Vaccine and Therapeutics for COVID-19: An overview

Rahul Kumar[1], Navneet Duggal[2], Chandan Mohapatra[3]

[1-3] School of Pharmaceutical sciences, Lovely Professional University, Punjab

Abstract

As COVID-19 has become one of the leading causes of death in the world, the world is plunged into one of the largest and most widespread public health crises in decades. SARS-CoV-2, the new coronavirus that causes COVID-19, unifies the scientific community to find treatment and prevention options. There are two main priorities at this time: First, reusing approved pharmacological agents or developing new therapies to reduce the morbidity and mortality associated with the continuously spreading virus. Second, the task of the broader scientific and pharmaceutical community is to develop, test and produce safe and effective vaccines as long-term solutions to prevent further spread and recurrence in the population. The purpose of this article is to review the latest public data on major drug therapies in clinical trials and vaccine candidates being developed to stop the COVID-19 threat.

1. Introduction

Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) is a sense-sense enveloped RNA\u03b2 coronavirus, which appeared in Wuhan, China in December 2019 [1]. According to the World Health Organization[2], this is the cause of the clinical disease called COVID-19, which has caused more than 50 million infections and more than 1.25 million deaths. COVID-19 is the third pandemic or respiratory epidemic caused by a new coronavirus infection. The first was SARS developed in Hong Kong in the early 2000s. It appeared on average 6 days after infection with fever, chills, headache, myalgia and cough. The main organ affected is the lungs, as shown by computed tomography (CT) images, the ligation evolves into pulmonary infiltration within 7 to 10 days. Many patients require mechanical ventilation, and by day 21 after the initial onset of SARS-CoV, most patients have recovered and the mortality rate is approximately 9.6% [3,4]. The second clinical epidemic caused by the new coronavirus is called Middle East Respiratory Syndrome (MERS) and appeared in and around the Arabian Peninsula in 2012. The disease is mainly related to fever, cough and shortness of breath, and the mortality rate. it is much higher, reaching 35% [4,5]. Although SARS-CoV-2 has sequence similarity to SARS-CoV (79%) and MERS-CoV (50%), it is more closely related to two SARS-like viruses of bat origin (bat-SL-CoVZC45 and bat). Close-SL-CoVZXC21, ~ 88% similarity) [1]. The new SARS-CoV-2 virus has been officially classified as the Sarbecovirus subgenus of the Beta coronavirus genus. Although it has many of the same characteristics as SARS, SARS-CoV-2 infection is unique in that it sheds virus particles in the pre-symptomatic stage of the infection [6], causing the virus to spread in large numbers around the world. In this article, we will first provide brief clinical symptoms, imaging findings, laboratory studies, histopathological findings and the course of the disease. Then, a detailed description of vaccine candidates and various treatment strategies currently in clinical trials, including drug therapy, convalescent plasma and monoclonal antibodies.

2. Clinical Symptoms

Patients with COVID-19 more often report fever, cough, myalgia, fatigue, dyspnea, dysosmia, and younger age [7,8]. In some cases, increased sputum secretion, headaches, hemoptysis, diarrhea, and myalgias [9-14], although it is believed that approximately 20% of patients are truly asymptomatic (see section "Disease course" below) [15].

3. Radiographic findings

Typical X-ray findings on chest X-rays or computed tomography (CT) scans indicate bilateral lung involvement, usually in the posterior lung area. Bilateral ground-glass opacity is common in the combined sub-segmental area (representing the area of interstitial inflammation), and it usually progresses to large, high-density lesions and shadows after the fifth day of clinical practice [14,16]. Cavitation, discrete pulmonary nodules, pleural effusion, emphysema and fibrosis are rare [17].

4.Laboratory studies

The most widely reported abnormal laboratory tests for COVID-19 include leukopenia, lymphopenia, and hypoalbuminemia [9,14]. As expected, there are elevated levels of cytokines and inflammatory markers, including erythrocyte sedimentation rate, C-reactive protein, and dimers [11]. These sometimes herald the development of cytokine release syndrome (CRS) in patients, greatly increasing the possibility of mortality and severe acute respiratory distress syndrome (ARDS) [18]. SARS-CoV-2 virus nucleic acid can be detected in the gastrointestinal tract, urine, and saliva [12], and abnormal liver function tests [10], including alanine and aspartic acid, are not uncommon. Elevated levels of transaminases (ALT, AST)), creatine kinase and lactate dehydrogenase [10,11,14]. Some laboratory markers have been shown to predict serious illness. One is the increased neutrophil to lymphocyte ratio (NLR), which is confirmed in patients requiring intensive care and ∨ or mechanical ventilation and patients with mild disease [19]. In addition, recent studies have shown that there is a link between blood type and the severity of COVID-19 symptoms, among which Rh negative blood type has the most protective effect on the outcome of serious illness [20].

5.Histopathological findings

Immunophenotyping of bronchoalveolar cells from COVID-19 patients showed that, in moderate to severe cases, macrophages of type M1 and M2 were observed, as well as an increase in inflammatory cytokines [21]. Direct measurement of circulating cytokines and chemokines in plasma showed higher levels of IL-1β, IL1RA, IL7, IL8, IL9, IL10 and, most importantly, IFN-γ and TNFα. Furthermore, FGF, CSF-3, CSF-2, CXCL10, CCL2, CCL3, CCL4, CCL8, CXCL2, CXCL8, CXCL9, CXCL16, PDGF, and VEGF are also elevated [9,14,22]. In addition to increased serum IL-6 and TNFα, serum IL-6 and TNFα were also found in spleen and lymph node samples, suggesting that IL-6 may play a role in mediating lymphopenia in severe cases. of COVID-19 [twenty Three]. Furthermore, single-cell analysis of individual bronchoalveolar immune cells showed that compared to moderate infections, the ratio of macrophages to neutrophils in severe COVID-19 cases was higher, and it also showed proliferation of CD8 T cells. And the heterotypic phenotype increases. , clonal expansion decreases. In addition, analysis of bronchiole lavage fluid in critically ill patients also reported higher levels of IL-8, IL-6, and IL-1β [21].

6.Disease course

Upon infection, one of the first symptoms that appears is a fever that can persist for up to 12 days. Dyspnea and cough can develop soon thereafter and follow a similar duration; in one study, cough persisted in 45% of survivors even after hospital discharge [24,25]. Both viral nucleic acid and replication-competent virus are detectable at the onset of symptoms in nasal swabs. Titers of replication-competent virus decline and active viral infection stops after 10 to 15 days [26]. It is important to note that the first complications not directly attributable to viral infection, such as sepsis, occur around day 9. More serious lung disease, including acute respiratory distress syndrome (ARDS) and CRS tend to occur in the second week of infection [27] and may require mechanical ventilatory support. In a few initial reports it was found that up to 20% of patients admitted to the hospital had to be put on a mechanical ventilatory support, although this has thankfully declined with improvements in treatment [13,28]. Additionally, acute cardiac and kidney injuries can appear in a 10–20 day period [24,25]. In the US, according to the CDC there have been more than 300,000 excess deaths, two-thirds of which are caused by COVID-19 [29]. In February of 2020, when COVID-19 spread through the US, the primary "at risk" group was identified as individuals over the age of 45, particularly those with multiple preexisting chronic medical conditions. In the initial CDC reports, this group represented almost 80% of deaths in the US [30]. However, as the pandemic has progressed and larger numbers of individuals were infected, an increasing number of 25 to 44 year-old individuals have succumbed, likely owing to social factors such as quarantine fatigue [29]. Interestingly, recent findings suggest that despite recovery and discharge from the hospital, COVID-19 patients may have some longterm health sequelae, including the induction of diabetes [31]. Furthermore, cerebral micro-structural changes that occur postrecovery in part explain widespread reports of prolonged anosmia and "brain fog" or difficulty with various cognitive tasks [32]. As the pandemic wears on and more data are collected, no doubt additional long-term consequences of COVID-19 will be identified.

7.Vaccines against SARS-CoV-2

Various vaccine methods against SARS-CoV-2 have been proposed. These methods include traditional methods: inactivation, attenuation, and protein∨adjuvant methods, as well as newer methods that have not been licensed so far: viral vectors and nucleic acids. This is a rapidly developing field, and some vaccines are more advanced than others. We focus on people who are undergoing clinical trials at the time of writing. Before any vaccine develops into widespread use, several factors need to be considered. The first is the safety and efficacy of the vaccine. Closely linked is the scope of world-scale production to produce sufficient doses to achieve herd immunity.

(i) Possible antigen

Peak protein (S). Before analyzing the platform under development, it is necessary to consider the antigens. Based on SARS-CoV-1 experience, most vaccines target the SARS-CoV-2 spike protein. Within the peak, the receptor binding domain (RBD) responsible for binding and entering the host cell is the main target of neutralizing antibodies [33], and some vaccines only include this region. However, a recent study of isolated monoclonal antibodies found that most of them are directed against regions other than RBD [34]. An important consideration is the correct folding of the protein, both during the production process and when storing the vaccine before it is deployed. Coronavirus peak is a type 1 fusion protein that is metastable and undergoes irreversible conformational changes to allow membrane fusion [51,35,36]. This affects the ability of the antigen to induce neutralizing antibodies. A similar effect has been observed with RSV fusion glycoprotein (F). Antibodies specific for F (pre-F) before fusion have a better neutralizing ability than specific antibodies for F after fusion [37-40]: The form of F before fusion may lead to a better response. Based on this, the pre-fused SARS-CoV-2 spike protein can trigger a stronger immune response, and a stable SARS-CoV-2 spike protein has been generated with stable proline mutations in the S2 domain.

(ii) Nucleocapsid protein (N)

The nucleocapsid (N) of the coronavirus is also immunogenic: in recovered patients, antibodies against SARS-CoV-1 protein N are more abundant and of longer duration than antibodies against protein S. Interestingly, protein N is used to the immune model of the SARS-CoV-1 system is related to vaccine-enhanced disease [41,42]. Although vaccine methods using whole viruses (inactivated virus or live attenuated vaccines) may contain N protein, it is unclear whether N protein can become a potential protective immunogen for SARS-CoV-2. In phase III trials of protein S-based vaccines, protein N can be used as a useful diagnostic method for infection.

(iii) T cell epitope

Although the focus is on the production of neutralizing antibodies, targeted T cell epitopes may provide additional protection [11]. In other respiratory viruses, such as RSV, T-cell-only strategies can potentiate disease [43], while T-cells in dengue can be harmful [44], although there is little evidence seen with influenza vaccine [Four. Five]. It is not clear if SARS-CoV-2 behaves more like RSV or influenza. Based on the information about SARS-CoV-1 and MERS-CoV, and using bioinformatics, it is possible to predict potential immunogenic epitopes in the SARS-CoV-2 proteome. A total of 781 class I human leukocyte antigens (HLA) and 418 common HLA class II epitopes were found between SARS-CoV-1 and SARS-CoV-2 [46]. It was found that the T cell response to SARS-CoV-1 structural proteins is more immunogenic than non-structural proteins [53].

(iv) Protein vaccine

Like other pathogens, the recombinant virus surface protein can be safely used as a vaccine for COVID-19. Although protein vaccines have good safety, their level of immunogenicity is low, which means that many vaccines require adjuvants to improve their efficacy. Although bacterial protein vaccines can be prepared by purifying entire pathogen preparations, viral subunit vaccines still require recombinant genetic engineering. Using a variety of expression systems, including insects, bacteria, yeast, and mammalian cells, it is possible to clone or synthesize genes that encode and select antigens for expression and purification [47]. Bacterial expression systems are commonly used because they have high levels of expression and are easy to expand, whereas fermenters are relatively easy to use. However, for viral antigens, where post-translational modification may be important, it is better to use insect cells or mammalian cells [48,49].

(v) Nanoparticles and virus-like particles

Virus-like particles (VLPs) are a subset of protein vaccines, which are artificially produced virus-like nanoparticles. The VLP does not form a single protein, but is composed of some or all of the proteins that make up the viral capsid [50]. They have some similarities with live attenuated vaccines or inactivated vaccines, and can produce a strong cellular and humoral immune response without the risk of reversal because they do not contain any genetic material of the virus. They can be used for various viruses including HPV, and the preclinical VLP of SARS-CoV-1 has been tested [57]. VLP nanoparticles are self-assembled protein particles, not necessarily derived from viral capsid proteins. Novavax, funded by CEPI and US Operation Warp Speed, has developed a recombinant nanoparticle vaccine (NVX-CoV2373) with SARS-CoV-2 spike protein [58,59]. This can happen with baculovirus engineered to infect Sf9 insect cells [60]. For clinical trials using NVX-CoV2373, Novavax is using its own saponin-based adjuvant Matrix-M (NCT04368988), which has recently been published [61]. The vaccine is immunogenic, but requires the addition of adjuvants to achieve 100% seroconversion. Two doses of neutralizing antibodies are required in all individuals. Animal models of immunization have developed spike protein-specific antibodies, which can prevent the spike protein from binding to host cell ACE-2 receptors and neutralizing wild-type viruses [62]. Another company (Medicago) is using the Nicotiana benthamiana plant system to produce VLPs [63] and is currently in clinical trials in combination with the adjuvant CpG or AS03 (NCT04450004). Other people in the preclinical stage include Saiba AG in Switzerland, who are using the cucumber mosaic virus VLP combined with SARS-CoV-2 RBD, which can induce neutralizing antibodies in mice [64].

(vi) Peptide vaccine

Peptide vaccination is based on the following concept: since a part of the intact protein can be used to induce T cell responses [65,66], it only needs to contain a minimal sequence of immunogenic peptides. By selecting for conserved epitopes, peptide vaccines can potentially induce broad spectrum immunity against multiple strains of given pathogens [67,68]. Peptides are easier to produce than intact protein antigens because they can be produced synthetically and do not need to be folded into a tertiary structure. However, peptide vaccines are generally weakly immunogenic. This is due to a variety of factors, including relatively small peptides and differences in MHC processing, which may require carrier proteins or adjuvants [69,70]. Several groups are exploring the use of multi-epitope peptide vaccines against SARS-CoV-2; After the prediction of immunogenic epitopes based on bioinformatics and immunoinformatics [71-74], research focuses on T-cell table rather than B-cell epitopes. OSE immunotherapy has used a multi-epitope peptide approach to induce T cell responses in mice [75]. Covaxx and the University of Nebraska Medical Center have recently registered a phase I clinical trial of a multi-epitope peptide vaccine (NCT04545749), and the Carrier Institute (NCT04527575) is currently in clinical trials.

(vii) Inactivated vaccine

Historically, the separation and inactivation of viruses with formaldehyde is one of the oldest methods of virus inoculation. Virus inactivation is effective against many different viruses. However, there have always been significant safety hazards associated with inactivated SARS-CoV-1 and MERS-CoV vaccines, reminiscent of FI-RSV, and these hazards also apply to SARS-CoV-2. For the \u03b3-irradiated MERS-CoV vaccine [56] and the UV-inactivated SARS-CoV-1 vaccine [76], pulmonary pathology has been observed in vaccinated animals. The choice of adjuvant and inactivator is important for the formation of an immune response. For

example, the formaldehyde-inactivated MERSCoV vaccine supplemented with alum and CpG showed improved protection without inducing eosinophil-mediated vaccine-related pathology [77].

(viii) Inactivated viral vaccines in development for SARS-CoV-2

There are four inactivated vaccine candidates in clinical trials. Sinovac Biotech is using the platform previously developed for SARS-CoV-1 [78]. The virus grows in Vero cells and is inactivated by \u03b2-propiolactone. The inactivated vaccine is safe and immunogenic in rhesus monkeys and provides complete protection against exposure to SARS-CoV-2, which has no virus detected in the pharynx or lungs [79]. Two different versions of this inactivated vaccine have been developed, with the adjuvant alum or CpG108. The vaccine has completed a phase II human trial (NCT04352608) in 600 healthy adults between 18 and 59 years old. After the second dose of the vaccine, 90% seroconversion was observed and some neutralizing antibodies were detected [80]. Interestingly, the virus production method was changed between phase I and phase II trials, which may have improved immunogenicity. The vaccine has entered phase III clinical trials in Brazil (NCT04456595) and Indonesia (NCT04508075). Sinopharm, in cooperation with Beijing Institute of Biological Products and Wuhan Institute of Biological Products, has also developed an inactivated vaccine. The vaccine has now been tested in a phase I\/II clinical trial (ChiCTR2000031809). In two different experiments, no serious adverse reactions were observed, and more than 95% of individuals were seroconverted with detectable neutralizing antibodies. After the second dose, antibodies were mainly observed. Two other organizations, the Bharat Biotechnology Company of India and the Institute of Medical Biology of the Chinese Academy of Medical Sciences, are carrying out clinical trials of inactivated vaccines, but they are still ongoing and there is no public data. Scotland-based Valneva has just expanded its BSL3 production capacity and has signed an agreement with the British government based on 100 million doses of CpG-assisted formaldehyde inactivated vaccine, which is based on its vaccine against Japanese encephalitis virus [82].

(ix) Live vaccine

The use of live viruses to prevent infection is the oldest vaccine method. The original vaccine vaccinia used this method to prevent smallpox. We bundle two methods into the live virus vaccine platform: virus attenuation or the use of viral vectors to deliver transgenes. Live attenuated vaccines Live attenuated vaccines are very similar to natural infections. As a result, they are usually immunogenic in a single dose without adjuvant [83]. One consideration is to balance attenuation and replication: an overly attenuated vaccine may not replicate enough to be immunogenic, and this balance may vary from individual to individual, especially very young or immunosuppressed individuals . Historically, serial passages have been used to attenuate mutations. For example, the live attenuated influenza vaccine (LAIV) is cold-adapted to restrict it to the upper respiratory tract. This method requires time and a lot of testing: the YF17D yellow fever vaccine has passed more than 200 times. In addition, attenuated viruses can be produced through reverse genetics [84], introducing site-directed mutations in genes related to virulence. Protein E targets both SARS-CoV-1 and MERS-CoV [85,86]. However, this method requires the identification of genes that attenuate viral replication and the inserted mutations are phenotypically stable [84]. A new method of codon pair deoptimization has been developed. The chemically synthesized codon deoptimized virus retains the same 100% amino acid sequence as the parent virus, but contains a greater number of CpG and UpA RNA dinucleotides to up-regulate the host response. Codon pair deoptimization has been used to attenuate RSV [87]. Codagenix and the Serum Institute of India are using codon deoptimization technology to develop a live attenuated vaccine against SARS-CoV-2 based on previous experience in RSV and influenza [88].

(x) DNA vaccine

Most DNA vaccines are constructed from plasmids that contain prokaryotic sequences that support propagation of the plasmid in E. coli and mammalian expression cassettes that control expression of the target transgene in the vaccinating organism. The expression cassette contains the upstream promoter that drives expression of the transgene, the Kozak sequence, the inserted transgene, and the 3 'polyadenylation (polyA) tail. After delivery, the DNA vaccine is taken up by host cells at the migrating APC or immune site [89]. To induce an adaptive immune response, DNA must enter the nucleus. When transported to the nucleus, the DNA induces an innate immune response through the inflamed cytoplasm detected by intracellular pattern recognition receptors (such as STING1 [89] or TBK1 [90]). The activation of innate immunity is essential to promote adaptive immunity to DNA vaccines. If APCs are directly transfected with DNA vaccines, they will load peptides encoded by the vaccines on MHC I and MHCII molecules and activate T cells [91]. The transfected stromal cells will produce an antigen, and after the antigen is released from the exosomes or apoptotic bodies, the APC and B cells will encounter the antigen. The efficiency of injecting naked nuclear DNA into the nucleus is very low, and most of the DNA does not pass through the cell membrane or nuclear membrane [92,93]. To alleviate this loss, the DNA vaccine program uses delivery platforms such as electroporation and bioinjection.

(xi) DNA vaccines – coronaviruses

Animal preclinical studies have shown that DNA vaccines encoding SARS-CoV-1 virus M, N, 3a or S protein can cause an immune response [94-96]. Multivalent DNA vaccines encoding S and M protein epitopes can prevent the cytopathic effects of SARS-CoV-1. Protein S is the only target of the SARS-CoV-1 DNA vaccine. The vaccine has been administered via a biosyringe and has entered phase I clinical trials. It is safe and can induce a neutralizing antibody response [97]. Inovio has developed a leading DNA vaccine against MERS-CoV (INO-4700). The phase I clinical trial was completed in 2019. The vaccine showed good safety and induced humoral immunity and multifunctional CD8 T cell response [98].

(xii) DNA vaccines in development for SARS-CoV-2

DNA vaccine is being developed The Inovio MERS INO4700 (GLS-5300) vaccine (NCT03721718), which is about to enter Phase II clinical trials, has now been redeployed as INO-4800 (NCT04336410) to begin clinical trials for SARS-CoV-2 protection. In the preclinical study of the INO4800 vaccine, neutralizing antibody and T cell responses were observed in mice, and the antibody response was blocked in vaccinated guinea pigs [99] and rhesus monkeys [100]. The first phase trial (NCT04336410) is underway, but the data has not yet been released. Genexine in South Korea (NCT04445389), Zydus Cadila in India (CTRI \/ 2020\/07\/026352) and Osaka University in Japan (NCT04463472) have begun phase I trials of DNA vaccines.

(Xiii) RNA vaccine RNA vaccines are based on the same premise as DNA vaccines that express vaccine antigen transgenes in host cells, but are one more step in the expression pathway, skipping the transcription step. Unlike DNA vaccines, RNA vaccines begin to express themselves once they enter the cytosol, which can increase the efficiency of expression. Like DNA vaccines, the presence of "foreign" RNA can be detected in both endosomes and cytoplasm [101], so RNA vaccines have a self-adjuvant effect [102]. However, the early trigger of the response to IFN type I can negatively regulate protein expression [103]. Modified nucleosides can be incorporated into mRNA products to produce a "silent" RNA vaccine that avoids detection by TLR and does not elicit a type I IFN response [104,105], but in vaccine constructs there is a balance between expression of antigens and sufficient triggering of inflammation to activate the immune response. This balance may be disturbed by the formulation of the vaccine administered, and may vary between different animal species, so it is difficult to predict based on preclinical studies. There are two main types of vaccine RNA mRNA and self-amplified mRNA (saRNA). Non-replicating mRNA vaccines are constructs designed to encode the target gene, usually with 5'borders, UTR and poly A tails located on both sides of the target gene. The 5'cap is an essential translational complex for the association of mRNA with eukaryotes. Choose UTR to optimize the expression of RNA protein and avoid including sequences that will cause translation difficulties [106,107]. RNA polymerase derived from bacteriophage and NTP are used to transcribe linearized DNA in vitro to prepare mRNA vaccine constructs. Self-amplified RNA vaccines are RNA replicons derived from alpha viruses, which can be modified to replace RNA structural proteins to encode target antigens. The viral replicon also contains an open reading frame (ORF), which encodes four alpha virus non-structural proteins (nsP1-4) and a subgenomic promoter. Nonstructural proteins form RNA-dependent RNA polymerase (RDRP). The RDRP complex transcribes more copies of the vaccine in the transfected cells. As a result, RNA vaccines express proteins at higher levels than non-replicating RNA and last longer [108].

8. Pharmacological therapies

(i) Remdesivir

Remdesivir (GS-5734) is a broad-spectrum antiviral drug that is a 1′ - substituted adenosine nucleotide analog. It has been proven an effective therapy against several RNA viruses, including Ebola, by competing with ATP for incorporation into RNA-dependent RNA polymerase [109]. During nascent viral RNA chain, RNA-dependent RNA polymerases are unable to process beyond the insertion of remdesivir, resulting in a chain elongation termination. Remdesivir has a conserved mode of action in a diverse group of RNA viruses [110], and was among the first antiviral agents to be tested for activity against SARS-CoV-2. Indeed, in initial in vitro testing, remdesivir inhibited SARS-CoV-2 infection in Vero E6 cells and human lung primary cell lines [111,112]. Consequently, many clinical trials are currently ongoing to test the drug's safety and efficacy for treatment against COVID-19 in human patients. A report from Beigel et al. showed that remdesivir successfully reduced the recovery time from 15 to 10 days on average in adults hospitalized with SARS-CoV-2 infection [113]. Additionally, the patients treated with remdesivir had almost a half reduced chance of mortality calculated by Kaplan-Meier estimates [113]. Even prior to the final report of the trial, the US Food and Drug Administration gave remdesivir Emergency Use Authorization (EUA) [114]; the drug label indicates a recommendation for use in patients 12 years of age and older and weighing at least 40 kg a 5-day course in mild to moderate infections and a 10-day course in severe infections requiring mechanical ventilation. Importantly, the maker of remdesivir (Gilead Sciences) has reported that parallel use of remdesivir and hydroxychloroquine might inhibit the effect of remdesivir and reduce both the rate and likelihood of recovery in patients receiving both drugs, a consideration given recent controversies surrounding the use of hydroxychloroquine in patients with COVID-19 [115].

(ii) Tocilizumab

Tocilizumab is a recombinant, fully humanized monoclonal antibody which targets both soluble and membrane-bound forms of the interleukin-6 receptor (IL-6R). It is used in clinical practice to treat adult patients with rheumatoid arthritis and pediatric patients with systemic juvenile idiopathic arthritis, although its effectiveness has recently been demonstrated in other systemic autoimmune and inflammatory conditions, including giant cell arteritis [35], multicentric Castleman's disease [36] and, importantly, was approved by the FDA in 2017 for the treatment of Cytokine Release Syndrome in chimeric antigen receptor (CAR) T-cell cancer therapy [37]. In COVID-19, elevated IL-6 levels have been correlated with increased mortality [55], sparking interest in the use of tocilizumab for COVID-19 therapy. There have been numerous case reports of tocilizumab improving oxygenation and reducing inflammatory biomarkers in hospitalized COVID-19 patients [119,120]. Although not yet associated with pathogenesis, an improvement in COVID-19-associated lymphopenia has also been reported following tocilizumab treatment [40]. A recent rapid meta-analysis of published case reports also demonstrated a reduction in mortality of 12% among COVID-19 patients treated with tocilizumab [122]. Despite these positive suggestions of efficacy, tocilizumab therapy for COVID-19 remains unproven; on August 27, 2020, the US National Institutes of Health (NIH) advised against the widespread use of anti-IL-6 receptor monoclonal antibodies (tocilizumab included) following conflicting published case reports and a lack of properly designed clinical trial data [123]. Very recently, a randomized double-blind placebo-controlled trial in hospitalized patients with severe COVID-19 showed that tocilizumab was ineffective in reducing mortality or the rate of intubation. Further, secondary end points including the need for supplemental oxygen and clinical worsening were not reduced by tocilizumab treatment compared to placebo [124].

(iii) Fostamatinib

Acute lung injury (ALI) and acute respiratory distress syndrome (ARDS) exhibited by a subset of patients suffering from COVID-19 and other respiratory ailments have been linked to the elevated circulating levels of mucin-1 (KL-6/MUC1) and MUC5AC [41,42]. As a transmembrane protein of the mucosal epithelial cells, MUC1 plays a critical role in the lining of the airway lumen. Overexpression of this protein and excessive mucus production have been shown to increase the duration of infections as well as mortality from respiratory diseases [45]. In an attempt to target MUC1, a large pharmacological screening study done by Alimova et al. 2020 identified Fostamatinib as a potential antagonist of MUC1 [46]. Fostamatinib is an inhibitor of the spleen tyrosine kinase (SYK), an enzyme known for its role in the adaptive immune receptor signaling, cellular adhesion, innate immune recognition, and platelet function. It has been used in the treatment of autoimmune diseases such as rheumatoid arthritis and immune thrombocytopenia (ITP) [47,48]. Moreover, the broad number of immune pathways involving SYK has been shown to play a role in the hyper-inflammatory response caused by anti-SARS-CoV-2-Spike IgG [49]. In particular, R406, an active component of Fostamatinib, has recently been confirmed to effectively block SYK and its downstream signaling and suggests potential efficacy of fostamatinib in the treatment of ALI and COVID-19 [46,49]. As it is already FDA approved, Fostamatinib has been accelerated into phase 2 clinical trials for COVID-19 (NCTNCT04579393 and NCT04581954).

Chloroquine/hydroxychloroquine (CQ/HCQ)

Quinine has been used as therapeutic agent for centuries. It was first introduced into the Western medical pharmacopeia in 1638 when the wife of the Spanish Viceroy of Peru, Countess Chincona, was given bark of the later-named Chincona tree by an Incan herbalist as a treatment for malaria. A derivative of quinine, chloroquine, saw significant use as an antimalarial drug during World War II. Its use to treat autoimmune disease, particularly systemic lupus erythematosus, began in the 1950s. Hydroxylation, leading to hydroxychloroquine, reduced systemic toxicities significantly and led to more widespread use in chronic diseases, including autoimmune diseases, particularly systemic lupus erythematosus and rheumatoid arthritis [125–127]. The possible mechanisms of action of chloroquine and its derivatives on coronavirus infection are several and not yet fully understood. Of relevance to coronavirus infection, these drugs increase endosomal pH, preventing acidification and reducing viral entry into cellular cytoplasm from endosomes [128]. Additionally, CQ inhibits glycosylation of the cellular ACE2 receptor, thereby interfering with SARS-CoV binding and infection [129]. As CQ/HCQ have been proven useful against SARS-CoV and other widely circulating human and bat coronaviruses (HCoV-229E and HCoV-O43) [111], it only seemed reasonable that it should work in a similar fashion with emerging SARS-CoV-2. Initial in vitro tests on Vero E6 cells showed promising viral inhibition, where both CQ and HCQ prevented the transport of virus from early endosomes to endolysosomes (suspected to be necessary for the release of viral genome) [128]. However, retrospective studies and clinical trial results show inconclusive evidence about the effect of these drugs against COVID-19. Initial reports from a multisite international randomized, double-blind, placebo-controlled trial showed that HCQ failed to produce a decrease in symptom presence and/or severity over a 14-day period [130]. Furthermore, additional observational and multi-center, randomized, controlled trial preliminary reports showed that HCQ did not significantly lower chances of required intubation or mortality rate [131,132]. Following these and other negative studies, treatment of COVID-19 with CQ and HCQ is not recommended by the NIH [133].

(iv) Corticosteroids

Considering that COVID-19 elicits a broad systemic inflammatory response, including inflammation-related damage of the lungs, corticosteroid therapy would seem an obvious therapeutic candidate. However, high doses and/or long-term use of corticosteroids are associated with a variety of deleterious effects, including metabolic derangements, an increased risk of infection, and bone abnormalities, among others [50]. Additionally, in MERS, corticosteroids have been linked to delayed viral clearance, which has also been reported in SARS-CoV-2 [57,59]. Despite this, several clinical trials are ongoing to evaluate the efficacy and appropriate timing of corticosteroid treatment for COVID-19. Perhaps the most influential of these, preliminary results of the RECOVERY trial published in mid-July 2020, reported a reduction in death rates associated with the administration of dexamethasone in hospitalized patients requiring supplemental oxygenation, but not among those receiving no respiratory support [60]. Interestingly, two previouslypublished retrospective analyses from Chinese cohorts had noted similar findings; the first study used methylprednisolone in severe COVID-19 cases presenting with acute respiratory distress syndrome [61], whereas the second study found a benefit in reducing length of hospitalization and improvement in chest imaging parameters in severe COVID-19 cases given methylprednisolone [62]. Additional studies report that low-dose, short-course corticosteroids might slow or prevent disease progression and reduce inflammation [63,64]. Based on current evidence, the NIH recommends the use of dexamethasone 6 mg per day for up to 10 days or until discharge for patients hospitalized with COVID-19 requiring supplemental oxygenation [65]. It should be noted that in milder COVID-19 there remains a possible risk that steroids might worsen the disease course, as steroids increase the risk of infection. Certainly, it should not be assumed that patients on chronic steroid therapy are immune from developing severe COVID-19 or that they are at a lesser risk of becoming infected by SARS-CoV-2

9.Conclusion

It is too early to know the best way to control COVID-19 with a vaccine. Speculating which vaccine platform is "better", although it is academically pleasing, is worthless here. So many new and old platforms are developing in the direction of efficacy research, which makes vaccination very exciting. There is no doubt that this outbreak will become a test case for new vaccine platforms, especially nucleic acid vaccines. So far, the platform has promised a lot, but has not yet obtained human use license. One question is whether vaccines will play a role in reducing the burden of the pandemic. Even if carried out at the fastest speed, the first efficacy test will begin 9 months after the pandemic begins and the first allowed dose may not be ready in 18 months. At this time, the virus will cause a large number of deaths and a large number of deaths. Global destruction. There are still important questions about what is a successful vaccine, how it should be implemented, and who should it be prioritized. Although the World Health Organization has

produced some draft guidelines [134], these will depend in part on the results of efficacy studies. Overall, this is an important chapter in vaccine development, with extensive cooperation and partnership in the race against the virus.

References

[1] R. Lu, X. Zhao, J. Li, P. Niu, B. Yang, H. Wu, W. Wang, H. Song, B. Huang, N. Zhu, Y. Bi, X. Ma, F. Zhan, L. Wang, T. Hu, H. Zhou, Z. Hu, W. Zhou, L. Zhao, J. Chen, Y. Meng, J. Wang, Y. Lin, J. Yuan, Z. Xie, J. Ma, W.J. Liu, D. Wang, W. Xu, E. C. Holmes, G.F. Gao, G. Wu, W. Chen, W. Shi, W. Tan, Genomic characterisation and epidemiology of 2019 novel coronavirus: implications for virus origins and receptor binding, Lancet 395 (2020) 565–574.

[2] Coronavirus disease (COVID-19) – World Health Organization, (n.d.). http s://www.who.int/emergencies/diseases/novel-coronavirus-2019 (accessed November 9, 2020).

[3] N. Lee, D. Hui, A. Wu, P. Chan, P. Cameron, G.M. Joynt, A. Ahuja, M.Y. Yung, C. B. Leung, K.F. To., S.F. Lui, C.C. Szeto, S. Chung, J.J.Y. Sung, A major outbreak of severe acute respiratory syndrome in Hong Kong, N. Engl. J. Med. 348 (2003) 1986–1994.

[4] M. Fani, A. Teimoori, S. Ghafari, Comparison of the COVID-2019 (SARS-CoV-2) pathogenesis with SARS-CoV and MERS-CoV infections, Futur. Virol. (2020), https://doi.org/10.2217/fvl-2020-0050.

[5] A. Zumla, D.S. Hui, S. Perlman, Middle East respiratory syndrome, Lancet. 386 (2015) 995–1007.

[6] S. Lee, T. Kim, E. Lee, C. Lee, H. Kim, H. Rhee, S.Y. Park, H.-J. Son, S. Yu, J. W. Park, E.J. Choo, S. Park, M. Loeb, T.H. Kim, Clinical course and molecular viral shedding among asymptomatic and symptomatic patients with SARS-CoV-2 infection in a community treatment Center in the Republic of Korea, JAMA Intern. Med. 180 (2020) 1447, https://doi.org/10.1001/ jamainternmed.2020.3862.

[7] D. Hornuss, B. Lange, N. Schroter, ¨ S. Rieg, W.V. Kern, D. Wagner, Anosmia in COVID-19 patients, Clin. Microbiol. Infect. 26 (2020) 1426–1427.

[8] L.A. Vaira, G. Salzano, G. Deiana, G. De Riu, Anosmia and Ageusia: common findings in COVID-19 patients, Laryngoscope. 130 (2020) 1787.

[9] C. Huang, Y. Wang, X. Li, L. Ren, J. Zhao, Y. Hu, L. Zhang, G. Fan, J. Xu, X. Gu, Z. Cheng, T. Yu, J. Xia, Y. Wei, W. Wu, X. Xie, W. Yin, H. Li, M. Liu, Y. Xiao, H. Gao, L. Guo, J. Xie, G. Wang, R. Jiang, Z. Gao, Q. Jin, J. Wang, B. Cao, Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China, Lancet. 395 (2020) 497–506.

[10] N. Chen, M. Zhou, X. Dong, J. Qu, F. Gong, Y. Han, Y. Qiu, J. Wang, Y. Liu, Y. Wei, J'an Xia, T. Yu, X. Zhang, L. Zhang, Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study, Lancet 395 (2020) 507–513.

[11] D. Wang, B. Hu, C. Hu, F. Zhu, X. Liu, J. Zhang, B. Wang, H. Xiang, Z. Cheng, Y. Xiong, Y. Zhao, Y. Li, X. Wang, Z. Peng, Clinical characteristics of 138hospitalized patients with 2019 novel coronavirus-infected pneumonia in Wuhan, China, JAMA (2020), https://doi.org/10.1001/jama.2020.1585.

[12] W.-J. Guan, Z.-Y. Ni, Y. Hu, W.-H. Liang, C.-Q. Ou, J.-X. He, L. Liu, H. Shan, C.- L. Lei, D.S.C. Hui, B. Du, L.-J. Li, G. Zeng, K.-Y. Yuen, R.-C. Chen, C.-L. Tang, T. Wang, P.-Y. Chen, J. Xiang, S.-Y. Li, J.-L. Wang, Z.-J. Liang, Y.-X. Peng, L. Wei, Y. Liu, Y.-H. Hu, P. Peng, J.-M. Wang, J.-Y. Liu, Z. Chen, G. Li, Z.-J. Zheng, S.- Q. Qiu, J. Luo, C.-J. Ye, S.-Y. Zhu, N.-S. Zhong, China medical treatment expert group for covid-19, clinical characteristics of coronavirus disease 2019 in China, N. Engl. J. Med. 382 (2020) 1708–1720.

[13] S. Richardson, J.S. Hirsch, M. Narasimhan, J.M. Crawford, T. McGinn, K. W. Davidson, the Northwell COVID-19 Research Consortium, D.P. Barnaby, L. B. Becker, J.D. Chelico, S.L. Cohen, J. Cookingham, K. Coppa, M.A. Diefenbach, A.J. Dominello, J. Duer-Hefele, L. Falzon, J. Gitlin, N. Hajizadeh, T.G. Harvin, D. A. Hirschwerk, E.J. Kim, Z.M. Kozel, L.M. Marrast, J.N. Mogavero, G.A. Osorio, M. Qiu, T.P. Zanos, Presenting characteristics, comorbidities, and outcomes among 5700 patients hospitalized with COVID-19 in the New York city area, JAMA (2020), https://doi.org/10.1001/jama.2020.6775.

[14] G. Chen, D. Wu, W. Guo, Y. Cao, D. Huang, H. Wang, T. Wang, X. Zhang, H. Chen, H. Yu, X. Zhang, M. Zhang, S. Wu, J. Song, T. Chen, M. Han, S. Li, X. Luo, J. Zhao, Q. Ning, Clinical and immunological features of severe and moderate coronavirus disease 2019, J. Clin. Invest. 130 (2020) 2620–2629.

[15] D. Buitrago-Garcia, D. Egli-Gany, M.J. Counotte, S. Hossmann, H. Imeri, A. M. Ipekci, G. Salanti, N. Low, Occurrence and transmission potential of asymptomatic and presymptomatic SARS-CoV-2 infections: a living systematic review and meta-analysis, PLoS Med. 17 (2020), e1003346.

[16] F. Song, N. Shi, F. Shan, Z. Zhang, J. Shen, H. Lu, Y. Ling, Y. Jiang, Y. Shi, Emerging 2019 novel coronavirus (2019-nCoV) pneumonia, Radiology. 295 (2020) 210–217.

[17] M. Chung, A. Bernheim, X. Mei, N. Zhang, M. Huang, X. Zeng, J. Cui, W. Xu, Y. Yang, Z.A. Fayad, A. Jacobi, K. Li, S. Li, H. Shan, CT imaging features of 2019 novel coronavirus (2019-nCoV), Radiology. 295 (2020) 202–207.

[18] Q. Ruan, K. Yang, W. Wang, L. Jiang, J. Song, Clinical predictors of mortality due to COVID-19 based on an analysis of data of 150 patients from Wuhan, China, Intensive Care Med. 46 (2020) 846–848.

[19] M. Kong, H. Zhang, X. Cao, X. Mao, Z. Lu, Higher level of neutrophil-tolymphocyte is associated with severe COVID-19, Epidemiol. Infect. 148 (2020), e139.

[20] M. Zietz, N.P. Tatonetti, Testing the association between blood type and COVID19 infection, intubation, and death, medRxiv (2020), https://doi.org/10.1101/ 2020.04.08.20058073.

[21] M. Liao, Y. Liu, J. Yuan, Y. Wen, G. Xu, J. Zhao, L. Cheng, J. Li, X. Wang, F. Wang, L. Liu, I. Amit, S. Zhang, Z. Zhang, Single-cell landscape of bronchoalveolar immune cells in patients with COVID-19, Nat. Med. 26 (2020) 842–844.

[22] D. Blanco-Melo, B.E. Nilsson-Payant, W.-C. Liu, S. Uhl, D. Hoagland, R. Møller, T. X. Jordan, K. Oishi, M. Panis, D. Sachs, T.T. Wang, R.E. Schwartz, J.K. Lim, R. A. Albrecht, B.R. ten Oever, Imbalanced host response to SARS-CoV-2 drives development of COVID-19, Cell 181 (2020), 1036–1045.e9.

[23] Y. Chen, Z. Feng, B. Diao, R. Wang, G. Wang, C. Wang, Y. Tan, L. Liu, C. Wang, Y. Liu, Y. Liu, Z. Yuan, L. Ren, Y. Wu, The Novel Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) Directly Decimates Human Spleens and Lymph Nodes, Infectious Diseases (except HIV/AIDS), 2020, https://doi.org/ 10.1101/2020.03.27.20045427.

[24] F. Zhou, T. Yu, R. Du, G. Fan, Y. Liu, Z. Liu, J. Xiang, Y. Wang, B. Song, X. Gu, L. Guan, Y. Wei, H. Li, X. Wu, J. Xu, S. Tu, Y. Zhang, H. Chen, B. Cao, Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study, Lancet. 395 (2020) 1054–1062.

[25] B.E. Young, S.W.X. Ong, S. Kalimuddin, J.G. Low, S.Y. Tan, J. Loh, O.-T. Ng, K. Marimuthu, L.W. Ang, T.M. Mak, S.K. Lau, D.E. Anderson, K.S. Chan, T.Y. Tan, T.Y. Ng, L. Cui, Z. Said, L. Kurupatham, M.I.-C. Chen, M. Chan, S. Vasoo, L.- F. Wang, B.H. Tan, R.T.P. Lin, V.J.M. Lee, Y.-S. Leo, D.C. Lye, Singapore 2019 novel coronavirus outbreak research team, epidemiologic features and clinical course of patients infected with SARS-CoV-2 in Singapore, JAMA. 323 (2020) 1488–1494.

[26] J.J.A. van Kampen, D.A.M.C. van de Vijver, P.L.A. Fraaij, B.L. Haagmans, M. M. Lamers, N. Okba, J.P.C. van den Akker, H. Endeman, D.A.M.P.J. Gommers, J. J. Cornelissen, R.A.S. Hoek, M.M. van der Eerden, D.A. Hesselink, H.J. Metselaar, A. Verbon, J.E.M. de Steenwinkel, G.I. Aron, E.C.M. van Gorp, S. van Boheemen, J.C. Voermans, C.A.B. Boucher, R. Molenkamp, M.P.G. Koopmans, C. Geurtsvankessel, A.A. van der Eijk, Shedding of infectious virus in hospitalized patients with coronavirus disease-2019 (COVID-19): duration and key determinants, in: Infectious Diseases (except HIV/AIDS), 2020, https://doi.org/ 10.1101/2020.06.08.20125310.

[27] X. Li, X. Ma, Acute respiratory failure in COVID-19: is it "typical" ARDS? Crit. Care 24 (2020) 198.

[28] C. Karagiannidis, C. Mostert, C. Hentschker, T. Voshaar, J. Malzahn, G. Schillinger, J. Klauber, U. Janssens, G. Marx, S. Weber-Carstens, S. Kluge, M. Pfeifer, L. Grabenhenrich, T. Welte, R. Busse, Case characteristics, resource use, and outcomes of 10 021 patients with COVID-19 admitted to 920 German hospitals: an observational study, Lancet Respir. Med. 8 (2020) 853–862.

[29] L.M. Rossen, A.M. Branum, F.B. Ahmad, P. Sutton, R.N. Anderson, Excess deaths associated with COVID-19, by age and race and ethnicity - United States, January 26-October 3, 2020, MMWR Morb. Mortal. Wkly Rep. 69 (2020) 1522–1527.

[30] Severe Outcomes Among Patients With Coronavirus Disease 2019 (COVID-19) — United States, February 12–March 16, 2020 68, 2020. https://stacks.cdc.gov/ view/cdc/85951 (accessed October 27, 2020).

[31] F. Rubino, S.A. Amiel, P. Zimmet, G. Alberti, S. Bornstein, R.H. Eckel, G. Mingrone, B. Boehm, M.E. Cooper, Z. Chai, S. Del Prato, L. Ji, D. Hopkins, W. H. Herman, K. Khunti, J.-C. Mbanya, E. Renard, New-onset diabetes in Covid-19, N. Engl. J. Med. 383 (2020) 789–790.

[32] Y. Lu, X. Li, D. Geng, N. Mei, P.-Y. Wu, C.-C. Huang, T. Jia, Y. Zhao, D. Wang, A. Xiao, B. Yin, Cerebral micro-structural changes in COVID-19 patients - an MRIbased 3-month follow-up study, EClinicalMedicine. 25 (2020) 100484.

[33] Zheng M, Song L. Novel antibody epitopes dominate the antigenicity of spike glycoprotein in SARS-CoV-2 compared to SARS-CoV. Cell Mol Immunol 2020; 17:536–8.

[34] Callow K, Parry H, Sergeant M, Tyrrell D. The time course of the immune response to experimental coronavirus infection of man. Epidemiol Infect 1990; 105:435–46

[35] Tortorici MA, Walls AC, Lang Y et al. Structural basis for human coronavirus attachment to sialic acid receptors. Nat Struct Mol Biol 2019; 26:481–9

[36] Li X, Giorgi EE, Marichannegowda MH et al. Emergence of SARS-CoV-2 through recombination and strong purifying selection. Sci Adv 2020:eabb9153

[37] Qiu T, Mao T, Wang Y et al. Identification of potential crossprotective epitope between a new type of coronavirus (2019- nCoV) and severe acute respiratory syndrome virus. J Genet Genomics 2020; 47:115–7.

[38] Tian X, Li C, Huang A et al. Potent binding of 2019 novel coronavirus spike protein by a SARS coronavirus-specific human monoclonal antibody. Emerg Microbes Infect 2020; 9:382–5.

[39] Seydoux E, Homad LJ, MacCamy AJ et al. Analysis of a SARSCoV-2-infected individual reveals development of potent neutralizing antibodies with limited somatic mutation. Immunity 2020; 53:98–105.e5

[40] Aziz M, Iheanacho F, Hashmi MF. Physiology, Antibody in StatsPearls. 2019. https://www.ncbi.nlm.nih.gov/books/NBK546670/

[41] Channappanavar R, Zhao J, Perlman S. T cell-mediated immune response to respiratory coronaviruses. Immunol Res 2014; 59:118–28.

[42] Yasui F, Kai C, Kitabatake M et al. Prior immunization with severe acute respiratory syndrome (SARS)-associated coronavirus (SARS-CoV) nucleocapsid protein causes severe pneumonia in mice infected with SARS-CoV. J Immunol 2008; 181:6337–48

[43] Openshaw PJ, Tregoning JS. Immune responses and disease enhancement during respiratory syncytial virus infection. Clin Microbiol Rev 2005; 18:541–55.

[44] Rothman AL. Immunity to dengue virus: a tale of original antigenic sin and tropical cytokine storms. Nat Rev Immunol 2011; 11:532–43.

[45] Gilbert SC. Influenza vaccines and immunopathology. Expert Rev Vaccin 2012; 11:873–5.

[46] Kiyotani K, Toyoshima Y, Nemoto K, Nakamura Y. Bioinformatic prediction of potential T cell epitopes for SARS-Cov-2. J Hum Genet 2020; 65:569–75.

[47] Wang M, Jiang S, Wang Y. Recent advances in the production of recombinant subunit vaccines in Pichia pastoris. Bioengineered 2016; 7:155–65.

[48] Nascimento I, Leite L. Recombinant vaccines and the development of new vaccine strategies. Braz J Med Biol Res 2012; 45:1102–11.

[49] Magistrelli G, Poitevin Y, Schlosser F et al. Optimizing assembly and production of native bispecific antibodies by codon deoptimization. MAbs 2017; 9:231–9.

[50] Syomin B, Ilyin Y. Virus-like particles as an instrument of vaccine production. Mol Biol 2019; 53:323–34

[51] Wrapp D, Wang N, Corbett KS et al. Cryo-EM structure of the 2019-nCoV spike in the prefusion conformation. Science 2020; 367:1260–3.

[52] Chen Z, John Wherry E. T cell responses in patients with COVID-19. Nat Rev Immunol 2020; 20:529–36.

[53] Kamepalli M, FIDSA C. How immune T-cell augmentation can help prevent COVID-19: a possible nutritional solution using ketogenic lifestyle. Univ Louisville J Respir Infect 2020

[54] Agrawal AS, Tao X, Algaissi A et al. Immunization with inactivated Middle East respiratory syndrome coronavirus vaccine leads to lung immunopathology on challenge with live virus. Hum Vaccin Immunother 2016; 12:2351–6.

[55] Yang X, Dai T, Zhou X et al. Analysis of adaptive immune cell populations and phenotypes in the patients infected by SARS-CoV-2. medRxiv 2020. https://doi.org/10.1101/2020.03. 23.20040675

[57] Ho Y, Lin P-H, Liu CY, Lee S-P, Chao Y-C. Assembly of human severe acute respiratory syndrome coronavirus-like particles. Biochem Biophys Res Comm 2004; 318:833–8

[58] Hodgson J. The pandemic pipeline. Nat Biotechnol News 2020. https://www.nature.com/articles/d41587-020-00005-z (accessed September 2020).

[59] Novavax. Novavax Awarded Funding from CEPI for COVID-19 Vaccine Development. 2020. Press release https://ir.novav ax.com/news-releases/news-release-details/novavax-awardedfunding-cepi-covid-19-vaccine-development (accessed September 2020).

[60] Novavax. Novavax Recombinant Protein Nanoparticle Vaccine Technology. 2020. Press release https://novavax.com/our-uniqu e-technology (accessed September 2020)

[61] Keech C, Albert G, Cho I, et al. Phase 1–2 trial of a SARSCoV-2 recombinant spike protein nanoparticle vaccine. NEJM. 2020. https://doi.org/10.1056/NEJMoa2026920

[62] Keech C, Albert G, Cho I, et al. Phase 1–2 trial of a SARSCoV-2 recombinant spike protein nanoparticle vaccine. NEJM. 2020. https://doi.org/10.1056/NEJMoa2026920

[63] Medicago. COVID-19: Medicago's Development Programs. 2020 Medicago. 2020. https://www.medicago.com/en/newsroom/ medicago-begins-phase-i-clinical-trials-for-its-covid-19-vacci ne-candidate/ (accessed September 2020)

[64] Zha L, Zhao H, Mohsen MO et al. Development of a COVID-19 vaccine based on the receptor binding domain displayed on virus-like particles. bioRxiv 2020. https://doi.org/10.1101/ 2020.05.06.079830

[65] Sesardic D. Synthetic peptide vaccines. J Med Microbiol 1993; 39:241–2.

[66] Bijker MS, Melief CJ, Offringa R, Van Der Burg SH. Design and development of synthetic peptide vaccines: past, present and future. Expert Rev Vaccin 2007; 6:591–603.

[67] Zhang H, Hong H, Li D et al. Comparing pooled peptides with intact protein for accessing cross-presentation pathways for protective CD8+ and CD4+ T cells. J Biol Chem 2009; 284:9184–91.

[68] Rosalia RA, Quakkelaar ED, Redeker A et al. Dendritic cells process synthetic long peptides better than whole protein, improving antigen presentation and T-cell activation. Eur J Immunol 2013; 43:2554–65

[69] Purcell AW, McCluskey J, Rossjohn J. More than one reason to rethink the use of peptides in vaccine design. Nat Rev Drug Discov 2007; 6:404–14.

[70] Li W, Joshi MD, Singhania S, Ramsey KH, Murthy AK. Peptide vaccine: progress and challenges. Vaccines 2014; 2:515–36.

[71] Ahmed SF, Quadeer AA, McKay MR. Preliminary identification of potential vaccine targets for the COVID-19 coronavirus (SARS-CoV-2) based on SARS-CoV immunological studies. Viruses 2020; 12:254

[72] Bhattacharya M, Sharma AR, Patra P et al. Development of epitope-based peptide vaccine against novel coronavirus 2019 (SARS-COV-2): immunoinformatics approach. J Med Virol 2020; 92:618–31.

[73] Kadam S, Sukhramani G, Bishnoi P, Pable A, Barvkar V. Molecular and Structural Insights into COVID-19 Pandemic. 2020. 2020050519. https://doi.org/10.20944/preprints202005. 0519.v1

[74] Kalita P, Padhi A, Zhang KY, Tripathi T. Design of a peptidebased subunit vaccine against novel coronavirus SARS-CoV-2. Microb Pathog 2020; 145:104236.

[75] Gauttier V, Morello A, Girault I et al. Tissue-resident memory CD8 T-cell responses elicited by a single injection of a multi-target COVID-19 vaccine. bioRxiv 2020:2020.08. 14.240093.

[76] Bolles M, Deming D, Long K et al. A double-inactivated severe acute respiratory syndrome coronavirus vaccine provides incomplete protection in mice and induces increased eosinophilic proinflammatory pulmonary response upon challenge. J Virol 2011; 85:12201–15.

[77] Deng Y, Lan J, Bao L et al. Enhanced protection in mice induced by immunization with inactivated whole viruses compare to spike protein of middle east respiratory syndrome coronavirus. Emerg Microbes Infect 2018; 7:1–10.

[78] Qin E, Shi H, Tang L et al. Immunogenicity and protective efficacy in monkeys of purified inactivated Vero-cell SARS vaccine. Vaccine 2006; 24:1028–34.

[79] Gao Q, Bao L, Mao H et al. Development of an inactivated vaccine candidate for SARS-CoV-2. Science 2020; 6499:77–81.

[80] Zhang Y-J, Zeng G, Pan H-X et al. Immunogenicity and safety of a SARS-CoV-2 inactivated vaccine in healthy adults aged 18–59 years: report of the randomized, double-blind, and placebo-controlled Phase 2 clinical trial. medRxiv 2020:2020. 07.31.20161216.

[81] Xia S, Duan K, Zhang Y et al. Effect of an inactivated vaccine against SARS-CoV-2 on safety and immunogenicity outcomes: interim analysis of 2 randomized clinical trials. JAMA 2020; 324:951–60

[82] Schlegl R, Weber M, Wruss J et al. Influence of elemental impurities in aluminum hydroxide adjuvant on the stability of inactivated Japanese Encephalitis vaccine, IXIARO®. Vaccine 2015; 33:5989–96.

[83] Yong CY, Ong HK, Yeap SK, Ho KL, Tan WS. Recent advances in the vaccine development against middle east respiratory syndrome – coronavirus. Front Microbiol 2019; 10:1781.

[84] Wareing M, Tannock G. Live attenuated vaccines against influenza; an historical review. Vaccine 2001; 19:3320–30.

[85] Regla-Nava JA, Nieto-Torres JL, Jimenez-Guardeño JM et al. Severe acute respiratory syndrome coronaviruses with mutations in the E protein are attenuated and promising vaccine candidates. J Virol 2015; 89:3870–87

[86] Almazán F, DeDiego M, Sola I et al. Engineering a replicationcompetent, propagation-defective Middle East respiratory syndrome coronavirus as a vaccine candidate. mBio 2013; 4:e00650-13.

[87] Mueller S, Stauft CB, Kalkeri R et al. A codon-pair deoptimized live-attenuated vaccine against respiratory syncytial virus is immunogenic and efficacious in non-human primates. Vaccine 2020; 38:2943–2948

[88] BioSpace. Codagenix and Serum Institute of India Initiate Co-Development of a Scalable, Live-Attenuated Vaccine Against the 2019 Novel Coronavirus, COVID-19. BioSpace, 2020. https:// www.biospace.com/article/releases/codagenix-and-serum-insti tute-of-india-initiate-co-development-of-a-scalable-live-atten uated-vaccine-against-the-2019-novel-coronavirus-covid-19/ (accessed September 2020).

[89] Porgador A, Irvine KR, Iwasaki A, Barber BH, Restifo NP, Germain RN. Predominant role for directly transfected dendritic cells in antigen presentation to CD8+ T cells after gene gun immunization. J Exp Med 1998; 188:1075–82.

[90] Ishikawa H, Ma Z, Barber GN. STING regulates intracellular DNA-mediated, type I interferon-dependent innate immunity. Nature 2009; 461:788–92

[91] Joffre O, Nolte MA, Sporri R, Reis e Sousa C. Inflammatory signals in dendritic cell activation and the induction of adaptive immunity. Immunol Rev 2009; 227:234–47

[92] Kim BM, Lee DS, Choi JH et al. In vivo kinetics and biodistribution of a HIV-1 DNA vaccine after administration in mice. Arch Pharm Res 2003; 26:493–8

[93] Capecchi MR. High efficiency transformation by direct microinjection of DNA into cultured mammalian cells. Cell 1980; 22:479–88.

[94] Okada M, Takemoto Y, Okuno Y et al. The development of vaccines against SARS corona virus in mice and SCID-PBL/ hu mice. Vaccine 2005; 23:2269–72.

[95] Lu B, Tao L, Wang T et al. Humoral and cellular immune responses induced by 3a DNA vaccines against severe acute respiratory syndrome (SARS) or SARS-like coronavirus in mice. Clin Vaccine Immunol 2009; 16:73–7.

[96] Hu H, Lu X, Tao L et al. Induction of specific immune responses by severe acute respiratory syndrome coronavirus spike DNA vaccine with or without interleukin-2 immunization using different vaccination routes in mice. Clin Vaccine Immunol 2007; 14:894–901

[97] Martin JE, Louder MK, Holman LA et al. A SARS DNA vaccine induces neutralizing antibody and cellular immune responses in healthy adults in a Phase I clinical trial. Vaccine 2008; 26:6338–43.

[98] Modjarrad K, Roberts CC, Mills KT et al. Safety and immunogenicity of an anti-Middle East respiratory syndrome coronavirus DNA vaccine: a phase 1, open-label, single-arm, dose-escalation trial. Lancet Infect Dis 2019; 19:1013–22.

[99] Smith TRF, Patel A, Ramos S et al. Immunogenicity of a DNA vaccine candidate for COVID-19. Nat Commun 2020; 11:2601.

[100] Patel A, Walters J, Reuschel EL et al. Intradermal-delivered DNA vaccine provides anamnestic protection in a rhesus macaque SARS-CoV-2 challenge model. bioRxiv 2020:2020.07.28.225649.

[101] Chen N, Xia P, Li S, Zhang T, Wang TT, Zhu J. RNA sensors of the innate immune system and their detection of pathogens. IUBMB Life 2017; 69:297–304.

[102] Edwards DK, Jasny E, Yoon H et al. Adjuvant effects of a sequence-engineered mRNA vaccine: translational profiling demonstrates similar human and murine innate response. J Transl Med 2017; 15:1.

[103] Pepini T, Pulichino AM, Carsillo T et al. Induction of an IFNmediated antiviral response by a self-amplifying RNA vaccine: implications for vaccine design. J Immunol 2017; 198:4012–24.

[104] Kariko K, Buckstein M, Ni H, Weissman D. Suppression of RNA recognition by Toll-like receptors: the impact of nucleoside modification and the evolutionary origin of RNA. Immunity 2005; 23:165–75.

[105] Kariko K, Muramatsu H, Welsh FA et al. Incorporation of pseudouridine into mRNA yields superior nonimmunogenic vector with increased translational capacity and biological stability. Mol Ther 2008; 16:1833–40.

[106] Guo H, Ingolia NT, Weissman JS, Bartel DP. Mammalian microRNAs predominantly act to decrease target mRNA levels. Nature 2010; 466:835–40.

[107] Barreau C, Paillard L, Osborne HB. AU-rich elements and associated factors: are there unifying principles? Nucleic Acids Res 2005; 33:7138–50.

[108] Vogel AB, Lambert L, Kinnear E et al. Self-amplifying RNA vaccines give equivalent protection against influenza to mRNA vaccines but at much lower doses. Mol Ther 2018; 26:446–55.

[109] Horby P, Lim WS, Emberson J et al. Effect of dexamethasone in hospitalized patients with COVID-19. Preliminary report. medRxiv 2020:2020.06.22.20137273.

[110] Spinner CD, Gottlieb RL, Criner GJet al, for the GSUSI. Effect of Remdesivir vs standard care on clinical status at 11 days in patients with moderate COVID-19: a randomized clinical trial. JAMA 2020; 324:1048–57.

[111] World Health Organization. Key criteria for the ethical acceptability of COVID-19 human challenge studies. 2020. https://www.who. int/ethics/publications/key-criteria-ethical-acceptability-of-covid -19-human-challenge/en/ (accessed September 2020).

[112] Deming ME, Michael NL, Robb M, Cohen MS, Neuzil KM. Accelerating development of SARS-CoV-2 vaccines – the role for controlled human infection models. N Engl J Med 2020; 383:e63. https://doi.org/10.1056/NEJMp2020076

[113] Mallory ML, Lindesmith LC, Baric RS. Vaccination-induced herd immunity: successes and challenges. J Allergy Clin Immunol 2018; 142:64–6.

[114] Scarbrough Lefebvre CD, Terlinden A, Standaert B. Dissecting the indirect effects caused by vaccines into the basic elements. Hum Vaccin Immunother 2015; 11:2142–57.

[115] Zheng M, Song L. Novel antibody epitopes dominate the antigenicity of spike glycoprotein in SARS-CoV-2 compared to SARS-CoV. Cell Mol Immunol 2020; 17:536–8.

[122] Berry JD, Jones S, Drebot MA et al. Development and characterisation of neutralising monoclonal antibody to the SARS-coronavirus. J Virol Methods 2004; 120:87–96.

[123] McKay PF, Hu K, Blakney AK et al. Self-amplifying RNA SARS-CoV-2 lipid nanoparticle vaccine candidate induces high neutralizing antibody titers in mice. Nat Commun 2020; 11:3523

[124] Wang Q, Li C, Zhang Q et al. Interactions of SARS coronavirus nucleocapsid protein with the host cell proteasome subunit p42. Virol J 2010; 7:99.

[125] Tang J, Zhang N, Tao X et al. Optimization of antigen dose for a receptor-binding domain-based subunit vaccine against MERS coronavirus. Hum Vaccin Immunother 2015; 11:1244–50

[126] Mou H, Raj VS, van Kuppeveld FJ, Rottier PJ, Haagmans BL, Bosch BJ. The receptor binding domain of the new Middle East respiratory syndrome coronavirus maps to a 231-residue region in the spike protein that efficiently elicits neutralizing antibodies. J Virol 2013; 87:9379–83

[127] Ma C, Wang L, Tao X et al. Searching for an ideal vaccine candidate among different MERS coronavirus receptor-binding fragments—the importance of immunofocusing in subunit vaccine design. Vaccine 2014; 32:6170–6.

[128] Keng C-T, Zhang A, Shen S et al. Amino acids 1055 to 1192 in the S2 region of severe acute respiratory syndrome coronavirus S protein induce neutralizing antibodies: implications for the development of vaccines and antiviral agents. J Virol 2005; 79:3289–96.

[129] Pogrebnyak N, Golovkin M, Andrianov V et al. Severe acute respiratory syndrome (SARS) S protein production in plants: development of recombinant vaccine. Proc Natl Acad Sci USA 2005; 102:9062–7.

[130] He Y, Lu H, Siddiqui P, Zhou Y, Jiang S. Receptor-binding domain of severe acute respiratory syndrome coronavirus spike protein contains multiple conformation-dependent epitopes that induce highly potent neutralizing antibodies. J Immunol 2005; 174:4908–15

[131] 119 Ma C, Li Y, Wang L et al. Intranasal vaccination with recombinant receptor-binding domain of MERS-CoV spike protein induces much stronger local mucosal immune responses than subcutaneous immunization: Implication for designing novel mucosal MERS vaccines. Vaccine 2014; 32:2100–8.

[132] PipelineReview. Clover Initiates Development of Recombinant Subunit-Trimer Vaccine for Wuhan Coronavirus (2019-nCoV). 2020. https://pipelinereview.com/index.php/2020012873644/ Vaccines/Clove r-Initi ates-Development-of-Recombinan t-Subunit-Trimer-Vaccine-for-Wuhan-Coronavirus-2019- nCoV.html (accessed September 2020).

[133] PipelineReview. Clover successfully produced 2019-nCoV subunit vaccine candidate and detected cross-reacting antibodies from sera of multiple infected patients. 2020. https://pipelinereview.com/index.php/2020021073754/Vacci nes/Clover-Successfully-Produced-2019-nCoV-Subunit-VaccineCandidate-and-Detected-Cross-Reacting-Antibodies-fromSera-of-Multiple-Infected-Patients.html (accessed September 2020)

[134] Phelan AL, Eccleston-Turner M, Rourke M, Maleche A, Wang C. Legal agreements: barriers and enablers to global equitable COVID-19 vaccine access. Lancet 2020; 396:800–2.