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SARS-CoV-2 vaccine development: where are we?

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Abstract. – The coronavirus disease 2019 (COVID-19) pandemic caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has proved to be one of the most challenging infectious diseases in the modern era, and despite several countermeasures to lessen its impact, the spread of the virus is still affecting most countries. This renders the goal of active immunization of the population through vaccination a worldwide public health priority. In fact, only when efficient vaccination programs will be successfully implemented, a return to pre-pandemic normality can be considered. The scientific community has made a tremendous effort to blow the lid off the pathogenesis of the disease, and unprecedented efforts are ongoing with governments, private organizations, and academics working together to expeditiously develop safe and efficacious vaccines. Previous research efforts in the development of vaccines for other coronaviruses (Severe Acute Respiratory Syndrome Coronavirus 1 and Middle East Respiratory Syndrome Coronavirus) as well other emerging viruses have opened the door for exploiting several strategies to design a new vaccine against the pandemic virus. Indeed, in a few months, a stunning number of vaccines have been proposed, and almost 50 putative vaccine candidates have entered clinical trials. The different vaccine candidates use different vaccine development platforms, from inactivated whole virus vaccine to subunit vaccine, nucleic acid, and vectored vaccines. In this review, we describe strengths, flaws, and potential pitfalls of each approach to understand their chances of success.

Key Words:
SARS-CoV-2, COVID-19, Vaccine, Pandemic, Immune response.

Introduction

Coronaviridae are a very intriguing family of positive single-stranded RNA viruses. They differ in many remarkable traits from all other positive single-stranded RNA viruses: i) their RNA genomes are substantially larger than the genomes of any other known positive-stranded RNA virus; ii) coronaviruses are unique in showing a helical symmetry of the nucleocapsid; and doubtless most peculiar, iii) coronaviruses replicate their positive-stranded RNA genomes by a strategy in which they give rise to a nested set of messenger RNAs (mRNAs) that have a common 3' end. Each of the mRNAs within the nested set contains multiple open reading frames (ORFs), where only the one at the 5' end of the molecule is translated[1]. This family of RNA viruses, though almost unknown to the large audience until recently, has been the object of extensive studies by scientists in the last few decades. Prior to 2003, only two strains of coronavirus, the Human coronavirus (HcoV) 229E and HCoV-OC43, were associated with a mild influenza-like illness in humans. Hence, researchers in the field were taken by surprise in 2003 when it was discovered that the causative agent for the new severe acute respiratory syndrome (SARS) epidemic was identified as a "new" coronavirus. The discovery of the SARS coronavirus (SARS-CoV) stimulated extensive research in the field of coronaviruses, as regards the molecular mechanisms necessary for the definition of target molecules, the development of antivirals and/or vaccines, and the identification of other novel coronaviruses in both humans and animals, leading to the discovery of two other coronaviruses that infect humans: HCoV-NL63 in 2004 and HCoV-HKU1 in 2005[2]. Unlike other human coronaviruses, SARS-CoV was capable of causing severe and life-threatening pneumonia in humans and was the most pathogenic coronavirus until a further coronavirus capable of causing fatal disease in humans, the Middle East respiratory

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syndrome coronavirus (MERS-CoV), appeared. Finally, to complete the picture of coronaviruses of human interest, a disease, named Coronavirus Disease 2019 (COVID-19), came to light in Wuhan, within the Hubei Province in China, in early December 2019, but in a flash, a pandemic magnitude was reached. As of 29th November 2020, a total number of 61,869,330 confirmed cases of COVID-19 have been reported, including 1,448,896 deaths[3]. Caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the pandemic represents a serious threat to public health since infection leads to a wide spectrum of clinical manifestations with an initial status of the asymptomatic subject, with progression to pneumonia which may further progress to acute respiratory distress syndrome (ARDS) followed by multi-organ failure (MOF) and death[4]. While the last three viruses with high pathogenicity and mortality (SARS-CoV, MERS-CoV, and SARS-CoV-2) have only recently emerged in the human population, HCoV-HKU1 and HCoV-NL63 have been circulating in humans for a long time. HCoV-HKU1 and HCoV-NL63 are both respiratory coronaviruses that are frequently associated with upper and lower respiratory tract diseases. Infections with these two human coronaviruses do not differ much from those caused by the "old" coronaviruses HCoV-229E and HCoV-OC43.

The first severe acute respiratory syndrome due to SARS-CoV spanned the world with more than 8,000 recognized cases and caused 774 deaths (almost 9% of infected cases) in less than a year and then disappeared[5]. The second outbreak in 2012, MERS-CoV, remained mainly localized in the Arabic peninsula, where 1,038 cases with 460 deaths were reported (approximately 37% mortality). Since then, it reappeared with small epidemics of mainly nosocomial infections in South Korea[6]. On the other hand, the present pandemic has swept the surface of the globe with an unprecedented and unforecastable spreading capacity, albeit with minor mortality compared to the previous two, and is still in a crescendo after almost a year from its emergence in the human population. As seen in the actual situation, we need to accept that the SARS-CoV-2 will remain a treat for a long time. Therefore, the best option for getting back to our usual social life (considering the measures adopted by most Governments worldwide of social distancing and more or less strict lockdowns) is represented by the widespread distribution and use of vaccines. This review addresses the key point of the immune responses deemed essential to provide protection against SARS-CoV-2. Moreover, it delves into the composition of the different vaccine platforms and the relative advantages and/or disadvantages of each approach.

Viral Structural Proteins and their Function

SARS-CoV-2 particles contain a positive-strand RNA genome of 29.9 kb in length. The genome is both capped and poly-adenylated, enabling SARS-CoV-2 to be translated upon its release within the cytoplasm right at the beginning of an infectious cycle.

The SARS-CoV-2 genome possess 14 ORFs encoding 27 proteins, and its genomic organization follows the common rules of other coronaviruses being: *5′-leader-UTR-replicase-Spike (S)-Envelope (E)-Membrane (M)-Nucleocapsid (N)-3′UTR-poly(A)tail*, with several accessory genes interspersed within the structural genes at the 3′ end of the genome[7]. The accessory genes are considered nonessential for *in vitro* replication but able to exert a suppressing effect on the antiviral-immune responses and also able to intensify pathogenesis[8]. A large portion of about two-thirds of the genome within the 5′ end is employed to encode two long ORFs named 1a and 1b, which produce the nonstructural proteins of the virus. Orfs 1a and 1b are translated first as polyprotein precursors named pp1a and pp1ab, where the latter results from a programmed –1 ribosomal frameshift event taking place at the short overlap at the end of ORF1a with ORF1b coding sequences[9]. The polyproteins include several viral proteases that together process pp1a and pp1ab into 16 nonstructural proteins (nsp1–16), which are subsequently required at diverse phases of SARS-CoV-2 replication. From a morphological point of view of the virus particles, the SARS-CoV-2 genome is associated with the N protein to form a helical nucleocapsid, which in turn is wrapped by a lipid membrane envelope containing the M, S, and E proteins (Figure 1). The trimeric S glycoprotein (~150 kDa) is the largest membrane protein forming 20 nm-long and prominent petal-shaped spikes and is involved in cell receptor binding of the virus to the host cell and in mediating the fusion mechanism able to allow the entry of the virus into cells.

The S glycoprotein is a class I fusion protein whose attachment to the host receptor angiotensin-converting enzyme 2 (ACE2) is able to trigger a cascade of cell membrane fusion events leading to viral entry[10-13]. Glycoprotein S is further

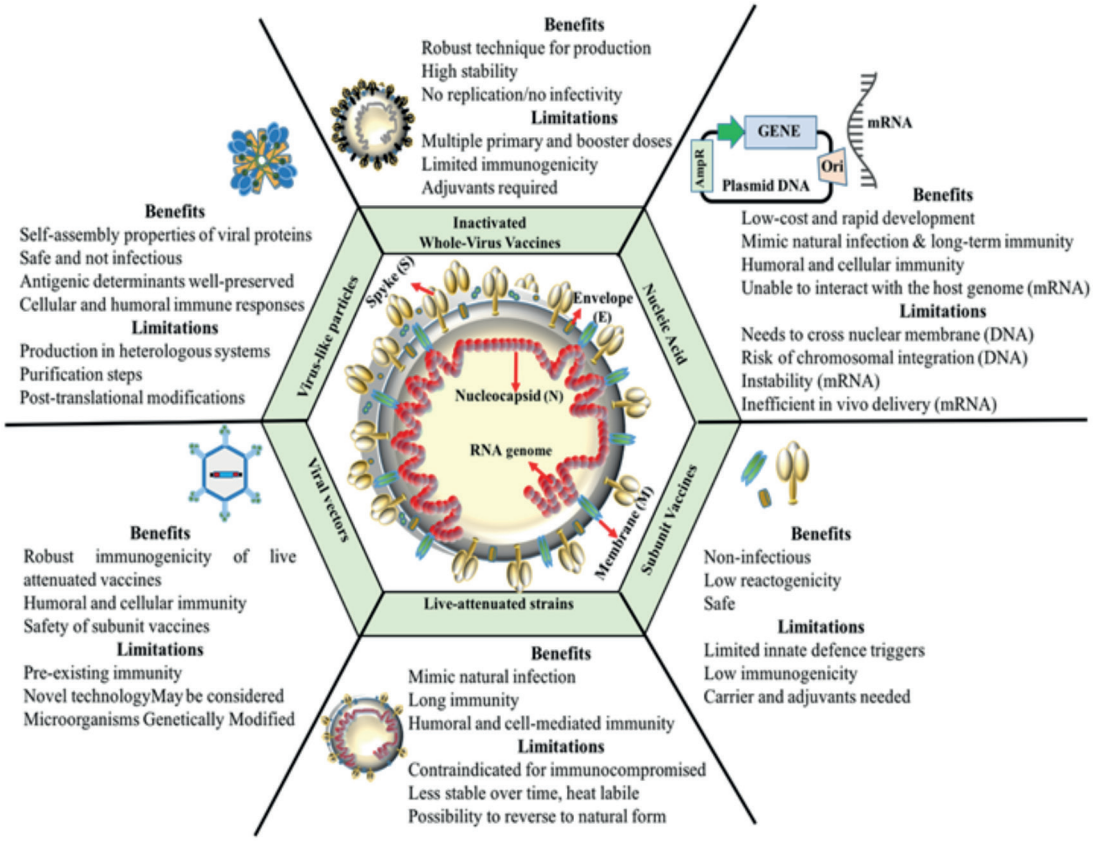

Figure 1. Vaccine platforms used for SARS-CoV-2 vaccine development.

cleaved by a host cellular protease into two separate polypeptides: a globular S1 domain at the N-terminal region, and the membrane-proximal S2 and transmembrane domains[14]. The receptor-binding domain (RBD) able to determine host range and cellular tropism is found within the S1 domain, while key features to activate membrane fusion are located within the S2 domain[15]. The S2 domain contains two coiled coil regions (the so-called heptad repeats), which give rise to a structure of trimeric hairpins, positioning the fusion peptide in close proximity to the C-terminal region of the ectodomain. The formation of this post-fusion structure is able to drive the apposition and subsequent merging of viral and target cell membranes[16]. On the contrary, the M protein (~25-30 kDa), with three transmembrane domains, differs from most other viral envelope glycoproteins in that only a short N-terminal domain is exposed on the exterior of the particle, while a large C-terminal domain is exposed beneath the envelope, facing the interior. The reason for this atypical orientation of the coronavirus M protein is to be found in its function in promoting membrane curvature as well as binding to the nucleocapsid; therefore, M protein fulfills the function of classical matrix protein of other enveloped viruses that contain helical nucleocapsids. Hence, the M protein defines the shape of the viral envelope. Finally, the E protein plays a crucial role in determining virus shape and driving assembly. It behaves as a viroporin forming pentameric protein-lipid pores that allow ion transport. Together, M and E are necessary and sufficient for an efficient assembly and release of SARS-CoV-2 virus-like particles (VLPs)[17].

Vaccine Platforms

It is a world high-priority to develop effective and safe vaccines to block COVID-19 pandemic, with the aim of limiting its inexorable spread, and ultimately to avoid any eventual future reappearance. In practice, we do not have to view vaccines as flawless weapons to ensure a COVID-19-free world, but the tasks of an efficient vaccine are to decrease disease severity, reduce viral spread,

and most importantly, person-to-person transmission. Currently, no vaccine has been licensed to prevent SARS-CoV-2 infection, but since the SARS-CoV-2 shares, considerable sequence similarity with the preceding two deadly coronaviruses (MERS-CoV and SARS-CoV), the studies to attempt to develop vaccines against MERS-CoV and SARS-CoV could represent a strong knowledge base to significantly accelerate the development of anti-COVID-19 vaccines[18]. Nevertheless, several factors require to be taken into consideration prior to any vaccine moving forward to a widespread usage to immunize vast populations. The pathway for a vaccine to reach the market is long and strewn with obstacles, and the need to proceed through an exploratory, a preclinical and a clinical stage. The exploratory stage represents the basic research conducted mainly in academic and research laboratories where the target antigen is discovered and analyzed. Therefore, it represents the first idea of the novel vaccine. This is followed by a preclinical stage where tissue-culture or cell-culture and animal testing are developed to evaluate the safety of the putative vaccine and the level of its immunogenicity. These studies are of great importance since they are able to produce invaluable data to allow a first prediction of the type of responses is possible to predict for human use. At this stage, researchers can start to envisage possible routes of administration and eventual dosage; indeed, more advanced studies also consider animal challenges with the infectious pathogen to find out the efficacy in preventing the infection. Once sufficient preclinical data become available clinical trials might be initiated, but very few putative candidates are, indeed, able to pass to the following stage of clinical testing. Generally, clinical trials are divided into Phase 1, Phase 2, and Phase 3 clinical trials, but in a period of great pressure due to the running pandemic, many companies have decided to use overlapping Phase 1 and Phase 2 trials to reduce the timing. In fact, the intrepid goal is to reduce to merely 12-18 months a process that prior to the pandemic used to take from 10 to 15 years. In Phase 1, a small number (less than 100) of healthy volunteers get administered the putative vaccine mainly to assess the safety and the quality of the immune response elicited; usually, this is not a blind experimentation, but both the volunteers and the researchers are informed on the protocol used. Only if the collected data are encouraging, the experimentation can proceed to the following phase, that is the Phase 2 vaccine trial, where a larger group of several hundred subjects are included in the trial. The Phase 2 trials involve randomized and well-controlled experimentation, with a placebo control group, in order to establish formulation and doses and prove efficacy. Finally, Phase 3 of the trials is used to definitely assess vaccine safety in a larger cohort of several thousands of individuals; all correlates of protection are analyzed (production of antibodies and cell-mediated immunity, immunological memory, and protection from disease). Once a successful Phase 3 is deemed terminated, there is the verification and approval by the licensing authority before entering the manufacturing and distribution to the public[19,20].

Great efforts around the globe and a commitment never seen before have allied many teams worldwide for the common scope to reduce the time for vaccine discovery and approval. Several platforms and a huge number of vaccines are being investigated, and in the present review, we have considered mainly 6 different platforms of vaccine development for distributing all candidate vaccine (196 vaccines), included the 6th, which comprises: live attenuated viruses (3 examples), replicating bacterial vectors (1 vaccine), and one T-cell based vaccine. The first category is dedicated to the inactivated whole SARS-CoV-2 virus with 21 examples, while the most prolific is the second category referred as "protein subunit" vaccines, which includes different technologies such as purified or expressed proteins and peptides with different carrier molecules. This category currently counts 68 candidate vaccines in development. The third category is made up of the nucleic acid platforms, which are further divided into RNA (24 candidates) and DNA (18 candidates). A fourth category is based on viral vectors either non-replicating (21 candidates) or replicating (21 candidates), and the last (5th) is represented by the vaccine based on virus-like-particles (VLP) or nanoparticles (18 candidates). Moreover, various adjuvant technologies like AS03 (GSK, Brentford, United Kingdom), MF-59 (Novartis, Basel, Switzerland), CpG 1018 (Dynavax, Emeryville, CA, United States) are now available to enhance the immunogenicity of any candidate vaccine to allow safe vaccine development[21]. What is really surprising is that 11 of such candidates have already reached Phase 3 clinical trials. The platforms on which these vaccines are based are inactivated, non-replicating viral vectors, protein subunits, and RNA-based vaccines as listed in Table I[22].

Table I. SARS-CoV-2 candidate vaccines in Phase 3 clinical trials.

COVID-19 vaccine developer/manufactures	Vaccine platform	Type of candidate vaccine	Current stage of clinical evaluation (estimated study completion date) (NA- Not Available)
Sinovac (Beijing, China)	Inactivated	Inactivated SARS-CoV-2 vaccine with aluminum hydroxide	NCT04456595 (October 2021) NCT04582344 (October 2021) NCT04617483 (May 2021)
Wuhan Institute of Biological products (Wuhan, China)/Sinopharm (Beijing, China)	Inactivated	Inactivated whole SARS-CoV-2	ChiCTR2000034780 (NA) NCT04612972 (September 2021)
Beijing Institute of Biological products/Sinopharm (Beijing, China)	Inactivated	Aluminum hydroxide adsorbed inactivated SARS-CoV-2 vaccine	NCT04560881 (December 2021) ChiCTR2000034780 (NA)
Bharat Biotech (Hyderabad, India)	Inactivated	Whole-Virion Inactivated	CTRI/2020/11/028976 (End 2021)
Novavax (Gaithersburg, MD, United States)	Protein Subunit	Full length recombinant SARS CoV-2 S prefusion glycoprotein adjuvanted with Matrix M protein	2020-004123-16 (NA) NCT04611802 (December 2022)
Moderna (Cambridge, MA, United States)/NIAID (Bethesda, MD, United States)	Nucleic Acid	LNP-encapsulated mRNA	NCT04470427 (October 2022)
BioNTech (Mainz, Germany)/Fosun Pharma (Shanghai, China)/Pfizer (New York, United States)	Nucleic Acid	Lipid-nanoparticle-formulated, nucleoside-modified mRNA vaccine that encodes the trimerized receptor-binding domain (RBD) of the spike glycoprotein of SARS-CoV-2.	NCT04368728 (December 2022)
University of Oxford (Oxford, United Kingdom)/AstraZeneca (Cambridge, United Kingdom)	Non-replicating viral vector	Recombinant replication-defective chimpanzee adenovirus expressing the S glycoprotein of SARS-CoV-2 (ChAdOx1-S)	NCT04540393 (March 2021) NCT04516746 (October 2022) CTRI/2020/08/027170 (NA)
CanSinoBiologicals InC (Tianjin, China)/Beijing Institute of Biotechnology (Beijing, China)	Non-replicating viral vector	Adenovirus type 5 vector carrying the S-protein of SARS-CoV-2	NCT04526990 (January 2022) NCT04540419 (July 2021)
Gamaleya Research Institute (Moscow, Russia)	Non-replicating viral vector	Combined adenovirus-based vector (rAd26-S+rAd5-S) of the S-protein of SARS-CoV-2	NCT04530396 (May 2021) NCT04564716 (April 2021)
Janssen Pharmaceuticals Companies (Beerse, Belgium)	Non-replicating viral vector	Adenovirus type 26 vector carrying the S1-subunit of SARS-CoV-2 (Ad26CoV-S1)	NCT04505722 (March 2023) NCT04614948 (May 2023)

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Inactivated Whole-Virus Vaccine

Commonly known as non-live vaccines, the main characteristic is their inability to replicate since they do not contain any living or infectious particles. Inactivated whole-virus vaccines are preparations comprising the entire disease-causing pathogen, which is inactivated physically (heat, radiation) or chemically (formalin or formaldehyde). Inactivation dismantles the virus's ability to replicate and provokes pathology, but foresees the maintenance of its immunogenicity, so that the immune system can still mount a defense against the pathogen[23]. A wide range of native viral antigens may be structurally conserved after inactivation to expose native epitope conformations; therefore, inactivated vaccines can induce

conformation-dependent antibody responses, and compared to many subunits or recombinant vaccines, the whole virus is presented to the immune system; therefore, immune responses are likely to be directed not only against the selected viral protein but also the against other structures such as matrix, envelope, and nucleoprotein. A satisfying safety profile is generally present, even in immunocompromised subjects. Per contra, a drawback of these vaccines is that immunogenicity and duration of protection tend to be of limited effectiveness, and they may require reminders of the immune system; in fact, the addition of adjuvants and repeated administrations to improve immunogenicity are generally necessary. Furthermore, inactivated viruses, with low production cost, safe, and not involving genetic manipulation, can be promptly developed and scaled up for a pandemic situation adopting infrastructure and methods proper of a well-established mature technology[24]. A list of inactivated vaccine candidates against SARS-CoV-2 is presented in Table II. Collectively, in recent years, we have witnessed a gradual shift of vaccination strategies from whole-virus vaccine to subunit, peptide and genetic vaccine mainly as a response to high reactogenicity generally associated with whole-virus vaccines. These more recently designed vaccines have been demonstrated to be less immunogenic if compared to whole-virus vaccines; in fact, they generally require adjuvants and/or multiple immunization regimes.

Nevertheless, inactivated whole-virus vaccines are considered trustful conventional vaccines with mature technology with likely possibilities to arrive first in the race for SARS-CoV-2 vaccine entering clinical use[25]. Out of the 11 candidate vaccines against SARS-CoV-2 that already reached phase 3 clinical trials, 4 are represented by inactivated whole-virus vaccines (Table I). These phase 3 clinical trials are done by the Beijing Institute of Biological Products (Beijing, China) (NCT04560881), Sinovac (Beijing, China) (NCT04456595, NCT04582344), Wuhan Institute of Biological Products (Wuhan, China) (ChiCTR2000034780) and Bharat Biotech (Hyderabad, India) (CTRI/2020/11/028976).

Inactivated vaccines are usually produced by growing SARS-CoV-2 in cell culture (Vero cells) followed by inactivation of the virus. BBIBP-CorV is an inactivated vaccine candidate, being developed by a Chinese state-owned Sinopharm together with the Beijing Institute of Biological Products. The vaccine has been produced by inactivation of the virus clinical isolates by β-propiolactone to eliminate viral infectivity and using aluminum hydroxide as adjuvant. Electron microscopy imaging showed proper viral particles with conserved spikes with diameters of approximately 100 nm, and the presence in vaccine stocks of viral structural proteins was demonstrated by western blots. Results from phase 2 clinical trials have shown that the inactivated SARS-CoV-2 vaccine BBIBP-CorV is safe, well-tolerated, and immunogenic in healthy individuals. Neutralizing antibodies in 100% of vaccine recipients have been obtained after two-dose immunizations in two age groups (18-59 years and ≥60 years), with the observation of only mild adverse reactions (pain and fever)[26]. In preclinical studies, immunization with BBIBP-CorV was able to induce high levels of neutralizing antibody titers in mice, rats, guinea pigs, rabbits, and non-human primates (cynomolgus monkeys and rhesus macaques) to provide protection against SARS-CoV-2. Moreover, in preclinical studies of BBIBP-CorV immunization followed by SARS-CoV-2 live virus challenge, no antibody-dependent enhancement was observed in rhesus macaques to confirm the safety of the vaccine[27]. A second vaccine candidate is the one produced by Sinopharm together with the Wuhan Institute of Biological Products with a similar methodology, and the preliminary results of their β-propiolactone-inactivated whole virus vaccine have shown that the vaccine was well tolerated in all dose groups under different injection procedures with no vaccine-related serious adverse events. A further Chinese purified inactivated SARS-CoV-2 virus vaccine candidate is the one produced by Sinovac and named PiCoVacc. This vaccine was shown to induce SARS-CoV-2-specific neutralizing antibodies in mice, rats, and non-human primates[28]. The vaccine manufactured by Sinovac contains 3 μg/0.5 mL of inactivated SARS-CoV-2 virus, and aluminum hydroxide as adjuvant and after promising phase 1/2 clinical trials is now being analyzed in phase 3 clinical studies[29,30]. The last candidate vaccine reaching the finish line is the one produced by the Indian company Bharat that has just entered the phase 3 clinical trial. The vaccine BBV152 has been produced through inactivation of the whole virus by β-propiolactone followed by a chromatographic purification step. Three different formulations have been prepared: i) BBV152A containing 3 g of antigen mixed with imidazoquinoline class TLR7/8 agonist (IMDG) adsorbed to aluminum hydroxide gel (Algel), ii) BBV152B con-

Table II. SARS-CoV-2 inactivated whole-virus candidate vaccines under development.

COVID-19 vaccine developer/manufactures	Vaccine platform	Type of candidate vaccine	Current stage of clinical evaluation (estimated study completion date) (NA-Not Available)
Institute of Medical Biology, Chinese Academy of Medical Sciences (Kunming, China)	Inactivated	Inactivated whole SARS-CoV-2	Phase 1/2: NCT04470609 (November 2021)
Research Institute for Biological Safety Problems (Gvardeyskiy, Kazakhstan)	Inactivated	Inactivated whole SARS-CoV-2	Phase 1/2: NCT04530357 (December 2020)
Beijing Minhai Biotechnology Co., Ltd. (Beijing, China)	Inactivated	Inactivated SARS-CoV-2 Vaccine (Vero Cells)	Phase 1: ChiC-TR2000038804 (NA)
Institute of Vaccines and Medical Biologicals (IVEC; Khánh Hòa, Vietnam)/Dynavax (Emeryville, CA, United States)/PATH; San Francisco, CA, United States)	Inactivated	Egg-based, inactivated, whole chimeric Newcastle Disease Virus (NDV) expressing membrane-anchored pre-fusion-stabilized trimeric SARS-CoV-2 S protein (Hexapro) + CpG 1018	Preclinical
Government Pharmaceutical Organization (GPO; Bangkok, Thailand)/Dynavax (Emeryville, CA, United States)/PATH; San Francisco, CA, United States)	Inactivated	Egg-based, inactivated, whole chimeric Newcastle Disease Virus (NDV) expressing membrane-anchored pre-fusion-stabilized trimeric SARS-CoV-2 S protein (Hexapro) + CpG 1018	Preclinical Preclinical
Institute Butantan (São Paulo, Brazil)/ Dynavax (Emeryville, CA, United States)/PATH; San Francisco, CA, United States)	Inactivated	Egg-based, inactivated, whole chimeric Newcastle Disease Virus (NDV) expressing membrane-anchored pre-fusion-stabilized trimeric SARS-CoV-2 S protein (Hexapro) + CpG 1018	Preclinical
KM Biologics (Kumamoto, Japan)	Inactivated	Inactivated + alum	Preclinical
Selcuk University (Selçuklu-Konya, Turkey)	Inactivated	Inactivated	Preclinical
Erciyes University (Talas/Kayseri, Turkey)	Inactivated	Inactivated	Preclinical
National Research Centre (Dokki, Egypt)	Inactivated	Inactivated	Preclinical
Osaka University/BIKEN/NIBIOHN (Osaka, Japan)	Inactivated	Inactivated	Preclinical
Sinovac (Beijing, China/Dynavax (Emeryville, CA, United States)	Inactivated	Inactivated + CpG 1018	Preclinical
Valneva (Saint-Herblain, France)/Dynavax (Emeryville, CA, United States)	Inactivated	Inactivated + CpG 1018	Preclinical
Shifa Pharmed (Tehran, Iran)	Inactivated	Inactivated + Alum	Preclinical
Zista Kian Azma Co. (Tehran, Iran)	Inactivated	Inactivated	Preclinical
Milad Pharmaceutics Co. Plymouth, MI, United States)	Inactivated	Inactivated	Preclinical
Kocak Farma Ilac ve Kimya San. A.S. (Istanbul, Turkey)	Inactivated	Inactivated	Preclinical

Modified from: DRAFT landscape of COVID-19 candidates vaccine ⇒ https://www.who.int/publications/m/item/draft-landscape-of-covid-19-candidate-vaccines

taining 6 μg of antigen mixed with Algel-IMDG, and iii) BBV152C with 6 μg of antigen adsorbed to Algel. In the ongoing clinical trial, BBV152B vaccine formulation will be administered as a two dose intramuscular injection 28 days apart[31]. The ongoing phase 3 clinical trials will provide more information on the safety and immunogenicity, doses, and possible immunization schedules of any of the inactivated whole-virus vaccine candidates that will maintain the actual promise for reaching the market.

Subunit Vaccines (Including Synthetic Peptides or Epitope Vaccines)

Subunit vaccines contain selected pathogen-derived proteins (antigens) or parts of them in place of the whole pathogen. In the case of bacterial pathogens, subunit vaccines can be obtained starting from conventional cultivation processes through the purification of pathogen preparations, while viral subunit vaccines require the use of recombinant DNA engineering[32]. The genes encoding the selected antigens are either cloned or synthesized and then are expressed using one of the available expression systems (insect, bacterial, yeast, and mammalian cells). One of the preferred systems is bacterial expression since they assure a very high level of expression and the scaling-up step is quite straightforward by fermentation technologies. Nevertheless, a major drawback of bacterial expression systems is the proper post-translational modifications; therefore, the use of insect cells or mammalian cells may be advantageous. The produced antigens can be easily purified and used in vaccine preparations. The absence of infectious virus improves safety patterns and wipes out any concern related to virulence reversion[33].

Compared to live or inactivated whole-organism vaccines, subunit vaccines are generally safer and causing less adverse effects, but are also consistently less immunogenic in view of the fact that they contain a reduced number of antigens and that following the purification process are devoid of the other viral components that are useful for the triggering of the immune response. Since the purified subunits are weak immunogens, to obtain vaccines with convenient efficacy, the antigens are conjugated with protein molecules, and adjuvant to enhance an immune response are required[34].

Within subunit vaccines, we also include vaccines based on synthetic peptide considering that minimal immunogenic peptide sequence may be sufficient to induce T cell responses, therefore using only fractions of the entire protein can provide several advantages[35,36]. In fact, fragments of antigen can display B and/or T cell epitope activity affecting the specificity of the immune response[37]. These peptide vaccines also have the advantage of being easily produced by chemical synthesis, do not undergo tertiary structure folding, and have the possibility to be used in several combinations to display multivalent antigens capable of eliciting strong humoral and cellular responses[38,39]. However, the low molecular weight of peptidic vaccines usually results in low immunogenicity; thus, structural modifications, multi-epitope delivery systems, and use of adjuvants are generally predicted[40].

Subunit vaccines represent the most common platform explored. There are 15 COVID-19 subunit vaccines in clinical trials (Tables I and III), with 55 more candidates under preclinical development. Many research institutions feature SARS-CoV-2 subunit vaccine and mainly use the spike glycoprotein S, and its fragments, such as S1, S2, receptor binding domain (RBD), and nucleocapsid protein as a prime target antigen[40]. Novavax, a North-American biotech company, has developed the COVID-19 subunit vaccine (NVX-CoV2373), which has already reached phase III clinical trials. The vaccine is made with the full-length SARS-CoV-2 spike (S) glycoprotein expressed in baculovirus *Spodoptera frugiperda* (Sf9) insect cells and stabilized in the prefusion conformation by two proline substitutions introduced at the S1/S2 furin cleavage site. NVX-CoV2373 with a Matrix-M saponin-based adjuvant induced a Th1 dominant B- and T-cell response with high titer anti-spike IgG able to block hACE2 binding and to neutralize infection in mice and non-human primate models. Moreover, immunized macaques showed protection against pulmonary disease after the SARS-CoV-2 challenge, and no evidence of vaccine-associated enhanced respiratory disease was present[41]. NVX-CoV2327 (two-dose regimens of 5 μg and 25 μg of plus the Matrix-M1 adjuvant) administered to healthy adults (18 to 59 years of age) showed acceptable safety and induced high immune responses, with levels of neutralizing antibodies that closely correlated with anti-spike IgG[42]. Anhui Zhifei Longcom Biopharmaceutical (China) has proposed a protein subunit vaccine comprising a tandem repeat single-chain dimer (sc-dimer) of the SARS-CoV-2 RBD to be produced (high yields, g/L level) in a Chinese hamster ovary (CHO) cell system[43].

Clover Biopharmaceuticals (Chengdu Shi, China) has employed a technology named 'Trimer-Tag' to produce a eukaryotic cell-derived trimeric subunit spike protein subunit vaccine. Immunization of S-Trimer with either AS03 (oil-in-water emulsion) or CpG 1018 (TLR9 agonist) with the addition of alum adjuvants generated high-levels of neutralizing antibodies and a Th1 immune response in animal models, and rhesus macaques were protected from SARS-CoV-2 challenge[44]. This vaccine is administered in conjunction with a CpG/Alum adjuvant and is actually being tested in phase 1 clinical trial (NCT04405908). Considering stabilization of the fusion proteins of enveloped viruses an important target for the creation of next generation vaccines, the University of Queensland (Brisbane, Australia) has proposed molecular clamp stabilized spike protein with MF59 adjuvant where the S protein has been "locked" in a prefusion conformation into the correct 3-dimensional shape. Therefore, this approach should allow the production of antibodies against a wide choice of conformational epitopes that are displayed on the virion surface[45]. Sanofi Pasteur (Lyon, France) has used a recombinant DNA *in vitro* platform (baculovirus) to produce a spike protein antigen in large quantities. The University of Pittsburgh (Pittsburgh, PA, United States) taking advantage of their previous experience on SARS and MERS vaccine development, has proposed a microneedle array (MNA) patch to deliver the antigen into the skin (PittCoVacc). The patch is applied as a band-aid, and the needles, which are entirely composed of sugar, enter the skin to allow diffusion of the antigen within tissues[42]. Other protein-based candidates in development are from Instituto Finlay de Vacunas (La Habana, Cuba) (two candidates: i) RBD plus adjuvant and ii) recombinant RBD produced in CHO-cell and chemically conjugated to tetanus toxoid), Medigen Vaccine Biologics Corporation (Taipei, Taiwan) (S-2P proteins plus CpG 1018), Vaxine Pty Ltd. (Adelaide, Australia) (Recombinant spike protein with AdvaxTM adjuvant), COVAXX/United Biomedical Inc. Asia (Long Island, NY, United States) (Multitope peptide-based S1 – RBD - protein vaccine), Generex (Miramar, FL, United States)/EpiVax (Providence, RI, United States) (using an innovative Ligand Epitope Antigen Presentation System to enhance the potency of peptide vaccines), Baiya Phytopharm (plant-based subunit RBD plus adjuvant), Quadram Institute Biosciences (Norwich, United Kingdom) (Bacterial Outer Membrane Vesicles based vaccine) and others (for a complete list see Table III).

Nucleic Acid Platform (DNA and mRNA)

Nucleic acid vaccines (based on DNA or RNA) have surged huge interest in the last decades for their potential in a pandemic crisis considering the low-cost and rapid development. They exploit either plasmid DNA or RNA encoding a specific target antigen that is delivered to the subject to vaccinate and is taken up by the cells where the antigenic sequence is expressed. The key point is that the human cells of the vaccinated subject process the viral protein in a very different way from what happens in a heterologous system for the production of subunits vaccines; therefore, the most important theoretical benefits of employing a nucleic acid into a vaccine seems to be the fact that the viral protein is processed by the same cellular apparatus that the virus would have directed the host to do in a real infection. This can be considered a close mimic of natural infection without the pathogenic damage[46]. Hypothetically, a single industrial plan may be able to produce any required nucleic acid vaccine and also scale up the production to satisfy a pandemic level demand. Vaccine platforms using recombinant DNA technology have a long history, whereas mRNA-based vaccines have only recently emerged as a novel option. As depicted in Table IV, there are 6 mRNA-based COVID-19 vaccines and 5 DNA-based COVID-19 vaccines in clinical trials, with 42 such vaccines (21 RNA-based and 21 DNA-based vaccines) under preclinical development.

DNA vaccines are routinely constructed from plasmid DNA molecules that encode one or more antigens. The plasmids generally contain prokaryotic sequences to drive propagation in Escherichia coli, a mammalian expression cassette able to direct the expression of the inserted genes in the subject inoculated with the vaccine. Once delivered, the plasmid DNA vaccine is internalized by host cells at the immunization site or by migrating antigen-presenting cells (APCs), where in order to induce an adaptive immune response, the DNA must enter the cell nucleus[47]. Finally, the target gene is expressed and translated into a protein. The nucleic acids guide host cells' activities to synthesize the target sequence beyond the obstacles of proper protein folding, purification, solubility and incorrect glycosylation of proteins that are usually a consequence of recombinant protein synthesis[48]. The manufacture of DNA vaccines is, to some extent, straightforward, and

Table III. SARS-CoV-2 protein subunit candidate vaccines under development.

COVID-19 vaccine developer/manufactures	Vaccine platform	Type of candidate vaccine	Current stage of clinical evaluation (estimated study completion date) (NA - Not Available)
Anhui Zhifei Longcom Biopharmaceutical/Chinese Academy of Sciences (Beijing, China)	Protein subunit	Recombinant SARS-CoV-2 RBD-Dimer protein subunit vaccine	Phase 2: NCT04466085 (December 2021)
Kentucky Bioprocessing, Inc (Owensboro, KY, United States)	Protein subunit	RBD-based	Phase 1/2: NCT04473690 (February 2022)
Sanofi Pasteur (Lyon, France)/ GSK (Brentford, United Kingdom)	Protein subunit	S protein (baculovirus production)	Phase 1/2: NCT04537208 (October 2021)
Biological E Ltd (Telangana, India)	Protein subunit	Adjuvanted protein subunit (RBD)	Phase 1/2: CTRI/2020/11/029032 (NA)
Clover Biopharmaceuticals Inc./GSK/Dynavax	Protein subunit	Recombinant SARS-CoV-2 trimeric S protein subunit vaccine	Phase 1: NCT04405908 (March 2021)
Vaxine Pty Ltd (Adelaide, Australia)/Medytox (Seoul, Korea)	Protein subunit	Recombinant spike protein with Advax™ adjuvant	NCT04453852 (July 2021)
University of Queensland (Brisbane, Australia)/CSL (Melbourne, Australia)/Seqirus (United Kingdom)	Protein subunit	Recombinant SARS-COV-2 spike protein 'molecular clamp' plus MF59 adjuvant	NCT04495933 (September 2021)
Medigen Vaccine Biologics Corporation/NIAID (Bethesda, MD, United States/Dynavax	Protein subunit	S-2P protein + CpG 1018	NCT04487210 (June 2021)
Instituto Finlay de Vacunas (Cuba)	Protein subunit	rRBD produced in CHO-cell chemically conjugated to tetanus toxoid	IFV/COR/06
Instituto Finlay de Vacunas (Cuba)	Protein subunit	RBD + adjuvant	IFV/COR/04 (February 2021)
FBRI SRC VB VECTOR, Rospotrebnadzor, Koltsovo	Peptide	Peptide	NCT04527575 (October 2020)
West China Hospital, Sichuan University (Chengdu, China)	Protein subunit	RBD (baculovirus production expressed in Sf9 cells)	ChiCTR2000037518 (NA)
University Hospital Tuebingen (Tübingen, Germany)	Peptide	SARS-CoV-2 HLA-DR peptides	NCT04546841 (December 2021)
COVAXX/United Biomedical Inc.	Protein subunit	S1-RBD-protein	NCT04545749 (August 2021)
Ohio State University (Columbus, OH, United States)/Kazakh National Agrarian University (Kazakhstan)	Protein subunit	RBD protein delivered in mannose-conjugated chitosan nanoparticle	Preclinical
Kazakh National Agrarian University (Kazakhstan)	Protein subunit	Recombinant spike protein with Essai O/W 1849101 adjuvant	Preclinical

Table continued

Table III. ***(Continued).*** SARS-CoV-2 protein subunit candidate vaccines under development.

COVID-19 vaccine developer/manufactures	Vaccine platform	Type of candidate vaccine	Current stage of clinical evaluation (estimated study completion date) (NA - Not Available)
Neo7Logic (Gaithersburg, MD, United States)	Peptide	Peptides	Preclinical
Kazakh National Agrarian University/National Scientific Center for Especially Dangerous Infections (Kazakhstan)	Protein subunit	Recombinant spike protein with Essai O/W 1849101 adjuvant	Preclinical
Max-Planck-Institute of Colloids and Interfaces (Potsdam, Germany)	Protein subunit	Recombinant S protein	Preclinical
Farmacológicos Veterinarios SAC (FARVET SAC)/Universidad Peruana Cayetano Heredia (UPCH)	Protein subunit	RBD protein (baculovirus production) + FAR-Squalene adjuvant	Preclinical
Research Institute for Biological Safety Problems (Kazakhstan)	Protein subunit	Protein Subunit	Preclinical
Mynvax (Bangalore, India)	Protein subunit	RBD-protein	Preclinical
Izmir Biomedicine and Genome Center (Izmir, Turkey)	Protein subunit	Recombinant S protein	Preclinical
Bogazici University (Instanbul, Turkey)	Peptide	Peptide + novel adjuvant	Preclinical
University of Virginia Charlottesville, VA, United States)	Protein subunit	S subunit intranasal liposomal formulation with GLA/3M052 adjs.	Preclinical
Helix Biogen Consult, Ogbomoso & Trinity Immonoefficient Laboratory (Ogbomoso, Oyo State, Nigeria)	Protein subunit	S-Protein (Subunit) + adjuvant, *E. coli* based Expression	Preclinical
National Research Centre (Egypt)	Protein subunit	Protein Subunit S, N, M&S1 protein	Preclinical
University of San Martin and CONICET (Argentina)	Protein subunit	Protein Subunit	Preclinical
Chulalongkorn University/GPO (Thailand)	Protein subunit	RBD protein fused with Fc of IgG + Adj.	Preclinical
AdaptVac (PREVENT-nCoV consortium)	Protein subunit	Capsid-like Particle	Preclinical
ExpreS2ion (Hørsholm, Denmark)	Protein subunit	Drosophila S2 insect cell expression system VLPs	Preclinical
IMV Inc (Dartmouth, Canada)	Peptide	Peptide antigens formulated in LNP	Preclinical
WRAIR/USAMRIID (MD, United States)	Protein subunit	S protein	Preclinical
National Institute of Infectious Disease/Shionogi/UMN Pharma (Japan)	Protein subunit	S protein + adjuvant	Preclinical

Table continued

Table III. ***(Continued).*** SARS-CoV-2 protein subunit candidate vaccines under development.

COVID-19 vaccine developer/manufactures	Vaccine platform	Type of candidate vaccine	Current stage of clinical evaluation (estimated study completion date) (NA - Not Available)
Osaka University/BIKEN/National Institutes of Biomedical Innovation (Japan)	Protein subunit	VLP-recombinant protein + adjuvant	Preclinical
University of Pittsburgh Pittsburgh, PA, United States)	Protein subunit	microneedle arrays S1 subunit	Preclinical
Vaxil Bio (Ontario, Canada)	Peptide	Peptide	Preclinical
Flow Pharma Inc (Palo Alto, CA, United States)	Peptide	Peptide	Preclinical
AJ Vaccines (Copenhagen, Denmark)	Protein subunit	S protein	Preclinical
Generex (Miramar, Florida, United States)/EpiVax (Providence, RI, United States)	Peptide	Ii-Key peptide	Preclinical
EpiVax/University of Georgia (Athens, GA, United States)	Protein subunit	S protein	Preclinical
EpiVax (Providence, RI, United States)	Protein subunit	Protein Subunit EPV-CoV-19	Preclinical
Heat Biologics (Morrisville, NC, United States)/University of Miami (Miami, FL, United States)	Protein subunit	gp-96 backbone	Preclinical
FBRI SRC VB VECTOR, Rospotrebnadzor, Koltsovo	Protein subunit	Subunit vaccine	Preclinical
Baylor College of Medicine (Houston, TX, United States)	Protein subunit	S1 or RBD protein	Preclinical
iBio/CC-Pharming (Newark, DE, United States)	Protein subunit	Subunit protein, plant produced	Preclinical
Saint-Petersburg Scientific Research Institute of Vaccines and Serums (Petersburg, Russia)	Protein subunit	Recombinant protein, nanoparticles (based on S-protein and other epitopes)	Preclinical
Innovax/Xiamen Univ./GSK	Protein subunit	COVID-19 XWG-03 truncated S (spike) proteins	Preclinical
VIDO-InterVac, University of Saskatchewan	Peptide	Adjuvanted microsphere peptide	Preclinical
OncoGen (Selangor, Malaysia)	Peptide	Synthetic Long Peptide Vaccine candidate for S and M proteins	Preclinical
MIGAL Galilee Research Institute (Kiryat Shmona, Israel)	Protein subunit	Oral *E. coli*-based protein expression system of S and N proteins	Preclinical
LakePharma, Inc. (San Carlos, CA, United States)	Protein subunit	Nanoparticle vaccine	Preclinical
Baiya Phytopharm/Chula Vaccine Research Center (Thailand)	Protein subunit	Plant-based subunit (RBD-Fc + adjuvant)	Preclinical
Quadram Institute Biosciences (Norwich, United Kingdom)	Protein subunit	OMV-based vaccine	Preclinical

Table continued

Table III. ***(Continued).*** SARS-CoV-2 protein subunit candidate vaccines under development.

COVID-19 vaccine developer/manufactures	Vaccine platform	Type of candidate vaccine	Current stage of clinical evaluation (estimated study completion date) (NA - Not Available)
BiOMViS Srl/University of Trento, Trento (Italy)	Protein subunit	OMV-based vaccine	Preclinical
Lomonosov Moscow State University (Moscow, Russia)	Protein subunit	Structurally modified spherical particles of the tobacco mosaic virus (TMV)	Preclinical
University of Alberta (Edmonton, Canada)	Protein subunit	Spike-based	Preclinical
AnyGo Technology (Shenzhen, China)	Protein subunit	Recombinant S1-Fc fusion protein	Preclinical
Yisheng Biopharma (Beijing, China)	Protein subunit	Recombinant protein	Preclinical
Vabiotech (Hanoi, Vietnam)	Protein subunit	Recombinant S protein in IC-BEVS	Preclinical
Applied Biotechnology Institute, Inc. (San Luis Obispo, CA, United States)	Protein subunit	Orally delivered, heat stable subunit	Preclinical
Axon Neuroscience SE (Slovakia)	Peptide	Peptides derived from Spike protein	Preclinical
MOGAM Institute for Biomedical Research, GC Pharma (Yongin, South Korea)	Protein subunit	Protein Subunit	Preclinical
Neovii (Rapperswil, Switzerland)/Tel Aviv University (Israel)	Protein subunit	RBD-based	Preclinical
Intravacc (Utrecht, Netherlands)/Epivax	Protein subunit	Outer Membrane Vesicle (OMV)-subunit	Preclinical
Intravacc/Epivax	Protein subunit	Outer Membrane Vesicle (OMV)-peptide	Preclinical
ImmunoPrecise (Victoria, Canada)/LiteVax BV (Ophemert, Netherlands)	Peptide	Spike-based (epitope screening)	Preclinical

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the product is considered fairly stable compared to mRNA-based vaccines. Plasmid DNA technology allows simple production of large quantities of vaccines with the possibility of conferring long-term immunity. An advantage of this kind of vaccine is the stimulation of both humoral and cellular immunity[49]. However, the disadvantages looming over DNA vaccines are due to their limitation of processing protein immunogen, the need to cross the nuclear membrane to become translated, and the risk of vector chromosomal integration and mutations in the host genome. Accordingly, for the optimal function of DNA vaccines, formulation and delivery strategies are of paramount importance[50].

Examples of DNA-based vaccines include the one proposed by Inovio (Plymouth Meeting, PA, United States), Genexine or Genexine. Inovio Pharmaceuticals developed a DNA vaccine candidate termed INO-4800 that has entered clinical phase 1/2 trial (NCT04336410 and NCT04447781), following the footsteps of the promising results obtained with INO-4700, a MERS-CoV candidate vaccine, which was able to produce a strong antibody and T cell responses and was well-tolerated[51]. Using Inovio's propri-

Table IV. SARS-CoV-2 nucleic acid candidate vaccines under development.

COVID-19 vaccine developer/manufactures	Vaccine platform	Type of candidate vaccine	Current stage of clinical evaluation (estimated study completion date) (NA - Not Available)
Curevac	RNA	mRNA	Phase 2: NCT04515147 (November 2021)
Inovio Pharmaceuticals/ International Vaccine Institute	DNA	DNA plasmid vaccine with electroporation using CELLECTRA® 2000 device	Phase 1/2: NCT04447781 (February 2022) Phase 1/2: NCT04336410 (July 2021)
Osaka University/AnGes/Takara Bio (Japan)	DNA	DNA plasmid vaccine + adjuvant	Phase 1/2: NCT04463472 (July 2021)
Cadila Healthcare Limited (Ahmedabad, India)	DNA	DNA plasmid vaccine	Phase 1/2: CTRI/2020/07/026352 (NA)
Genexine Consortium	DNA	DNA Vaccine (GX-19)	Phase 1/2: NCT04445389 (June 2022)
Arcturus/Duke-NUS (Singapore, Malaysia)	RNA	saRNA	Phase 1/2: NCT04480957 (January 2021)
Symvivo	DNA	bacTRL-Spike	Phase 1: NCT04334980
Imperial College London (London, United Kingdom)	RNA	LNP-nCoVsaRNA	Phase 1: ISRCTN17072692 (Nd)
People's Liberation Army (PLA) Academy of Military Sciences (Beijing, China)/Walvax Biotech. (China)	RNA	mRNA	Phase 1: ChiCTR2000034112 (Nd)
Globe Biotech Limited (Bangladesh)	DNA	DNA plasmid vaccine	Preclinical
National institute of Chemistry (Slovenia)	DNA	Plasmid DNA, nanostructured RBD	Preclinical
DIOSynVax Ltd/University of Cambridge (Cambridge, United Kingdom)	DNA	DNA, engineered vaccine inserts compatible with multiple delivery systems	Preclinical
Ege University (İzmir, Turkey)	DNA	DNA vaccine	Preclinical
Scancell/University of Nottingham/Nottingham Trent University (Nottingham, United Kingdom)	DNA	DNA plasmid vaccine RBD&N	Preclinical
National Research Centre (Egypt)	DNA	DNA plasmid vaccine S, S1, S2, RBD & N	Preclinical
Karolinska Institute (Sweden)/ Cobra Biologics (OPENCORONA Project) (Newcastle, United Kingdom)	DNA	DNA with electroporation	Preclinical
Chula Vaccine Research Center	DNA	DNA with electroporation	Preclinical
Takis (Rome, Italy)/Applied DNA Sciences (Stony Brook, New York, United States)/ Evvivax (Rome, Italy)	DNA	DNA	Preclinical
Immunomic Therapeutics, Inc. (Rockville, MD, United States)/ EpiVax, Inc./PharmaJet, (Golden, CO, United States)	DNA	Plasmid DNA, Needle-Free Delivery	Preclinical
BioNet Asia (Bangkok, Thailandia)	DNA	DNA vaccine	Preclinical
Entos Pharmaceuticals (Edmonton, Canada)	DNA	DNA vaccine	Preclinical

Table continued

Table IV. ***(Continued).*** SARS-CoV-2 nucleic acid candidate vaccines under development.

COVID-19 vaccine developer/manufactures	Vaccine platform	Type of candidate vaccine	Current stage of clinical evaluation (estimated study completion date) (NA - Not Available)
Globe Biotech Ltd (Dhaka, Bangladesh)	RNA	D614G variant LNP-encapsulated mRNA	Preclinical
Infectious Disease Research Institute (Washington, United States)/Amyris, Inc. (Emeryville, CA, United States)	RNA	saRNA formulated in a NLC	Preclinical
Max-Planck-Institute of Colloids and Interfaces	RNA	LNP-encapsulated mRNA encoding S	Preclinical
Gennova (Maharashtra, India)	RNA	Self-amplifying RNA	Preclinical
Selcuk University (Konya, Turkey)	RNA	mRNA	Preclinical
Translate Bio (Lexington, MA, United States)/Sanofi Pasteur (Lyon, France)	RNA	LNP-mRNA	Preclinical
CanSino Biologics (China)/ Precision NanoSystems (Vancouver, Canada)	RNA	LNP-mRNA	Preclinical
Fudan University/Shanghai JiaoTong University/RNACure Biopharma (Shanghai, China)	RNA	LNP-encapsulated mRNA encoding RBD	Preclinical
Centro Nacional Biotecnología (CNB-CSIC) (Spain)	RNA	Replicating Defective SARS-CoV-2 derived RNAs	Preclinical
University of Tokyo/ Daiichi-Sankyo (Tokyo, Japan)	RNA	LNP-encapsulated mRNA	Preclinical
BIOCAD (Russia)	RNA	Liposome-encapsulated mRNA	Preclinical
RNAimmune, Inc. (MD, United States)	RNA	Several mRNA candidates	Preclinical
FBRI SRC VB VECTOR, Rospotrebnadzor, Koltsovo	RNA	mRNA	Preclinical
China CDC/Tongji University/ Stermina (China)	RNA	mRNA	Preclinical
Chula Vaccine Research Center/University of Pennsylvania, PA, United States	RNA	LNP-mRNA	Preclinical
eTheRNA (Niel, Belgium)	RNA	mRNA in an intranasal delivery system	Preclinical
Greenlight Biosciences (Medford, MA, United States)	RNA	mRNA	Preclinical
IDIBAPS-Hospital Clinic (Spain)	RNA	mRNA	Preclinical

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etary *in silico* Gene Optimization Algorithm an optimized DNA plasmid was designed to improve expression and immunogenicity of the DNA vaccine. This DNA vaccine is administered intradermally by CELLECTRA® electroporation. *in vitro* studies showed proper expression of the S protein and its RNA after transfection with the plasmid constructs of cell lines, and in *in vivo* animal models, humoral and T cell responses were observed[52], so initial results were highly suggestive

of the immunogenicity of Inovio's COVID-19 vaccine candidate. Symvivo Corporation (Burnaby, Canada) is conducting a phase 1 clinical trial to analyze the safety and immunogenicity profiles of their bacTRL-spike vaccine against SARS-CoV-2 (NCT04334980). This vaccine is constituted by *Bifidobacterium longum* engineered to deliver synthetic DNA encoding the spike protein from SARS-CoV-2 contained in a plasmid vector. The vaccine is orally administered, and the gut colonization by *B. longum* should provide continuous delivery and expression of SARS-CoV-2S protein encoding plasmids (Symvivo, Covid-19 Program Vision, 2019). A mucosal, systemic humoral, and cell-mediated immune response is foreseen as a result of the translation of this plasmid within the gastrointestinal lymphoid tissues.

Biotech firm Genexine Inc. (Seongnam, South Korea) has launched a human clinical trial of their COVID-19 vaccine constituted by a synthetic soluble spike DNA-based candidate named GX-19. The ectodomain of the S gene has been codon optimized for increased antigen expression in mammalian cells and subcloned into the plasmid pGX27 vector. Preliminary studies have shown that electroporation-enhanced GX-19 induced robust antibody and T cell responses. Furthermore, vaccination of GX-19 was shown to confer effective protection against SARS-CoV-2 challenge at 10 weeks following the last vaccination in immunized non-human primates supporting further expectations for GX-19 as a vaccine candidate against SARS- CoV-2 in ongoing human clinical trials[53].

Messenger RNA (mRNA) is an intermediate carrier of genetic information acting as a template for protein synthesis on cellular translating ribosomes in the vaccinated organism. mRNA vaccines are able to induce strong cellular and humoral immune responses. These vaccines are relatively safe and effective because of their activity as transient carriers of messages that are unable to interact with the host genome. Moreover, mRNA vaccines do not require the use of the whole virus[54]. There are two types of mRNA vaccines platform: non-replicating mRNA and self-amplifying mRNA (saRNA). Non-replicating mRNA vaccines contain the sequence of the selected target antigen flanked by 5′ and 3′ untranslated regions (UTRs). They typically have a 5' cap essential for mRNA to associate with the eukaryotic translation complex; therefore, mRNA triggers rapid and immediate antigen expression in the cytoplasm without the need for crossing the nuclear membrane. Self-amplifying RNA vaccines are most commonly based on alphavirus-derived RNA replicons modified to encode the antigen of choice instead of viral structural proteins. The replicon maintains the ability to encodes alphavirus non-structural proteins (nsP1-4) and a sub genomic promoter; therefore, an RNA-dependent RNA polymerase (RdRP) is produced and used to transcribes more copies of the vaccine in the transfected cell. Consequently, saRNA vaccines express protein at higher levels and persist longer than non-replicating RNA. mRNA vaccine is a promising alternative to traditional vaccine approaches due to their safety, potency, quick vaccine-development time, and low-cost production[55]. The main advantages of mRNA are the ability to generate a rapid antigen expression in the cell cytoplasm without the burden of the necessity of crossing the nuclear membrane to become active. Moreover, the risk of infection or insertional mutagenesis is near zero since mRNA expression is rapid and transient as mRNA are safely degraded by normal cellular processes. This involves concerns related to mRNA vaccine instability and inefficient *in vivo* delivery[49].

As soon as March 2020, a first candidate vaccine (mRNA-1273) was announced by the NIAID (Bethesda, MD, United States) and Moderna (Cambridge, MA, United States) to be evaluated in a clinical trial (NCT04283461) on the basis of the prior preclinical and clinical data gathered with studies developed by Moderna on CMV, Zika virus, H7N9, hMPV, and RSV[56]. The vaccine includes synthetic mRNA coding for the full-length, pre-fusion stabilized spike protein (S) of SARS-CoV-2 that has been encapsulated in lipid nanoparticle (LNP) composed of ionizable lipid, distearoyl phosphatidylcholine, cholesterol, and polyethylene glycol lipid. mRNA-1273 has been shown to induce potent neutralizing antibody responses and to both wild-type (D614) and D614G mutant SARS-CoV-2[57]. The delivered mRNA is also able to protect against live virus challenge in mice without evidence of immunopathology[58]. Also studies on non-human primates, receiving 10 or 100 μg of mRNA-1273, showed satisfactory antibody and T-cell responses demonstrating the induction of a robust SARS-CoV-2 neutralizing activity, rapid protection in the upper and lower airways without pathologic effect in the lung[59]. In clinical trials, the results presented so far describe that using 100-μg dose is possible to induce higher binding-antibody and neutralizing-antibody titers than the 25-μg dose, which lent credibility to the strategy to use the 100-μg dose in the ongoing

phase 3 vaccine trial (NCT04470427). Moreover, no trial-limiting safety concerns were identified so far, with adverse events associated with the mRNA-1273 vaccine being mainly mild or moderate[60].

Pfizer (New York, NY, United States), in collaboration with BioNTech (Mainz, Germany), is developing two vaccines named BNT162b1 and BNT162b2. Like mRNA-1273 from Moderna, BNT162b is a lipid nanoparticle encapsulating mRNA encoding for SARS-CoV-2 antigens. One of these candidates, BNT162b1, encodes the SARS-CoV-2 RBD trimerized by the addition of a T4 fibritin domain intended to improve immunogenic effect in view of the multivalent display, while the other, BNT162b2, has full- length spike envelope protein, but modified by the insertion of two proline to block the glycoprotein in its prefusion conformation to allow a stronger resemblance to the intact virus particles[61,62]. BNT162b1, at multiple dose levels, has been assessed in healthy adults from 18 to 55 years of age, indicating that dose levels of BNT162b1 that elicited an acceptable level of reactogenicity also efficiently elicited antibody titers similar to SARS-CoV-2 human convalescent patients. These antibodies were also found to be broadly neutralizing using several SARS-CoV-2 pseudoviruses based on circulating strains. A CD4+ and CD8+ responses were also shown[63,64]. Both vaccine candidates have also been tested in a further clinical trial to assess the safety and immunogenicity of three dose levels of BNT162b1 and BNT162b2 and showed good immunogenicity[65]. BNT162b1 was associated with a higher incidence of systemic reactions compared to BNT162b2, especially in older adults, supporting the selection of BNT162b2 for the ongoing phase 3 clinical trial (NCT04368728).

CureVac AG (Tübingen, Germany) has developed a vaccine candidate based on the spike glycoprotein of SARS-CoV-2, which includes a 5′ cap structure, a GC-enriched open reading frame (ORF), 3′ UTR, polyA tail and does not use chemically modified nucleosides. The lipid nanoparticles are made of ionizable amino lipid, phospholipid, cholesterol, and a PEGylated lipid[66]. This vaccine has been evaluated in various animal models, and the obtained data indicated that the induced neutralizing antibody titers were comparable to sera from patients who recovered from COVID-19. The vaccine was generally well tolerated across the tested dose range of 2-12 μg.

Arcturus (San Diego, CA, United States), with the collaboration of Duke University and the National University of Singapore, proposes a self-replicating RNA (saRNA) construct encoding an alphavirus-based replicon and the SARS-CoV-2 full length spike glycoprotein. Translation of the replicon generates a replicase complex able to amplify and extend the expression of the antigenic protein. The saRNA is encapsulated with a lipid-enabled and unlocked nucleic acid modified RNA (LUNAR), a safe ionizable and biodegradable LNP platform for effective LNP mRNA delivery[67]. Mice vaccination showed vigorous neutralizing antibody responses, and cell-mediated immunity produced a strong viral antigen specific CD8+ T lymphocyte response. Following wild-type SARS-CoV-2 challenge, a single vaccine inoculation of 2 μg or 10 μg doses fully protected human ACE2 transgenic mice[68].

A similar approach has also been pursued by Imperial College London, which used a plasmid vector to synthesize a self-amplifying RNA (saRNA) replicon based on alphavirus genome where the viral structural proteins have been replaced by the surface glycoprotein S of SARS-CoV-2 sequence modified by proline mutations to stabilize the protein in its pre-fusion state[69]. The saRNA LNP vaccine elicited robust antibody and cellular responses, and human trials of the vaccine started in June 2020 to assess the safety of the vaccine and its effects on the immune system.

Viral vectors

Viral vector-based vaccines are live viruses (the vector itself) engineered to express heterologous antigens by carrying a foreign DNA sequence. Their main property is the combination of the robust immunogenicity of live attenuated vaccines and the safety of subunit vaccines since only one or few antigenic proteins are present. Their similarity to live attenuated strains favor a proper immunological response by eliciting both cell-mediated and humoral immunity *in vivo*. Several viral vectors have been exploited for vaccine development, and the most common include vaccinia virus, modified vaccinia virus Ankara (MVA), adenovirus (Ad), adeno-associated virus (AAV), retrovirus/lentivirus, alphavirus, herpes virus, Newcastle disease virus, poxvirus, and many others. These recombinant viral-vectored vaccines can be built on either a replication-deficient viral backbone or an attenuated replication-competent viral backbone where the latter is engineered through recombinant DNA technology to present antigens derived from the target pathogen within a different replicating virus[70]. Replication-deficient

viral vectors generally lack early genes essential for the reproduction of the virus; therefore, they are only able to deliver the antigen gene within host cells as Trojan horses without replicating in the vaccinated individual[60]. Usual viral vectors employed in vaccine developments are adenoviruses (Ad) and modified vaccinia virus Ankara (MVA). They are very common (at least 50 human subtypes available) and account for a big part of the methodology, with Ad serotype 5 (Ad5) showing to be a stable, non-replicating virus used in different vaccine platforms. Some difficulties arise from the preexisting immunity against human Ad5 being quite widespread in the population, therefore interfering with its broad use for novel vaccine development[71]. Some have opted for chimpanzee adenovirus (ChAdOx1) to find an alternative to the human Ad vector due to its lack of preexisting immunity in humans, and results seem to be very promising[60,72]. Several vectors have been developed on replication-competent attenuated strains such as measles derived from the original 1954 vaccine strain, the live-attenuated yellow fever 17D (YF17D) vaccine or Newcastle disease virus (NDV)[60].

Currently, adenoviruses are the most commonly used vectors for building replication-deficient viral vectors for vaccination purposes. Recombinant Ad vectors are widely used because of their high transduction efficiency, high level of transgene expression, and a broad range of viral tropism. Four different companies have already reached phase 3 clinical trials; their vectors are based on Ad5 and Ad26 in three cases (CanSino Biological Inc., Gamaleya Research Institute and Janssen Pharmaceuticals Companies) while the remaining proposed vaccine from the partnership between the Jenner Institute of Oxford University (Oxford, United Kingdom) and AstraZeneca (Cambridge, United Kingdom) is based on a chimpanzee adenovirus ChAdOx1 (Table I).

Ad5-nCoV from CanSino Biologics (China) is a recombinant, replication-defective adenovirus type-5 vector (Ad5) expressing the recombinant spike protein of SARS-CoV-2. The sequences of the whole gene coding for the S protein with the plasminogen activator signal peptide gene have been codon-optimized and cloned into the Ad5 virus vector with deleted E1 and E2 genes. In an open-label, non-randomized, phase 1 clinical trial, the Ad5 vectored vaccine against SARS-CoV-2 has shown to be well tolerated and immunogenic in healthy adults. A single vaccine dose induced rapid, specific T-cell and humoral responses within 14 days and specific humoral responses peaked at day 28 post-vaccination[73]. The phase 1 trial has been followed by the phase 2 trial, where the candidate vaccine has confirmed a good safety profile, with only mild, transient adverse events related to vaccination and no serious adverse events. Moreover, the administration of 5×10^{10} viral particles is safe and induced significant immune responses in the majority of recipients after a single immunization, to warrant the move into international multicenter, randomized, double-blind, controlled phase 3 effectiveness trials, namely NCT04540419 expected to end in July 2021 and NCT04526990 expected to end in January 2022, to further evaluate the efficacy of the vaccine. Janssen Vaccines (Beerse, Belgium) has opted for the use of a different adenovirus type, namely Ad26, to use as a basis for its candidate vaccine, based on the long experience in the field against other viral diseases. A replication-incompetent Ad26 vector with deletions in E1/E3 structural proteins was engineered using the AdVac system[74], using a single plasmid method with the Ad26 vector genome including a transgene expression cassette. Within the expression cassette, the codon-optimized SARS-COV-2 Spike gene has been inserted in the E1 position under the control of the HCMV promoter and the SV-40 polyadenylation sequence. The candidate vaccine Ad26.COV2.S elicited potent neutralizing humoral immunity and cellular immunity in mice[74] and has proved to provide robust protection against severe clinical disease after high-dose SARS-CoV-2 infection in hamsters[75]. The efficiency of the Ad26.COV2.S vaccine in eliciting protective immunity against SARS-CoV-2 infection was successfully demonstrated in a non-human primate challenge model where vaccinated animals developed humoral and cellular immune responses. After vaccine inoculation, animals were challenged with SARS-CoV-2, and a net decrease of median viral loads in bronchoalveolar lavage and nasal mucosa was observed. Vaccine-elicited neutralizing antibody titers are well correlated with the detected protective efficacy confirming protection against SARS-CoV-2 in non-human primates[76,77]. The preclinical data supported the Phase 1/2 randomized, double-blinded, placebo-controlled clinical study to assess the safety, reactogenicity and immunogenicity of Ad26.COV2.S. The study indicated that a single dose of Ad26.COV2.S ($5x10^{10}$ viral particles or $1x10^{11}$ viral particles, is safe, well-tolerated, and high-

ly immunogenic[78]. Phase 3 clinical trial studies (NCT04505722 and NCT04614948) are ongoing.

One other non-replicating vectored vaccine that has abundantly debated on mass media is from the Gamaleya Research Institute (Moscow, Russia), which was named Sputnik V. This vaccine combines two distinct adenovirus vectors, namely Ad26 and Ad5, both carrying the gene for the full-length SARS-CoV-2 spike glycoprotein (rAd26-S and rAd5-S). Thus, the use of a heterologous prime-boost immunization, when rAd26-S is injected in the priming phase, and rAd5-S is after used for boosting, is analyzed as a different approach to elicit a robust immune response to SARS-CoV-2 and to reduce the immune response that is possibly mounted against the components of the viral vector. Preclinical analysis of the vaccine (though unpublished) claimed evident humoral and cellular immune responses in non-human primates, which were protected from SARS-CoV-2 infection. Immunosuppressed hamsters inoculated with the vaccine were protected with a success of 100% in a lethal model of SARS-CoV-2 challenge and antibody-dependent enhancement of infection was not reported. Safety and immunogenicity of two formulations (frozen and lyophilized) of this vaccine have been analyzed in NCT04436471, NCT04437875, and NCT04587219 phase 1 and 2 clinical trials in healthy adult volunteers. Trial participants were injected with a prime-boost vaccination consisting of a single dose of intramuscular rAd26-S on day 0 and a subsequent dose of intramuscular rAd5-S on day 21. The vaccine produced immune responses that showed to be adequate and comprising both the humoral and cellular arms in healthy adults, besides being well tolerated. Antibodies against the SARS-CoV-2 spike and neutralizing antibodies considerably increased at day 14 and kept increasing throughout the observation period. Specific T-cell responses peaked on day 28 after vaccination. The reported adverse events were considered mild (pain at the injection site, hyperthermia, headache, asthenia, and muscle and joint pain) and also typical for vaccines based on recombinant viral vectors[79].

Considering that the use of Ad5 as a vector has the drawback of the presence of a preexisting immunity from natural exposure (adenoviruses are frequent causes of common colds) to Ad5 can depress cellular immune responses to any heterologous antigen introduced in the vector, Oxford University, and AstraZeneca have developed a recombinant adenovirus vaccine using codon optimized S glycoprotein which has been synthesized with the tissue plasminogen activator (tPA) leader sequence at its 5' end. The vector genome was constructed by the methodology of bacterial artificial chromosomes by inserting the SARS-CoV-2 S gene into the E1 locus of chimpanzee adenovirus (ChAdOx1) genome. Humans have low seroprevalence for ChAd, therefore low likelihood to promote any immunogenicity to the vector as a consequence of vaccination[80]. The development of ChAdOx1-based vaccine, named AZD1222, is based on the encouraging results from human studies with ChAdOx1-MERS vaccine[81,82]. In preclinical studies, the AZD1222 vaccine was shown to be immunogenic in mice and pigs. The experimental plan conceived one or two doses of AZD1222 in both animal models and the results showed a good immunization already with the single dose, but a booster immunization clearly enhanced antibody responses, especially in pigs, with a mighty increment in viral neutralization[83]. In rhesus macaques, vaccination with ChAdOx1 vectored SARS-CoV-2 vaccine induced a balanced humoral and cellular immune response of type-1 and type-2 T helper cells and managed to constitutively reduce viral loads in the bronchoalveolar lavage fluids and lower respiratory tract tissues. No signs of pneumonia could be detected after live virus challenge as well as no evidence of immune-enhanced disease was reported[84]. Finally, all data actually available from human clinical trials have shown an acceptable safety profile and homologous boosting increased antibody responses, that together with the results showing a proper induction of humoral and cellular immune responses warrant the expectation of the ongoing phase 3 clinical trials (NCT04540393, NCT04516746, and CTRI/2020/08/027170)[85].

Also, ReiThera Srl (Rome, Italy), a biotech company, in cooperation with the Lazzaro Spallanzani National Institute for Infectious Diseases (Istituto Nazionale per le Malattie Infettive – INMI) (Rome, Italy), is running a phase 1 clinical study on a proprietary replication-defective simian (gorilla) adenoviral vector (called GRAd) encoding the full-length coronavirus spike protein (GRAd-COV2). GRAd vector belongs to C adenovirus species that have been indicated as potent vaccine carriers; like other simian adenoviruses, GRAd has low seroprevalence in humans. Therefore, GRAd vaccine immunogenicity is not likely to be reduced by preexisting anti-human adenovirus antibodies. Preliminary results from the trial have shown that the candidate vaccine is well tolerat-

ed and induces an evident immune response in healthy subjects from 18 to 55 years of age.

Within non-replicating viral vectors, of interest seems to be the one from the Icahn School of Medicine at Mount Sinai (New York, United States) where a Newcastle disease virus (NDV) vector expressing the SARS-CoV-2 S protein has been constructed. NDV is an avian pathogen belonging to the family of Paramyxoviridae, generally not infectious for humans, which lack preexisting immunity toward this virus. The strain selected as a starting point for the vaccine construction is the LaSota (LS) strain that, besides being avirulent in birds, has already been utilized for applications for delivery of oncolytic agents and vaccines. NDV vectors were built up to express two forms of the SARS-CoV-2 glycoprotein, one with the full-length wild type protein (NDV_LS_S) and the other expressing a chimera composed of the ectodomain of S (deleted of the polybasic cleavage site at the interface between S1 and S2) attached on the cytoplasmic and transmembrane domain of the NDV fusion protein F (NDV_LS_S-F). Both constructs were properly displayed on the surface of NDV and injected in mice stimulated the production of high titers of binding and neutralizing antibodies, besides fully protecting mice from challenge with a SARS-CoV-2 mouse-adapted strain[86].

In the group of replication active viral vectors, we can describe the vaccine candidate developed by Beijing Wantai in association with academia and based on the established flu-based DelNS1 live attenuated influenza virus (LAIV) platform[87]. Deletion of NS1, a key virulence factor, renders safer the live attenuated influenza virus; the addition of the surface protein of SARS-CoV-2 should confer specificity and immunogenicity against the RBD of SARS-CoV-2. This candidate is now being analyzed in phase 2 clinical trials (ChiCTR2000039715) as an intranasal flu-based-RBD SARS-CoV-2 vaccine. The Pasteur Institute, Themis Bioscience GmbH, and the University of Pittsburgh Center for Vaccine Research (Pittsburgh, PA, United States) are developing a measles virus vectored vaccine able to expresses the SARS-CoV-2 S protein, and similarly are doing other companies like Zydus Cadila, which is developing a live attenuated recombinant measles virus vectored vaccine produced by reverse genetics and expressing codon-optimized proteins. A further vector extensively used as a basis for producing replicating-competent vectored vaccines is the vesicular stomatitis virus (VSV), which is under investigation by the Israel Institute for Biological Research with its phase 1/2 clinical trial NCT04608305 expected to end in June 2022, and by the IAVI and Merck association which is exploiting the experience gained for the production of the rVSV-based vaccine for Ebola Zaire and conducting a phase 1 clinical trial (NCT04569786) expected to be close to the end (December 2021) (Table V).

Virus-like particles and nanoparticles

Virus-like particles (VLP) vaccines are based on the consideration that heterologous expression of specific viral proteins is able to exploit the self-assembly properties of viral proteins to direct the spontaneous symmetric aggregation of particles with a strong structural similarity to the original viruses. Notably, VLPs are not infectious because they are empty shells lacking the viral genome but can mimic the morphology of the whole virus. However, the native conformation of the antigenic proteins is well preserved, and the molecular weight is well above the monomeric counterpart, which improves VLPs immunogenicity compared to free proteins. VLPs are generally produced by encoding the viral structural proteins and expressing them in heterologous systems, such as recombinant vaccinia virus, mammalian cells (293T, CHO), baculovirus, yeast expression systems and plant expression vectors[88]. In practice, VLPs-based vaccines are similar to whole inactivated virus vaccines, but the antigenic proteins may be better preserved and exposed to the immune system since no inactivation step is performed. Therefore, it is less likely to affect the immunogenicity of viral proteins due to surface epitopes destruction. Moreover, since no live virus is used in any steps for the production, VLPs are conveniently accomplished in low-containment manufacture settings[89]. VLP-based vaccines have been proved to produce strong cellular and humoral immune responses[90]. The best-known examples of VLP-based vaccines are Cervarix® (human papillomavirus) and Engerix® (hepatitis B virus) by GlaxoSmithKline (Brentford, United Kingdom), but also the corresponding vaccines produced by Merck and Co., Inc. (Kenilworth, NJ, United Kingdom) against hepatitis B virus (Recombivax HB®) and human papillomavirus (Gardasil®)[91]. SpyBiotech (Oxford, United Kingdom) and the Serum Institute of India (Pune, India) are conducting a phase ½ clinical trial (ACTRN12620000817943) with the investigational vaccine based on their proprietary technology

Table V. SARS-CoV-2 viral-vectored candidate vaccines under development.

COVID-19 vaccine developer/manufactures	Vaccine platform	Type of candidate vaccine	Current stage of clinical evaluation (estimated study completion date) (NA - Not Available)
ImmunityBio, Inc. & NantKwest Inc. (CA, United States)	Non-Replicating Viral Vector	hAd5 S+N 2nd Generation Human Adenovirus Type 5 Vector (hAd5) Spike (S) + Nucleocapsid (N)	Phase 1: NCT04591717 (November 2021)
ReiThera (Rome, Italy)/LEUKOCARE (Planegg, Germany)/ Univercells (Charleroi, Belgium)	Non-Replicating Viral Vector	Replication defective Simian Adenovirus (GRAd) encoding S	Phase 1: NCT04528641 (July 2021)
CanSino Biological Inc/Institute of Biotechnology, Academy of Military Medical Sciences, PLA of China	Non-Replicating Viral Vector	Ad5-nCoV	Phase 1: NCT04552366 (June 2021)
Vaxart (San Francisco, CA, United States	Non-Replicating Viral Vector	Recombinant Adenovirus Type 5 adjuvanted Oral vaccine *a. rAd-S b. rAd-S-N c. rAd-S1-N*	Phase 1: NCT04563702 (October 2021)
Ludwig-Maximilians, University of Munich	Non-Replicating Viral Vector	Adenovirus-based NasoVAX expressing SARS2-CoV S protein	Phase 1: NCT04569383 (May 2021)
University of Helsinki & University of Eastern Finland	Non-Replicating Viral Vector	Ad 5 vector for intranasal administration	Preclinical
Globe Biotech Limited (Bangladesh)	Non-Replicating Viral Vector	Adenovirus Type 5 Vector	Preclinical
ID Pharma (Tsukuba, Japan)	Non-Replicating Viral Vector	Sendai virus vector	Preclinical
Massachusetts Eye and Ear/ assachusetts General Hospital/ AveXis (Bannockburn, United Kingdom)	Non-Replicating Viral Vector	Adeno-associated virus vector (AAVCOVID)	Preclinical
Ankara University (Ankara, Turkey)	Non-Replicating Viral Vector	Adenovirus-based	Preclinical
GeoVax (Atlanta, GA, United States)/BravoVax (Wuhan, China)	Non-Replicating Viral Vector	Recombinant Modified Vaccinia Virus Ankara - encoded VLP	Preclinical
DZIF – German Center for Infection Research/IDT Biologika GmbH (Germany)	Non-Replicating Viral Vector	Recombinant Modified Vaccinia Virus Ankara – SARS-CoV-2 Spike encoded	Preclinical
IDIBAPS-Hospital Clinic (Spain)	Non-Replicating Viral Vector	Recombinant Modified Vaccinia Virus Ankara – SARS-CoV-2 Spike protein	Preclinical
Altimmune (Gaithersburg, MD, United States)	Non-Replicating Viral Vector	Adenovirus-based NasoVAX expressing SARS2-CoV spike protein	Preclinical
Greffex (Aurora, CO, United States)	Non-Replicating Viral Vector	Ad5 S (GREVAXTM platform)	Preclinical
Stabilitech Biopharma Ltd (United Kingdom)	Non-Replicating Viral Vector	Oral Ad5 S	Preclinical
Valo Therapeutics Ltd (Oxford, United Kingdom)	Non-Replicating Viral Vector	Adenovirus-based + HLA-matched peptides	Preclinical

Table continued

Table V. ***(Continued).*** SARS-CoV-2 viral-vectored candidate vaccines under development.

COVID-19 vaccine developer/manufactures	Vaccine platform	Type of candidate vaccine	Current stage of clinical evaluation (estimated study completion date) (NA - Not Available)
Centro Nacional Biotecnología (CNB-CSIC) (Spain)	Non-Replicating Viral Vector	MVA expressing structural proteins	Preclinical
Erciyes University (Kayseri, Turkey)	Non-Replicating Viral Vector	Adeno5-based	Preclinical
National Research Centre (Egypt)	Non-Replicating Viral Vector	Influenza A H1N1 vector	Preclinical
Icahn School of Medicine at Mount Sinai (New York, United States)	Non-Replicating Viral Vector	Newcastle disease virus expressing S	Preclinical
Beijing Wantai Biological Pharmacy/Xiamen University (China)	Replicating Viral Vector	Intranasal flu-based-RBD	Phase 2: ChiCTR2000039715 (NA)
Israel Institute for Biological Research (Israel)	Replicating Viral Vector	VSV-S	Phase 1/2: NCT04608305 (June 2022)
Merck Sharp & Dohme (Kenilworth, NJ, United States)/ IAVI (New York, United States)	Replicating Viral Vector	Replication-competent VSV delivering the SARS-CoV-2 Spike	Phase 1: NCT04569786 (December 2021)
Institute Pasteur (Paris, France)/ Themis/University of Pittsburg CVR/Merck Sharp & Dohme (United States)	Replicating Viral Vector	Measles-vector based	Phase 1: NCT04497298 (October 2021)
Farmacológicos Veterinarios SAC (FARVET SAC)/ Universidad Peruana SARS-CoV2 Preclinical Cayetano Heredia (UPCH)	Replicating Viral Vector	Intranasal Newcastle disease virus vector (rNDV-FARVET) expressing RBD	Preclinical
KU Leuven (Leuven, Belgium)	Replicating Viral Vector	YF17D Vector	Preclinical
Cadila Healthcare Limited (Ahmedabad, India)	Replicating Viral Vector	Measles Vector	Preclinical
FBRI SRC VB VECTOR, Rospotrebnadzor, Koltsovo	Replicating Viral Vector	Measles Vector	Preclinical
DZIF – German Center for Infection Research/CanVirex AG	Replicating Viral Vector	Measles Virus (S, N targets)	Preclinical
Tonix Pharma (Chatham, NJ, United States)/Southern Research (Birmingham, AL, United States)	Replicating Viral Vector	Horsepox vector expressing S protein	Preclinical
BiOCAD (Moscow, Russia) and IEM (Morrisville, NC, United States)	Replicating Viral Vector	Live viral vectored vaccine based on attenuated influenza virus backbone (intranasal)	Preclinical
FBRI SRC VB VECTOR, Rospotrebnadzor, Koltsovo	Replicating Viral Vector	Recombinant vaccine based on Influenza A virus, for the prevention of COVID-19 (intranasal)	Preclinical
Fundação Oswaldo Cruz and SARS-CoV2 Preclinical Instituto Buntantan (Brazil)	Replicating Viral Vector	Attenuated Influenza expressing an antigenic portion of the Spike protein	Preclinical
University of Hong Kong (China)	Replicating Viral Vector	Influenza vector expressing RBD	Preclinical

Table V. ***(Continued).*** SARS-CoV-2 viral-vectored candidate vaccines under development.

COVID-19 vaccine developer/manufactures	Vaccine platform	Type of candidate vaccine	Current stage of clinical evaluation (estimated study completion date) (NA - Not Available)
University of Manitoba (Winnipeg, Canada)	Replicating Viral Vector	Replicating VSV vector-based DC-targeting	Preclinical
FBRI SRC VB VECTOR, Rospotrebnadzor, Koltsovo	Replicating Viral Vector	VSV vector	Preclinical
University of Western Ontario (London, Canada)	Replicating Viral Vector	VSV-S	Preclinical
Aurobindo (Hyderabad, India)	Replicating Viral Vector	VSV-S	Preclinical
UW–Madison (Madison, WI, United States)/FluGen (WI, United States)/Bharat Biotech (Hyderabad, India)	Replicating Viral Vector	M2-deficient single replication (M2SR) influenza vector	Preclinical
Intravacc/Wageningen Bioveterinary Research (Lelystad, Netherlands)/Utrecht University (Netherlands)	Replicating Viral Vector	Newcastle disease virus vector (NDV-SARS- CoV-2/Spike)	Preclinical
The Lancaster University (United Kingdom)	Replicating Viral Vector	Avian paramyxovirus vector (APMV)	Preclinical

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named SpyCatcher/SpyTag platform. This technology directs antigens to be displayed onto VLPs with a covalent, irreversible bond in a high density, stable and specific orientated presentation of epitope[92]. The VLP vaccine candidate is based on the hepatitis B surface antigen (HBsAg) fused to the SpyCatcher protein to which the RBD of SARS-CoV-2 can be easily displayed on the VLP. Several companies are developing VLP based coronavirus vaccines, such as ARTES Biotechnology (Langenfeld, Germany) using the proprietary platform METAVAX® for the development of vaccines built on enveloped virus-like particle nanostructures (eVLPs) based on the duck Hepatitis B small surface antigen presenting domains of the spike protein of SARS-CoV 2, or the Imophoron Ltd applying their fine technology named ADDomer (VRAGNIAU C, 2019) where on a single VLP particle hundreds of epitopes (the parts mediating SARS-CoV-2 entry into cells) can be accommodated (Table VI).

Other

The use of a live virus to prevent infection is one of the most widely used methods and ancient vaccination approach. Absolutely, the first vaccine in history is the one produced against smallpox and used this approach based on the concept of a virus capable of infecting a species other than its original one but which retained a certain level of serological correlation. Therefore, the bovine virus inoculated in humans was capable of producing only a reduced local replication but not a systemic disease (attenuation of virulence). The conservation of different immunological epitopes allowed for an efficient immune response[93]. Historically, many other successful human vaccines have been based on empirically attenuated strains of the actual pathogen, with deletion or mutation of virulence genes through a serial passage into animal models or tissue cultures. At each "passage", the selected viruses improve in infecting and replicating in the selected cell cultures but more and more their ability to enter and replicate in their original human host is lost. Attenuation can also be reached by growing microorganisms in suboptimal conditions (i.e., low temperature passages) allowing the selection of less virulent strains[94]. This method selects viruses that replicate well in a colder environment but less well at body temperature, thus decreasing their pathogenicity in the human host, resulting in an attenua-

Table VI. SARS-CoV-2 virus-like particles candidate vaccines under development.

COVID-19 vaccine developer/manufactures	Vaccine platform	Type of candidate vaccine	Current stage of clinical evaluation (estimated study completion date) (NA - Not Available)
SpyBiotech (Oxford, United Kingdom)/Serum Institute of India (Pune, India)	VLP	RBD-HBsAg VLPs	Phase 1/2: ACTRN12620000817943
Medicago Inc. (Quebec City, Canada)	VLP	Plant-derived VLP adjuvanted with GSK or Dynavax adjs.	Phase: NCT04450004 (December 2021)
Shiraz University (Shiraz, Iran)	VLP	Plant derived VLP VLP	Preclinical
Tampere University (Tampere, Finland)	VLP	VLPs produced in BEVS	Preclinical
Max Planck Institute for Dynamics of Complex Technical Systems (Magdeburg, Germany)	VLP	VLP	Preclinical.
University of Manitoba (Winnipeg, Canada)	VLP	Virus-Like particle-based Dendritic cell (DC)-targeting vaccine	Preclinical
Bezmialem Vakif University (Instanbul, Turkey)	VLP	VLP	Preclinical
Middle East Technical University (Ankara, Turkey)	VLP	VLP	Preclinical
VBI Vaccines Inc. (MA, United States)	VLP	Envelope Virus-like particles (eVLP)	Preclinical
IrsiCaixa AIDS Research/ IRTA-CReSA/Barcelona Supercomputing Centre/Grifols (Barcelona, Spain)	VLP	S protein integrated in HIV VLPs	Preclinical
Mahidol University/The GPO/ Siriraj Hospital (Thailand)	VLP	VLP + adjuvant	Preclinical
Navarrabiomed, Oncoimmunology group (Pamplona, Spain)	VLP	Virus-like particles, lentivirus and baculovirus vehicles	Preclinical
Saiba GmbH (Pfäffikon, Switzeland)	VLP	Virus-like particle, based on RBD	Preclinical
Imophoron Ltd and Bristol University's Max Planck (United Kingdom)	VLP	ADDomer™ multiepitope dysplay	Preclinical
Doherty Institute (Melbourne, Australia)	VLP	Unknown	Preclinical
OSIVAX (Paris, France)	VLP	VLP	Preclinical
ARTES Biotechnology (Langenfeld, Germany)	VLP	eVLP	Preclinical
University of Sao Paulo (Brazil)		VLPs peptides/whole virus	Preclinical

Modified from: DRAFT landscape of COVID-19 candidates vaccine ⇒ https://www.who.int/publications/m/item/draft-landscape-of-covid-19-candidate.

tion of virulence while maintaining the ability to induce the immune response[95]. Examples of such vaccines that have revolutionized human history are for example: the measles vaccine, the bacillus Calmette-Guérin (BCG) vaccine for tuberculosis (TB), the rabies vaccine, the yellow fever vaccine and the vaccine against poliovirus, some of which are still in use nowadays. Some of these vaccines have demonstrated impressive efficacy and have provided the backbone for vaccine development for other pathogens using genetic engineering techniques. A successful example is the YF-17D strain of the yellow fever vaccine. It was developed by Max Theiler in the 1930s by attenuating a viral strain of yellow fever by more than 200 serial passages through monkeys and cultures of mouse and chicken embryo tissues. All yellow fever vaccines currently in use are derived from this attenuated strain[23]. Furthermore, YF-17D has been employed to obtain vaccines against two other flaviviruses, namely, the Japanese encephalitis and dengue, by substituting the genes encoding for the major antigenic proteins of yellow fever virus with their functional homologs of these other viruses[96,97]. Technological evolution for the production of attenuated vaccines has made it possible to rationally design attenuated virus strains by mutating or eliminating virulence genes by means of genome engineering. The deletion of non-structural proteins, but also structural proteins such as protein E, has been proposed to design vaccine strains of various zoonotic and veterinary coronaviruses[98-100]. The deletion of protein E contributes to the attenuation of virulence and allows the formulation of efficient vaccines, but as a negative effect, there was the possible reversion of the attenuated phenotype[101]. Indeed, live attenuated vaccines are highly immunogenic and do not require adjuvants to obtain an optimal response by virtue of their effectiveness in inducing excellent immunity dictated by a close imitation of natural infection. However, live attenuated vaccines have shown limitations that hold back their development and use today. In fact, vaccine-induced symptoms that are normally much milder than those of natural infection are still a major problem for immunocompromised individuals who may be at risk for unregulated pathogenic replication that can lead to severe infection or death. Furthermore, it should always be kept in mind that live attenuated vaccines have the potential to revert to a disease-causing form[102]. Furthermore, the generation of an attenuated strain for vaccine use demands that the key mutation is phenotypically stable by showing its inability to genetically return back to its pathogenic status[103]. This is remarkably arduous for coronaviruses as they are prone to recombine in nature; therefore, any attenuated vaccine strain could, at least in theory, recombine with wildtype coronaviruses to give rise to a further pathogenic strain[104].

New strategies by which changes to the synonym coding are introduced to transform codon utilization have the advantage that the attenuation of the resulting virus relies on a broad amount of mutations, each of which only slightly reduces the replicative capacity, but taken together, they produce an evident attenuation together with a significantly improved genetic stability[105].

This new codon pair deoptimization method produces a chemically synthesized genome with the amino acid sequence identical to the original virus but containing a greater number of CpG and UpA RNA dinucleotides to upregulate host responses by swapping optimized and non-optimized codons[106]. Codon pair deoptimization (CPD) was developed as a procedure for producing a vast number of other live attenuated virus vaccines, including influenza A virus, porcine reproductive and respiratory syndrome virus (PRRSV), human immunodeficiency virus type 1 (HIV-1), respiratory syncytial virus, chikungunya virus, enterovirus A71, zika virus, Marek's disease virus, lassa fever virus, and lymphocytic choriomeningitis virus[107-118].

So far, there are only three attenuated SARS-CoV-2 vaccines generated by codon deoptimization in preclinical development, from Mehmet Ali Aydinlar University (Turkey), Codagenix and Serum Institute of India, and Indian Immunologicals Ltd and Griffith University (Brisbane, Australia)[119]. Codagenix and the Serum Institute of India are involved in the development of a live attenuated SARS-CoV-2 vaccine, using codon deoptimization technology, following on their previous experience with vaccines against RSV and influenza using the same technology[120] (Table VII).

Conclusions

The entire world is in a desperate search for the optimal safe and effective vaccine against SARS-CoV-2. Several R&D institutions and pharmaceutical companies have started a race for the rapid production of these vaccines, with the results of more than 200 vaccine candidates being devel-

Table VII. Other SARS-CoV-2 candidate vaccines under development.

COVID-19 vaccine developer/manufactures	Vaccine platform	Type of candidate vaccine	Current stage of clinical evaluation (estimated study completion date)
OSE immunotherapeutics (Nantes, France)	T-cell based	CD8 T cell peptide targeting (S, M, N) and (NSPs) SARS-CoV-2 proteins	Preclinical
Farmacológicos Veterinarios SAC (FARVET SAC) (Ica, Perù)/Universidad Peruana (Lima, Perù)	Replicating Bacteria Vector	Oral Salmonella enteritidis (3934Vac) based protein exprvremession system of RBD	Preclinical
Mehmet Ali Aydinlar University/Acıbadem Labmed Health Services A.S. (Istanbul, Turkey)	Live Attenuated Virus	Codon deoptimized live attenuated vaccines	Preclinical
Codagenix (Melville, NY, United States)/Serum Institute of India (Pune, India)	Live Attenuated Virus	Codon deoptimized live attenuated vaccines	Preclinical
Indian Immunologicals Ltd (Hyderabad, India)/Griffith University (South East Queensland, Australia)	Live Attenuated Virus	Codon deoptimized live attenuated vaccines	Preclinical

Modified from: DRAFT landscape of COVID-19 candidates vaccine ⇒ https://www.who.int/publications/m/item/draft-landscape-of-covid-19-candidate-vaccines.

oped and almost 50 having entered phase 1, 2, and 3 clinical trials within less than a year from the emergence of the pandemic[121,122]. This has been rendered possible by the nowadays high specialized scientific knowledge and development of the vaccinology field that has exploited the technologies available and sometimes well-documented and previously used for contrasting many other infectious diseases. Several vaccine platforms have been extensively explored for other infections and cancer and often parallel other technologies used in gene therapy. However, it must be borne in mind that the development of a vaccine must necessarily follow strict safety rules and cannot be achieved overnight. After the design and preparation of the vaccine, the phase of evaluating the efficacy and safety in preclinical studies is necessary for the definition of quality standards before entering clinical studies. In fact, while usual therapeutic drugs are administered to patients already suffering from specific pathologies, vaccines, by definition, are given in advance to healthy subjects and therefore require very accurate safety levels. The different platforms and strategies for vaccine development described in this paper and in the published literature[123-129] have specific immunological advantages and disadvantages (summarized in Figure 1 and in several detailed review articles)[130-132], but all data collected so far indicate the possibility that vaccines require both humoral and cellular responses to provide an adequate level of protection and to induce a durable and robust immunological response. At present, many questions are still unanswered, such as the actual safety and efficacy of vaccines, which will not be available until the Phase 3 clinical trial is completed and the data is properly reviewed. It is still debatable whether neutralizing antibodies are sufficient and what are the critical quantity and quality of protective antibodies. Clinical trials will also establish other critically important data, such as: i) whether humoral and/or cellular cytotoxic responses are needed, ii) which helper T cell types are most effective (i.e., Th1 *vs.* Th2 *vs.* Th17) and iii) which kind of antibody response (i.e., IgG *vs.* IgA) is most effective to protect against this virus. A key point actually addressed by many studies is a better definition of the amount of antigen dose to be administered, the number of doses needed, the duration of immunity, and the need for boosters that depend on the technology used to produce each vaccine preparation. The need to develop an effective COVID-19 vaccine is so demanding that it is likely that unless one vaccine is much more effective than any other, more candidate vaccines will gain approval in different geographic

regions. It is also likely that as early as the start of 2021, more than one candidate vaccine will be authorized for human use, and although the global spread of SARS-CoV-2 may begin to fall under control before a successful vaccine will be ready for distribution, we are confident that efforts put forward by the global scientific community and by governments will be of inestimable value as the most successful vaccines could serve as models for future vaccines that can prevent outbreaks from other SARS-like viruses. Hopefully, in the future, a single vaccine may be able to provide broad coverage against more than one of these pathogenic viruses and represent a pan-coronavirus vaccine.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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References

1) Masters PS, Perlman S. Coronaviridae. Field Virology 2013; pp. 825-858.
2) Izzi A, Messina V, Rinaldi L, Maggi P. Editorial - Sofosbuvir/Velpatasvir as a combination with strong potential activity against SARS-CoV2 (COVID-19) infection: how to use direct-acting antivirals as broad-spectrum antiviral agents. Eur Rev Med Pharmacol Sci 2020; 24: 5193-5194.
3) World Health Organization. Novel-coronavirus-2019. Available at: https://www.who.int/emergencies/diseases/novel-coronavirus-2019. [Accessed 22 December 2020].
4) Lescure FX, Bouadma L, Nguyen D, Parisey M, Wicky PH, Behillil S, Gaymard A, Bouscambert-Duchamp M, Donati F, Le Hingrat Q, Enouf V, Houhou-Fidouh N, Valette M, Mailles A, Lucet JC, Mentre F, Duval X, Descamps D, Malvy D, Timsit JF, Lina B, van-der-Werf S, Yazdanpanah Y. Clinical and virological data of the first cases of COVID-19 in Europe: a case series. Lancet Infect Dis 2020; 20: 697-706.
5) Carta MG, Scano A, Lindert J, Bonanno S, Rinaldi L, Fais S, Orrù G. Association between the spread of COVID-19 and weather - climatic parameters. Eur Rev Sci 2020; 24: 8226-8231.
6) De wit E, Van Doremalen N, Falzarano D, Munster VJ. SARS and MERS: recent insights into emerging coronaviruses. Nat Rev Microbiol 2016; 14: 523-534.
7) Wu A, Peng Y, Huang B, Ding X, Wang X, Niu P, Meng J, Zhu Z, Zhang Z, Wang J, Sheng J, Quan L, Xia Z, Tan W, Cheng G, Jiang T. Genome composition and divergence of the novel coronavirus (2019-nCoV) originating in China. Cell Host Microbe 2020; 27: 325-328.
8) Fehr AR, Perlman S. Coronaviruses: an overview of their replication and pathogenesis, in Coronaviruses. Methods Mol Biol 2015; 1282: 1-23.
9) Finkel Y, Mizrahi O, Nachshon A, Weingarten-Gabbay S, Morgenstern D, Yahalom-Ronen Y, Tamir H, Achdout H, Stein D, Israeli O, Beth-Din A, Melamed S, Weiss S, Israely T, Paran N, Schwartz M, Stern-Ginossar N. The coding capacity of SARS-CoV-2. Nature 2021; 589: 125-130.
10) Rey FA, Lok SM. Common features of enveloped viruses and implications for immunogen design for next-generation vaccines. Cell 2018; 172: 1319-1334.
11) Harrison SC. Viral membrane fusion. Virology 2015; 479: 498-507.
12) White JM, Delos SE, Brecher M, Schornberg K. Structures and mechanisms of viral membrane fusion proteins: multiple variations on a common theme. Crit Rev Biochem Mol Biol 2008; 43: 189-219.
13) Hoffmann M, Kleine-Weber H, Schroeder S, Krüger N, Herrler T, Erichsen S, Schiergens TS, Herrler G, Wu NH, Nitsche A, Müller MA, Drosten C, Pöhlmann S. SARS-CoV-2 cell entry depends on ACE2 and TMPRSS2 and is blocked by a clinically proven protease inhibitor. Cell 2020; 181: 271-280.e8.
14) Walls AC, Park YJ, Tortorici MA, Wall A, Mcguire AT, Veesler D. Structure, function, and antigenicity of the SARS-CoV-2 spike glycoprotein. Cell 2020; 181: 281-292.
15) Wrapp D, Wang N, Corbett KS, Goldsmith JA, Hsieh CL, Abiona O, Graham BS, Mclellan JS. Cryo-EM structure of the 2019-nCoV spike in the prefusion conformation. Science 2020; 367: 1260-1263.
16) V'Kovski P, Kratzel A, Steiner S, Stalder H, Thiel V. Coronavirus biology and replication: implications for SARS-CoV-2. Nat Rev Microbiol 2021; 19: 155-170.
17) Xu R, Shi M, Li J, Song P, Li N. Construction of SARS-CoV-2 virus-like particles by mammalian expression system. Front Bioeng Biotechnol 2020; 8: 862
18) 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.

19) Valdenassi L, Franzini M, Ricevuti G, Rinaldi L, Galoforo AG, Tirelli U. Potential mechanisms by which the oxygen-ozone (O2-O3) therapy could contribute to the treatment against the coronavirus COVID-19. 24: 4059-4061.

20) Amanat F, Krammer F. SARS-CoV-2 vaccines: status report. Immunity 2020; 52: 583-589.

21) Liang Z, Zhu H, Wang X, Jing B, Li Z, Xia X, Sun H, Yang Y, Zhang W, Shi L, Zeng H, Sun B. Adjuvants for Coronavirus vaccines. Front Immunol 2020; 11: 589833.

22) World Health Organization. Draft landscape of COVID-19 candidate vaccines. Available at: https://www.who.int/publications/m/item/draft-landscape-of-covid-19-candidate-vaccines. [Accessed 22 December 2020].

23) Vetter V, Denizer G, Friedland LR, Krishnan J, Shapiro M. Understanding modern-day vaccines: what you need to know. Ann Med 2018; 50: 110-120.

24) Plotkin SA, Plotkin SL. The development of vaccines: how the past led to the future. Nat Rev Microbiol 2011; 9: 889-893.

25) Furuya Y. Return of inactivated whole-virus vaccine for superior efficacy. Immunol Cell Biol 2012; 90: 571-578.

26) Xia S, Zhang Y, Wang Y, Wang H, Yang Y, Gao GF, Tan W, Wu G, Xu M, Lou Z, Huang W, Xu W, Huang B, Wang H, Wang W, Zhang W, Li N, Xie Z, Ding L, You W, Zhao Y, Yang X, Liu Y, Wang Q, Huang L, Yang Y, Xu G, Luo B, Wang W, Liu P, Guo W, Yang X. Safety and immunogenicity of an inactivated SARS-CoV-2 vaccine, BBIBP-CorV: a randomised, double-blind, placebo-controlled, phase 1/2 trial. Lancet Infect Dis 2021; 21: 39-51.

27) Wang H, Zhang Y, Huang B, Deng W, Quan Y, Wang W, Xu W, Zhao Y, Li N, Zhang J, Liang H, Bao L, Xu Y, Ding L, Zhou W, Gao H, Liu J, Niu P, Zhao L, Zhen W, Fu H, Yu S, Zhang Z, Xu G, Li C, Lou Z, Xu M, Qin C, Wu G, Gao GF, Tan W, Yang X. Development of an inactivated vaccine candidate, BBIBP-CorV, with potent protection against SARS-CoV-2. Cell 2020; 182: 713-721.

28) Gao Q, Bao L, Mao H, Wang L, Xu K, Yang M, Li Y, Zhu L, Wang N, Lu Z, Gao H, Ge X, Kan B, Hu Y, Liu J, Cai F, Jiang D, Yin Y, Qin C, Li J, Gong X, Lou X, Shi W, Wu D, Zhang H, Zhu L, Deng W, Li Y, Lu J, Li C, Wang X, Yin W, Zhang Y, Qin C. Development of an inactivated vaccine candidate for SARS-CoV-2. Science 2020; 369: 77-81.

29) Palacios R, Patiño EG, De Oliveira Piorelli R, Conde MTRP, Batista AP, Zeng G, Xin Q, Kallas EG, Flores J, Ockenhouse CF, Gast C. Double-blind, randomized, placebo-controlled Phase III clinical trial to evaluate the efficacy and safety of treating healthcare professionals with the adsorbed COVID-19 (inactivated) vaccine manufactured by Sinovac–PROFISCOV: a structured summary of a study protocol for a randomised controlled trial. Trials 2020; 21: 853.

30) Zhang Y, Zeng G, Pan H, Li C, Hu Y, Chu K, Han W, Chen Z, Tang R, Yin W, Chen X, Hu Y, Liu X, Jiang C, Li J, Yang M, Song Y, Wang X, Gao Q, Zhu F. Safety, tolerability, and immunogenicity of an inactivated SARS-CoV-2 vaccine in healthy adults aged 18-59 years: a randomised, double-blind, placebo-controlled, phase 1/2 clinical trial. Lancet Infect Dis 2021; 21: 181-192.

31) Ganneru B, Jogdand H, Dharam VK, Molugu NR, Prasad SD, Vellimudu S, Ella KM, Ravikrishnan R, Awasthi A, Jose J. Evaluation of safety and immunogenicity of an adjuvanted, TH-1 Skewed, whole virion inactivated SARS-CoV-2 vaccine-BBV152. bioRxiv 2020;

32) Kleerekoper M, Ibels LS, Ingham JP, McCarthy SW, Mahony JF, Stewart JH, Posen S. Hyperparathyroidism after renal transplantation. Br Med J 1975; 3: 680-682.

33) Moyle PM, Toth I. Modern subunit vaccines: development, components, and research opportunities. Chem Med Chem 2013; 8: 360-376.

34) Schiller JT, Lowy DR. Raising expectations for subunit vaccine. J Infect Dis 2015; 211: 1373-1375.

35) Bijker MS, Melief CJ, Offringa R, van der Burg SH. Design and development of synthetic peptide vaccines: past, present and future. Expert Rev Vaccines 2007; 6: 591-603.

36) Franci G, Falanga A, Zannella C, Folliero V, Martora F, Galdiero M, Galdiero S, Morelli G, Galdiero M. Infectivity inhibition by overlapping synthetic peptides derived from the gH/gL heterodimer of herpes simplex virus type 1. J Pept Sci 2017; 23: 311-319.

37) Zhang H, Hong H, Li D, Ma S, Di Y, Stoten A, Haig N, Di Gleria K, Yu Z, Xu XN, McMichael A, Jiang S. Comparing pooled peptides with intact protein for accessing cross-presentation pathways for protective CD8+ and CD4+ T cells. J Biol Chem 2009; 284: 9184-9191.

38) Rosalia RA, Quakkelaar ED, Redeker A, Khan S, Camps M, Drijfhout JW, Silva AL, Jiskoot W, van Hall T, van Veelen PA, Janssen G, Franken K, Cruz LJ, Tromp A, Oostendorp J, van der Burg SH, Ossendorp F, Melief CJ. Dendritic cells process synthetic long peptides better than whole protein, improving antigen presentation and T-cell activation. Eur J Immunol 2013; 43: 2554-2565.

39) Czechowicz JK, Raczkowska M. Digital image transducer applied for evaluation of optic disc and optic cup in patients with glaucoma. Klin Oczna 1978; 48: 275-277.

40) Li W, Joshi MD, Singhania S, Ramsey KH, Murthy AK. Peptide vaccine: progress and challenges. Vaccines 2014; 2: 515-536.

41) Guebre XM, Patel N, Tian JH, Zhou B, Maciejewski S, Lam K, Portnoff AD, Massare MJ, Frieman MB, Piedra PA, Ellingsworth L, Glenn G, Smith G. NVX-CoV2373 vaccine protects cynomolgus macaque upper and lower airways against SARS-CoV-2 challenge. Vaccine 2020; 38: 7892-7896.

42) Kim E, Erdos G, Huang S, Kenniston TW, Balmert SC, Carey CD, Raj VS, Epperly MW, Klimstra WB,

Haagmans BL, Korkmaz E, Falo LD Jr, Gambotto A. Microneedle array delivered recombinant coronavirus vaccines: immunogenicity and rapid translational development. EBioMedicine 2020; 55: 102743.

43) Dai L, Zheng T, Xu K, Han Y, Xu L, Huang E, An Y, Cheng Y, Li S, Liu M, Yang M, Li Y, Cheng H, Yuan Y, Zhang W, Ke C, Wong G, Qi J, Qin C, Yan J, Gao GF. A universal design of betacoronavirus vaccines against COVID-19, MERS, and SARS. Cell 2020; 182: 722-733.e11.

44) Wang X, Guo X, Xin Q, Pan Y, Hu Y, Li J, Chu Y, Feng Y, Wang Q. Neutralizing antibody responses to SARS-CoV-2 in COVID-19 inpatients and convalescent patients. Clin Infect Dis 2020; 71: 2688-2694.

45) Watterson D, Wijesundara D, Modhiran N, Mordant F, Li Z, Avumegah M, McMillan C, Lackenby J, Guilfoyle K, van Amerongen G, Stittelaar K, Cheung S, Bibby S, Daleris M, Hoger K, Gillard M, Radunz E, Jones M, Hughes K, Hughes B, Goh J, Edwards D, Scoble J, Pearce L, Kowalczyk L, Phan T, La M, Lu L, Pham T, Zhou Q, Brockman D, Morgan S, Lau C, Tran M, Tapley P, Letelier FV, Barnes J, Young A, Jaberolansar N, Scott C, Isaacs A, Amarilla A, Khromykh A, Reading P, Ranasinghe C, Subbarao K, Munro T, Young P, Chappell K. Molecular clamp stabilised Spike protein for protection against SARS-CoV-2. Preprint from Research Square 2020. DOI: 10.21203/rs.-68892/v1.

46) Liu MA. A comparison of plasmid DNA and mrna as vaccine technologies. Vaccines 2019; 7: 37.

47) 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: 1075-1082.

48) Leitner WW, Ying H, Restifo NP. DNA and RNA-based vaccines: principles, progress and prospects. Vaccine 1999; 18: 765-777.

49) Rauch S, Jasny E, Schmidt KE, Petsch B. New vaccine technologies to combat outbreak situations. Front Immunol 2018; 9: 1963.

50) Kutzler MA, Weiner DB. DNA vaccines: ready for prime time? Nat Rev Genet 2008; 9: 776-788.

51) Modjarrad K, Roberts CC, Mills KT, Castellano AR, Paolino K, Muthumani K, Reuschel EL, Robb ML, Racine T, Oh MD, Lamarre C, Zaidi FI, Boyer J, Kudchodkar SB, Jeong M, Darden JM, Park YK, Scott PT, Remigio C, Parikh AP, Wise MC, Patel A, Duperret EK, Kim KY, Choi H, White S, Bagarazzi M, May JM, Kane D, Lee H, Kobinger G, Michael NL, Weiner DB, Thomas SJ, Maslow JN. 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: 1013-1022.

52) Smith TRF, Patel A, Ramos S, Elwood D, Zhu X, Yan J, Gary EN, Walker SN, Schultheis K, Purwar M, Xu Z, Walters J, Bhojnagarwala P, Yang M, Chokkalingam N, Pezzoli P, Parzych E, Reuschel EL, Doan A, Tursi N, Vasquez M, Choi J, Tello RE, Maricic I, Bah MA, Wu y, Amante D, Park DH, Dia Y, Ali AR, Zaidi FI, Generotti A, Kim KY, Herring TA, Reeder S, Andrade VM, Buttigieg K, Zhao G, Wu JM, Li D, Bao L, Liu J, Deng W, Qin C, Brown AS, Khoshnejad M, Wang N, Chu J, Wrapp D, McLellan JS, Muthumani K, Wang B, Carroll MW, Kim JJ, Boyer J, Kulp DW, Humeau L, Weiner DB, Broderick KE. Immunogenicity of a DNA vaccine candidate for COVID-19. Nat Commun 2020; 11: 2601.

53) Seo YB, Suh YS, Ryu JI, Jang H, Oh H, Koo BS, Seo SH, Hong JJ, Song M, Kim SJ. Soluble Spike DNA vaccine provides long-term protective immunity against SAR-CoV-2 in mice and nonhuman primates. bioRxiv 2020.

54) Zhang C, Maruggi G, Shan H, Li J. Advances in mRNA vaccines for infectious diseases. Front Immunol 2019; 10: 594.

55) Pardi N, Hogan MJ, Porter FW, Weissman D. mRNA vaccines–a new era in vaccinology. Nat Rev Drug Discov 2018; 17: 261.

56) Espeseth AS, Cejas PJ, Citron MP, Wang D, DiStefano DJ, Callahan C, Donnell GO, Galli JD, Swoyer R, Touch S, Wen Z, Antonello J, Zhang L, Flynn JA, Cox KS, Freed DC, Vora KA, Bahl K, Latham AH, Smith JS, Gindy ME, Ciaramella G, Hazuda D, Shaw CA, Bett AJ. Modified mRNA/lipid nanoparticle-based vaccines expressing respiratory syncytial virus F protein variants are immunogenic and protective in rodent models of RSV infection. NPJ Vaccines 2020; 5: 16.

57) Nastri BM, Zannella C, Folliero V, Rinaldi L, Restivo L, Stelitano D, Sperlongano R, Adinolfi LE, Franci G. Editorial - Role of Highly Active Antiretroviral Therapy (HAART) for the COVID-19 treatment. Eur Rev Med Pharmacol Sci 2020; 24: 11982-11984.

58) Corbett KS, Edwards D, Leist SR, Abiona OM, Boyoglu BS, Gillespie RA, Himansu S, Schafer A, Ziwawo CT, Dipiazza AT, Dinnon KH, Elbashir SM, Shaw CA, Woods A, Fritch EJ, Martinez DR, Bock KW, Minai M, Nagata BM, Hutchinson GB, Bahl K, Garcia Dominguez D, Ma L, Renzi I, Kong WP, Schmidt SD, Wang L, Zhang Y, Stevens LJ, Phung E, Chang LA, Loomis RJ, Altaras NE, Narayanan E, Metkar M, Presnyak V, Liu C, Louder MK, Shi W, Leung K, Yang ES, West A, Gully KL, Wang N, Wrapp D, Doria RNA, Stewart JG, Bennett H, Nason MC, Ruckwardt TJ, Mclellan JS, Denison MR, Chappell JD, Moore IN, Morabito KM, Mascola JR, Baric RS, Carfi A, Graham BS. SARS-CoV-2 mRNA Vaccine development enabled by prototype pathogen preparedness. bioRxiv 2020.

59) Corbett KS, Flynn B, Foulds KE, Francica JR, Boyoglu BS, Werner AP, Flach B, O'Connell S, Bock KW, Minai M, Nagata BM, Andersen H, Martinez DR, Noe AT, Douek N, Donaldson MM, Nji NN, Alvarado GS, Edwards DK, Flebbe DR, Lamb E, Doria RNA, Lin BC, Louder MK, O'Dell S, Schmidt SD, Phung E, Chang LA, Yap C, Todd JM, Pessaint L, Van Ry A, Browne S, Greenhouse

J, Putman TT, Strasbaugh A, Campbell TA, Cook A, Dodson A, Steingrebe K, Shi W, Zhang Y, Abiona OM, Wang L, Pegu A, Yang ES, Leung K, Zhou T, Teng IT, Widge A, Gordon I, Novik L, Gillespie RA, Loomis RJ, Moliva JI, Stewart JG, Himansu S, Kong WP, Nason MC, Morabito KM, Ruckwardt TJ, Ledgerwood JE, Gaudinski MR, Kwong PD, Mascola JR, Carfi A, Lewis MG, Baric RS, McDermott A, Moore IN, Sullivan NJ, Roederer M, Seder RA, Graham BS. Evaluation of the mRNA-1273 Vaccine against SARS-CoV-2 in Nonhuman Primates. N Engl J Med 2020; 383: 1544-1555.

60) Jackson LA, Anderson EJ, Rouphael NG, Roberts PC, Makhene M, Coler RN, McCullough MP, Chappell JD, Denison MR, Stevens LJ, Pruijssers AJ, McDermott A, Flach B, Doria-Rose NA, Corbett KS, Morabito KM, O'Dell S, Schmidt SD, Swanson PA 2nd, Padilla M, Mascola JR, Neuzil KM, Bennett H, Sun W, Peters E, Makowski M, Albert J, Cross K, Buchanan W, Pikaart-Tautges R, Ledgerwood JE, Graham BS, Beigel JH; mRNA-1273 Study Group. An mRNA vaccine against SARS-CoV-2 – Preliminary report. N Engl J Med 2020; 383: 1920-1931.

61) Guthe S, Kapinos L, Moglich A, Meier S, Grzesiek S, Kiefhaber T. Very fast folding and association of a trimerization domain from bacteriophage T4 fibritin. J Mol Biol 2004; 337: 905-915.

62) Wrapp D, Wang N, Corbett KS, Goldsmith JA, Hsieh CL, Abiona O, Graham BS, Mclellan JS. Cryo-EM structure of the 2019-nCoV spike in the prefusion conformation. Science 2020; 367: 1260-1263.

63) Sahin U, Muik A, Derhovanessian E, Vogler I, Kranz LM, Vormehr M, Baum A, Pascal K, Quandt J, Maurus D, Brachtendorf S, Lorks V, Sikorski J, Hilker R, Becker D, Eller AK, Grutzner J, Boesler C, Rosenbaum C, Kuhnle MC, Luxemburger U, Kemmer BA, Langer D, Bexon M, Bolte S, Kariko K, Palanche T, Fischer B, Schultz A, Shi PY, Fontes GC, Perez JL, Swanson KA, Loschko J, Scully IL, Cutler M, Kalina W, Kyratsous CA, Cooper D, Dormitzer PR, Jansen KU, Tureci O. COVID-19 vaccine BNT162b1 elicits human antibody and TH1 T cell responses. Nature 2020; 586: 594-599.

64) Mulligan MJ, Lyke KE, Kitchin N, Absalon J, Gurtman A, Lockhart S, Neuzil K, Raabe V, Bailey R, Swanson KA, Li P, Koury K, Kalina W, Cooper D, Fontes GC, Shi PY, Tureci O, Tompkins KR, Walsh EE, Frenck R, Falsey AR, Dormitzer PR, Gruber WC, Sahin U, Jansen KU. Phase I/II study of COVID-19 RNA vaccine BNT162b1 in adults. Nature 2020; 586: 589-593.

65) Walsh EE, Frenck RWJR, Falsey AR, Kitchin N, Absalon J, Gurtman A, Lockhart S, Neuzil K, Mulligan MJ, Bailey R, Swanson KA, Li P, Koury K, Kalina W, Cooper D, Fontes GC, Shi PY, Tureci O, Tompkins KR, Lyke KE, Raabe V, Dormitzer PR, Jansen KU, Sahin U, Gruber WC. Safety and immunogenicity of two RNA-based Covid-19 vaccine candidates. N Engl J Med 2020; 9: e64496.

66) Rauch S, Roth N, Schwendt K, Fotin Mleczek M, Mueller SO, Petsch B. mRNA based SARS-CoV-2 vaccine candidate CVnCoV induces high levels of virus neutralizing antibodies and mediates protection in rodents. bioRxiv 2020. bioRxiv 2020.10.23.351775;

67) Ramaswamy S, Tonnu N, Tachikawa K, Limphong P, Vega JB, Karmali PP, Chivukula P, Verma IM. Systemic delivery of factor IX messenger RNA for protein replacement therapy. Proc Natl Acad Sci USA 2017; 114: e1941-e1950.

68) De alwis RM, Gan ES, Chen S, Leong YS, Tan HC, Zhang SL, Yau C, Matsuda D, Allen E, Hartman P, Park J, Alayyoubi M, Bhaskaran H, Dukanovic A, Bao B, Clemente B, Vega J, Roberts S, Gonzalez JA, Sablad M, Yelin R, Taylor W, Tachikawa K, Parker S, Karmali P, Davis J, Sullivan SM, Hughes SG, Chivukula P, Oi EE. A single dose of self-transcribing and replicating RNA based SARS-CoV-2 vaccine produces protective adaptive immunity in mice. bioRxiv 2020.

69) McKay PF, Hu K, Blakney AK, Samnuan K, Brown JC, Penn R, Zhou J, Bouton CR, Rogers P, Polra K, Lin PJC, Barbosa C, Tam YK, Barclay WS, Shattock RJ. Self-amplifying RNA SARS-CoV-2 lipid nanoparticle vaccine candidate induces high neutralizing antibody titers in mice. Nat Commun 2020; 11: 3523.

70) Matthews QL, Gu L, Krendelchtchikov A, Li ZC, Ming W. Novel gene therapy approaches. IntechOpen, 2013.

71) Holman DH, Penn Nicholson A, Wang D, Woraratanadharm J, Harr MK, Luo M, Maher EM, Holbrook MR, Dong JY. A complex adenovirus-vectored vaccine against Rift Valley fever virus protects mice against lethal infection in the presence of preexisting vector immunity. Clin Vaccine Immunol 2009; 16: 1624-1632.

72) Chinnakannan SK, Cargill TN, Donnison TA, Ansari MA, Sebastian S, Lee LN, Hutchings C, Klenerman P, Maini MK, Evans T, Barnes E. The design and development of a multi-HBV antigen encoded in chimpanzee adenoviral and modified vaccinia Ankara viral vectors; a novel therapeutic vaccine strategy against HBV. Vaccines 2020; 8: 184.

73) Zhu FC, Li YH, Guan XH, Hou LH, Wang WJ, Li JX, Wu SP, Wang BS, Wang Z, Wang L, Jia SY, Jiang HD, Wang L, Jiang T, Hu Y, Gou JB, Xu SB, Xu JJ, Wang XW, Wang W, Chen W. Safety, tolerability, and immunogenicity of a recombinant adenovirus type-5 vectored COVID-19 vaccine: a dose-escalation, open-label, non-randomised, first-in-human trial. Lancet 2020; 395: 1845-1854.

74) Abbink P, Lemckert AA, Ewald BA, Lynch DM, Denholtz M, Smits S, Holterman L, Damen I, Vogels R, Thorner AR, O'Brien KL, Carville A, Mansfield KG, Goudsmit J, Havenga MJ, Barouch DH. Comparative seroprevalence and immunogenicity of six rare serotype recombinant adenovirus vaccine vectors from subgroups B and D. J Virol 2007; 81: 4654-4663.

75) Tostanoski LH, Wegmann F, Martinot AJ, Loos C, McMahan K, Mercado NB, Yu J, Chan CN, Bondoc S, Starke CE, Nekorchuk M, Busman Sahay K, Piedra MC, Wrijil LM, Ducat S, Custers J, Atyeo C, Fischinger S, Burke JS, Feldman J,

Hauser BM, Caradonna TM, Bondzie EA, Dagotto G, Gebre MS, Jacob DC, Lin Z, Mahrokhian SH, Nampanya F, Nityanandam R, Pessaint L, Porto M, Ali V, Benetiene D, Tevi K, Andersen H, Lewis MG, Schmidt AG, Lauffenburger DA, Alter G, Estes JD, Schuitemaker H, Zahn R, Barouch DH. Ad26 vaccine protects against SARS-CoV-2 severe clinical disease in hamsters. Nat Med 2020; 26: 1694-1700.

76) Mercado NB, Zahn R, Wegmann F, Loos C, Chandrashekar A, Yu J, Liu J, Peter L, McMahan K, Tostanoski LH, He X, Martinez DR, Rutten L, Bos R, Van Manen D, Vellinga J, Custers J, Langedijk JP, Kwaks T, Bakkers MJG, Zuijdgeest D, Rosendahl Huber SK, Atyeo C, Fischinger S, Burke JS, Feldman J, Hauser BM, Caradonna TM, Bondzie EA, Dagotto G, Gebre MS, Hoffman E, Jacob Dolan C, Kirilova M, Li Z, Lin Z, Mahrokhian SH, Maxfield LF, Nampanya F, Nityanandam R, Nkolola JP, Patel S, Ventura JD, Verrington K, Wan H, Pessaint L, Van RY A, Blade K, Strasbaugh A, Cabus M, Brown R, Cook A, Zouantchangadou S, Teow E, Andersen H, Lewis MG, Cai Y, Chen B, Schmidt AG, Reeves RK, Baric RS, Lauffenburger DA, Alter G, Stoffels P, Mammen M, Van Hoof J, Schuitemaker H, Barouch DH. Single-shot Ad26 vaccine protects against SARS-CoV-2 in rhesus macaques. Nature 2020; 586: 583-588.

77) Yu J, Tostanoski LH, Peter L, Mercado NB, Mcmahan K, Mahrokhian SH, Nkolola JP, Liu J, Li Z, Chandrashekar A, Martinez DR, Loos C, Atyeo C, Fischinger S, Burke JS, Slein MD, Chen Y, Zuiani A, Lelis FJN, Travers M, Habibi S, Pessaint L, Van Ry A, Blade K, Brown R, Cook A, Finneyfrock B, Dodson A, Teow E, Velasco J, Zahn R, Wegmann F, Bondzie EA, Dagotto G, Gebre MS, He X, Jacob Dolan C, Kirilova M, Kordana N, Lin Z, Maxfield LF, Nampanya F, Nityanandam R, Ventura JD, Wan H, Cai Y, Chen B, Schmidt AG, Wesemann DR, Baric RS, Alter G, Andersen H, Lewis MG, Barouch DH. DNA vaccine protection against SARS-CoV-2 in rhesus macaques. Science 2020; 369: 806-811.

78) Sadoff J, LE Gars M, Shukarev G, Heerwegh D, Truyers C, De Groot AM, Stoop J, Tete S, Van Damme W, Leroux Roels I, Berghmans P-J, Kimmel M, Van Damme P, De Hoon J, Smith W, Stephenson K, Barouch D, De Rosa S, Cohen K, McElrath J, Cormier E, Scheper G, Hendriks J, Struyf F, Douoguih M, Van Hoof J, Schuitemaker H. Safety and immunogenicity of the Ad26. COV2. S COVID-19 vaccine candidate: interim results of a Phase 1/2a, double-blind, randomized, placebo-controlled trial. medRxiv 2020.09.23.20199604; doi: https://doi.org/10.1101/2020.09.23.20199604.

79) Logunov DY, Dolzhikova IV, Zubkova OV, Tukhvatullin AI, Shcheblyakov DV, Dzharullaeva AS, Grousova DM, Erokhova AS, Kovyrshina AV, Botikov AG, Izhaeva FM, Popova O, Ozharovskaya TA, Esmagambetov IB, Favorskaya IA, Zrelkin DI, Voronina DV, Shcherbinin DN, Semikhin AS, Simakova YV, Tokarskaya EA, Lubenets NL, Egorova DA, Shmarov MM, Nikitenko NA, Morozova LF, Smolyarchuk EA, Kryukov EV, Babira VF, Borisevich SV, Naroditsky BS, Gintsburg AL. Safety and immunogenicity of an rAd26 and rAd5 vector-based heterologous prime-boost COVID-19 vaccine in two formulations: two open, non-randomised phase 1/2 studies from Russia. Lancet 2020; 396: 887-897.

80) Dicks MD, Spencer AJ, Edwards NJ, Wadell G, Bojang K, Gilbert SC, Hill AV, Cottingham MG. A novel chimpanzee adenovirus vector with low human seroprevalence: improved systems for vector derivation and comparative immunogenicity. PLoS One 2012; 7: e40385.

81) Ewer K, Sebastian S, Spencer AJ, Gilbert S, Hill AVS, Lambe T. Chimpanzee adenoviral vectors as vaccines for outbreak pathogens. Hum Vaccin Immunother 2017; 13: 3020-3032.

82) Ewer K, Rampling T, Venkatraman N, bowyer G, Wright D, Lambe T, Imoukhuede EB, Payne R, Fehling SK, Strecker T, Biedenkopf N, Krahling V, Tully CM, Edwards NJ, Bentley EM, Samuel D, Labbe G, Jin J, Gibani M, Minhinnick A, Wilkie M, Poulton I, Lella N, Roberts R, Hartnell F, Bliss C, Sierra Davidson K, Powlson J, Berrie E, Tedder R, Roman F, De Ryck I, Nicosia A, Sullivan NJ, Stanley DA, Mbaya OT, Ledgerwood JE, Schwartz RM, Siani L, Colloca S, Folgori A, Di Marco S, Cortese R, Wright E, Becker S, Graham BS, Koup RA, Levine MM, Volkmann A, Chaplin P, Pollard AJ, Draper SJ, Ballou WR, Lawrie A, Gilbert SC, Hill AV. A Monovalent Chimpanzee Adenovirus Ebola Vaccine Boosted with MVA. N Engl J Med 2016; 374: 1635-1646.

83) Graham SP, McLean RK, Spencer AJ, Belij Rammerstorfer S, Wright D, Ulaszewska M, Edwards JC, Hayes JWP, Martini V, Thakur N, Conceicao C, Dietrich I, Shelton H, Waters R, Ludi A, Wilsden G, Browning C, Bialy D, Bhat S, Stevenson Leggett P, Hollinghurst P, GILBride C, Pulido D, Moffat K, Sharpe H, Allen E, Mioulet V, Chiu C, Newman J, Asfor AS, Burman A, Crossley S, Huo J, Owens RJ, Carroll M, Hammond JA, Tchilian E, Bailey D, Charleston B, Gilbert SC, Tuthill TJ, Lambe T. Evaluation of the immunogenicity of prime-boost vaccination with the replication-deficient viral vectored COVID-19 vaccine candidate ChAdOx1 nCoV-19. NPJ Vaccines 2020; 5: 69.

84) Van Doremalen N, Lambe T, Spencer A, Belij Rammerstorfer S, Purushotham JN, Port JR, Avanzato VA, Bushmaker T, Flaxman A, Ulaszewska M, Feldmann F, Allen ER, Sharpe H, Schulz J, Holbrook M, Okumura A, Meade White K, Perez L, Edwards NJ, Wright D, Bissett C, Gilbride C, Williamson BN, Rosenke R, Long D, Ishwarbhai A, Kailath R, Rose L, Morris S, Powers C, Lovaglio J, Hanley PW, Scott D, Saturday G, De Wit E, Gilbert SC, Munster VJ. ChAdOx1 nCoV-19 vaccine prevents SARS-CoV-2 pneumonia in rhesus macaques. Nature 2020; 586: 578-582.

85) Folegatti PM, Ewer KJ, Aley PK, Angus B, Becker S, Belij-Rammerstorfer S, Bellamy D, Bibi S, Bittaye M, Clutterbuck EA, Dold C, Faust SN, Finn A, Flaxman AL, Hallis B, Heath P, Jenkin D, Lazarus

R, Makinson R, Minassian AM, Pollock KM, Ramasamy M, Robinson H, Snape M, Tarrant R, Voysey M, Green C, Douglas AD, Hill AVS, Lambe T, Gilbert SC, Pollard AJ; Oxford COVID Vaccine Trial Group. Safety and immunogenicity of the ChAdOx1 nCoV-19 vaccine against SARS-CoV-2: a preliminary report of a phase 1/2, single-blind, randomised controlled trial. Lancet 2020; 396: 467-478.

86) Sun W, Leist SR, McCroskery S, Liu Y, Slamanig S, Oliva J, Amanat F, Schafer A, Dinnon KH, Garcia Sastre A, Krammer F, Baric RS, Palese P. Newcastle disease virus (NDV) expressing the spike protein of SARS-CoV-2 as a live virus vaccine candidate. EBioMedicine 2020; 62: 103132.

87) Wang P, Zheng M, Lau SY, Chen P, Mok BW, Liu S, Liu H, Huang X, Cremin CJ, Song W, Chen Y, Wong YC, Huang H, To KK, Chen Z, Xia N, Yuen KY, Chen H. Generation of DelNS1 influenza viruses: a strategy for optimizing live attenuated influenza vaccines. mBio 2019; 10: e02180-02199.

88) Mohsen MO, Zha L, Cabral Miranda G, Bachmann MF. Major findings and recent advances in virus-like particle (VLP)-based vaccines. Semin Immunol 2017; 34: 123-132.

89) Kushnir N, Streatfield SJ, Yusibov V. Virus-like particles as a highly efficient vaccine platform: diversity of targets and production systems and advances in clinical development. Vaccine 2012; 31: 58-83.

90) Sarkar B, Islam SS, Zohora US, Ullah MA. Virus like particles-A recent advancement in vaccine development. Korean J. Microbiol 2019; 55: 327-343.

91) Roldão A, Mellado MCM, Castilho LR, Carrondo MJ, Alves PM. Virus-like particles in vaccine development. Expert Rev Vaccines 2010; 9: 1149-1176.

92) Marini A, Zhou Y, li Y, Taylor IJ, Leneghan DB, Jin J, Zaric M, Mekhaiel D, Long CA, Miura K, Biswas S. A universal plug-and-display vaccine carrier based on HBsAg VLP to maximize effective antibody response. Front Immunol 2019; 10: 2931.

93) Riedel S. Edward Jenner and the history of smallpox and vaccination. Proc (Bayl Univ Med Cent) 2005; 18: 21-25.

94) Plotkin S. History of vaccination. Proc Natl Acad Sci USA 2014; 111: 12283-12287.

95) Lauring AS, Jones JO, Andino R. Rationalizing the development of live attenuated virus vaccines. Nat Biotechnol 2010; 28: 573-579.

96) Yun SI, Lee YM. Japanese encephalitis: the virus and vaccines. Hum Vaccin Immunother 2014; 10: 263-279.

97) Guy B, Briand O, Lang J, Saville M, Jackson N. Development of the Sanofi Pasteur tetravalent dengue vaccine: one more step forward. Vaccine 2015; 33: 7100-7111.

98) Netland J, Dediego ML, Zhao J, Fett C, Álvarez E, Nieto Torres JL, Enjuanes L, Perlman S. Immunization with an attenuated severe acute respiratory syndrome coronavirus deleted in E protein protects against lethal respiratory disease. Virology 2010; 399: 120-128.

99) Almazán F, Dediego ML, Sola I, Zuñiga S, Nieto Torres JL, Marquez Jurado S, Andrés G, Enjuanes L. Engineering a replication-competent, propagation-defective Middle East respiratory syndrome coronavirus as a vaccine candidate. mBio 2013; 4: e00650-13.

100) Hou Y, Meulia T, Gao X, Saif LJ, Wang Q. Deletion of both the tyrosine-based endocytosis signal and the endoplasmic reticulum retrieval signal in the cytoplasmic tail of spike protein attenuates porcine epidemic diarrhea virus in pigs. J Virol 2019; 93: e01758-18.

101) Jimenez Guardeño JM, Regla Nava JA, Nieto Torres JL, Dediego ML, Castaño Rodriguez C, Fernandez Delgado R, Perlman S, Enjuanes L. Identification of the mechanisms causing reversion to virulence in an attenuated SARS-CoV for the design of a genetically stable vaccine. PLoS Pathog 2015; 11: e1005215.

102) Minor PD. Live attenuated vaccines: historical successes and current challenges. Virol J 2015; 479: 379-392.

103) Hanley KA. The double-edged sword: how evolution can make or break a live-attenuated virus vaccine. Evolution (NY) 2011; 4: 635.

104) Tao Y, Shi M, Chommanard C, Queen K, Zhang J, Markotter W, Kuzmin IV, Holmes EC, Tong S. Surveillance of bat coronaviruses in Kenya identifies relatives of human coronaviruses NL63 and 229E and their recombination history. J Virol 2017; 91: e01953-16.

105) Tulloch F, Atkinson NJ, Evans DJ, Ryan MD, Simmonds P. RNA virus attenuation by codon pair deoptimisation is an artefact of increases in CpG/UpA dinucleotide frequencies. Elife 2014; 3: e04531.

106) Coleman JR, Papamichail D, Skiena S, Futcher B, Wimmer E, Mueller S. Virus attenuation by genome-scale changes in codon pair bias. Science 2008; 320: 1784-1787.

107) Mueller S, Coleman JR, Papamichail D, Ward CB, Nimnual A, Futcher B, Skiena S, Wimmer E. Live attenuated influenza virus vaccines by computer-aided rational design. Nat Biotechnol 2010; 28: 723-726.

108) Yang C, Skiena S, Futcher B, Mueller S, Wimmer E. Deliberate reduction of hemagglutinin and neuraminidase expression of influenza virus leads to an ultraprotective live vaccine in mice. Proc Natl Acad Sci USA 2013; 110: 9481-9486.

109) Ni YY, Zhao Z, Opriessnig T, Subramaniam S, Zhou L, Cao D, Cao Q, Yang H, Meng XJ. Computer-aided codon-pairs deoptimization of the major envelope GP5 gene attenuates porcine reproductive and respiratory syndrome virus. Virology 2014; 450-451: 132-139.

110) Martrus G, Nevot M, Andres C, Clotet B, Martinez MA. Changes in codon-pair bias of human immu-

nodeficiency virus type 1 have profound effects on virus replication in cell culture. Retrovirology 2013; 10: 78.

111) Le Nouën C, Brock LG, Luongo C, McCarty T, Yang L, Mehedi M, Wimmer E, Mueller S, Collins PL, Buchholz UJ, DiNapoli JM. Attenuation of human respiratory syncytial virus by genome-scale codon-pair deoptimization. Proc Natl Acad Sci USA 2014; 111: 13169-13174.

112) Le Nouën C, Collins PL, Buchholz UJ. Attenuation of human respiratory viruses by synonymous genome recoding. Front Immunol 2019; 10: 1250.

113) Carrau L, Rezelj VV, Noval MG, Levi LI, Megrian D, Blanc H, Weger Lucarelli J, Moratorio G, Stapleford KA, Vignuzzi M. Chikungunya virus vaccine candidates with decreased mutational robustness are attenuated in vivo and have compromised transmissibility. J Virol 2019; 93: e00775-19.

114) Tsai YH, Huang SW, Hsieh WS, Cheng CK, Chang CF, Wang YF, Wang JR. Enterovirus A71 containing codon-deoptimized VP1 and high-fidelity polymerase as next-generation vaccine candidate. J Virol 2019; 93: e02308-18.

115) Li P, Ke X, Wang T, Tan Z, Luo D, Miao Y, Sun J, Zhang Y, Liu Y, Hu Q, Xu F, Wang H, Zheng Z. Zika virus attenuation by codon pair deoptimization induces sterilizing immunity in mouse models. J Virol 2018; 92: e00701-18.

116) Eschke K, Trimpert J, Osterrieder N, Kunec D. Attenuation of a very virulent Marek's disease herpesvirus (MDV) by codon pair bias deoptimization. PLoS Pathog 2018; 14: e1006857.

117) Cai Y, Ye C, Cheng B, Nogales A, Iwasaki M, Yu S, Cooper K, Liu DX, Hart R, Adams R, Brady T, Postnikova EN, Kurtz J, St Claire M, Kuhn JH, de la Torre JC, Martínez-Sobrido L. A Lassa fever live-attenuated vaccine based on codon deoptimization of the viral glycoprotein gene. mBio 2020; 11: e00039-20.

118) Cheng BY, Nogales A, De La Torre JC, Martínez-Sobrido L. Development of live-attenuated arenavirus vaccines based on codon deoptimization of the viral glycoprotein. Virology 2017; 501: 35-46.

119) World Health Organization. Draft landscape of COVID-19 candidate vaccines. Available at: https://www.who.int/publications/m/item/draft-landscape-of-covid-19-candidate-vaccines. [Accessed 12 November 2020].

120) Tregoning JS, Brown ES, Cheeseman HM, Flight KE, Higham SL, Lemm NM, Pierce BF, Stirling DC, Wang Z, Pollock KM. Vaccines for COVID-19. Clin Exp Immunol 2020; 202: 162-192.

121) Perrella A, Carannante N, Berretta M, Rinaldi M, Maturo N, Rinaldi L. Novel Coronavirus 2019 (Sars-CoV2): a global emergency that needs new approaches? Eur Rev Med Pharmacol Sci 2020; 24: 2162-2164.

122) Khan MNM, Sarker MS. A review of coronavirus 2019 (covid-19), a life threating disease all over the world. Indian J Pediatr 2020; 87: 281-286.

123) Chung YH, Beiss V, Fiering SN, Steinmetz NF. COVID-19 vaccine frontrunners and their nanotechnology design. ACS Nano 2020; 14: 12522-12537.

124) Poland GA, Ovsyannikova IG, Crooke SN, Kennedy RB. SARS-CoV-2 vaccine development: current status. Mayo Clin Proc 2020; 95: 2172-2188.

125) Alturki SO, Alturki SO, Connors J, Cusimano G, Kutzler MA, Izmirly AM, Haddad EK. The 2020 pandemic: current SARS-CoV-2 vaccine development. Front Immunol 2020; 11: 1880.

126) Krammer F. SARS-CoV-2 vaccines in development. Nature 2020; 586: 516-527.

127) Kaur SP, Gupta V. COVID-19 vaccine: a comprehensive status report. Virus Res 2020; 288: 198114.

128) Ye T, Zhong Z, Garcia Sastre A, Schotsaert M, De Geest BG. Current status of COVID-19 (pre) clinical vaccine development. Angew Chem Int Ed Engl 2020; 59: 18885-18897.

129) Funk CD, Laferriere C, Ardakani A. A snapshot of the global race for vaccines targeting SARS-CoV-2 and the COVID-19 pandemic. Front Pharmacol 2020; 11: 937.

130) Dong Y, Dai T, Wei Y, Zhang L, Zheng M, Zhou F. A systematic review of SARS-CoV-2 vaccine candidates. Signal Transduct Target Ther 2020; 5: 237.

131) Kim KD, Hwang I, Ku KB, Lee S, Kim SJ, Kim C. Progress and challenges in the development of COVID-19 vaccines and current understanding of SARS-CoV-2 – Specific immune responses. J Microbiol Biotechnol 2020; 30: 1109-1115.

132) Calina D, Docea AO, Petrakis D, Egorov AM, Ishmukhametov AA, Gabibov AG, Shtilman MI, Kostoff R, Carvalho F, Vinceti M, Spandidos DA, Tsatsakis A. Towards effective COVID19 vaccines: updates, perspectives and challenges (Review). Int J Mol Med 2020; 46: 3-16.