibodies. The RBD antigen expressed by BNT162B1 is fused to the T4 fibrin-derived "fold" trimer domain, which increases its immunogenicity through multivalent display. Meanwhile, the BNT162B1 vaccine conjugates 1-methyl-pseuduracil to inhibit innate immune response and increase mRNA translation in vivo [14].

In a phase 1/2 placebo-controlled trial, in order to demonstrate dose-dependent response, 45 adults were randomized to 2 doses of 10,30 or 100 μg. Doses were 10 μg, 30 μg and 100 μg [15]. No serious adverse events were recorded. Local injection site reactions and systemic events (mainly influenza-like symptoms) are dose-dependent, generally mild to moderate, and transient. The antibodies, which recognize the receptor binding domain and the neutralizing antibodies, are also activated in a dose-dependent manner, and increased after the second dose. Serum RBD binding immunoglobulin G (IgG) concentration and SARS-CoV-2 neutralization titer also increased with the augment of the dose and after the second dose. After 14 days of dose increase, the geometric mean neutralization titer reached 1.9-4.6 times that of COVID-19 human convalescence serum (HCS). The vaccine was then compared with a second version of the stable, membrane-anchored full-length prefusion, BNT162B2. Interestingly, both vaccines produced similar dose responses in both younger and older adults, and BNT162B2 was selected for further clinical studies due to the higher safety [11].

Similarly, mRmRNA-1273 is also suitable for stable perfusion. A phase 1 dose escalation open label trial demonstrated the safety and reactivity of this vaccine and induced neutralizing antibodies after two injections. The experiment involved 45 healthy adults aged from 18 to 55 years who received two mRmRNA-1273 doses of 25, 100, or 250 μg, 28 days apart. There were 15 participants in each dose group [16]. The safety of the vaccine was also tested in elderly people aged among 56 to 70 years and with a BBB of 0 or above, with two doses of either 25 μg or 100 μg. In this population, adverse events at both doses were moderate. The vaccine also induces a CD4+ T cell response, including production of IFN-γ, IL-2, and TNF-α [17].

2.4 Clinical Data

A Phase 1/2/3 global clinical trial of BNT162B2 vaccine, funded by Biotech and Pfizer, evaluated the safety, immunogenicity, and efficacy of 30 μg BNT162B2 against COVID-19 in people from 16 years and older. Results of Phase 2/3 safety and efficacy of BNT162B2 vaccine were shown in the trial.

A total of 43,548 participants were randomized, with 21,720 receiving the BNT162B2 vaccine and 21,728 receiving a placebo. A total of 170 infected people were negative after the second injection, of which 8 were in BNT162B2 recipients and 162 were in placebo recipients. Of the 10 cases of severe COVID-19 that developed after the first injection, 9 of them were in placebo recipients and 1 was in BNT162B2 recipients. BNT162B2 was 95% effective in preventing COVID-19 (95% confidence interval 90.3 to 97.6). Similar vaccine potency (typically 90-100%) was observed in age, sex, race, ethnicity, baseline body mass index, and subgroups with or without comorbidities. The safety profile of BNT162B2 was short-term, mild to moderate pain, fatigue, and headache at the injection site. The incidence of serious adverse events was lower and similar in the vaccine and placebo groups. Therefore, one draws the conclusion that BNT162B2 two-dose regimen provides 95% protection against COVIAD-19 in people aged 16 years and older. The median safety of 2 months was similar to that of other viral vaccines [18].

A phase 3 clinical trial of the MRA-1273 vaccine, funded by the Biomedical Advanced Research and Development Agency as well as the National Institute of Allergy and Infectious Diseases, has demonstrated an evaluation of the safety and efficacy of the MRA-1273 vaccine in preventing SARS-CoV-2 infection(table2). The trial enrolled 30,420 volunteers, who were randomly assigned in a 1:1 ratio to receive either the vaccine or a placebo (15,210 participants in each group). More than 96% of participants received both injections, and 2.2% had evidence (serological, virology, or both) of SARS-CoV-2 infection at baseline. Symptomatic COVID-19 disease was confirmed in 185 participants in the placebo group (56.5/1000 person-years; 95% confidence interval [CI], 48.7 to 65.3) and mRNA 1273 for 11 participants (3.3/1000 person-years; 95% confidence interval, 1.7 to 6.0). The efficacy of the vaccine was 94.1% (95%

confidence interval, 89.3-96.8%; P < 0.001). In key secondary analyses, efficacy was similar, including assessments 14 days after the first injection, analyses of participants who had evidence of SARS-COV-2 infection at baseline, and participants over 65 years old. 30 participants developed severe COVID-19 and 1 died, which were all from the placebo group. Moderate and transient reactivity after vaccination was more common in the mRNA-1273 group. Serious adverse events were rare and the incidence was similar between the two groups. There may be transient local and systemic reactions after mRNA-1273 injection. Whereas no safety concerns have been found from the others. To sum up, the mRNA-1273 vaccine showed 94.1% efficacy in preventing COVID-19 disease, including severe disease [19].

Table2: The progress of COVID-19 vaccine

Organizer	name	Technical route	Clinical number	Clinical stage	Starting time	Number of people
Moderna	mRNA-1273	RNA	NCT0470427	III	2020727	30000
CNBG	——	Inactivated	Chi CTR200034780	III	2020/7/18	15000
sinovac	——	Inactivated vaccine	NCT04456595	III	2020/7/1	8870
AstraZeneca	COV003	Virus vector	ISRCTN89951424	III	2020/5/28	2000
BioNTech	BNT162b28	RNA	——	III	2020/7/27	30000
cansinotech	Ad5-nCov	Virus vector	ChicTR2000031781	II	2020/4/12	500
Academy of Military Medical Sciences	——	recombination	NCT04341389	II	2020/4/12	508

3 Inactivated whole-virus vaccines

3.1 Introduction of inactivated vaccine

An inactivated vaccine is one in which a virus or bacterium is grown and then inactivated with heat or a chemical usually formalin. An inactivated vaccine can be composed of whole viruses or bacteria, or of their lysed fragments, as a lysed vaccine. Lytic vaccines are produced by further purification of the microorganism until the vaccine contains only the desired antigenic components, such as pneumococcal polysaccharides.

Inactivated vaccines often require multiple doses since a single dose is unable to produce protective immunity but merely "initializes" the immune system. A second or third dose is required to produce protective immunity. The immune response is usually humoral immunity. Besides, the antibodies it produces have the function of neutralizing and eliminating pathogenic microorganisms as well as their toxins, but rarely or not causes cellular immunity. Antibody titers produced by vaccination with inactivated vaccines decrease with time, i.e., some inactivated vaccines require periodic booster vaccination. Inactivated vaccines, which are usually unaffected by circulating antibodies and can be administered even if antibodies are present in the blood, cannot replicate in the body and can be used in immunocompromised people. The advantage of inactivated vaccines is that they are safe to use, easy to save, no pollution risk, and insensitive to the neutralization of maternal antibodies. Which shows the high feasibility for the product of COVID-19 vaccines by this means.

3.2 Clinical Data

Coronavac, the COVID-19 inactivated, and aluminum adjuvant vaccine produced by China Kehua Biological Products Co., Ltd., has completed a phase I/II efficacy and safety, double-blind, randomized, placebo-controlled clinical trial [20] (as shown in Table2) . The phase II trial involved 600 adult participants (ages 18 to 59). Participants were randomly assigned to one of two dual-dose programs, 0 to 14 days, and 0 to 28 days. In each plan, 120 participants were given a 3 μg dose, 120 participants were given a 6 μg dose, and 60 participants were given a placebo. Local adverse events such as pain and swelling were mild to moderate in the treatment regimen. No serious grade 3 adverse events have been reported. In both regimens, participants who were given 3 and 6 μg had high NAB responses. At 28 days after the second administration, NAB levels in the 0- and 14-day groups were stable, but significantly increased in the 0 and 28 day groups. A similar pattern was observed for specific antibodies. In addition, NAB levels decreased with age, suggesting that older people need to fortify the dose [21].

The COVID-19 inactivated and aluminum adjuvant vaccine Coronavac is currently in phase III clinical trials. It documented the occurrence of adverse events associated with two doses of COVID-19 adsorbed vaccine (inactivated vaccine) produced by Sinovac within 1 week of vaccination in adults (18-59 years old) and older adults (60 years old or older). Its phase 3 clinical trials are being conducted in Brazil and Indonesia due to the low number of active cases in China [20].

Except for Coronavac, Sinopharm is working with the Wuhan Institute of Biological Products and the Beijing Institute of Biological Products to develop an inactivated vaccine. The vaccine candidate is currently in phase 3 trials [22]. According to Wuhan Biologics Research, a

preliminary assessment for the safety and immunogenicity of a candidate inactivated vaccine in healthy Chinese adults was reported after 28 days of vaccination. It contains the phase I and phase II double-blind randomized clinical trial. The vaccine was given three doses in the phase I trial and two in the phase II trial. The trials were conducted in Henan Province, China. The first phase involved 96 healthy adults aged from 18 to 59. The second phase consisted of 224 participants of the same condition.

A total of 320 randomized patients completed two phases of the trial within 28 days of full vaccination. The average age of the participants was 42.8 years. In the phase 1 trial, 96 participants were assigned to one of three dose groups, 2.5, 5, and 10 μg/ dose, the aluminum hydroxide (alum) adjuvant only group, and received three intramuscular injections on days 0, 28, and 56. High NAB response and seroconversion were observed in all participants in the low-dose and high-dose groups 14 days after the third vaccination (Day 70), with 23 of them (95.8%) in the medium-dose group. In the phase 1 trial, high levels of specific antibody responses were also produced. Besides, seroconversion was observed in all participants. The most common adverse reactions were pain at the injection site and self-relieving fever.

In the phase 2 trial, 224 adults were randomly assigned to two treatment groups, 0 and 14 days after injection, and 0 and 21 days after injection. In each treatment group, 84 people were assigned to the medium-dose (5 μg) vaccine group and 28 to the aluminum adjuvant placebo group. Five patients (6%) in the 5 μg group and 4 patients (14.3%) in the placebo group experienced adverse reactions during 0 and 14 days of treatment. 16 patients (19%) in the 5 μg group and 5 patients (17.9%) in the placebo group had adverse events during the 0 and 28 days of treatment. Both treatments had higher NAB response and the serum conversion rate was 97.6%. In addition, for specific antibody responses, 0 - and 21-day antibody responses were much higher than 0 - and 14-day antibody cases. Seroconversion was also relatively low at 0 - and 14-day treatments, at 85.7%, compared with 100% at 0 - and 21-day treatments. Adverse reactions, as in the phase 1 trial, were injection site pain and self-relieving fever [23]. In this interim report on the Phase I and Phase II trials of COVID-19 inactivated vaccine, patients had a low adverse event rate and showed immunogenicity, which indicates great potential for development.

4 Adenovirus Vector Vaccine

4.1 Introduction of adenovirus

Adenovirus (Ad) is an unencapsulated DNA virus with an icosahedral capsid and a diameter of about 90 nm. Rowe and his colleagues first discovered this in 1953, during an experiment to grow adenoid tissue [24]. Subsequently, in 1993, the first ADS-based study of human gene therapy was performed with the first in vivo gene therapy in a 23-year-old homozygous cystic fibrosis patient. He was given the E1-E3 deletion RAD vector of normal human CFTR. In the 2000s, this technology was widely applied since Ads could cause the reaction of T cells and B cells. In addition, Ad is capable of inducing apoptosis of tumor cells (also known as oncolytic cells). Ad has great potential in the development of vaccines to prevent infectious pathogens or cancer treatments [25]. On account of its high safety, Ad is able to replicate almost all living cells and can be absorbed without adjuvants via oral, intranasal, or intramuscular routes. At the same time, Ad infection is usually mild in humans but can sometimes be life-threatening, especially in immunocompromised individuals.

The AD genomic DNA is about 26-45kb, with two 100-140bp inverted terminal repeats at each end. The genes expressed in the ADS life cycle are generally divided into two types: early genes and late genes. Early genes promote ADS replication by changing the expression level of host related genes. Late genes assist in the cleavage of host cells and the assembly as well as release of virions. Ad virions are mainly composed of two proteins, the capsid protein and core protein. Core proteins act as DNA-related proteins, including V, VII, and X proteins [26]. Among them, VII protein plays a key role in DNA manipulation, e.g., DNA binding, initiation of DNA replication, protection of viral genome, etc. Capsid proteins consist of Hexon, Penton, fiber, IIIA, VIII, and IX [27]. Hexons are the main structural proteins on the capsid with 240 hexagonal trimers located on the surface of Ad virions. There are several hypervariable regions on the Hexons, which are the main neutralization sites of Ad and can be replaced by other foreign antigens as potential vaccine vectors. The IIIa protein is located on the surface of the undergarment and Ad in the proper assembly of the virus, the stabilization of the vertex region, and the assembly of the encapsulating genome. VI protein is in the capsid and serves as the key cleavage factor of AdS in the process of endosomal destruction. The VIII protein provides the bonding between the surrounding pentagonal prism and hexahedrons, maintaining the stability of the capsid. The function of IX protein is to inhibit the innate immune response and maintain the virality and stability of the capsid [28].

4.2 Adenovirus Vector Vaccine

Ad vector is one of the most effective vectors for the delivery of foreign antigen to host cells. Therefore, the strategy of viral vector vaccination has obvious advantages. Besides, among the virus vectors under study, adenovirus (AdV) is the most promising vaccine vector. As a vector for gene therapy, Adenoviral-Vec vaccines have the inherent ability to deliver genes to mammalian cells while Adv is the vector for live vaccines [29]. As a popular vaccine vector, AdV has high safety, high gene transfer potential, broad cell orientation, and well-characterized genomes as well as their operational protocols [30].

AdV stands out for its safety as a vaccine. Early in the 1970s, the U.S. Army developed the AdV Serotype 4 (HAdV-4) and HAdV-7 vaccines for export to prevent acute respiratory disease caused by ADHU4 and ADHU7 with no side effects [31]. Meanwhile, the AdV vector has high immunogenicity. They can transduce APCs and

induce humoral and cellular immune responses, which may be enhanced by the initial induction of a strong innate response. First, after APCs ingest virions, intracellular viral proteins are processed and presented to CD8+ T cells along with major histocompatibility complex (MHC) class I molecules. This leads to the production of Ad-specific cytotoxic T lymphocytes (CTL), which clear AD-transduced cells. Furthermore, MHC molecules presented with de novo synthesis of viral proteins lead to type 1 activation of CD4 + T-helper (Th1 cells). Th1 cells secrete cytokines, such as interleukin-2 (IL-2) and interferon-γ (IFN-γ), induce further proliferation of AD-specific CTLs. Moreover, IFN-γ can up-regulate the expression of MHC class I molecules in Ad-transduced cells and promote their recognition by CTL. AdV enters B cells and is subsequently processed to input virion coat proteins, which are present on the surface of B cells along with MHC class II molecules, activating Th2 cells to secrete cytokines such as IL-4, IL-5, IL-6, and IL-10. These cytokines induce the differentiation of these B cells into Ad specific antibody secretory plasma cells. Overall, the inherent immunogenicity of AdV makes it an attractive vaccine vector. Nevertheless, it also creates barriers to its usage. In detail, Anti-AdV immunity, whether pre-existing or caused by the AdV vaccine, may impair vaccine effectiveness [32].

4.3 Clinical Data

A recombinant adenovirus type 5 vector Covid-19 vaccine, funded by the National Key R&D Programme of China, the National Science and Technology Major Project, and Cansino Biologics, has been tested in phase 2 immunogenicity and safety trials in healthy adults aged 18 years or older.

The randomized, double-blind, placebo-controlled clinical trial was conducted at a center in Wuhan, China. Participants were all healthy adults over18 years old with a total of 508 participants. The mean age of participants was 39·7 years (SD: 12·5; Age range 18-83), 309 (61%) 18-44 years old, 134 (26%) 45-54 years old, and 65 (13%) 55 years old and older. 253 were randomly assigned to the 1 × 1011 particle dose group, 129 to the 5 × 1010 particle dose group, and 126 to the placebo group. Among 129 subjects in the 1 × 1011 and 5 × 1010 particle dose groups, 227 (90%, 95% CI 85-93) and 113 (88%, 81-92) IFN-γ ELISA responses were observed after vaccination. Adverse events were reported in 183 (72%) of 253 patients and 96 (74%) of 129 patients in the 1 × 1011 and 5 × 1010 particle dose groups. Severe adverse reactions occurred in 24 cases (9%) of the 1 × 1011 virus particle group and 1 case (1%) of the 5 × 1010 virus particle group. The results showed that the 5×1010 particle Ad5 vector Covid-19 vaccine was safe and induced a significant immune response in most recipients after a single dose of immunization [33].

In addition, a phase 2/3 controlled trial conducted by U. K. Research and Innovation, National Institutes for Health Research (NIHR), Coalition for Epidemic Prepared Innovations, NIHR Oxford Biomedical Research Centre, Thames Valley and South Midlands NIHR Clinical Research Network, and AstraZeneca, to document the safety and immunogenicity of ChAdOx1 nCoV-19 vaccine in young and older adults.

There were 560 participants in the study. Of the 160 participants aged from 18-55 years, 100 were assigned to Chadox1 nCOV-19 and 60 to Menacwy. Among 160 participants aged from 56 to 69 years, 120 were assigned to Chadox1 nCoV-19 and 40 to Menacwy. With regard to 240 participants over 70 years of age, 200 were assigned to Chadox1 nCOV-19 and 40 were assigned to Menacwy. A total of 552 participants were analyzed, of which 280 (50%) were female. Local and systemic reactions were more common in participants who received the Chadox1 Novel Coronavirus-19 compared to those who received the control vaccine. The presenting of them contain injection site pain, fever, muscle pain, and headache, but were less common in the elderly (age ≥56 years) than in younger participants. In those who received two standard doses of Chadox1 NCOV-19, post-vaccination local reactions were reported in 43 (88%) participants in the 18-55 years group,22(73%) in the 56-69 years group and 49(61%) in the elderly population aged 70 years and older, in 42(86%) and systemic reactions in participants in the 18-55 years group, 23(77%) in the 56-69 years group and 32 70 years and older group (65%). As of 26 October 2020, there have been no serious adverse events related to the investigational vaccine.

Among participants who received two doses of vaccine, the median 28-day anti-spike SARS-CoV-2 IgG response was similar across three age groups (standard dose group :18-55 years, 20 713 arbitrary units [AU]/mL [IQR 13 898-33 550], n=39; 56-69, 16 170 Au /mL [10 233-40 353], n=26; ≥70 years 17 561 Au /mL [9705-37 796], n=47; P = 0 · 68). Neutralizing antibody titers after the enhanced dose were similar in all age groups (median MNA80 on day 42 in the standard dose group :18 -- 55 years, 193 [IQR 113-238], n=39; 56-69 years old, 144 [119-347], n=20; ≥70 years old, 161 [73-323], n=47; P = 0 · 40). After 14 days of booster administration, 208 of 209 enhanced participants (>99%) had a neutralizing antibody response. T cell response reached the peak on day 14 after a single standard dose of Chadox1 nCoV-19 (18--55 years: median 1187 speckle-forming cells per million peripheral blood mononuclear cells [IQR 841-2428], n=24; 56-69 years :797 square feet [383-1817], n=29; ≥70 years :977 SFCS [458-1914], n=48). These results denoted that ChAdOx1 nCoV-19 vaccine appeared to be better tolerated in older adults than in younger adults and had similar immunogenicity in all age groups increased doses. Further evaluation of the effectiveness of the vaccine is still needed [34].

5 Recombinant protein vaccine

5.1 Introduction of recombinant protein vaccine

Recombinant protein vaccine is a kind of virus target antigen gene constructed on the expression vector. The constructed expression protein vector is transformed into bacteria, yeast or mammal or insect cells, under certain induction conditions, which expresses many antigen

proteins through the purification of vaccine preparation. The cost of producing and purifying immunogens is high. Besides, the production of large quantities of immunogenic proteins with sufficient purity after identification of immunogenic proteins is also an important part of vaccine manufacturing. The advent of recombinant DNA technology means that foreign genes can be inserted into expression vectors and then introduced into cells to aid in the production of foreign proteins. In many cases, this provides a vast and inexpensive source of protein for vaccination research [35].

As for bacterial expression, it uses Escherichia coli to establish the first recombinant expression system. This expression system can provide a relatively large number of defined proteins. However, expressed proteins are often misfolded because prokaryotic cells have different processing and transport mechanisms. One of the first successfully produced recombinant veterinary vaccines was based on the gp70 surface glycoprotein or p45 protein of FeLV expressed in E. coli [36]. With regard to yeast expression, which is an industrial microorganism, the widespread use of Saccharomyces cerevisiae (Baker's yeast) makes it the alternative antigen protein expression system of choice. It makes the recombinant protein more likely to fold properly than the prokaryotic system. Moreover, the expression system of insect cells was established by Spodoptera frugiperda infected with a baculovirus vector, Autographa californica nuclear polyhedrosis virus [37]. In addition, mammalian cell expression, due to the infection and replication of many veterinary pathogens in cultured mammalian cells [38]. Finally, plant cell expression, an additional emerging expression system worthy of mention. The first approved vaccine for the expression system is a vaccine against Newcastle disease (NDV) infection in poultry. It is being studied for other vaccine applications, including infectious bronchitis virus, infectious bursal disease virus, ETEC, BVD and bovine herpes virus [39].

5.2 Clinical Data

A phase I-II trial of SARS-CoV-2 polypeptide Spike Protein Nanoparticle Vaccine, NVX-COV2373, designed by Novavax and funded by the Coalition for Epidemic Prepared Innovations, was completed. NVX CoV2373 is a candidate vaccine engineered from a novel coronavirus gene. It was produced using Novavax's recombinant nanoparticle technology to generate antigens for Novel Coronavirus spikes. It also contains Novavax's proprietary saponin-based Matrix-M adjuvant, which enhances the immune response and stimulates high levels of neutralizing antibody production. To be more specific, it stimulates the entry of antigen-presenting cells into the injection site and enhances antigen presentation in local lymph nodes.

In Phase 1 clinical trials, a total of 131 adults aged from 18 to 59 years received two vaccine injections to evaluate the safety and immunogenicity of rSARS-CoV-2 vaccine. Participants were randomly assigned to receive the adjuvant vaccine, with 83 participants assigned to receive the adjuvant vaccine, 25 to not receive the adjuvant vaccine, and 23 to receive a placebo. All the subjects developed IgG antibodies against spines after a single vaccination and many of them also produced a neutralizing antibody response against the wild-type virus. After the second dose, 100% of subjects developed a neutralizing antibody response. Two doses of 5 g vaccine produced a mean geometric titer (GMT) of neutralizing antibodies of about 3900, four times higher than in patients recovering from COVID-19. Most participants were nonreactive or mildly reactive and the duration of adverse reactions was short (mean, ≤2 days). One participant had a mild fever that lasted 1 day. In addition, the researchers examined the participants' CD4-positive T cell responses. The results illustrated that NVX CoV2373 will induce antigen specific multifunctional CD4 positive T cell response. Moreover, the immune response is strongly biased towards Th1 phenotype (IFN-γ, IL-2, and TNF-α). The activation of CD4 positive T cells is conducive to the long-term production of highly effective neutralizing antibodies, which indicates promising results for the generation of long-term immunity. The results proved that NVX-COV2373 appeared to be safe at day 35, triggering an immune response that exceeded the level of COVID-19 recovery serum. CD4+ T cell response induced by matrix - M1 adjuvant was biased towards the Th1 phenotype [40].

6 Conclusion

Recently, positive results have been reported from Phase III clinical trials of the new coronavirus in China, the United States, Russia, the United Kingdom and other countries. Due to the great success of the research and development of the new coronavirus on different technical routes, many countries around the world have begun to carry out large-scale vaccination work. Globally, orders for COVID-19 vaccines total more than 10 billion doses, according to national press data.

In terms of vaccine capacity, the United States has the highest capacity. Novavax's vaccines, Pfizer's, Modena's and Johnson & Johnson's have a capacity of at least 4.8 billion doses. Orders for the four vaccines totaled 6 billion yuan. The UK's AstraZeneca vaccine is also seen as a promising alternative to the US vaccine, with orders totaling more than 2 billion doses. In addition, Russia has received orders for 1.2 billion doses of vaccines from more than 50 countries, according to data released by the country. In the end, China laid out a total of five technical routes to develop the new coronavirus. The total vaccine capacity of China mRNA new coronavirus vaccine, Kangtai Biotech's adenovirus technology vaccine, Zhifang Biotech's phase 2 clinical recombinant protein new coronavirus vaccine, and the inactivated vaccine produced by Sinopharmaceutical Beijing Co., Ltd., Sinopharmaceutical Wuhan Co., Ltd., and Beijing Kexingzhongwei Co., Ltd., will exceed 2.2 billion doses. And the total number of vaccine orders in China is 500 million.

This is a great blessing for the society, which is now in a state of great depression. After the mass production and

injection of the novel coronavirus, the novel coronavirus is bound to be controlled to a great extent, and the society and economy will recover steadily. This is the future that the whole world is looking forward to and longing for.

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