Review

COVID-19 animal models and vaccines: current landscape and future prospects

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Abstract: The worldwide pandemic of coronavirus disease 2019 (COVID-19) has become an unprecedented challenge to global public health. With the intensification of the COVID-19 epidemic, the development of vaccines and therapeutic drugs against the etiological agent severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is also widespread. To prove the effectiveness and safety of these preventive vaccines and therapeutic drugs, available animal models that faithfully recapitulate clinical hallmarks of COVID-19 are urgently needed. Currently, animal models including mice, golden hamsters, ferrets, nonhuman primates and other susceptible animals have been involved in the study of COVID-19. 92 vaccine candidates have entered clinical trials after the primary evaluation in animal models, of which inactivated vaccines, subunit vaccines, virus-vectored vaccines and messenger ribonucleic acid (mRNA) vaccines are promising vaccine candidates. In this review, we summarize the landscape of animal models and advanced vaccines with efficacy range from about 50% to more than 95%. In addition, we point out future directions for animal models and vaccine development, aiming at providing valuable information and accelerating the breakthroughs confronting SARS-CoV-2.

Keywords: COVID-19; SARS-CoV-2; animal models; vaccines; future prospects

1. Introduction

In December 2019, a previously unknown beta coronavirus causing human pneumonia emerged and was soon isolated, named 2019-nCoV [1]. Subsequently, the virus was renamed as SARS-CoV-2 and the syndrome was named COVID-19 by the World Health Organization (WHO) [2]. By July 4, 2021, over 184 billion COVID-19 cases have been confirmed, causing 3.98 billion deaths worldwide [3]. The progression and dissemination of COVID-19 seriously threatened the international health security and caused an immeasurable loss on the global economy, hence scientists all over the world are embarking on prophylactic and therapeutic research on SARS-CoV-2.

SARS-CoV-2 is an enveloped, positive-sense, single-stranded ribonucleic acid (RNA) virus encoding 16 non-structural proteins (nsp1-nsp16), several accessory proteins and four structural proteins, including spike surface glycoprotein (S), matrix protein (M), small envelope protein (E) and nucleocapsid protein (N) [4]. S protein plays a key role in mediating the virus entry via interacting with the receptors of the host cell and is considered as the main target to induce neutralizing antibodies (nAbs). S protein is composed of S1 and S2 subunits, of which S1 subunit functions as receptor-binding subunit while S2 subunit mediates membrane fusion. Within S1, a region with 194 residues named receptor binding domain (RBD) is identified as the core sequence for SARS-CoV-2 binding to host cell. RBD shows a high binding affinity to human angiotensin-converting enzyme 2 (hACE2) and serves the entry and infection of SARS-CoV-2

[5,6]. Whilst some enzymes, including transmembrane protease serine 2 (TMPRSS2), cathepsin B/L and RNA-dependent RNA polymerase (RdRp) are key regulators of viral entry, replication and transcription [5,7].

This life-threatening COVID-19 is characterized by symptoms of viral pneumonia, including fever, cough and chest discomfort. In severe cases, dyspnea and bilateral lung infiltration are observed [1,8]. Generally speaking, elderly patients with comorbidities face higher risk for SARS-CoV-2 infection and unfavorable prognosis. Besides the above clinical characteristics, animal models recapitulating the transmission characteristic, pathology and corresponding immunological response to COVID-19 are foundational and urgent need. Although great progress has been achieved in the development of prophylactic and therapeutic measures, only a few products have been proven effective. Currently, animal models involved in the study of COVID-19 include mice, golden hamsters, ferrets, nonhuman primates and pigs. More than 216 vaccine candidates are under development, of which 92 of them have successfully entered clinical trials [9]. Herein, we briefly discuss popular animal models and cutting-edge vaccines of COVID-19, followed by a detailed discussion of their commonalities and personalities, pros and cons. We aim to grasp key information of animal models and vaccine research of COVID-19 and provide reference for subsequent breakthroughs.

2. Animal Models for SARS-CoV-2 countermeasures Evaluation

In the background of the COVID-19 pandemic, the development of vaccines, antibodies and drugs of COVID-19 was also at high speed. Before clinical trails, the above prophylactic and therapeutic countermeasures are in need of pre-clinical evaluation to ensure their safety and efficacy. COVID-19 animal models are fundamental and essential need in this phase. In the evaluation of countermeasures, even in the study of the SARS-CoV-2 related mechanism, animal models are required to have some common characteristics. For example, the susceptibility to SARS-CoV-2 and the similarity SARS-CoV-2 post-infection immune response like human beings. Here, we retrospected the basic background and the development paths of COVID-19 animal models. More importantly, we paid close attention to the unique features that animal models needed for vaccine evaluation. We have summarized the animal models as well as susceptible animals (potential animal models) of COVID-19 in figure 1.

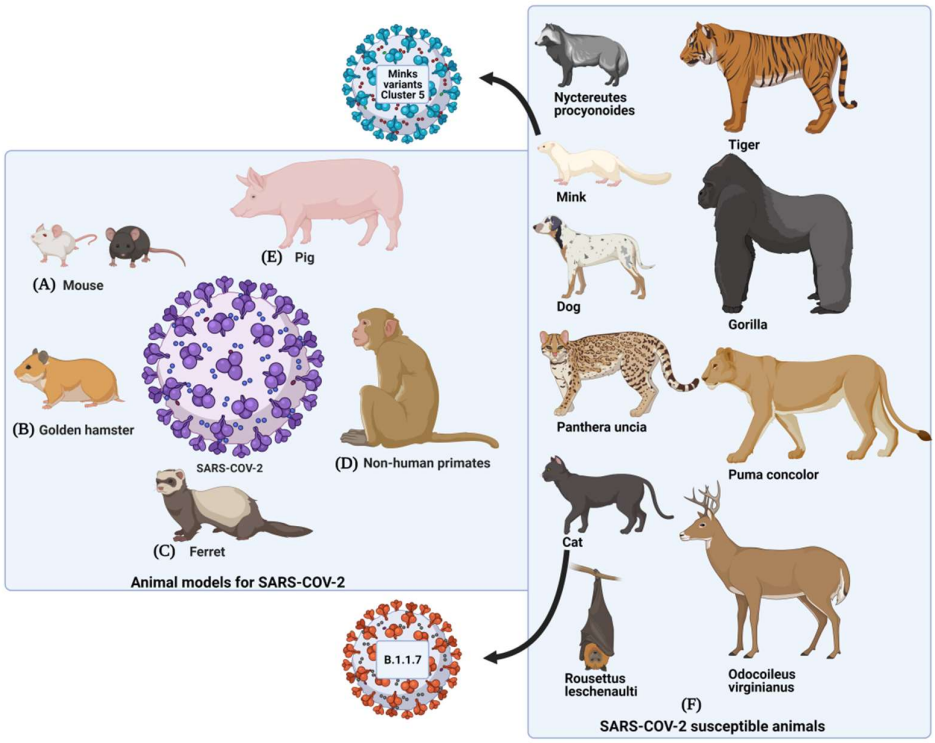

Figure 1. COVID-19 animal models and susceptible animals. **(A)** Mouse models: hACE2 transgenic mouse, hACE2-transdued mouse and mouse-adapted SARS-CoV-2. **(B)** Golden hamster model. **(C)** Ferret model. **(D)** No human primates: Rheus macaque model and Cynomolgus macaque model. **(E)** Pig model. **(F)** Other susceptible animals

2.1. Mouse models

Laboratory mice are the most frequently used animal models in the preclinical study of vaccines. Economical, abundant, well characterized, easy to handle and manipulate, easily accessible reagents and tools, these characteristics support extensive development of mouse models. However, SARS-CoV-2 shows limited affinity to murine ACE2 [10], which hinders the application of mouse models in SARS-CoV-2 research. Scientists overcome this issue from three directions: hACE2 transgenic mice, hACE2 transduced mice as well as mouse-adapted SARS-CoV-2.

To enhance the replication capability of SARS-CoV-2 in mice, Jiang et.al developed a hACE2 transgenic C3B6 mouse infection model with similar pneumonia and pathology to COVID-19 patients [11]. High viral load was detectable in lungs of hACE2 transgenic C3B6 mouse. Pre-exposure to SARS-CoV-2 protected mice from severe pneumonia, indicating the production of protective immunity. In addition, using CRISPR/Cas9 knock-in technology, Sun et.al generated a transgenic C57BL/6 mouse model whose mACE2 gene was completely replaced with hACE2 (termed hACE2 mice) [12]. High viral loads in lung, trachea and brain were detectable, followed with interstitial pneumonia and elevated cytokines occurring in SARS-CoV-2 infected hACE2 mice. In hACE2 mice, viral RNA load in lungs was much higher and the distribution of hACE2 in various tissues was more in line with human conditions in comparison to other hACE2 genetically

engineered mice generated by pronuclear microinjection [11,13]. In particular, the pathological changes observed in 30 weeks old hACE2 mice were similar to those observed in aged COVID-19 patients. Interestingly, intragastric inoculation of SARS-CoV-2 caused digestive tract infection and resulted in pulmonary pathological changes in 4.5 weeks old hACE2 mice, which were consistent with the other previous study [14]. The effective replication of SARS-CoV-2 in human intestinal organoid suggested that intestinal tract may be another transmission route of SARS-CoV-2. These results indicated that an infection through the digestive tract was established in hACE2 mice, which provided a reference for developing oral vaccines. More recently, a transgenic hACE2 mouse model with typical pathological changes in lungs for SARS-CoV-2-induced acute respiratory was established [15]. It is of significance for the future evaluation of vaccines and drugs against SARS-CoV-2-induced acute respiratory distress syndrome (ARDS).

Meanwhile, two independent research groups reported hACE2 receptor-transduced BALB/c and C57BL/6 mice models using replication-defective adenoviruses [16,17]. Corresponding clinical signs and virus replication were observed after the SARS-CoV-2 infection. These models have been applied to assess the effectiveness of vaccine candidates and antiviral therapies including convalescent plasma, monoclonal antibody (mAb) and chemical drugs etc. Beyond that, exogenous delivery of hACE2 through Venezuelan equine encephalitis replicon particles (VEEV-VRP-hACE2) has been reported [18].

The mouse-adapted SARS-CoV-2 was obtained by serial passages. Mouse-adapted SARS-CoV-2 MASCp6 was achieved after 6 passages of SARS-CoV-2 in 9 months old BALB/c mice. MASCp6 efficiently infected both the aged and 6 weeks old BALB/c mice, it replicated efficiently in the lung and trachea resulting in moderate pneumonia as well as inflammatory responses [19]. A key substitution of N501Y in RBD was predicted to contribute to the enhanced infectivity of MACSp6 in mice. A RBD-based SARS-CoV-2 subunit vaccine has been proved to have promising protective efficacy in this model. Compared with young (6 weeks old) mice, aged (9 months old) mice exhibited more severe lung damage after MASCp6 infection. In another previous study, a mouse-adapted SARS-CoV-2 HRB26M efficiently infected the upper and lower respiratory tract of 4~6 weeks old BALB/c mice and C57BL/6J mice [20]. 8~9 months old adult BALB/c mice infected with HRB26M showed more extensive pathological changes in the respiratory tract than those of 10 weeks old BALB/c mice. Subsequently, a lethal mouse-adapted SARS-CoV-2 MA10 which caused acute lung injury (ALI) in 10 weeks old and 1 year old BALB/c mice have been isolated after 10 passages in 10 weeks old BALB/c mice. It exhibited the epidemiological characteristics of COVID-19 disease as well as aspects of host genetics, age, cellular tropisms, elevated Th1 cytokines, and loss of surfactant expression and pulmonary function linked to pathological features of ALI [21]. Interestingly, SARS-CoV-2 MA10 showed no mortality in 10 weeks old C57BL/6J mice. The process of adaptation introduces multiple point mutations into the virus genome that are responsible for increasing the virulence, yet whether this artificially introduced genetic divergence compromises the relevance of the adapted viruses in the first place remains to be fully elucidated.

The development of genetically modified mouse models is time-consuming, hard-operating and costly. As for hACE2 transduced mouse models, pathological changes in mice due to adenovirus vectors should be taken into account. Besides, mice transduced with Ad5-hACE2 do not develop extrapulmonary manifestations, no severe disease was observed. Lethal mouse-adapted SARS-CoV-2 has achieved breakthrough, with 100% fatality and clear mutation site (unpublished data). Thus mice-adapted SARS-COV-2 mice models provide relatively inexpensive and easily accessible animal models for vaccine evaluation.

2.2. Golden hamster models

Golden hamsters have previously shown susceptibility to SARS-CoV [22]. They were another potential small animal model to study the pathogenesis and transmission

of SARS-CoV-2 [23]. Apparent weight loss was observed in the SARS-CoV-2 infected hamsters. Viral antigens were observed in nasal mucosa, bronchial epithelial cells, duodenum epithelial cells and lung consolidation. SARS-CoV-2 inoculated hamsters efficiently transmit SARS-CoV-2 to naïve hamsters by direct contact and via aerosols. Transmission caused by fomites from soiled cages was less efficient. Although viral RNA was continuously detectable in inoculated hamsters until 14 days post infection (dpi), the SARS-CoV-2 inoculated donors have a short communicable period of less than 6 days and correlated with the detection of infectious virus rather than viral RNA. All recovered animals produce SARS-CoV-2 nAbs . SARS-CoV-2 infection in golden Syrian hamsters resembled features of mild COVID-19 patients. Beyond that, clinical signs of rapid breathing could be observed [24]. In hamsters, immunoprophylaxis with early convalescent serum significantly decreased virus load in lungs but not prevented or ameliorated lung pathology. SARS-CoV-2 infection triggers bronchopneumonia and a strong inflammatory response in the lungs with neutrophil infiltration and edema [25]. Most importantly, by using hamsters with ablated signal transducer and activator of transcription 2 (STAT2$^{-/-}$) and Interleukin 28R (IL28R-a$^{-/-}$) expression, STAT2 signaling pathway was confirmed to play a contradictory role in COVID-19 associated immune pathogenesis by driving severe lung injury and restricting systemic virus dissemination as well. Several vaccine candidates have been tested in hamsters. Vaccinated hamsters showed reduced or disappeared infectious virus in the lungs and less body weight loss as well as less histopathological changes of pneumonia. Vaccinated hamsters produced a level of nAbs against SARS-CoV-2 [26-29]. In short, golden hamsters reflect certain clinical manifestations and immune response after the vaccination of COVID-19 vaccines.

2.3. Ferret models

The domestic ferret (*Mustela putorius furo*) is naturally susceptible to many viruses including bunyaviruses, paramyxoviruses, rhabdoviruses and togaviruses [30]. Besides, ferret has been used as a model system for respiratory diseases [31]. It is naturally susceptible to influenza A virus and recapitulates many aspects of influenza infection. In the past decades ferret was commonly utilized to the prophylactic and therapeutic research of influenza. In addition, ferret has been applied in coronaviruses related research [31]. Ferrets recapitulated some typical disease features of COVID-19 observed in humans. Naturally infected ferrets rapidly transmitted SARS-CoV-2 to the entire population via direct or indirect contact [32]. Infected ferrets exhibited elevated body temperatures and virus replication. Virus shedding was confirmed in nasal washes, saliva, urine, and feces while infectious viruses were detected in nasal turbinate, trachea, lungs, and intestine with acute bronchiolitis present in infected lungs. Thus, ferret represented an ideal animal model for virus shedding and transmission. However, mild clinical symptoms and relatively lower virus titers in lungs hindered the fully application of ferret models. Due to the moderate susceptibility to SARS-CoV-2, ferrets were considered a mild clinical disease model of COVID-19. As showed in another previous study [33], Viral RNA shedding in the upper respiratory tract (URT) was observed in all ferrets (6/6) after a high (5×10^6 pfu) dose of SARS-CoV-2 challenge, while only 1/6 ferrets showed similar signs after low dose (5×10^2 pfu) challenge. According to the above discussed dose-dependent response to the infection of SARS-CoV-2, the application of ferrets in vaccine evaluation is relatively limited.

2.4. Nonhuman primate models

Due to the similar biological characteristics of non-human primates (NHPs) and humans, NHPs are golden standard models of many emerging infectious diseases, including Ebola, Lassa fever, etc [33-40].

In rhesus macaques models**,** infectious SARS-CoV-2 were detectable in nose, throat as well as bronchoalveolar. Viral replication in 15 years old animals were more active

than those of 3~5 years old animals after SARS-CoV-2 challenge, which was consistence of the susceptibility characteristic of humans [41]. Infected animals developed typical interstitial pneumonia, especially in old animals. They exhibited diffuse severe interstitial pneumonia. The respiratory disease caused by SARS-CoV-2 lasted 1~2 weeks in infected rhesus macaques [42]. Signs of disease including changes in respiratory patterns and piloerection could be observed. Similar to humans, infiltration could be seen in lung radiographs of all infected animals. In another previous study, SARS-CoV-2 infected rhesus macaques exhibited modestly decreased appetite and responsiveness suggestive of mild clinical disease as well as mild transient neutropenia and lymphopenia in the high dose group. Obvious clinical symptoms were not observed [43]. After infection, virus-specific antibody and nAbs responses started to appear at 10 dpi, animals with the lowest and tardiest nAbs response showed prolonged viral shedding from the intestinal tract, which suggested that nAbs plays a role in the control of infection. SARS-CoV-2 challenge and rechallenge trails in rhesus macaque models suggested that all exposed macaques developed binding antibody response and nAbs to SARS-CoV-2 [43] [44], a large part of animals developed cellular immunity. SARS-CoV-2 rechallenged animals showed reductions in median viral loads compared with their primary infection. Challenged rhesus monkeys were almost completely protected from the reinfection of SARS-CoV-2, indicating the production of protective immunity. Overall, rhesus macaques successfully recapitulated many hallmark features of human COVID-19 and triggered certain immune responses after SARS-CoV-2 infection. Inhibited virus replication and the production of certain humoral and cellular immunity against SARS-CoV-2 are two remarkable features exhibited in rhesus macaques after the vaccination of COVID-19 vaccines [45-51]. Antibodies decreased at 7 months post-priming [45], which is similar to human beings. From this point of view, the post-vaccination immune response in rhesus monkeys can better reflect the actual situation that in human beings.

Similarly, cynomolgus macaques were permissive to SARS-CoV-2 infection and displayed COVID-19-like disease [52], SARS-CoV-2 replicated efficiently in respiratory epithelial cells throughout the respiratory tract. Prolonged viral shedding was observed in the URT of aged animals. All SARS-CoV-2 post challenge remaining animals produced SARS-CoV-2–specific antibodies against the virus S1 domain and N proteins. In SARS-CoV infected cynomolgus macaques, lung lesions were typically more severe compared with SARS-CoV-2 infection. Cynomolgus macaques have the potential to be an animal model for emerging beta coronavirus including SARS-CoV, Middle East Respiratory Syndrome (MERS-CoV) and SARS-CoV-2. However, the data should be interpreted cautiously since there are differences between SARS-CoV-2 infection in macaques and humans with many parameters yet to be defined. In the evaluation of COVID-19 vaccines, vaccinated cynomolgus macaques produced high levels of RBD-specific immunoglobulin G (IgG) and potent nAbs, they exhibited lower or no viral RNA copies and very mild histopathological changes in lung [53,54].

2.5. Other susceptible animals

In addition to the above commonly used laboratory animal models, susceptibility of other animals to SARS-CoV-2 has been reported, including nyctereutes procyonoides [55], minks [56-59], dogs [60], panthera onca, cats [61] , rousettus leschenaulti [62], tiger, gorilla, odocoileus virginianus [63], etc. In Netherlands, United States, and Denmark, millions of minks have been culled over concerns that the animals transmit SARS-CoV-2 to human beings, subsequently cases of mink-to-human transmission were confirmed [56-59]. COVID-19 affected minks in a similar way to humans, causing respiratory problems and lung lesions that tend to be worse in older animals. In theory, animals susceptible to SARS-CoV-2 are potential animal models. On the other hand, the broad spectrum of SARS-CoV-2 infections is an overall concern, attention should be paid to the prevention and control of SARS-CoV-2 infection in animals.

2.6. Future prospects

We have summarized the characteristics of reported animal models in table 1. At present, no animal model recapitulates all aspects of human COVID-19, so the choice of animal models is determined by the purpose of the study. Mouse-adapted SARS-CoV-2 infection model is an economic, productive and available small animal model. Virus replicate, certain pneumonia, pathology and lethality were observed. However, it is still unclear the extent that the immune response to COVID-19 in the mouse models could be exquisitely reflected in humans although some drugs and vaccines have been tested in this animal model. Another small animal model, golden Syrian hamster, has been utilized in SARS-CoV-2 pathogenesis mechanism and transmissibility study. Similar to hamsters, ferrets successfully recapitulated human infection and transmission, with a clinical symptom of fever. In NHPs, no obvious clinical signs were observed after SARS-CoV-2 infection. Beyond that, NHPs are limited to be widely and largely used in terms of ethics, high cost and spacious feeding space. However, more severe interstitial pneumonia and prolonged viral shedding are observed in the URT of aged individuals, which indicates that aged NHPs were ideal animal models for recapitulating the representative characteristics of aged COVID-19 patients. Besides, the immune response in NHPs is similar to that of human beings, which laied the pre-eminence of NHPs in the evaluation of the immunogenicity of vaccine. At present, mice and NHPs are the most frequently used in pre-clinical vaccine evaluation.

Table 1. Characterizations of animal models for COVID-19

Animals/design	Challenge Dose	Route	Lethality	Clinical features	Infected Organs	Reference
hACE2 transgenic C3B6 mice	3×10^4 $TCID_{50}$	i.n.	No	Interstitial pneumonia and pathology, lose weight	Lungs, eye, heart, and brain	[11]
hACE2 transgenic C57BL/6 mice	4×10^5 PFU	i.n./i.g.	No	Interstitial pneumonia, pathology and elevated cytokines	Lung, trachea and brain	[12]
	40 μl, 10^7PFU/ml	i.t.	No	ARDS, lung pathology, neutrophilic infiltration		[15]
hACE2-transduced BALB/c and C57BL/6 mice	10^5 PFU	i.n.+i.t.	No	Pneumonia, lung pathology and weight loss	Lung, heart, spleen, and brain	[16,17]
BALB/c or C57BL/6	7.2×10^5PFU	i.n.	No	Moderate pneumonia and inflammatory responses	Lung, upper and lower respiratory tract	[64]
mouse-adapted SARS-CoV-2	$10^{6.2}$ PFU/$10^{4.4}$PFU	i.n.	No	/		[20]
	10^2~10^5 PFU	i.n.	Yes	ALI, lung disease, elevated cytokines		[21]
Golden hamsters	8×10^4 $TCID_{50}$/10^5PFU	i.n.	No	Lung pathology, weight loss, rapid breathing	URT, duodenum epithelial cells and lung consolidation areas	[23-25]
Ferrets	$10^{5.5}TCID_{50}$	i.n.	No	Elevated body temperature, acute bronchiolitis	Nasal turbinate, trachea, lungs, and intestine	[32]
Cynomolgus macaques	/	i.n.+i.t.	No	Lung pathology, no overt clinical signs	Nose, throat trachea, bronchi, and lung lobes	[52]
Rhesus macaques	10^6 $TCID_{50}$	i.n.	No	Interstitial pneumonia and pathology, weight loss, asthenia, respiratory disease	Nose, throat, lung and anus	[41-43]

Note: $TCID_{50}$: median tissue culture infective dose; **PFU:** plaque forming unit; **i.n.:** Intranasal inoculation; **i.g.:** intragastric inoculation; **i.t.:** intratrachea inoculation

3. Vaccines for SARS-CoV-2 Prevention

We have summarized the most sophisticated COVID-19 vaccines including inactived vaccines, protein subunit vaccines, virus-vectored vaccines and nucleic acid vaccines (detailed information in table 2).

3.1. Inactivated vaccines

Using radiation techniques and chemical substances for viral inactivation, the inactivated vaccines are a member of the first generation vaccine that has been widely used for decades, promising a mature solution. The complete antigen epitopes of COVID-19 inactivated vaccine enables the fully expose of immune epitopes other than S protein. However, the production of COVID-19 inactivated vaccine must be handled in the biosafety level 3 workshop, this poses the primary challenge. At present, three inactivated vaccines have been approved in China whilst an inactivated vaccine named COVAXIN® has been approved in India [65]. The research of inactivated vaccines in other countries is rarely reported.

Sinovac Biotech Ltd has developed an inactivated SARS-CoV-2 vaccine named CoronaVac, which is capable of inducing S protein or N protein-specific antibodies and nAbs in mice, rats and NHPs [66]. Antibodies elicited by CoronaVac exhibited potent neutralization activities against 10 SARS-Cov-2 strains circulating worldwide (including D614G variants). CoronaVac completely protected macaques from the SARS-CoV-2 challenge without the antibody-dependent enhancement (ADE) effect. Most notably, the high dose group showed a more potent viral load decrease than the low dose group. Furthermore, the perspective of safety and immunogenicity of CoronaVac in the elderly was encouraging in phase I/II clinical trials [67]. The Phase III clinical trial results in Brazil showed that the protective efficacy of CoronaVac against COVID-19 at 14 days post vaccination (dpv), the protection efficacy of cases requiring medical attention and the protective efficacy of hospitalized cases were 50.65%, 83.70% and 100.00%, respectively. In Turkish, the protective efficacy of CoronaVac 14 dpv against COVID-19 was 91.25% [68]. Sinovac Biotech Ltd has established two production lines, which ensured mass production of vaccines. On February 5, 2021, the conditional marketing application for CoronaVac was approved [69]. The immunization schedule was 2 doses (0.5 ml per dose) for humans with an interval of 14 to 28 days. Subsequently, CoronaVac was also approved for emergency use in more than 10 countries and regions including Hungary, Seychelles, Pakistan, and Morocco, etc and was registered and listed in the United Arab Emirates and Bahrain.

Developed by the Chinese Center for Disease Control and Prevention and Beijing Institute of Biological Products Company Limited, another inactivated vaccine candidate termed BBIBP-CorV induced high levels of nAbs in mice, rats, guinea pigs, rabbits and NHPs [70]. Two-dose of BBIBP-CorV completely cleared SARS-CoV-2 in rhesus macaques. In addition, BBIBP-CorV exhibited genetic stability to be efficiently cultured for vaccine manufacturing. It's safety was proven in phase III clinical trial. On December 31, 2020, BBIBP-CorV became the first inactivated vaccine been approved for human use in China. After two dose vaccinations, all individuals produced high titers of antibody, nAbs positive conversion rate was 99.52%, the protection efficacy of BBIBP-CorV vaccine was up to 79.34% (unpublished data).

Sinopharm Wuhan Institute of Biological Products developed another inactivated vaccine, termed COVILO. On June 16, 2020, the phase I/II clinical trial of COVILO was unblinding [71]. COVILO exhibited good safety without any serious adverse reaction, the adverse reactions were mild or moderate, injection site pain was the most frequently observed, followed by fever. Vaccinated volunteers produced high titers of antibodies. After two doses of immunization at day 0 and day 28, nAbs reached 100% positive conversion [72]. On February 28, 2021, COVILO became the third COVID-19 vaccine licensed in China. The above three inactivated vaccines will be popularly vaccinated in China and become the main force of COVID-19 vaccines in China.

3.2. Protein subunit vaccines

Subunit vaccines are composed of immunogenic proteins or peptides of specific antigens. Characteristics such as safety (absence of side effects), immunogenic, and flexibility shed light on the development of protein subunit vaccines [73]. The S protein of SARS-CoV-2 with stabilized trimeric form (S-Trimer) has been widely used for designing vaccines for SARS-CoV and MERS-CoV [74-76].

Clover Biopharmaceuticals developed an S-Trimer subunit vaccine named SCB-2019 [77]. In phase I clinical trial, around 30% of individuals reported adverse effects after the second dose. Two serious adverse events were recorded both in older adults, including cellulitis and hyponatraemia. SCB-2019 should be combined with AS03 or CpG/Alum adjuvants to accomplish a promising immune response. Developed by Novavax, NVX-CoV is another distinguished nanoparticle vaccine composed of S-Trimers [78]. No obvious serious adverse events were observed in phase I clinical trial of NVX-CoV. The combination with adjuvant Matrix-M1 resulted in enhanced immune responses. A Two-dose regimen of NVX-CoV induced robust S protein specific IgG and nAbs. The immune response is more inclined to Th1 immune response. Of note, S-Trimer can be stored for a long term at 2~8°C and remain stable for two months at room temperature.

To combat the possible pandemic of emerging beta coronavirus, a flexible vaccine design strategy is needed. Chinese scholars have described a dimeric form of MERS-CoV RBD that overcame the limited immunogenicity of monomeric RBD [79]. RBD-dimer fully exposed dual receptor-binding motifs, the major target for nAbs. Mice vaccinated with the RBD-dimer showed significantly increased nAbs against MERS-CoV infection compared to those who receive conventional monomeric RBD. When RBD-dimer was applied to SARS-CoV-2 and SARS-CoV vaccines development, 10~100 folds enhancement of nAbs was achieving. This team has developed another tandem-repeat dimeric RBD protein-based COVID-19 vaccine, named ZF2001 [80], which has been authorized by Uzbekistan for emergency use. RBD-dimers in pilot-scale production yielded high yields, supporting their scalability for further clinical development.

Walls, A.C., et al. developed a structure-based nanoparticle vaccine displaying 60 copies of the SARS-CoV-2 RBD in a highly immunogenic array [81]. This vaccine candidate induced robust nAbs target multiple distinct epitopes on the RBD, which means that it may not be easily susceptible to escape mutations. Minimize antigen structure, maximize antigens amounts, nanoparticle vaccines are a potential candidate to overcome the ADE effect. In addition, the manufacture of nanoparticle vaccines based on antigen display platforms is highly scalable.

3.3. Virus-vectored vaccines

Viral vectored vaccines have been widely used for the prevention of emerging infectious diseases [82]. They are immunogenic due to their ability to enhance both humoral and cellular immune response. Nevertheless, safety issues and pre-existing immunity against the vectors are the major concerns for viral vectored vaccines [83]. At present, adenovirus vector, vesicular stomatitis virus, attenuated rabies virus, and modified vaccinia virus are the most popular virus vectors [84]. Herein, we mainly introduced the advances of the above viral vectored vaccines of COVID-19.

3.3.1. Adenovirus vector

Adenovirus type 5 (Ad5) has several unique advantages as a vaccine vector, including prominent immunogenicity, easy manipulation, strong ability to express exogenous genes and to elicite humoral and cellular immune response. Ad5-EBOV is one of the most advanced Ebola vaccines [85-88]. The Ad5-nCoV vaccine was developed by CanSino Biological Inc. and Beijing Institute of Biotechnology, which was designed to deliver the gene of the SARS-CoV-2 S protein into human cells. Ad5-nCoV is the first single-dose vaccine candidate to enter clinical trials. In phase I clinical trial [89], Ad5-nCoV was tolerable in healthy adults, adverse reactions were mild or moderate, in-

cluding fever, fatigue, headache, and muscle pain, no serious adverse event was noted. S-specific antibodies and nAbs increased significantly at 14 dpv, and peaked at 28 dpv. SARS-CoV-2 specific T-cell response peaked at 14 dpv. However, high pre-existing Ad5 nAbs compromised the seroconversion of SARS-CoV-2 nAbs and reduced the peak of post-vaccination T-cell responses. SARS-CoV-2 nAbs titers were relatively low, ranging from 14.5 to 34 at four weeks post infection. In phase II clinical trial [90], safety and immunogenicity were further assessed, especially in participants over 55. nAbs were consistent with the results in phase I clinical trial. The results of phase III clinical trial suggested that 14 or 28 days after a single dose injection of the vaccine, the overall protective efficacy was 68.83% and 65.28%, respectively. The protective efficacy against the occurrence of severe illnesses 14 or 28 dpv was 95.47% and 90.07% respectively [91]. On February 5, 2021, the conditional marketing application for Ad5-nCoV was approved.

Ad26-vectored vaccines have also been evaluated in clinical trials, among them Ad26-Ebola was confirmed to be a safe vaccine for humans with good immunogenicity [92]. Harvard Medical School constructed an Ad26-vectored vaccine expressing SARS-CoV-2 variants S with different leader sequences, antigen forms, and stabilization mutations [93]. NHPs experiment demonstrated the optimal vaccine candidate, termed Ad26.COV2.S, which contained the wildtype leader sequence, the full-length membrane-bound S with a mutation in the furin cleavage site and two proline stabilizing mutations. A single shot of Ad26.COV2.S induced robust nAbs and provided complete or near-complete protection in bronchoalveolar lavage and nasal swabs following the SARS-CoV-2 challenge. Ad26.COV2.S is currently being evaluated in phase III clinical trials.

Replication-deficient chimpanzee adenovirus type 1 (ChAdOx1) has been utilized for MERS vaccine development [94,95]. The University of Oxford developed a ChAdOx1-vectored SARS-CoV-2 vaccine encoding a codon-optimized full-length S gene [96]. ChAdOx1-S induced robust humoral and cellular immune response in mice. IgG subclasses were predominantly Th1-biased. A single dose of ChAdOx1-S induced immune response and reduced viral loads in rhesus macaques, but virus titers remained high in the upper and lower respiratory tract after the high dose challenge. Three monkeys showed accelerated breathing and obvious symptoms after challenge, no difference of the viral load in the nasal swabs when compared with the control. Although no pneumonia or immune-enhanced diseases was observed, the efficacy of the vaccine was still questionable. In another study, ChAdOx1 nCoV-19 was tested in mice (BALB/c and CD1) and pigs [97]. A prime-boost strategy significantly enhanced antibody and T cell responses in pigs but not in mice compared to the single dose group. Similar to mice, pigs' T cell responses showed a dominant Th1-type cytokines but with a higher frequency of S-specific $CD4^+$ T cells compared to $CD8^+$ T cells. This study indicated that immunogenicity data in mice were distributed at the upper end of the dose response curve, which may saturate the immune response and largely obscure the difference between different regimens. Compared with the mouse, pig may be a better animal model for further evaluation of the immunogenicity of ChAdOx1 nCoV-19 and other COVID-19 vaccines. In phase I/II and II/III clinical trials [98,99], ChAdOx1 nCoV-19 was tolerable, humoral and cellular immune responses were observed in a large part of volunteers. Homologous boosting increased antibody responses and protective efficacy, especially in those with a longer prime-boost interval (≥12 weeks). No hospital admissions for COVID-19 in the vaccination group after the initial 21-day exclusion period. Antibody and protection efficacy last at least 3-month. The UK regulatory authority has approved AZD1222 for emergency use. For participants who received two standard doses and participants who received a low dose followed by a standard dose, the reported efficacy was 62.1% and 90.0% respectively, resulting in an overall efficacy of 70.4% [100]. However, research in South Africa indicated that the efficacy of ChAdOx1 nCoV-19 has been affected by the variant 501Y.V2, it failed to resist the occurrence of mild or moderate COVID-19 in some cases. What's more, on September 10, 2020, the Phase III clinical trial was once called off

due to a case report of transverse myelitis in one volunteer. Subsequently, the European Drug Administration (EMA), the British drug regulatory agency (MHRA) and WHO issued statements that ChAdOx1 nCoV-19 may be associated with rare thrombosis, so the safety of ChAdOx1 needs further evaluation.

Heterologous prime-boost strategy is an effective scheme to reduce the pre-existing adenovirus immunity. Russia has developed a rAd26 and rAd5 vector-based heterologous prime-boost strategy, termed Sputnik V [101]. Compared with single dose strategy, the heterologous rAd26 and rAd5 vector-based COVID-19 vaccine induced stronger humoral and cellular immune responses in participants. Beyond that, there are two formulations available, including frozen and lyophilized.

3.3.2. Vesicular stomatitis virus vector

Rapid replication, high growth titer, multi antigens expression ability, single dose use, these characteristics render vesicular stomatitis virus (VSV)-vectored vaccine very popular in recent years. One of the most advanced Ebola vaccines VSV-EBOV was developed based on this platform expressing EBOV GP and achieved promising results in clinical trials [102]. Case et.al constructed a replication-competent VSV-vectored vaccine that expressed a modified form of the SARS-CoV-2 S gene in place of the native glycoprotein gene (VSV-eGFP-SARS-CoV-2). Vaccinated mice produced a high level of nAbs and showed significantly reduced SARS-CoV-2 infection and inflammation in lung. Passive transfer of immunization sera conferred protection for naïve mice from the SARS-CoV-2 challenge. In addition to acting as a vaccine vector, VSV has been successfully applied in neutralization assay at biosafety level 2 and the mechanism study of SARS-CoV-2 infection [103,104]. Unfortunately, the clinical trials of VSV-vectored COVID-19 vaccine progressed slowly due to safety issues.

3.3.3. Rabies virus vector

Rabies virus (RABV)-vectored vaccine is usually used in the form of inactivation. Some progress has been achieved in the research of other coronaviruses including SARS and MERS [105-108]. Wirblich, C. et al. developed a RABV-vectored vaccine candidate CORAVAX™ containing the SARS-CoV S1 domain fused to the C-terminus of the RABV G protein [105,109]. Both live and inactivated candidates induced potent virus nAbs at much higher levels than that of convalescent patients. According to previous experience in virus hemorrhagic fever, multi-dose inactivated vaccines based on rabies virus vectors were ideal in terms of the durability of immune protection [110-112].

3.4 Nucleic Acid Vaccines

Nucleic acid vaccines include DNA vaccines and RNA vaccines. As these are not virus-containing vaccines, limited risk associated with virulence exists upon application. While this new technology brings surprises, there are many issues unknown. Whilst the strategy to improve in vivo transfection efficiency of nucleic acid vaccines is another challenge.

Inovio Pharmaceuticals was the first company to begin pre-clinical and clinical trials of a DNA vaccine (named as INO-4800) against COVID-19. This vaccine transfers DNA plasmids expressing the SARS-CoV-2 S proteins, which holds the preponderance of producing therapeutic antibodies and activating immune cells. DNA vaccine was delivered to patients through the skin. INO-4800 induced T cell responses and nAbs responses against both the D614 and G614 SARS-CoV-2 S proteins [113]. Macaques vaccinated with two doses of INO-4800 were challenged 13 weeks after the second dose. During this period, the initial antibody and T cell immune response triggered by the vaccine had already declined. INO-4800 triggered a strong memory immune response to SARS-CoV-2. Although the immune response generated by memory B cells and T cells after challenge could clear the virus from the upper and lower respiratory tract more quickly, it could not completely prevent the infection and replication of virus in cells, indicating that this vaccine may help reduce the symptoms of COVID-19 in patients, but it failed to completely prevent the spread of the SARS-CoV-2.

Yu et.al developed several prototype DNA vaccine candidates expressing different forms of the SARS-CoV-2 S protein and evaluated their immunogenicity and protective efficacy in rhesus macaques [114]. Vaccinated macaques developed humoral and cellular immune responses. nAbs titers in the vaccinated macaques were comparable in magnitude to that of convalescent macaques and humans. A trend toward higher ADCD (antibody-dependent complement deposition) responses was observed in the S and S.dCT (deletion of the cytoplasmic tail) groups while higher NK cell activation was observed in the RBD (receptor-binding domain with a folden trimerization tag) and S.dTM.PP (a perfusion stabilized soluble ectodomain with deletion of the furn cleavage site, two proline mutations, and a folden trimerization tag) group. SARS-CoV-2 challenged animals who vaccinated with the vaccine encoding the full-length S protein resulted in reductions in median viral loads and increased cellular and humoral responses compared with sham controls. Vaccine-elicited nAb titers correlated with protective efficacy. However, mild symptoms and low level of virus replication were still observed in vaccinated macaques, which indicated that sterile immunity is not achieved. In the near future, the protective efficacy of the DNA vaccine needs to be further improved.

The mRNA molecule of mRNA vaccine is rigorously modified and delivered via lipid nanoparticle systems. Based on the capacity of the individual to translate the encoding mRNA to specific antigens, mRNA vaccine exhibits high expression efficiency, economical, cell-free and scalable production capabilities. It is a hopeful alternative to traditional vaccine [115]. However, mRNA vaccine shows poor stability, difficulty in administration, and relatively high probability of safety issues due to the unclear interaction of mRNA with the human body. Pfizer, Moderna, BioNTech, CureVac, Arcturus and many other biological companies have established different types of mRNA platforms.

mRNA-1273 was developed by Moderna in collaboration with the US National Institute of Allergy and Infectious Diseases. Encapsulated by lipid nanoparticle, mRNA-1273 encodes a perfusion-stabilized viral S protein of SARS-CoV-2. mRNA-1273 induced robust SARS-CoV-2 nAbs in nonhuman primates and achieve rapid protection in the upper and lower airways [116]. In a dose-escalation phase I clinical trial, Virus-specific antibodies increased with the dose, with the highest geometric mean titer (GMT) 213, 526 observed in the 250-μg group. nAbs were comparable to those of higher titers in convalescent patients. Systemic and severe adverse events were more likely to happen after the second vaccination and in the high dose group. In phase I/II clinical trials, 119 days after the first vaccination (two doses, 100 μg per dose, 28 days apart), binding and nAbs remained elevated in all participants 3 months after the booster vaccination. In phase III trial, mRNA-1273 showed a 94.1% protective rate in an interim analysis [117,118].

Pfizer, in concert with BioNTech, developed another mRNA platform-based vaccines included: BNT162b1, which encodes a secreted trimerized SARS-CoV-2 RBD; or BNT162b2 encodes a membrane-anchored SARS-CoV-2 full length spike, stabilized in the prefusion conformation [119-121]. In phase I clinical trial, BNT162b2 was safer than BNT162b1. Dose-dependent SARS-CoV-2 nAbs geometric mean titers were observed in both BNT162b1 and BNT162b2, with GMT comparable or higher than that of SARS-CoV-2 convalescent serum samples. Considering several factors including safety and immunogenicity in the two phase I/II trials and NHPs challenge studies, BNT162b2 was finally selected for follow-up study. Despite lower than the nAbs titers against USA-WA1/2020 strain, BNT162b2 induced nAbs against engineered spike glycoproteins of emerged Delta variants at GMT of more than 40 [122]. BNT162b2 was reported a 95% protective efficacy in phase III clinical trial involving 43,548 participants [121]. After two doses vaccinations, protective efficacy ranged from 90 to 100% across subgroups defined by age, sex, race, etc. No differences existed between vaccine and placebo groups in terms of serious adverse events.

Qin et.al developed a lipid nanoparticle-encapsulated mRNA (mRNA-LNP) encoding a fragment of the RBD of SARS-CoV-2 (termed ARCoV) [123]. ARCoV induced

nAbs as well as Th1-biased cellular response in mice and NHPs. Two doses of ARCoV vaccinated in mice accomplished protection against the challenge of a mouse-adapted SARS-CoV-2. Of note, ARCoV is thermostable and overcome the dependence of the cold chain.

McKay et.al presented a self-amplifying RNA (saRNA) vaccine encoding the S protein of SARS-CoV-2 encapsulated within a lipid nanoparticle (LNP), termed saRNA LNP [124]. Mice vaccinated with saRNA LNP produced high levels of SARS-CoV-2 specific antibody and robust nAbs. The antibody response is Th1-biased, no ADE effects was observed. Even at dose as low as 0.01μg, mice vaccinated with saRNA LNP produced higher quantities of SARS-CoV-2 specific IgG and nAbs compared to COVID-19 convalescents. The authors confirmed the high correlation between SARS-CoV-2 specific IgG and viral neutralization activity in both mice and human patients. High cellular responses, characterized by high IFN-γ secretion, were induced upon re-stimulation with SARS-CoV-2 peptides. However, this vaccine candidate is unlikely to be manufactured on a large scale and costly.

Table 2. Details of vaccines for COVID-19

Vaccine Dsign	Name	Country	Stage	Dose	nAbs (GMT)	Efficacy	Note	Referencce
Inactived vaccine	CoronaVac	China	Phase III	2	23.8~44.1	50.65%~91.25%	Safe in the elderly and juveniles	[66] [67]
	BBIBP-CorV	China	Phase III	2	/	79.34%	Approved, Safe, pilot-scale production	[70]
	COVILO	China	Phase IV	2	121~247	72.51%	Safe	[72]
	Covaxin	India	Phase III	2	/	81%	/	[65]
Virus-vectored vaccine	AZD1222	Britain	Phase IV	2	274(232~542)	66.7%	Reduced efficacy in the variants, adverse effects	[96,99,100]
	Convidicea	China	Phase III	1	18.3~19.5	70.4%	Tolerable, Safe in elder people, pre-existing Ad5 immunity	[89] [90]
	Ad26-S	America	Phase III	1	113/600	66%	Adverse effects	[93]
	Sputnik V	Russia	Phase III	2	44.5(31.8–62.2)	91.6%	Immunogenic in older	[101] [125]
	CORAVAX™	America	Phase I/II	1/ 3	/	/	Safe, long lasting protection.	[109]
Nucleotide vaccine	INO-4800	America	Phase I/II	2	PNT:70~170	/	Antibody responses against both the D614 and G614 SARS-CoV-2	[114]
	bacTRL-S-1	America	Phase I/II	2	IC_{50}:P:~27(W12)	/	Multiforms, reduce median viral loads	[113]
	mRNA-1273	America	Phase IV	2	$PRNT_{80}$: 339.7; 654.3	94.5%	Antibodies remained more than 3 months	[117,118]
	BNT162b2	America	Phase IV	2	NT:540; PNT:10000	95%	Antibody persisted for at least 70 days	[126]
	ARCoV	China	Phase I/II	2	NT_{50}: ~1/699, ~1/6482	/	Completely protect mice against the challenge, thermostable	[123]
	saRNA LNP	Britain	Phase I/II	2	NT: 80 to 20480	/	Highly immunogentic	[124]
	Nanoparticle	America	Phase I/II	2	IC_{50} : 3×10^3 to 7×10^3	/	Robust nAbs targeting distinct epitopes, stability, highly scalable	[81]
Subunit vaccine	SCB-2019	Australia	Phase II/III	2	1280~3948/ 1076~3320	/	Need adjuvant, robust immune responses	[77]
	NVX-CoV2373	America	Phase III	2	3906	96.4%	/	[78]

Note: GMT: Geometric mean titer; **IC_{50}:** The half maximal inhibitory concentration; **NT:** neutralizing titers; **NT_{50}:** Half maximum neutralization potency; **PNT:** Pseudovirus neutralizing titers; **$PRNT_{80}$:** plaque-reduction neutralization testing assay that shows reduction in SARS-CoV-2 infectivity by 80% or more; **nAbs titers list from low to high dose.**

4. Future prospects for vaccine development

2021 will be an extraordinary year for the development and full deployment of COVID-19 vaccine. We summarize future prospects of vaccine development into five aspects (Figure 2). Based on traditional issues including safety, efficacy and durability, there are three innovative directions and one problem urgently to be solved. In terms of technology roadmap, the emerging and mature technology routes exist simultaneously; as for the route of inoculation, mucosal immunity will be a convenient and feasible choice; in terms of design strategy, a universal β coronavirus vaccine is a pressing need. Finally, attention should be paid to contain the frequent mutations of SARS-CoV-2.

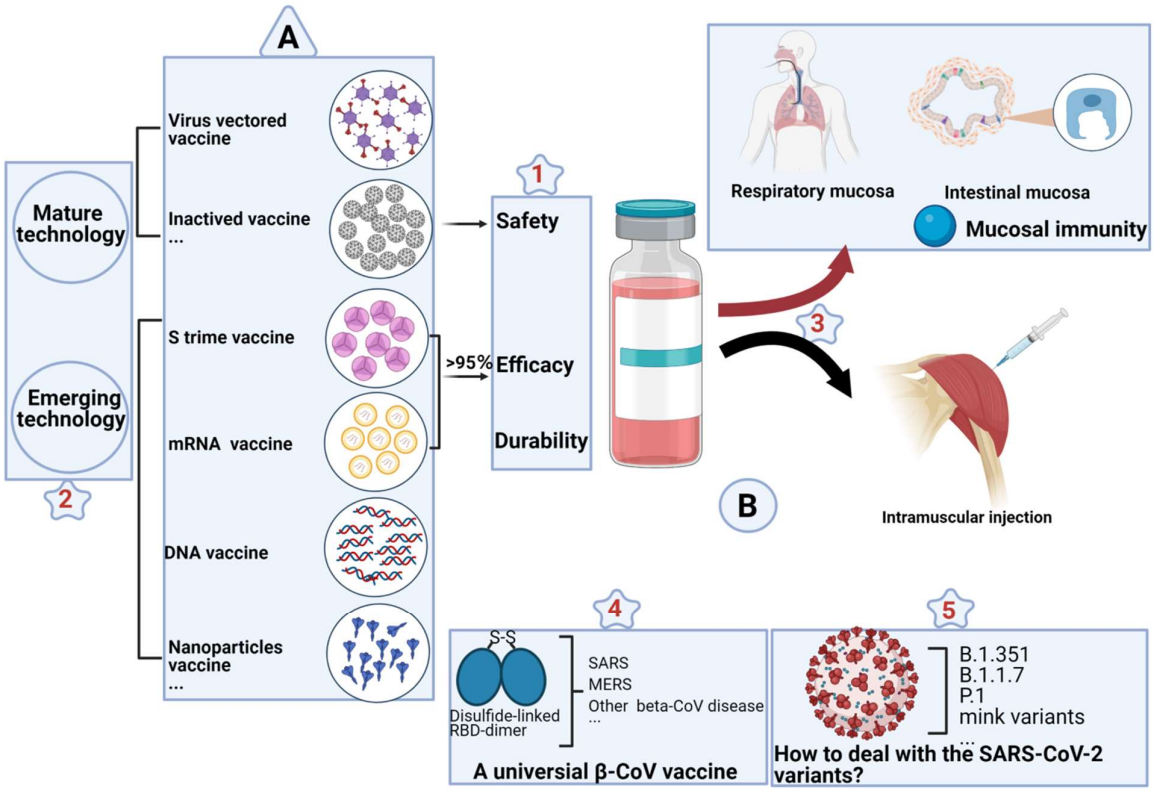

Figure 2. The landscapes of COVID-19 vaccine and five aspects of future development. A: frontier COVID-19 vaccines include inactivated vaccine, viral vector vaccine, etc. B: five aspects of future development: 1, A delicate balance between safety, immunogenicity and durability; 2, Alternation of old and new, common development of multiple lines; 3, Vaccine based on the mucosal immune pathway; 4, Universal coronavirus vaccine designs; 5, *Battle with* SARS-CoV-2 variants

4.1 A delicate balance between safety, immunogenicity and durability.

Safety issues are the primary concerns in vaccine development. Cytokine storm accounts for the severity of the clinical SARS and MERS disease [127,128]. The elevated exhaustion levels and reduced functional diversity of T cells in peripheral blood induced by excessive T-cell responses contribute to the pathogenesis of COVID-19 [129]. Thus, all COVID-19 vaccines and therapies require careful safety evaluations for immunopotentiation that could lead to increased infectivity or eosinophilic infiltration, especially ADE effect. From experience of vaccine research of SARS and other pathogens, multiple vaccines based on different design strategies are all possible to cause ADE [130,131]. In

May 2021, ADE about SARS-CoV-2 was clarified for the first time [132]. N-terminal-domain (NTD) targeted monoclonal antibodies who recognize specific sites was screened, they enhanced the binding capacity of the spike protein to ACE2 and infectivity of SARS-CoV-2. The above antibodies were detected at high levels in severe patients, they were also found in uninfected donors. The above study raised the concerns of ADE. A key measure to prevent ADE is to select the appropriate target antigen and reduce the non-nAbs induction area, which means that a delicate balance between the size and the immunogenicity of antigen should be achieved. High titers of nAbs and moderate cellar immunity increase the chance of treatment and reduce the risk of ADE while antigens that induce large amounts of Th2 cytokines (such as IL-5 and IL-13) are prone to cause ADE [133,134].

When it comes to immunogenicity, the elucidation of protective immunity is significant. In NHP models, the lowest and tardiest nAbs accompanied with prolonged viral shedding, which suggests that nAbs plays a role in the control of infection [42,93,114]. Innate immune effector functions such as ADCD may provide support [114]. Beyond humoral immunity, broad and strong memory CD4+ and CD8+ T cells are detected in convalescent COVID-19 patients [135]. However, it is uncertain the role of virus-specific T cells in control and resolution of SARS-CoV-2 infections. In severe COVID-19 patients with acute respiratory distress syndrome, the strongest T-cell responses were directed to S surface glycoprotein, and SARS-CoV-2-specific T cells predominantly produced effector and Th1 cytokines [136]. SARS-CoV-2-specific T cells are present relatively early and increase over time. The potential variations in T-cell responses may serve as a function of disease severity, an indicator to understand the potential role of immunopathology in the disease, and information for vaccine design and evaluation. In current opinion, robust humoral and moderate cellular immunogenicity are all needed to increase the likelihood of inducing protection.

Since the first vaccine entered the clinic has only been tested for one year, data about the durability is very limited. At least for now it is certain that the vast majority of infected individuals with mild-to-moderate COVID-19 experience robust IgG antibody responses and nAbs persist 6-8 months after infection [137,138].

4.2 Alternation of old and new, common development of multiple lines.

For emerging infectious diseases, preexisting sophisticated technology is sure to play a key role. Several inactivated vaccines developed in China are always staying in the frontier of vaccine echelon and have been licensed in view of their safety and immunogenicity. Despite the existence of uncertainty and adverse effects, emerging technology like mRNA and S trimer vaccine brings us the strongest immune response and up to 95% efficacy. They may be the best choice in terms of protective efficacy. From this point of view, the multi-line development of vaccine candidates enables specific people with specific choices. For frontline healthcare workers with corresponding medical conditions, 2 doses of inactive vaccine or mRNA vaccine would be a better choice. For underdeveloped regions like West Africa with poor medical conditions, maybe a single dose Ad5-S vaccine is more feasible. According to the *Industry Guidelines: Development and Licensing of COVID-19 Preventive Vaccine* issued by the FDA Center for Biological Products Evaluation and Research (CBER), particular people groups including people with underlying disease, subjects without a history of SARS-CoV-2 infection, asymptomatic SARS-CoV-2 positive subjects as well as children, elders, pregnant women, etc. should be taken into consideration, multi-line development is the only way to consider all groups of people.

4.3 Vaccine based on the mucosal immune pathway.

Currently, most of the COVID-19 vaccines are delivered by intramuscular injection. However, there has been reported that COVID-19 vaccines may not be able to inhibit the

infection of the virus in the upper respiratory tract [139]. In additon, ACE2 is found to be highly concentrated in the oronasal epithelium and the lowest in the alveoli [140], which explained the profound viral replication in the mucosal sites (oral/nasal). In the situation, mucosal immunity is essential for blocking the viral entry through oro-respiratory tracts.

Although oral vaccines lag behind, inspired by the oral polio vaccine, several sprays or oral vaccines have been reported. Vaxart has recently developed an enteric-coated tablet vaccine containing an adenoviral-vector that encodes the S and the N gene of the SARS-CoV-2 [141]. In addition, there is the idea of recombinant poliovirus Sabin as a delivery vector [142]. Our team is doing the same work. For our technical route, we package our antigen through poly (lactic-co-glycolic acid) (PLGA), an FDA approved cutting-edge drug adjuvant material. The formulation of PLGA with antigens and traditional Chinese medicine such as Dendrobium polysaccharides allows not only the delivery of antigens but also an adjuvant effect itself [143]. PLGA is modified by Polyethyleneimine, and linked with the targeted peptide (as described in figure 3). The enteric coating prevents the contents' active ingredient from the stomach's acidic environment, so it reaches the intestinal tract and targets M cells. After ingestion, it is transferred to antigen-presenting cells to initiate an immune response. In addition, related research on respiratory viruses as vaccine vectors provides the possibility for the respiratory pathway immunity of COVID-19 vaccine [144].

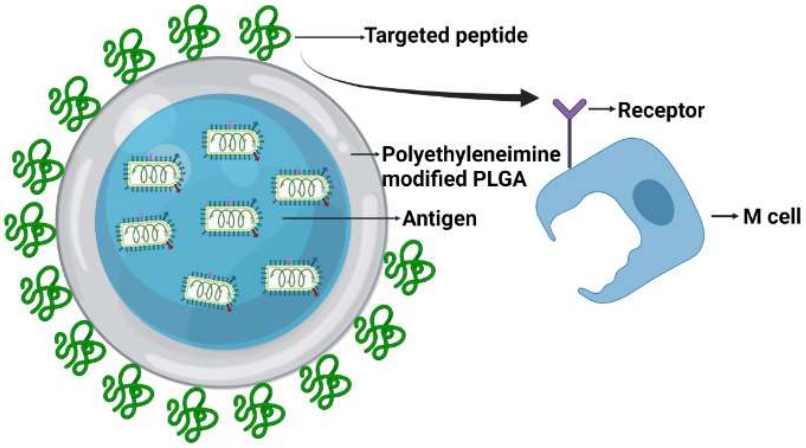

Figure 3. Schematic design of PLGA encapsulated oral COVID-19 vaccine: immunogenic antigen are packaged with PLGA in the form of water-oil-water, then modified with M cell receptor targeted peptide, targeting M cell in the small intestine and stimulating mucosal immune response

4.4 Universal coronavirus vaccine design.

SARS emerged in 2003 [145], just over a decade SARS-CoV-2 was reported [1], the frequent outbreaks of β-coronavirus have had a profound impact on human health around the world and global economy. In this situation, a universal vaccine expressing the common epitope of the beta coronavirus is of great significance to the prevention and control of SARS-CoV-2 and even new coronavirus infections that may occur at any time in the future. RBD-dimer [79]and multi peptide [146] vaccines are promising choices. Beyond that, virus-vectored vaccine also has corresponding potential. Refer to the experience on viral hemorrhagic fever vaccine research, VSV vector can effectively express multiple antigens without interfering with each other [147-149], therefore, it is feasible to develop a VSV-vectored multi-linked vaccine for the emerging β-coronavirus.

4.5 Battle with SARS-CoV-2 variants.

The incidence of convalescent patients re-infected SARS-CoV-2 posed a challenge for all human beings, among them virus mutation was considered to be responsible for

this phenomenon [150]. In March 2020, an increasingly prevalent SARS-CoV-2 variant encoding a D614G mutation in the viral S gene was reported and soon became the main strain. D614G is located in one of the predicted B-cell epitopes of SARS-CoV-2 S protein, and this is a highly immunodominant region [151]. The S-G614 protein contains a novel serine protease cleavage site, so it could be cleaved by serine protease elastase-2 more efficiently, thus entry efficiency is increased, that explains why it transmit more efficiently by enhancing viral infectivity and behaves more effective at transducing cells [152-155]. G614 is a more pathogenic characterized by an increasing case fatality rate [156]. More recently, several new *SARS-CoV-2* variants were reported including United Kingdom variant N501Y.V1 (B.1.1.7)[157], South Africa variant N501Y.V2 (B.1.351) [158], Brazil variant 501Y.V3 (P.1), Indian variant Delta (B.1.617.2) as well as an animal variants cluster 5 in domestic minks. Several previous studies have confirmed that the above *SARS-CoV-2* variants reduce the neutralizing activity of antibodies to different degrees [159-164]. Among them the Delta variant is spreading all over the world, it's spike P681R mutation accelerates and enhances S-mediated fusion [164], which may explains the shorter incubation period of Delta variant infection [165]. The control of Delta variant is vital to curb the second wave of the epidemic. Although the mutation of SARS-CoV-2 has not yet had a disruptive impact on the effectiveness of the vaccine [122,166,167], preparations should be made right now to deal with *SARS-CoV-2* variants from two aspects. On one hand, closely monitoring the protective ability of existing vaccines on SARS-CoV-2 variants. Once the vaccine loses its protective efficacy on emerging variants, mature technology route should be adopted again. For example, when new variants are added at the feeding end, COVID-19 inactivated vaccine confronting SARS-CoV-2 variants is produced without any changes in production process. On the other hand, the emerging vaccine production technology discussed above may play a role in the rapid response of SARS-CoV-2 variants. Moderna has already initiated development of an updated version of vaccine confronting the SARS-COV-2 mutation. To sum up, mature and flexible vaccine development technologies are key issues in the *battle with SARS-CoV-2* variants.

5. Conclusions

This review summarizes current available animal models and promising vaccines including their current stage, progress has been achieved and problems to be solved urgently. The core goal of this review is to grasp the universal connection of things and integrate all aspects of SARS-CoV-2 vaccine development into a whole, provide really useful access information and accelerate the elimination of COVID-19 in the near future. Once vaccination starts and herd immunity is established, the elimination of SARS-CoV -2 is in the near future.

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