VOLUME 32, NUMBERS 11 AND 12, JUNE 2021

ISSN: 1043-0342

Human Gene Therapy

Mary Ann Liebert, Inc. publishers

www.liebertpub.com/hgt

REVIEW

HGT

Current Update on Severe Acute Respiratory Syndrome Coronavirus 2 Vaccine Development with a Special Emphasis on Gene Therapy Viral Vector Design and Construction for Vaccination

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Severe acute respiratory syndrome (SARS) is a newly emerging infectious disease (COVID-19) caused by the novel coronavirus SARS-coronavirus 2 (CoV-2). To combat the devastating spread of SARS-CoV-2, extraordinary efforts from numerous laboratories have focused on the development of effective and safe vaccines. Traditional live-attenuated or inactivated viral vaccines are not recommended for immunocompromised patients as the attenuated virus can still cause disease via phenotypic or genotypic reversion. Subunit vaccines require repeated dosing and adjuvant use to be effective, and DNA vaccines exhibit lower immune responses. mRNA vaccines can be highly unstable under physiological conditions. On the contrary, naturally antigenic viral vectors with well-characterized structure and safety profile serve as among the most effective gene carriers to provoke immune response via heterologous gene transfer. Viral vector-based vaccines induce both an effective cellular immune response and a humoral immune response owing to their natural adjuvant properties via transduction of immune cells. Consequently, viral vectored vaccines carrying the SARS-CoV-2 spike protein have recently been generated and successfully used to activate cytotoxic T cells and develop a neutralizing antibody response. Recent progress in SARS-CoV-2 vaccines, with an emphasis on gene therapy viral vector-based vaccine development, is discussed in this review.

Keywords: SARS-CoV-2, COVID-19, vaccines, viral vectors

INTRODUCTION

THE NOVEL CORONAVIRUS named severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) first emerged in December 2019 in China as an outbreak of pneumonia, but soon spread to other countries leading to a serious worldwide pandemic.[1,2] The outbreak was declared a public health emergency of international concern on January 30, 2020, and a pandemic on March 11, 2020, by The World Health Organization. Because of its genetic similarity to bat coronaviruses, the virus is believed to have emerged from a bat-borne virus with a zoonotic origin.[3] Based on epidemiological studies conducted with the Wuhan strain, each infection is estimated to result in 5.7 new ones in the absence of immunity or vaccination.[4] COVID-19 disease symptoms include (but are not limited to) fever, dry cough, breathing difficulties (dyspnoea), headache, and pneumonia leading to eventual death (Fig. 1).[5] Acute lung injury, systemic inflammatory response syndrome, and acute respiratory distress syndrome (ARDS) have been observed in patients with COVID-19 similar to what has been described in SARS-CoV and Middle East Respiratory Syndrome (MERS)-CoV-infected patients.[6] Patients with severe COVID-19 exhibited a cytokine storm characterized by the overproduction of inflammatory cytokines such as interleukin (IL)-1β, IL-6, IL-12, interferon (IFN)-γ, and tumor necrosis factor-α.[5] The cytokine storm is responsible for causing ARDS, a systemic inflammatory response, and multiple organ failure.[7] Contrary to an anticipated inflammatory response as a result of a viral infection, a cytokine storm can cause serious damages to patients' health leading to high mortality rates if not handled properly.[8] As of April 2021, more than 133 million coronavirus cases in 220 countries were reported with 2.9 million causalities around the world.

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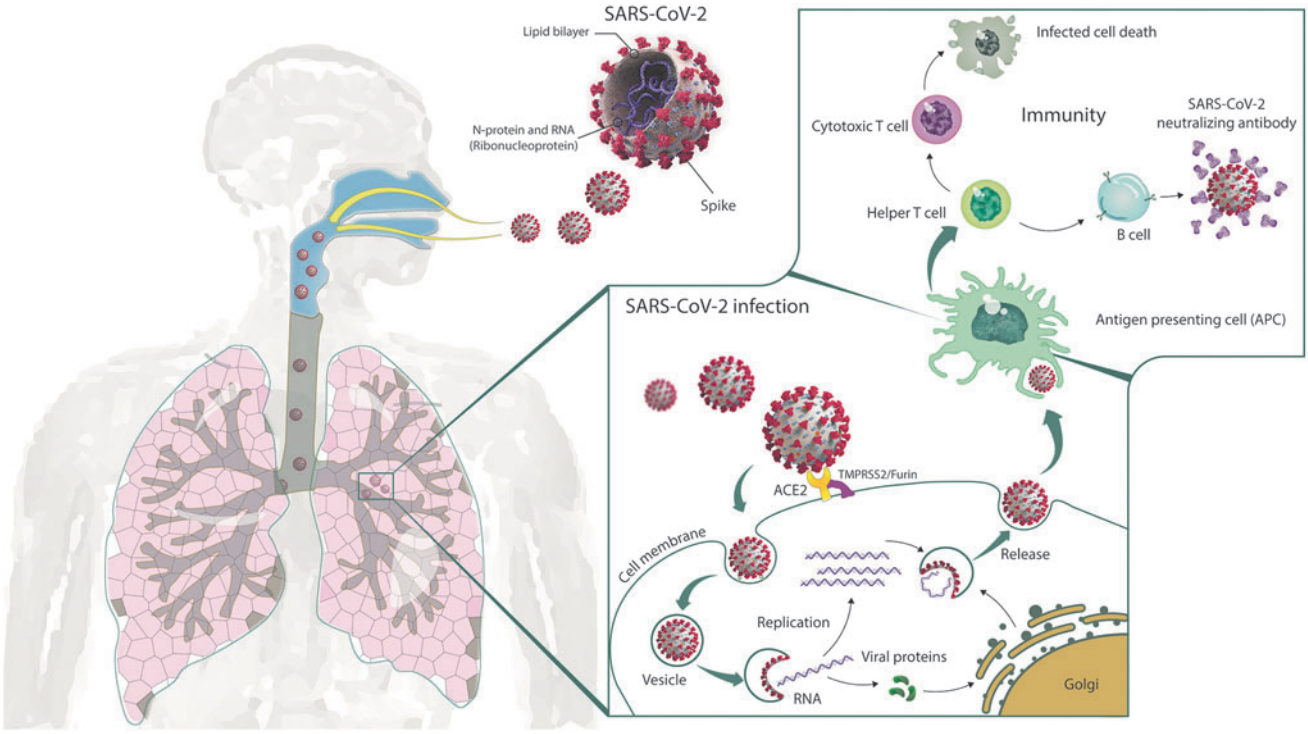

Figure 1. The mechanisms of host cell entry and immune system activation by SARS-CoV-2.[113] The life cycle and main route of transmission of SARS-CoV-2 are also depicted. Direct, indirect, or close contact with infected people may result in the transmission of SARS-CoV-2 via respiratory droplets expelled from an infected person through coughing, sneezing, or talking. Angiotensin I-converting enzyme-2 (ACE2) and transmembrane protease serine 2 (TMPRSS2) act as receptors involved in SARS-CoV-2 (or 2019-nCoV) entry into host target cells. Spike is cleaved by TMPRSS2 protease on the cell surface to ensure virus and host-cell membrane fusion. Then SARS-CoV-2 virus replicates within the cell and passes to the lower airways potentially leading to severe pneumonia. Antigen-presenting cells deliver viral antigens to immune cells for the activation of humoral and cellular immune responses. Helper T cells mediate cytotoxic T cell generation and activation leading to the destruction of SARS-CoV-2 infected cells. Neutralizing antibodies produced from the activated B cells bind and interfere with the SARS-CoV-2 binding to the targeted respiratory cells. The duration of protection depends on the presence and longevity of memory T and B cells in circulation. SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

Vaccination is the most effective way for protection against infectious diseases, and the development of a vaccine against SARS-CoV-2 has been a priority.[9] A guidance document on COVID-19 vaccines has recently been issued by The United States Food and Drug Administration.[10] An ideal vaccine is expected to have an excellent safety profile across multiple population groups (*e.g.*, immunocompromised individuals, children, older adults, pregnant women, and so on). It should ideally induce protective immunity within 2 weeks, and it should demonstrate at least 70% efficacy with minimal adverse events. Furthermore, long-lasting protection that engages both humoral and cellular immune responses lasting for at least 1 year is desired. The vaccine should also be stable at room temperature to avoid cold chain and transportation issues, and it should be amenable to mass production. Even though more than 90 SARS-CoV-2 vaccines have been under development by scientists in companies and universities around the globe, the process of vaccine development can not be rushed and may take even longer than expected. It is noteworthy to mention that more than 70% of the scientists leading vaccine research are from industrial or private firms. Furthermore, vaccine development might be hampered due to differences in severity, transmission rate, and the geographic distribution of different viral strains. To date, 149 mutations have been detected in 103 SARS-CoV2 strains sequenced, revealing L and S subtypes and these numbers are increasing each day.[11] For example, SARS-CoV-2 with a D614G substitution in the spike protein appeared in early 2020.[12] Soon after, the D614G mutation successfully substituted the SARS-CoV-2 strain originally discovered in China, becoming the dominant form of the virus around the globe.[13] Despite the increased infectivity and transmission rate, the strain with the D614G substitution neither caused severe illness nor modified the effectiveness of vaccines or therapeutics.[14] Another SARS-CoV-2 variant namely SARS-CoV-2 VOC 202012/01 recently emerged in the United Kingdom on December 14, 2020. This variant possessed 23 nucleotide substitutions leading to increased transmissibility without affecting disease severity. By the end of 2020, VOC-202012/01 variant (lineage B.1.1.7) had been reported in 31 other countries.[15] The other observed variants such as B.1.351 (501Y.V2, South African variant)[16] and P.1 (501Y.V3, Brazilian variant)[17] were also claimed to be more transmissible. Despite the variants, B.1.1.7, B.1.351,

and P.1 did not manifest enhanced host cell entry, variants B.1.351 and P.1 but not B.1.1.7 could escape from therapeutic antibodies (monoclonal antibodies with emergency use authorization [EUA] for COVID-19 treatment) or antibodies induced by natural infection or vaccination (convalescent plasma and sera).[18] These results suggest that the B.1.351 and P.1 variants might be able to successfully spread even in vaccinated individuals or convalescent patients elevating the risk for human health.

The development of an effective SARS-CoV-2 vaccine also requires the understanding of the molecular structure of the virus, since proper antigen and the vector type are essential to inducing optimum immune responses following vaccination.[19] SARS-CoV-2 is a member of Betacoronaviruses sharing 79.5% sequence identity to SARS-CoV and 50% homology to the MERS coronavirus. SARS-CoV-2 carries a positive-strand RNA genome of nearly 30 kbp in length (Fig. 2A, B). The 5′ end of the genome contains a long ORF1ab polyprotein consisting of 15–16 nonstructural proteins,[20] while the 3′ end of the genome encodes four major structural proteins, including the spike (S), nucleocapsid (N), membrane (M), and the envelope (E).[21] Importantly, all of these viral structural proteins may serve as antigen candidates for vaccine development as described below.

Assessment of viral structural proteins for vaccination studies

Even though SARS-CoV-2 has an affinity for angiotensin-converting enzyme 2 (ACE2)-expressing epithelial cells of the respiratory tract, systemic hyperinflammation coordinated by the adaptive immune system involving T cell-mediated cellular and B cell-mediated humoral immunity has been observed in patients with severe COVID-19.[22] Following infection with the virus, viral peptides are loaded and presented on the major histocompatibility complex (MHC) I of epithelial cells, monocytes, and dendritic cells (DCs). Cytotoxic T lymphocytes recognize the viral peptides and kill virus-infected cells by inducing apoptosis. Viral peptides presented on MHC II activate CD4+ helper T cells leading to the release of cytokines such as IL-2 and IL-6. Activation and proliferation of virus-specific B cells generate plasma cells secreting immunoglobulin M, immunoglobulin G (IgG), and immunoglobulin A antibodies that mediate

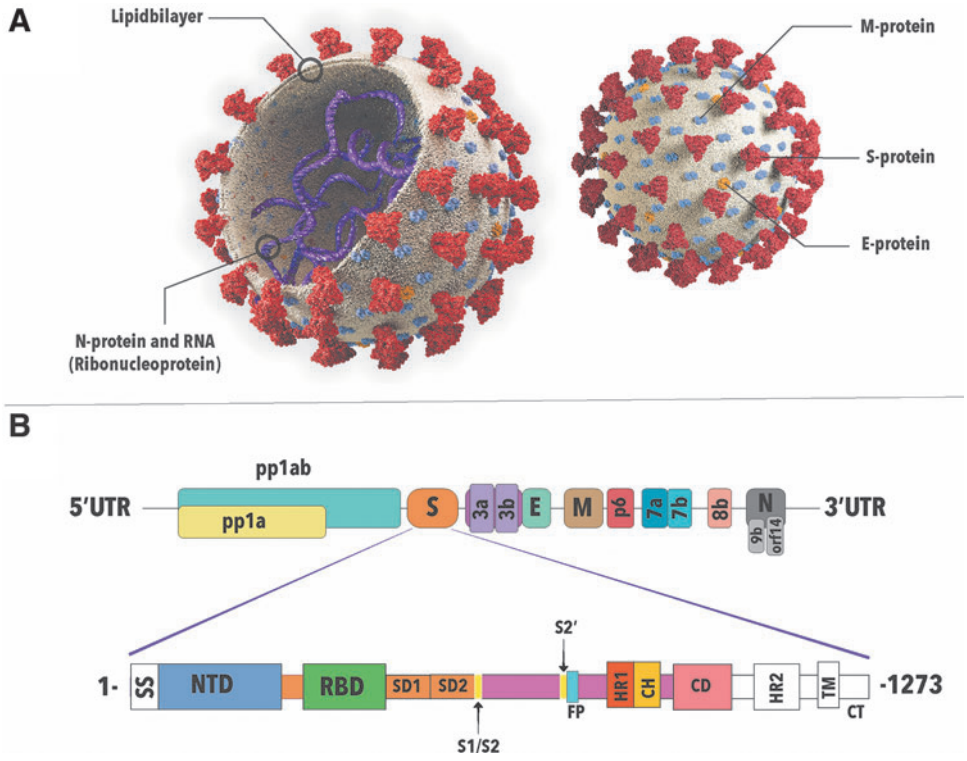

Figure 2. Virion structure and genome composition of SARS-CoV-2 virus.[187] **(A)** Schematic of SARS-CoV-2 virion structure. Locations of Spike, Envelope, Membrane, and Ribonucleoproteins are shown. **(B)** The genome of SARS-CoV-2 is ~30 kb in length. Fourteen ORFs are encoded by the RNA genome of SARS-CoV-2.[188] 5′ region encodes polyprotein (pp1a/ab) needed for virus replication and synthesis of structural proteins for the spike (S), envelop (E), membrane (M), and nucleoprotein (N). Accessory genes (3a, 3b, p6, 7a, 7b, 8b, 9b, and orf14) are not required for virus replication and they are located at the 3′ terminus. The composition of the SARS-CoV-2 Spike protein is given under the SARS-CoV-2 genome.[34] NTD, N-terminal domain; RBD, receptor-binding domain; SD1, subdomain 1; SD2, subdomain 2; SS, signal sequence; S2′, S2′ protease cleavage site; FP, fusion peptide; HR1, heptad repeat 1; CH, central helix; CD, connector domain; HR2, heptad repeat 2; TM, transmembrane domain; CT, cytoplasmic tail. *Arrows* denote protease cleavage sites. ORF, open reading frame.

virus neutralization. During SARS-CoV-2 infection, an antigen-specific immune response generated by activated T and B cells is essential for clearing the virus and protecting the patient from a deadly infection. As a result of uncontrolled virus replication, SARS-CoV-2 dampens antiviral IFN responses to evade the innate immune response. The infiltration of monocytes/macrophages, neutrophils, and adaptive immune cells results in the overproduction of proinflammatory cytokines.[23] Systemic inflammation eventually leads to vasodilation resulting in inflammatory lymphocytic and monocytic infiltration of the lung and heart. Pathogenic T cells secreting granulocyte-macrophage colony-stimulating factor have been implicated in the recruitment of inflammatory IL-6-secreting monocytes and severe lung pathology in patients with COVID-19.[24] The severity of the inflammation has been attributed to the magnitude of the cytokine storm—especially IL-1β, IFN-γ, CXCL10, and CCL2.[25] Thus, the analysis of SARS-CoV-2 structural protein-derived antigenic peptides is essential to produce a vaccine to achieve optimum stimulation of innate and adaptive immune responses to restrain the spread of SARS-CoV-2.[26]

Whole-cell antigens (WCA), such as proteins, lipids, polysaccharides, and nucleic acids, of the virus are mainly used in the development of either dead or live-attenuated vaccines.[27,28] Although WCA is a quick and easy way of producing a vaccine and routinely used in many laboratories worldwide, quality assurance and efficacy studies are unreliable due to their complex nature. Conversely, the spike protein appears to be the most promising antigen candidate in SARS-CoV-2 vaccine development for the following reasons. First, spike proteins of SARS-CoV and MERS-CoV viruses have already been shown to be effective for vaccination.[29–33] Second, the spike protein is present on the virus surface, which can easily be recognized by the host defense system resulting in the activation of a robust immune response against the virus itself.[34] Finally, the spike protein mediates viral transduction via interaction with the ACE2 receptors followed by endocytosis.[35] These results suggested that vaccines based on the spike protein could induce antibodies that prevent the virus from both binding to the host cell and fusing with the host cell membrane. Compared to all structural proteins of SARS-CoV, the spike protein appeared to be the main immunogenic protein capable of inducing both cellular and humoral immunity against virus infection. Intriguingly, the structure of spike protein carries some clues for the development of an effective vaccine against SARS-CoV-2 as explained below.

SARS-CoV-2 spike protein, in monomeric form, consists of 1,273 amino acids and has a molecular weight of about 140 kDa (Fig. 2B). Spike protein contains two subunits (S1 and S2) and can naturally form a trimer. Moreover, the spike protein plays essential roles in viral transduction and pathogenesis; specifically, the S1 subunit recognizes and binds to host ACE2 receptors, and then the S2 subunit undergoes conformational changes resulting in the fusion of the viral envelope with the host cell membrane.[30] While the receptor-binding domain (RBD) of the spike protein is located at the C-terminal domain of the S1 subunit, membrane fusion is mediated by the fusion peptide (FP) located in the S2 subunit.[36] The discovery of prefusion conformation of spike protein in trimeric form and the interaction of RBD with ACE2 receptors drastically influenced vaccine design against SARS-CoV-2.[34,35]

Various fragments of the spike protein (the full-length spike protein, the RBD domain, the S1 subunit, N-terminal domain [NTD], and FP) have been all individually tested as antigens in vaccine development with varying degrees of success. Full-length spike proteins in their natural forms are expected to serve as better antigens as compared to truncated versions due to the presentation of more immunogenic epitopes. As expected, numerous vaccine candidates based on the full-length spike protein of SARS-CoV have already been reported. For example, the full-length spike protein SARS-CoV Urbani strain encoded by a DNA vaccine generated both protective neutralizing antibody and T cell responses in mice.[37] Mice immunized with baculovirus-produced spike protein complexed with nanoparticles in an alum adjuvant formulation yielded a high titer of neutralizing antibodies.[38] Vaccination of mice or monkeys with attenuated modified vaccinia Ankara (MVA) virus encoding the full-length spike protein of the SARS-CoV Urbani strain or HKU39849 strain-induced neutralizing antibodies and decreased viral titers in the respiratory tracts of animals after homologous SARS-CoV challenge.[39,40] Mice, camels, and rhesus macaques immunized with a DNA vaccine encoding MERS-CoV spike protein had reduced typical clinical symptoms of the disease during the infection.[41] Finally, vaccination of mice and hamsters with a full-length S protein trimer protected the animals from the homologous SARS-CoV (HKU39849 strain) challenge.[42] These data collectively highlight the potential for the highly immunogenic full-length S protein to elicit neutralizing antibodies capable of suppressing virus proliferation and protection against SARS-CoV challenge. However, it is important to keep in mind that SARS vaccines encoding the full-length S protein may also activate destructive immune responses yielding liver and lung damage of the vaccinated animals by antibody-dependent enhancement (ADE).[30,43–45] The region of the SARS-CoV-2 spike protein responsible for the generation of harmful immune responses remains unknown, and ADE has not been detected in cases where effective neutralizing antibody formation was achieved.

Since the RBD of the spike protein in the S1 subunit interacts with ACE2 receptors on the host cell surface, antibodies generated by RBD-based vaccines are expec-

ted to interfere with virus binding and entry into the cells.[46] Most of the SARS-CoV[47] and MERS-CoV[48] subunit vaccines were developed using the RBD domain carrying multiple conformational neutralizing epitopes as an antigen.[49] Consequently, intramuscular and mucosal administration of an adeno-associated virus (AAV)-based vaccine encoding RBD (RBD-rAAV) stimulated satisfactory neutralizing antibody production that inhibited homologous SARS-CoV (GZ50) challenge in mice.[50,51] However, this approach has been disputed since coronaviruses can display antibody escape mutations in the RBD domain.[52] Because of this reason, the use of different epitopes of SARS-CoV-2 to induce an effective immune response has been advised to avoid the immune escape of the virus. Other virus surface proteins, except the E Protein (E Protein), are suitable antigens with varying degrees of effectiveness for vaccine development, such as the NTDs, S1 Subunit, the FP domain of the S2 subunit, N Protein, and M Protein.[19]

SARS-CoV-2 VACCINES UNDER DEVELOPMENT

Live-attenuated or inactivated vaccines

Live-attenuated vaccines prepared from weakened viruses under laboratory conditions have been used since the 1950s.[53] These vaccines can replicate in a vaccinated individual and produce an immune response with mild or no disease. Compared to an infection with the wild-type virus, live-attenuated viral vaccines can generate an exceptional immune response providing recurrent antigenic stimulation needed for the generation of memory cells. However, there are some safety concerns regarding the use of these vaccines. Live-attenuated pathogens have the potential (although rare) to revert to a pathogenic form and cause disease in vaccinated individuals. Furthermore, individuals with compromised immune systems may not be able to respond effectively. For instance, the formalin-inactivated poliovirus vaccine (IPV) developed by Jonas Salk was licensed for use in 1955.[54] While polio was finally brought under control after implementing the use of IPV, making Jonas Salk a national hero in the United States, failure to properly inactivate IPV in the early batches resulted in vaccine-associated outbreaks of polio—known as Cutter incident.[55] In the 1960s, Albert Sabin developed attenuated versions of the oral poliovirus vaccine (OPV) by passaging virulent viruses through different animals and cells.[56] However, the Sabin infectious/attenuated poliovirus vaccines also caused vaccine-associated paralytic poliomyelitis in a small number of recipients.[57] Since properly inactivated Salk vaccine did not accidentally cause poliomyelitis, OPV was eventually replaced with Salk's vaccine as recommended by federal authorities in 1999.[58] Nevertheless, Codagenix, Inc., in collaboration with the Serum Institute of India, Ltd. agreed to develop a live-attenuated vaccine against SARS-CoV-2. COVI-VAC, which is a single-dose intranasal, live-attenuated vaccine against SARS-CoV-2, was developed with Codagenix's Synthetic Attenuated Virus Engineering (SAVE) platform using synthetic biology to recode the genes of viruses into a safe and stable vaccine.[59] This vaccine is currently being tested in a randomized, double-blind, placebo-controlled, dose-escalation trial involving 48 volunteers.[60]

Whole-cell killed/inactivated vaccines are made from viruses that have been killed through physical (heat) or chemical (formaldehyde) processes. Individuals vaccinated with whole-cell killed or live-attenuated vaccines may display diverse immune responses against the pathogen since these vaccines present various immunogenic components to the host.[61] Because whole-cell killed/inactivated vaccines are considered to be traditional vaccines, easy to make and produce, they can quickly enter into clinical trials. Several institutions around the world have successfully isolated SARS-CoV-2 strains and immediately initiated traditional vaccine development to save time. China approved a clinical trial for a candidate COVID-19 vaccine named CoronaVac developed by Sinovac in mid-April 2020.[62] CoronaVac, which is a chemically inactivated whole virus vaccine for COVID-19, has since entered Phase 3 clinical trials in Brazil, Chile, Indonesia, Philippines, and Turkey.[9] The Butantan Institute has revealed the vaccine efficacy to be 50.4% in Brazil.[63] Another inactivated virus-based COVID-19 vaccine known as Covaxin (BBV152) has been developed by Bharat Biotech in collaboration with the Indian Council of Medical Research.[64] This whole-virion inactivated SARS-CoV-2 vaccine produced in Vero cells was prepared with a Toll-like receptor7/8 agonist molecule adsorbed to alum. The safety and tolerability of Covaxin were revealed in the phase 1 vaccine trial, which evaluated 375 participants in four groups.[65] Tolerable safety outcomes and enhanced humoral- and cell-mediated immune responses were observed in phase 2 clinical trial involving 380 participants.[66] A randomized, double-blinded, placebo-controlled Phase 3 study involving 25,800 participants between 18 and 98 years of age covering a total of 22 sites across India was initiated in November 2020. On January 3, 2021, the Drugs Controller General of India granted the EUA for the vaccine, which is still in clinical trials. As of March 2021, the interim efficacy rate for the phase 3 trial was reported to be $\sim$81%.[67]

mRNA vaccines

Nucleic acid therapeutics, particularly those involving mRNA constructs, have appeared as promising alternatives to traditional vaccine approaches in recent years. Major technological innovation and research in mRNA synthesis, modification, and delivery technology have allowed mRNA to become a promising therapeutic approach in vaccine development. Compared to live-attenuated, whole-cell killed/inactivated vaccines, including DNA-

based approaches, mRNA-based vaccines offer several benefits. Because mRNA is noninfectious and does not integrate into the genome, there is no potential risk of infection or insertional mutagenesis. The half-life and immunogenicity of the mRNA in cells can easily be modulated through genetic modification and delivery methods.[68] Covalent conjugates, protamine complexes, nanoparticles based on lipids or polymers, and hybrid formulations can be formulated into carrier molecules for efficient *in vivo* delivery of mRNA.[69] Furthermore, mRNA vaccines can be administered repeatedly without any concern over antivector immunity. Rapid, inexpensive, and scalable manufacturing of mRNA are the other benefits of using mRNA-based vaccines as nucleic acid therapeutics.[70] mRNA-based vaccine development involves the selection of antigen, sequence optimization, and modification, assessment of immune response, and safety following administration.[71] Storage and handling requirements related to the need for freezing temperatures due to rapid degradation and increased reactogenicity in a subset of vaccinated individuals regarding side effects such as fever, muscle aches, and fatigue appeared to be the main limitations to mRNA-based vaccine platforms.[72]

BioNTech in cooperation with Pfizer has developed an mRNA-based COVID-19 vaccine sold under the brand name Comirnaty (Tozinameran code-named BNT162b2). The Pfizer-BioNTech COVID-19 vaccine is composed of nucleoside-modified mRNA (modRNA) encoding a mutated form of the spike protein of SARS-CoV-2 (D614G), which is encapsulated in lipid nanoparticles. Modification of select nucleosides was necessary to suppress intrinsic immunogenic properties of the mRNA of interest. Structural engineering of the modRNA and nucleoside modification converted a virus mRNA encoding spike protein into a surrogate cellular mRNA with higher protein expression efficacy. BioNTech has demonstrated that the modification of select nucleosides in the manufactured mRNA suppresses its intrinsic immunogenic properties while resulting in superior protein production. Clinical trials of the Pfizer-BioNTech COVID-19 vaccine began in April 2020.[73] After testing a total of 43,548 participants at 152 sites worldwide by December 2020, a two-dose regimen of the Pfizer-BioNTech COVID-19 vaccine conferred 95% protection against Covid-19 in persons 16 years of age or older.[74] This vaccine became the first COVID-19 vaccine to be authorized by a stringent regulatory authority for emergency use in individuals 16 years of age and older on December 11, 2020 and was the first to be cleared for regular use.[75]

The Moderna COVID-19 vaccine, known as mRNA-1273, is an mRNA-based COVID-19 vaccine developed by the National Institute of Allergy and Infectious Diseases, Moderna, and the Biomedical Advanced Research and Development Authority. This vaccine uses a technology involving a modRNA compound to induce immunity to SARS-CoV-2 by encoding a prefusion-stabilized spike protein naturally present on the surface of SARS-CoV-2 particles.[76] On December 30, 2020, the Phase 3 randomized, observer-blinded, placebo-controlled clinical trial enrolling 30,420 volunteers was conducted at 99 centers across the United States demonstrating 94.1% efficacy in preventing COVID-19 infection, including severe disease.[77] Apart from transient local and systemic reactions, no safety concerns were revealed. The Moderna COVID-19 vaccine was issued an EUA by the United States Food and Drug Administration (FDA) on December 18, 2020. Despite the Moderna and the Pfizer-BioNTech vaccines having similar efficacy, the Moderna vaccine requires storage at the temperature of 2–8°C for up to 30 days or −20°C for up to 4 months, whereas the Pfizer-BioNTech vaccine requires ultracold freezer storage between −80°C and −60°C. Although differences in lipid nanoparticle formulations or mRNA secondary structures were reasoned for the thermostability differences between the two vaccines, variations in storage requirements and shelf lives do not negatively impact their efficacy.[78]

DNA vaccines

DNA vaccines offer several benefits compared with conventional live-attenuated vaccines; for example, they induce both cellular and humoral immunity without replication, enable the creation of a vector encoding different antigens in a single preparation, and large-scale but low-cost manufacture.[79] Preserving the quality of the live vaccines requires cold storage, but DNA vaccines are highly stable without the need for refrigeration at low temperatures. DNA-based vaccines comprised plasmids encoding one or more antigens. Compared to mRNA-based vaccines, they are more stable, but carry insertional mutagenesis risks due to vector integration.[80] Intriguingly, all current clinical trials of DNA vaccines utilize the delivery of the spike protein as the antigen.[81] Inovio Pharmaceuticals (Plymouth Meeting, PA) developed a DNA vaccine using a plasmid pGX9501 encoding the SARS-CoV-2 spike protein as the antigen (INO-4800). The vaccine administered intradermally through electroporation is now under a phase 1 open-label study to evaluate the safety, tolerability, and immunogenicity of INO-4800.

Subunit vaccines

Similar to inactivated whole-cell vaccines, subunit vaccines do not contain live virus particles and are considered very safe. However, they only contain the antigenic parts of the virus to elicit a protective immune response different from the inactivated whole-cell vaccines. To achieve this, antigenic properties of the various subunits of the virus must first be studied in detail to determine which particular mixtures will yield an effective immune response. Nevertheless, the generation of a strong protective immune response requires the addition of adjuvants.

Currently, almost all SARS-CoV-2 subunit vaccines under testing use the spike protein as the antigen. Clover Biopharmaceuticals, Inc., developed a subunit vaccine candidate against SARS-CoV-2 using the Trimer-Tag technology for the expression of trimeric spike protein in mammalian cells.[82] After completing primate studies, a Phase 1 randomized, observer-blind, placebo-controlled clinical trial involving 150 adult and elderly participants was started in June 2020 to assess the safety and immunogenicity of the adjuvanted COVID-19 S-Trimer vaccine at multiple-dose levels. A similar subunit vaccine based on the ''molecular clamp'' technology was also developed by the University of Queensland in collaboration with a global biotech company CSL.[83] A molecular clamp is a polypeptide-based technology designed to preserve the prefusion configuration of peptides to generate a better immune response in experimental vaccines. However, this vaccine failed to proceed to further clinical trials due to ''false positive'' results to human immunodeficiency virus (HIV) tests.[84]

Peptide-based vaccines are chemically synthesized small fragments of intact antigens. They are very easy to make and produce in large quantities. Unfortunately, due to their small size, they often exhibit weak immunogenicity requiring detailed structural modification and the addition of adjuvants.[85] Currently, many peptide-based SARS-CoV-2 vaccine candidates are being used in clinical trials.[86]

Viral vector-based vaccines

One of the effective tools for controlling infectious diseases is genetic immunization by way of induction of strong protective immunity. DCs acting as professional antigen-presenting cells (APCs) trigger strong T and B cell-mediated immunity. Delivery of antigens into DCs *ex vivo*, and then transfer into patients was the first method used in DC-based vaccines developed against infectious diseases. The discovery of viral vectors paved the way to the virus-mediated direct transfer of antigens *in vivo* leading to prolonged immunity.[87] Viral vector-based vaccine development against infectious agents involves the introduction of antigenic full-length or truncated viral surface protein-encoding genes (*e.g.*, SARS-COV2 spike) into viral expression vectors. After verification of viral vector-driven antigen expression in cell lines, immunization in preclinical animal models is carried out to assess the vaccine efficacy before conducting clinical trials.[88] In this scenario, viral vectors serve as gene transfer vehicles generating immune responses by way of heterologous gene transfer. Because they are naturally immunogenic and well-characterized, they soon became the preferred vector of choice in vaccine development.[89] The most used viral vectors for vaccination studies are adenovirus (Ad), AAV, poxvirus, and lentiviruses. Viral vector-based vaccines generate a very strong cellular immune response, as infection of immune cells leads to the stimulation of humoral immune response due to their inherent adjuvant properties. Compared to conventional vaccines, they can be modified for specific targeting and provide prolonged antigen presentation. The potential application of viral vectors in humans ranges from infectious diseases to cancer treatments. High levels of recombinant protein expression is achievable by viral vectors, which provided the basis for modern vaccine development. One benefit of using viral vectors to transport vaccine antigens is that the live viral vectors may generate robust mucosal humoral immune response[90] much better than what is accomplished by other vaccine candidates, such as protein and DNA vaccines.[91,92]

Ad structure and vector design strategy

Ad is a nonenveloped, double-stranded DNA virus with a 90–100 nm icosahedral capsid composed of penton and hexon subunits. Attachment to host cells is mediated by fiber and knob domains, while the penton base is involved in the secondary interactions required for virus entry into the cell (Fig. 3A). Based on the virus serotype, the affinity of the knob domain varies primarily depending on the use of coxsackievirus Ad receptor, CD46, and various integrins for cellular entry. Ads possess a linear dsDNA genome of 36 kb (26–48 kb), categorized into early (*E1*, *E2A*, *E2B*, *E3*, and *E4*), intermediate (*IVa2* and *IX*), and late (*L1*, *L2*, *L3*, *L4*, and *L5*) genes flanked by inverted terminal repeats (ITRs) sequences (Fig. 3B). Since *E1* is involved in virus replication and *E3* interferes with the host immune response, *E1* deletion in association with *E3* results in the generation of replication-deficient Ad called first-generation vectors creating a transgene capacity of 6.5 kb for cloning, just as the *E1A/E1B* deletion frees up space for a transgene that is 4.5 kb in length.[93] The *E2* and *E4* regions of Ad play essential roles in viral DNA replication and viral mRNA export, respectively. Deletion of these regions in addition to *E1* and *E3* reduces immunogenicity and prevents leaky viral gene expression, which led to the second-generation Ad vectors. Unfortunately, apart from increasing transgene capacity to 10.5 kb, second-generation Ad vectors have had limited use since these vectors do not provide much benefit over their first-generation counterparts. Despite prolonged transgene expression, second-generation Ad vectors can still trigger host immunogenicity and cellular toxicity.[94] The removal of the entire protein-coding region of the Ad backbone, except for the ψ packaging signal and flanking ITR sequences, results in the generation of gutless viruses called third-generation vectors. These changes are essential to decrease the immunogenicity of the vector while prolonging transgene expression and improving the safety of Ad vectors. Gutless vectors have a cargo-carrying capacity of 36 kb with a minimized chance of producing replication-competent virus. Current adenoviral vectors specific for COVID-19 primarily use the first-generation Ad, which only supplies short-term transgene expression *in vivo*. However, if prolonged transgene expression is desired without sacrificing the natural adjuvant property

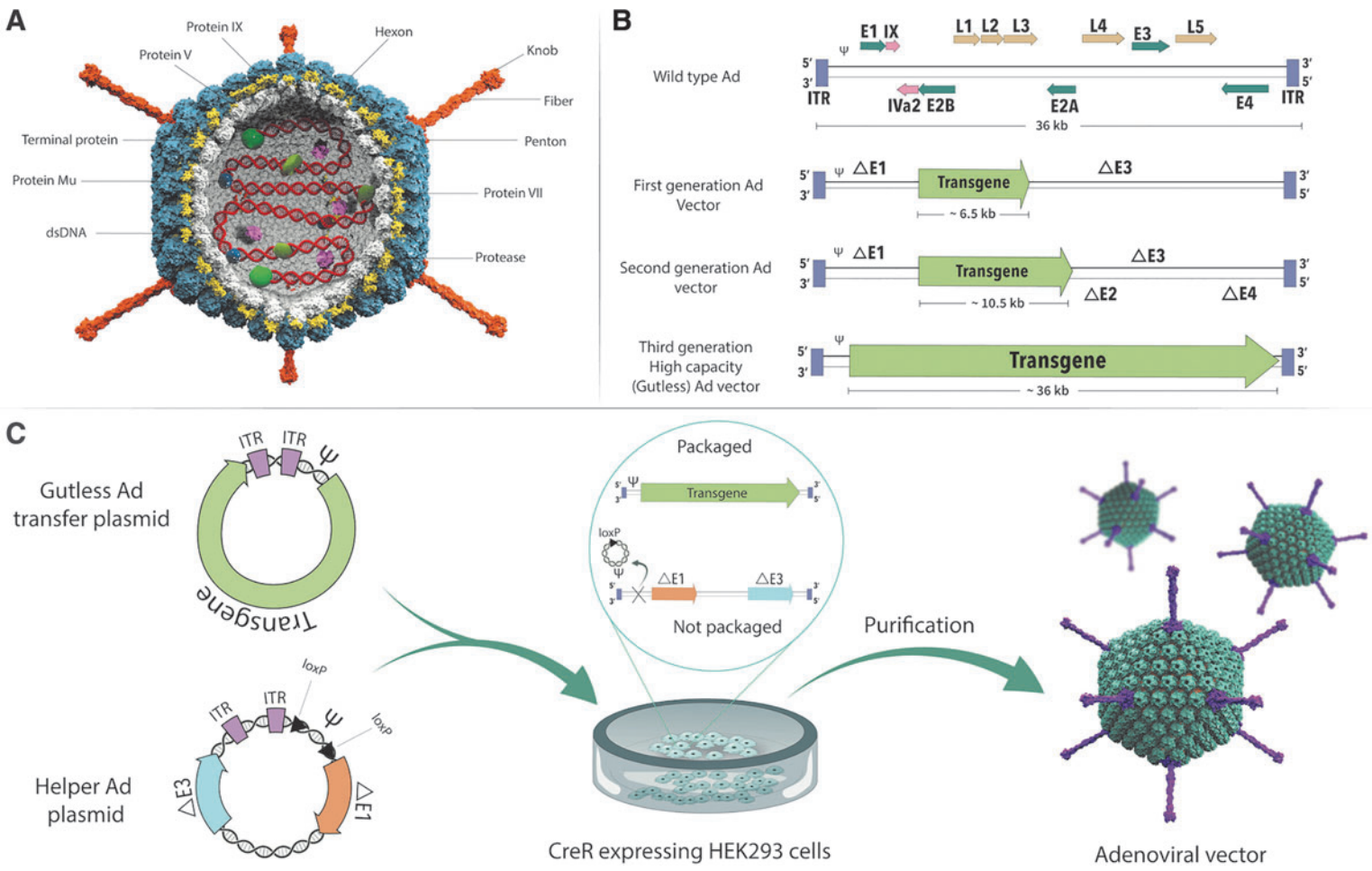

Figure 3. Ad structure and vector design strategy.[189] **(A)** Molecular structure of Ad displaying a linear dsDNA genome, hexon capsids, penton bases, fiber, and knob domains of spike proteins. **(B)** Comparative analysis of wild-type Ad genome with first, second, and third generations of adenoviral vectors.[93] Genomic locations of early and late genes, flanked by ITR, and ψ packaging signal are provided for wild-type Ad. While first-generation adenoviral vectors carry deletions at the *E1* and/or *E3* regions; additional genes, *E2* and *E4* are deleted in addition to *E1* and *E3* in the second-generation vectors.[96] All coding viral regions are removed in the third-generation vectors, also called gutless or gutted Ad. **(C)** Gutless Ad production scheme.[95] The transfer vector carries the transgene, packaging signal, and ITRs. A helper Ad plasmid, which is similar to that of the first-generation adenoviral vector with packaging signal flanking loxP sites is utilized to produce the gutless adenoviral vector. Transient transfection of Cre recombinase (CreR) expressing HEK293 cells with the transfer vector and the helper Ad encoding plasmid exclusively results in the packaging of gutless Ad vectors but not the helper virus genome. Ad, adenovirus; ITR, inverted terminal repeat.

of the virus, then gutless adenoviral vectors might be preferred so the vector-transduced cells only express the vaccine antigen (spike) and not Ad antigens (Fig. 3C).

Among the gene therapy vectors tested, Ad vectors are the most effective gene delivery vehicles in carrying foreign antigens to host cells.[95] Compared to other virus-based gene delivery systems such as lentivirus, retrovirus, and AAVs, they are more immunogenic and activate both the innate and adaptive immune response.[96] Moreover, they are the preferred vector of choice in cancer gene therapy applications.[97–105] Contrary to stable and high levels of transgene expression required in target cells for classical gene therapy approaches, transient antigen expression might be clinically more beneficial for vaccination purposes. In comparison to short-term overexpression, prolonged low levels of antigen expression minimize antigen-induced apoptosis of T cells and increase the persistence of memory T cells.[106] By this token, Ad vectors provide only transient gene expression due to their antigenicity. Ad-based vectors can function as a ''self-adjuvant,'' leading to the activation of multiple innate immunostimulatory pathways following infection, which may boost the immunogenicity of the encoded antigen. Conversely, the triggering of undesired innate immunity pathways also has the potential to be damaging to their existence resulting in the clearance of Ad-transduced cells.[107] Vigilant engineering of adenoviral vectors is needed to use Ad vectors for both gene therapy and vaccination purposes. Besides, their natural affinity for upper airway epithelial cells makes them useful in intranasal vaccination against mucosal surface-infecting viruses such as influenza and SARS-CoV-2. Ad-based vaccines can be used as nasal sprays making them practical to use in mass.[108]

Ad-based vaccines

Compared to other viral vectors, Ad vector-based vaccines are easy to design and produce on a mass scale, which is of paramount significance for clinical use. In addition to inherent adjuvant properties, and the ability to induce robust transgene-specific T and B cell responses, Ad vectors are easy to purify to high titer, are genetically stable, and can be delivered via oral, intramuscular, intradermal, and aerosol routes.[109] Ad triggers several innate immune signaling pathways resulting in the secretion of several proinflammatory cytokines. These proinflammatory cytokines pave the way to effective immune cell stimulation leading to the induction of robust adaptive humoral and cellular immune responses. Recent studies have demonstrated that Ad-vector vaccines induced better humoral and cellular immune responses than recombinant protein vaccines, plasmid-based DNA vaccines, and other recombinant vector systems currently available (Table 1). Since the nasal mucosa is the first line of defense against SARS-CoV-2 before viral spread to the lung, Ad-mediated intranasal vaccination might be an attractive strategy to prevent COVID-19. An intranasal Ad type 5 (Ad5)-vectored vaccine encoding the RBD of the SARS-CoV-2 spike protein called AdCOVID has activated both systemic and local immune responses successfully inducing mucosal immunity against COVID-19 in mice.[110]

Building on CanSinoBIO's Ad-based viral vector vaccine technology platform, Convidicea (Ad5-nCoV) has become the first novel coronavirus vaccine for COVID-19 in a clinical trial in China.[111] This vaccine is a recombinant Ad5-vectored COVID-19 vaccine expressing the spike glycoprotein of SARS-CoV-2. Immunogenicity and safety of a recombinant Ad5-vectored COVID-19 vaccine in healthy adults aged 18 years or older were assessed in a randomized, double-blind, placebo-controlled phase 2 trial involving 508 eligible participants.[112] The Ad5-vectored COVID-19 vaccine (consisting of 5×10^{10} viral particles) was safe and induced significant immune responses in the majority of recipients after a single immunization. In February 2021, data obtained from an interim analysis of Phase 3 trials involving 30,000 participants and 101 COVID cases revealed that the vaccine had an efficacy of 65.7% at preventing moderate cases of COVID-19 and 90.98% efficacy at preventing severe cases. This

Table 1. *Adenovirus-based severe acute respiratory syndrome coronavirus 2 vaccines under development*

Vaccine Brand Name	*Vector Type*	*Clinical Efficacy (% Protection) Moderate/Severe Cases*	*Company Name*	*EUA Approval Status*
Ad5-nCoV Convidicea	Ad type-5	65.7/91	CanSinoBIO/China	Approved in China, Mexico and Pakistan
Ad26.COV2-S Janssen COVID-19	Ad type-26	66/85	Johnson and Johnson (J&J) and Janssen Pharm, United States	FDA approved
ChAdOx1 AZD1222	Simian adenovirus (ChAd) serotype Y25	62/90	Jenner Institute, University of Oxford, United Kingdom	European Medicines Agency approved
Gam-COVID-Vac Sputnik V	Ad types-26 and 5	91.6	Gamaleya Research Institute Russia	Approved in Russia and 21 other countries
VXA-CoV2-1	Ad type-5 adjuvanted oral tableted vaccine	NA	Vaxart, United States	In Phase 1 trial (NCT04563702)
AdCOVID	Ad type-5 intranasal vaccine	NA	Altimmune, Inc., United States	In Phase 1 trial (NCT04679909)

Ad, adenovirus; EUA, emergency use authorization; FDA, Food and Drug Administration; NA, not available.

vaccine is currently licensed for use in the Chinese military. In February 2021, China approved the vaccine for general use.

Johnson and Johnson (J&J) and Janssen Pharmaceutical research teams in collaboration with Beth Israel Deaconess Medical Center, constructed a vaccine candidate Ad26.COV2-S (Ad26COVS1; Janssen COVID-19), a nonreplicating Ad26-based vector expressing the stabilized prefusion spike protein for the prevention of coronavirus infection.[113] A multicenter phase 1/2a randomized, double-blinded, placebo-controlled clinical study to assess the safety, reactogenicity, and immunogenicity of Ad26.COV2.S was designed (NCT04436276). The safety profile and immunogenicity after only a single dose were supportive for further clinical development of Ad26.COV2.S at a dose level of 5×10^{10} virus particles, as a potentially protective vaccine against COVID-19. On September 23, 2020, J&J announced the launch of its large-scale, pivotal, multicountry Phase 3 trial (ENSEMBLE) for its COVID-19 vaccine candidate (JNJ-78436735) developed by its Janssen Pharmaceutical Companies. On February 27, 2021, the United States FDA issued an EUA for the third vaccine (the Janssen COVID-19 vaccine) for the prevention of COVID-19. The safety data were obtained from 43,783 participants enrolled in an ongoing randomized, placebo-controlled study being conducted in South Africa, in South America, Mexico, and the United States 21,895 vaccine recipients and 21,888 saline placebo volunteers were tracked for a median of 8 weeks after vaccination. The Janssen COVID-19 vaccine was nearly 66% effective in preventing symptomatic COVID-19 and 85% effective in stopping severe COVID-19. The EUA permits the Janssen COVID-19 vaccine, which is administered as a single dose to be circulated in the United States for use in individuals 18 years of age and older.

The University of Oxford in collaboration with AstraZeneca has developed a replication-deficient simian Ad vector (ChAdOx1) carrying the full-length codon-optimized coding sequence of the structural spike protein of SARS-CoV-2 with a tissue plasminogen activator (tPA) leader sequence as an nCoV-19 vaccine (AZD1222). The safety, reactogenicity, and immunogenicity of a chimpanzee Ad-vectored vaccine (ChAdOx1 nCoV-19) expressing the spike protein of SARS-CoV-2 were assessed in a phase 1/2, single-blind, randomized controlled trial in healthy adults aged 18–55 years in five trial sites across the United Kingdom.[114] Results indicated the ChAdOx1 nCoV-19 vaccine given at a dose of 5×10^{10} viral particles was safe, well-tolerated, and immunogenic. Even though a single dose was sufficient to provoke both humoral and cellular responses against SARS-CoV-2 without serious adverse reactions, booster immunization was needed to enhance neutralizing antibody titers paving the way for phase 3 trials. Based on this encouraging data, phase 3 trials were launched in the United Kingdom (2020-001228-32), Brazil (ISRCTN89951424), United States (NCT04516746), Russia (NCT04540393), and India (CTRI/2020/08/027170).[115] The interim analysis of the clinical trial in the United Kingdom (2020-001228-32) and Brazil (ISRCTN89951424) demonstrated the nCoV-19 vaccine ChAdOx1 to have an average efficacy of 70%, based on analyzing a total of 131 COVID-19 cases from 11,636 volunteers as revealed on November 23, 2020. However, 90% efficacy was achieved using the dosing regimen where the vaccine was initially given as a half dose followed by a second full dose ($n = 2{,}741$). This finding is very intriguing considering that the regimen consisting of two full doses had only 62% efficacy ($n = 8{,}895$). The most suitable regimen of AZD1222 awaited further clinical trials with the hope of resolving the dose-response discrepancy between different subgroups. Due to a severe adverse reaction (transverse myelitis) observed in a previously healthy 37-year-old woman, AstraZeneca gave a voluntary pause to its phase 3 COVID-19 vaccine trial in the United Kingdom on September 9, 2020. Accordingly, scientists advised caution and pointed out the necessity for addressing the safety concerns.[116] After a brief pause, AstraZeneca resumed the trial in the United Kingdom on September 12, 2020. The vaccine has been approved by the United Kingdom, Argentina, El Salvador, India, and the Dominican Republic regulatory authorities for emergency usage. However, there are still some concerns regarding the possibility of dominant immunogenicity toward the Ad vector genes rather than the transgenes. On January 29, 2021, Oxford/AstraZeneca vaccine was recommended for the European Medicines Agency approval for granting conditional marketing authorization for people 18 years of age and older. Even though both the AZD1222 and Janssen COVID-19 vaccines use spike protein as an antigen, differences in transgene design (tPA attached spike vs the stabilized prefusion spike) and vector choice (chimpanzee vs. human) might account for differences in efficacy and dose requirements (one vs. two-dose regiments) to achieve protection from COVID-19.

Gam-COVID-Vac, trade-named Sputnik V and developed by Gamaleya Research Institute Epidemiology and Microbiology, Health Ministry of the Russian Federation, is a viral two-vector vaccine (rAd26-S + rAd5-S) based on two human Ad vectors, which encode spike protein of SARS-CoV-2 to stimulate an antiviral immune response. The Ad26-based vaccine is used for the initial immunization, and the Ad5 vaccine is given 21 days later to boost the immune response. The safety and immunogenicity of an rAd26 and rAd5 vector-based heterologous prime-boost COVID-19 vaccine were studied in Phase 1–2 trials involving 76 participants from Russia. The results suggested that the heterologous rAd26 and rAd5 vector-based COVID-19 vaccine has a good safety profile and induced strong humoral and cellular immune responses in partici-

pants. On August 11, 2020, Russia was the first country to approve a vaccine against COVID-19 (Sputnik V). However, scientists around the world have raised concerns about the clinical trials conducted to evaluate its safety and efficacy.[117] As of December 2020, only Belarus and Argentina have received EUAs for Gam-COVID-Vac outside of the Russian Federation. The federal authorities announced the start of a large-scale free-of-charge vaccination program with Gam-COVID-Vac for Russian citizens in early December 2020. Currently, Gam-COVID-Vac is in Phase 3 trials involving more than 40,000 participants in Russia and Belarus with the intent of expanding to various other countries such as UAE, India, Venezuela, Egypt, and Brazil. An interim analysis of the safety and efficacy of an rAd26 and rAd5 vector-based heterologous prime-boost COVID-19 vaccine was recently revealed in a randomized controlled phase 3 trial (NCT04530396) involving 25 hospitals and polyclinics in Moscow, Russia.[118] 91.6% efficacy against COVID-19 was reported in this interim analysis of Gam-COVID-Vac study involving 21,977 enrolled adults between September 7 and November 24, 2020.

Lentivirus structure and vector design

Lentiviruses comprising nine species (seven animal and two human lentiviruses) are enveloped single-stranded RNA viruses belonging to the Retroviridae family (Fig. 4A). HIV-1 is the most extensively characterized human lentivirus used as the backbone for lentiviral-vectored gene therapies.[119,120] Compared to retroviral vectors, lentiviral vectors have superior properties, such as the ability to transduce diving and nondividing cells, high infection efficiency, and low probability of insertional mutagenesis.[121] Contrary to AAV (weakly immunogenic) and Ad-vectored (highly immunogenic) therapies, lentiviral vectors are fairly nonimmunogenic and provide long-term gene expression due to permanent integration into the host genome. Two copies of the viral RNA genome and some other viral enzymes such as reverse transcriptase and integrase are present within the icosahedral viral capsid. The viral RNA genome is $\sim$9.7 kb in size and encodes a total of nine genes flanked by 5′ and 3′ long terminal repeat (LTR) sequences. Structural proteins such as the viral nucleocapsid and matrix are encoded by GAG, while reverse transcriptase, integrase, and protease functions are encoded by POL. Surface glycoproteins necessary for host cell receptor recognition are encoded by ENV (Fig. 4B). The regulatory genes *tat* and *rev* play essential roles during viral replication, whereas the remaining accessory genes *nef*, *vif*, *vpr*, and *vpu* are needed to increase infectivity, but they are dispensable for viral vector preparation.

First-generation lentiviral vector production involves the use of three plasmids, including a transfer plasmid, a packaging plasmid, and an envelope plasmid. Transfer plasmid contains the lentiviral vector genome composed of the wild-type 5′ and′ LTRs, the ψ sequence, a part of the *env* gene containing the rev response element, an internal promoter, and the transgene.[122] The packaging plasmid harbors the HIV-1 genome with all viral genes except the *env* gene. The envelope plasmid contains envelope proteins from another virus such as the vesicular stomatitis virus G (VSV-G) protein that improves the stability and widens the cellular tropism of the viral particles produced. Unfortunately, the first-generation vector system is considered to have an unacceptably high risk of generating replication-competent viral particles (RCLs) via recombination.[123] Second-generation vectors were developed by the removal of virulence factors (*nef*, *vif*, *vpr*, and *vpu*) after the discovery that these accessory proteins were not needed for efficient viral replication and production.[124] Removal of the accessory genes does not interfere with the transfer of genetic material to the host cell. Second-generation lentiviral vectors are safer than first-generation vectors and can be used routinely in a scientific research laboratory. To rule out even the theoretical possibility of creating RCL, third-generation vectors were developed to use lentiviral vectors not only in a laboratory but also in a clinical setting (Fig. 4C).[125] In this scenario, the promoter of the 5′LTR has been deleted to reduce its activity and Rev is expressed from a separate plasmid in addition to the removal of the HIV Tat gene used to drive expression from the LTRs. Furthermore, an RSV or CMV promoter is commonly inserted in this LTR to transcribe the viral genome in producing cells. Deletion of the promoter/enhancer elements in the 3′ LTR resulted in self-inactivating (SIN) third-generation vectors unable to transcribe full-length RNA after integration, further improving safety. Since third-generation vectors require only three HIV-1 genes (*gag*, *pol*, and *rev*) for production,[126,127] this system offers the best safety profile.[128] Consequently, VSV-G pseudotyped third-generation SIN lentiviral vectors became the preferred vector of choice to achieve long-term gene expression *in vivo*.[129,130] Integration-deficient lentiviral vectors were developed to rule out the risk of insertional mutagenesis completely.[131] These integrase-deficient lentiviral vectors have mutations in the catalytic domain of the viral integrase, preventing the integration of vector cDNA into the host genome. Instead, they produce circular forms, which are lost eventually due to cell division, since they do not replicate inside the cell.

Lentivirus-based vaccines

DCs are the most effective immune-stimulatory cells that induce antigen-specific T cell responses against infectious pathogens *in vivo*. Contrary to nonviral gene delivery approaches, viral vectors are the most effective gene delivery vehicles for stimulating T cell response.[132] However, the dominant immunity against the viral antigens might represent a clear handicap to establish optimal

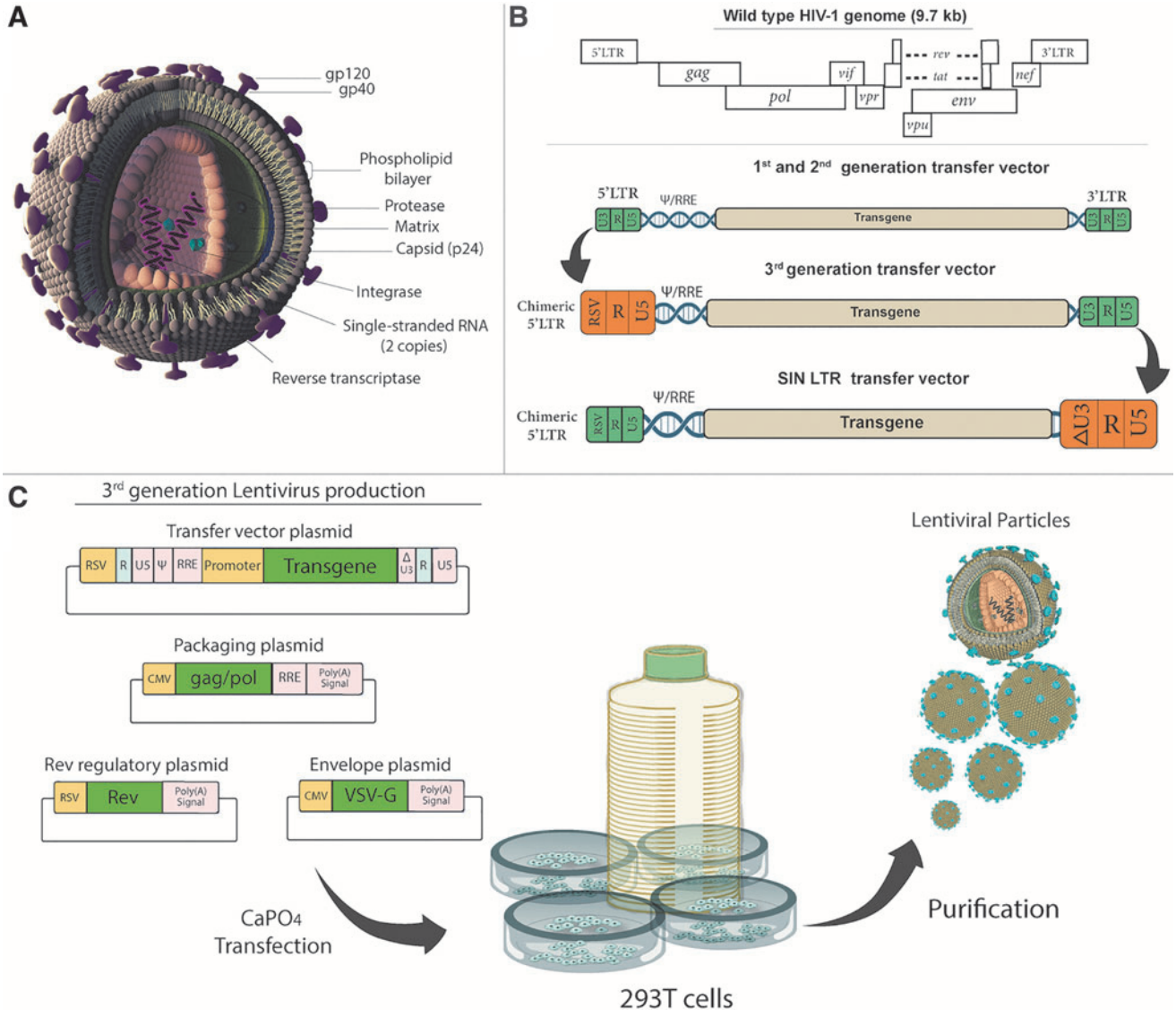

Figure 4. Lentivirus structure and vector design strategy. **(A)** Computer-generated image of the lentivirus structure. **(B)** Various lentiviral vector designs in comparison to wild-type HIV.[189] First-generation lentiviral vectors carry a substantial portion of the HIV genome, including the *gag* and *pol* genes, in addition to accessory genes (*vif*, *vpr*, *vpu*, and *nef*) and regulatory genes (*tat* and *rev*). Second-generation lentiviral vectors lack accessory virulence genes (*vif*, *vpr*, *vpu*, and *nef*). In the third-generation system, *gag* and *pol* genes in addition to the *rev* or *env* genes are encoded by three separate plasmids containing the necessary viral sequences for packaging. The third-generation lentiviral vectors lack the *tat* gene due to the addition of a constitutively active promoter into the upstream LTRs of the transfer plasmid. Self-inactivating lentiviral vectors interrupting the promoter/enhancer activity of the LTR are generated by deletions in the 3′LTR of the viral genome to further improve safety. **(C)** Lentivirus production scheme. Transient transfection of transfer plasmid in association with packaging plasmids (*gag/pol*, *rev*, and *vsv-g*) results in the production of a lentivirus from 293T cells.[126,127] LTR, long terminal repeat; HIV, human immunodeficiency virus.

transgene-specific immune response. Even though higher levels of transgene expression are desired for optimal immunogenicity, the efficacy of Ad-vectored vaccines in clinics is limited due to preexisting and/or acquired immunity to Ad, that is, following natural infection or previous vaccination. In contrast, lentiviruses demonstrated superior properties at inducing T cell-mediated immune response compared to other viral vectors (*e.g.*, Ad), such that 2- to 10-fold higher transduction levels were achieved in human and murine DCs using lentiviral vectors[133,134] without interfering with the antigen presentation machinery of DCs.[135] Based on these studies, 10- to 100-fold more Ad vector was needed to achieve the same levels of transduction attained with lentivirus vectors. In conclusion, lentivirus-mediated gene transfer to DCs results in effective antigen presentation and activation of transgene-specific cytotoxic T cells.[133,136] The immune responses elicited via direct injection of lentivirus vectors appear to be less dependent on CD4+ T cells in terms of primary and memory CTL response.[137] Compared to vaccinia vectors, lentivirus-mediated CD8+ T cell responses have been reported to last longer.[138] The fact that lentivirus vectored

immunization yielded more CD127+ antigen-specific CD8+ T cells, compared to peptide-based applications, suggests lentiviral vectors generate an effective memory T cell response.[139]

Since antigen-specific T cells not only eliminate cancer cells but also virally infected cells, the expansion of a large population of T cells with anti-SARS-CoV-2 viral antigen specificity might be helpful to stop an ongoing COVID-19 infection.[140] Similarly, genetically modified APCs expressing the conserved domains of the viral structural proteins carried by the lentivirus are expected to stimulate the differentiation of naive T cells into effector cytotoxic T cells. By this token, clinical trials are underway evaluating the safety and efficacy of genetically modified APCs alone and in combination with antigen-specific cytotoxic T cells (NCT04299724, NCT04276896). These trials involve the testing of multiple Covid-19 minigenes using a lentiviral vector system to express viral proteins and immune-modulatory genes to modify DCs leading to the activation of T cells.

Preclinical studies of lentivirus-mediated spike gene delivery against COVID-19 have recently begun. A lentiviral vector encoding the full-length, membrane-anchored form of SARS-CoV-2 spike glycoprotein was generated to test the vaccine efficacy of lentivirus-mediated spike gene delivery in a preclinical animal model of COVID-19.[141] The animal model was generated by transduction of respiratory tract cells using Ad expressing the SARS-CoV-2 receptor hACE2. Systemic injection of lentivirus carrying spike gene in mice resulted only in partial protection despite high levels of serum neutralizing activity and T cell responses. Intriguingly, an intranasal boost strategy generated a stronger localized immune response in the upper respiratory tract yielding complete protection against SARS-CoV-2 in rodents.

AAV structure and vector design

AAVs are naturally nonpathogenic, nonenveloped, and small DNA viruses (25 nm in size) of the Parvoviridae family (Fig. 5A).[142] AAV is a replication-defective virus requiring a helper virus such as Ad to initiate productive infection. AAV2 genome size is nearly 4.7 kbp and the genome encodes four open reading frames (ORFs), *Rep* (Replication), *Cap* (Capsid), *aap* (Assembly), and *maap* (membrane-associated accessory protein) flanked by two 145 base ITRs (Fig. 5B).[143,144] The complementary DNA strand is synthesized from these ITRs.[145] At least 13 serotypes of AAV have been identified,[146] but most vector systems utilize AAV2 ITR as the packaging signal. Type 2 ITRs can be packaged with a variety of capsid types leading to further refinement of the tissue tropism of AAV vectors.[147–149] Translation of Rep and Cap using different promoters and alternative splicing generates multiple distinct gene products (Rep78, Rep68, Rep52, and Rep40—required for the AAV life cycle; VP1, VP2, and VP3-capsid proteins).[150] Four replication proteins are encoded from the first ORF, while the second ORF encodes three capsid proteins. The assembly-activating protein (AAP) and the recently discovered membrane-associated accessory protein (MAAP) with unknown function are encoded from the third and fourth ORFs, respectively.[93] AAP is derived from the *aap* gene in an alternate reading frame overlapping the cap gene. The expression of the AAP together with capsid proteins VP1, VP2, and VP3 is needed for AAV capsid assembly.[151] AAV transfer plasmid is constructed by placing the transgene between the two ITRs and cotransfection of the transfer plasmid with Rep and Cap supplied in *trans* in addition to an adenoviral helper plasmid is needed to produce AAV particles in 293 cells (Fig. 5C).

The conversion of ssDNA genomes to dsDNA is one of the rate-limiting steps in AAV transduction.[152] This causes delays in transgene expression because the vector depends on the DNA replication machinery of the host cell to synthesize the complementary strand. Consequently, second-generation self-complimentary (sc) AAV vectors were developed to resolve the inefficient process of second-strand DNA synthesis causing low transduction efficiency with AAV vectors.[153] scAAV vectors possess complementary sequences capable of spontaneously annealing following transduction, which abolishes the requirement for host cell DNA synthesis. However, this approach reduces the packaging capacity of AAV to 2.2 kb (Fig. 5B). Trans-splicing of AAV vectors allowed for the cloning of larger transgenes into AAV vectors.[154] In this strategy, the transgene is cloned into two AAV transfer plasmids, the first with a 3′ splice donor and the second with a 5′ splice acceptor. Following cotransduction of target cells by these two AAV vectors, viral genomes form concatemers through trans-splicing allowing for the full-length transgene expression. Homologous recombination relying on substantial sequence overlap between two transfer plasmids is another method used to increase the packaging capacity of AAV vectors.

AAV-based vaccines

AAV-based gene therapy vectors have a significant potential for treating viral infectious diseases. The rAAV-based vaccine (RBD-rAAV) encoding the RBD of SARS-CoV S protein, which is a major target of neutralizing antibodies, was generated.[155] A single dose of RBD-rAAV in BALB/c mice resulted in the production of sufficient neutralizing antibodies to protect against SARS-CoV infection. Compared to intramuscular injection, a single intranasal prime dose vaccination with RBD-rAAV could induce a detectable systemic humoral immune response, but the addition of a booster immunization resulted in a much stronger and prolonged mucosal immune response with neutralizing activity.[51] Furthermore, RBD-rAAV priming and boosting with RBD-specific peptides for

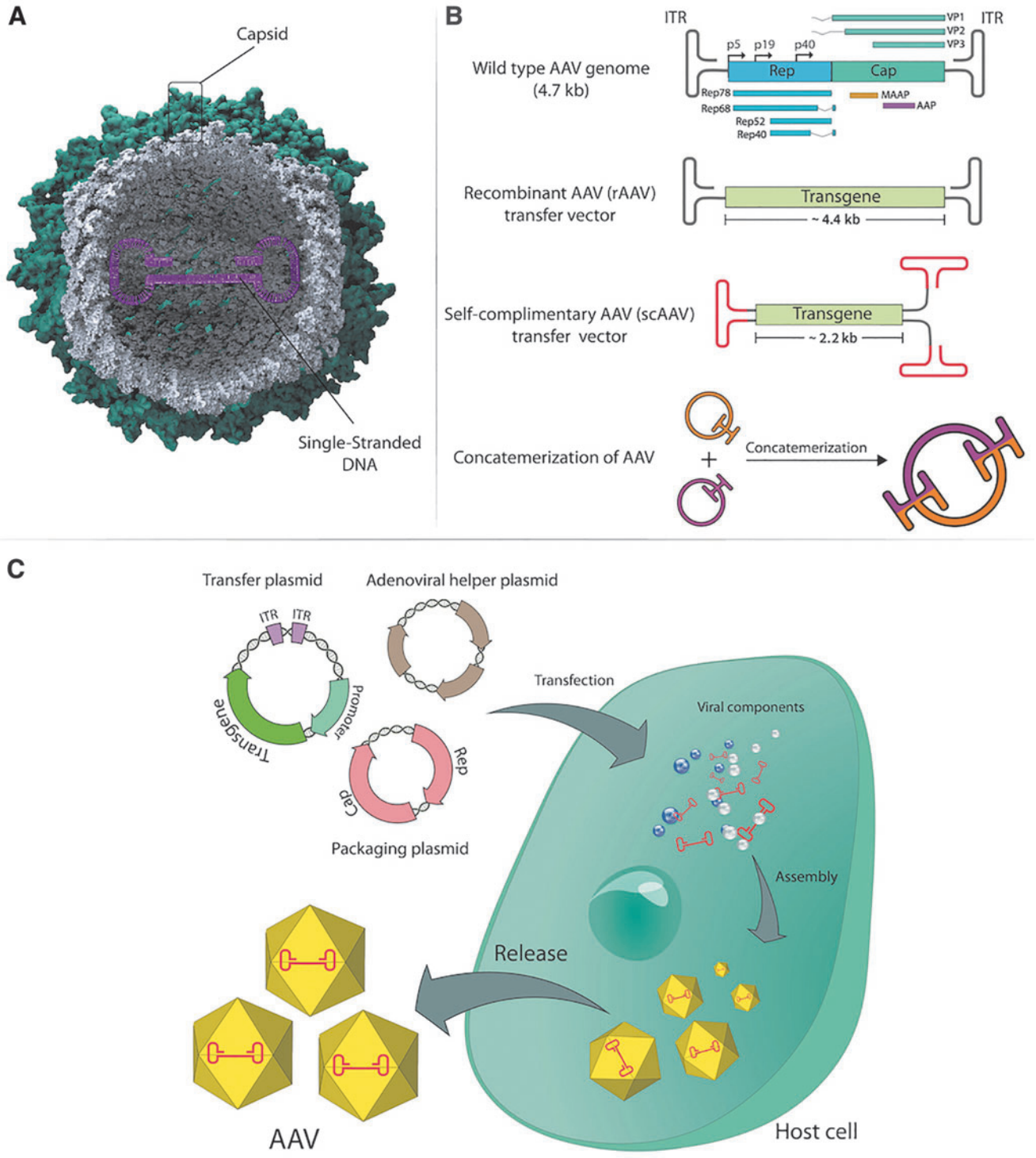

Figure 5. AAV structure and vector design strategy. **(A)** Computer-generated AAV virus structure. **(B)** Wild-type AAV genome is a single-stranded DNA encoding two ORF's, REP and CAP flanked by ITR sequences. All protein-coding genes except for the flanking ITR sequences are removed from the first-generation AAV vectors.[190] Compared to AAV vectors with ss DNA genomes, sc AAV vectors do not require *de novo* second-strand DNA synthesis for transcription.[191] Cloning of large transgenes bigger than 4.7 kb was resolved by the generation of high-capacity split genome AAV vectors. In this strategy, the large transgene is divided into different AAV vectors; head-to-tail recombination of ITR sequences within the cell reconstitutes the transgene in one piece following transduction.[192] **(C)** AAV production scheme. AAV production requires delivery of transfer plasmid with the transgene of interest, packaging plasmids encoding *rep* and *cap*, and helper plasmid carrying adenoviral vector genome into HEK293 cells.[142] AAV, adeno-associated virus.

T cell epitopes increased both humoral and cellular immune responses against SARS-CoV infection in female BALB/c mice.[50]

AAV-based COVID-19 vaccines are currently in early-stage development. On May 5, 2020, Massachusetts Eye and Ear and Massachusetts General Hospital delivered a press release on the testing of an experimental vaccine called AAVCOVID, which is being developed in the laboratory of Luk H. Vandenberghe, PhD, director of the Grousbeck Gene Therapy Center at Massachusetts Eye and Ear at Harvard Medical School. The AAVCOVID vaccine candidate uses AAV vector technology to deliver the SARS-CoV-2 spike antigen-encoding gene to induce a protective immune response against COVID-19. The preclinical safety and immunogenicity of two novel AAV vector-based COVID-19 vaccines were recently reported.[156] In this particular study, AAVrh32.33, a novel vector developed from rhesus macaques isolates with highly reduced antibody prevalence in humans,[157] was utilized to express a full-length SARS-CoV-2 S protein locked in a prefusion conformation and a truncated version of SARS-CoV-2 S protein designed to be secreted. Both vaccine candidates manifested robust long-lived humoral and cellular immunogenic responses following a single intramuscular injection in rodents and nonhuman primates. Furthermore, AAVCOVID vaccine candidates could be stored at room temperature for up to 1 month without losing their potency and effectiveness. These vaccine candidates are currently in preclinical trials and testing in humans is expected following the completion of animal experiments.

In November, a cocktail of two of Regeneron's laboratory-made antibodies, casirivimab and imdevimab, received an EUA from the FDA to treat COVID-19 via intravenous infusion.[158] This is a recombinant monoclonal antibody cocktail intended to yield resistance to the SARS-CoV-2 coronavirus. The University of Pennsylvania in collaboration with Regeneron Pharmaceuticals, Inc., agreed to test whether Regeneron's casirivimab and imdevimab investigational antibody cocktail could stop COVID-19 transmission when delivered intranasally using AAV vectors.[159] In cases where vaccines are not effective due to mutations, delivering the AAV vector through a nasal spray was proposed to express well-characterized anti-SARS-CoV-2 antibodies in cells that line the nose and throat to neutralize COVID-19.

Current hurdles regarding immune responses to gene therapy viral vectors used in vaccination

The suitability of a viral vector for gene therapy relies on many factors, including intrinsic immunotoxicity of the vector, *in vivo* versus *ex vivo* gene transfer, target organs, tissue tropism, cargo capacity, and the potential for genome integration concerning insertional mutagenesis. Viral vectors used in gene therapy might be indistinguishable from their wild-type counterparts to the immune system exposing them to similar innate and adaptive immune responses. Thus, the immune response to viral vectors represents one of the most important hurdles to clinical gene therapy.[160] Upon recognition of viral structures such as nucleic acids and capsids, innate immune cells stimulate the secretion of IFN-α/β resulting in reducing viral transduction and activation of the adaptive immune response. Activation of DCs and antigen presentation lead to further expansion and differentiation of T cells. While MHC I-restricted CD8+ T cells recognize and lyse virus-infected cells, MHC II-restricted CD4+ T cells (helper T cells) participate in the generation of memory CD8+ T cells and B cells leading to antibody production.

Ad is one of the first viruses to be explored as a potential gene therapy vector due to its high transduction efficiency and packaging capacity. Despite high levels of transgene expression, severe immunotoxicity associated with a high degree of inflammatory response resulted in transient gene expression and limited use against genetic diseases.[161] On the contrary, since they strongly activate CD8+ T cells, adenoviral vectors are favored in vaccination and cancer gene therapy applications. Ease of manipulation, their capacity for high titer growth, and intrinsic antigenic properties have made nonreplicating Ad vectors a preferred vector for vaccination studies.[95] However, preexisting immunity to Ad-based vectors also represents a handicap for repeated use *in vivo*. To overcome this, a variety of Ads isolated from humans (Ad types-26 and 5) and chimpanzees (simian adenovirus ChAd serotype Y25), which have low seroprevalence in humans, have been cloned, modified as a gene transfer vector, and tested as vaccines.[107] Compared to other viral vectors, AAV manifested superior qualities for *in vivo* gene transfer for vaccination due to the availability of a variety of viral capsids with diverse tropism, low immunogenicity, and satisfactory safety profile.[162] Despite these features, the seroprevalence issue is still a great concern in AAV-mediated gene therapy or vaccination approaches. This is why patients are screened for neutralizing Ab titers against the AAV vector capsid before enrollment in clinical trials. In addition to alternative serotypes or capsid modifications, the use of decoy capsids or plasmapheresis has been advised to overcome preexisting neutralizing Ab.[163,164] On the contrary, Ad type-26 (Janssen COVID-19 vaccine) and simian adenovirus ChAd serotype Y25 (AstraZeneca-AZD1222) vaccinated individuals do not require screening for preexisting neutralizing antibody due to low seroprevalence of vaccine vectors in the human population.

In vivo gene transfer to DCs is a new strategy in vaccine development, and HIV-based lentiviral vectors are also becoming very popular owing to their effectiveness in DC transduction.[165] Generation of integrase deficient len-

tiviral vectors for vaccination was essential to relieve safety concerns regarding integration.[166] Pseudotyping of lentiviral vectors appeared to be the main method of targeting and induction of immune response to vaccine antigens.[167] Finally, preexisting immunity to lentiviral vectors is low in humans, which further increases their appeal for use in vaccination.

Other viral vectors under development against the COVID-19

Live-attenuated viral vectors have the potential to induce long-lasting protective immunity after a single dose and are low-cost to manufacture at a large scale.[168] Owing to safe history, well-established manufacturing processes, and induction of strong, long-lasting immunity, the measles virus-vectored (MeV) vaccine is an attractive candidate for the development of an effective vaccine against COVID-19. Thus, MeV vaccine candidates expressing the full-length SARS-CoV-2 spike glycoprotein have been generated.[169] The SARS-CoV-2 S protein-encoding gene sequence was inserted into two different positions of the MeV genome to control antigen gene expression. Both protective neutralizing IgG antibody responses and cytotoxic T cell responses with S protein-specific killing activity were detected in vaccinated hamsters and mice. In another study, the MeV-based SARS-CoV-2 vaccine using the prefusion stabilized, full-length spike antigen, but not the S2 subunit, generated Th1-dominant T cell and neutralizing antibody responses leading to long-term immunity and protection from SARS-CoV-2 challenge in mice.[170] A Randomized, Placebo-controlled Phase 1 trial is underway to evaluate the safety and immunogenicity of the Measles Vector-based Vaccine Candidate Against COVID-19 in Healthy Volunteers (NCT04497298).

MVA is a vastly weakened poxvirus vector broadly used to develop vaccines for infectious diseases and cancer. Poxviruses are easy to modify since they require a little manipulation to yield robust protein expression. A novel vaccine platform based on a unique three-plasmid system to effectively produce recombinant MVA vectors from chemically synthesized DNA (sMVA) was generated in response to the ongoing global pandemic caused by SARS-CoV-2. Strong SARS-CoV-2 antigen-specific humoral and cellular immune responses in addition to potent neutralizing antibodies were obtained from mice immunized with these sMVA vectors.[171] The safety, tolerability, and immunogenicity of the Candidate Vaccine MVA-SARS-2-S is underway in an open, single-center Phase 1 trial (NCT04569383).

VSV, belonging to the Rhabdoviridae family, is a nonsegmented single-stranded negative-sense RNA virus. Due to their replicating nature, VSV-based vaccines can be deployed as a single-dose regimen to efficiently stimulate both humoral and cellular immunity. Recombinant VSV-based vaccines encoding viral glycoproteins manifest several advantages as a vaccine platform such as efficient, simple, and large-scale production in mammalian cell culture in addition to the absence of preexisting immunity to the vector. The immunogenicity and *in vivo* efficacy of a replication-competent VSV-eGFP-SARS-CoV-2 was tested in a mouse model of COVID-19.[172] A single dose of VSV-eGFP-SARS-CoV-2 injection was sufficient to produce a vigorous neutralizing antibody response in BALB/c mice that neutralized SARS-CoV-2 infection and decreased viral load in lung and peripheral organs. In another study, a recombinant replication-competent VSV-based vaccine candidate expressing the SARS-CoV-2 S protein (rVSV-ΔG-spike), in which the glycoprotein of VSV is replaced by the spike protein of SARS-CoV-2, was generated.[173] A single-dose vaccination of the golden Syrian hamster, which is used as a model for SARS-CoV-2 pathogenesis and transmission, with rVSV-ΔG-spike revealed a safe, effective, and sufficient neutralizing antibody response against SARS-CoV-2 challenge paving the way for clinical trials (NCT04608305). The International AIDS Vaccine Initiative, a nonprofit scientific research organization, and Merck developed SARS-CoV-2 vaccine candidate V590 using the rVSV vector-based platform. Due to inferior immune responses observed in Phase 1 clinical trial (NCT04569786, unpublished results) compared to those seen following natural infection and to those reported for other SARS-CoV-2 vaccines, the development of the SARS-CoV-2 vaccine candidate V590 was discontinued.

REMAINING CONCERNS RELEVANT TO COVID-19 VACCINE DEVELOPMENT AND CONCLUDING REMARKS

Due to the COVID-19 pandemic, we are experiencing an accelerated track for vaccine development. We are not even sure if the SARS-CoV-2 infection will protect us from future infection and how long protection will last. Currently, it is unclear if vaccine-induced immune responses are long- or short-lived than immune responses induced by natural infection. What is clear is that the best vaccine candidates are expected to induce durable, long-term protective cellular and humoral immune responses to prevent the need for boost injections over time. Vaccine candidates demonstrating short-term immunogenicity might undermine the feasibility of vaccine application in humans. A single dose vaccine regiment is preferred compared to a multiple-dose regimen to reduce the cost, increase compliance and minimize the manufacturing requirements. Most of the currently developed vaccines against COVID-19 utilize spike protein of the SARS-CoV-2 virus. However, RNA viruses accumulate mutations that can sometimes interfere with vaccine-induced immunity, as previously seen with influenza viruses. The recent unexpected high mutation rate of the SARS-CoV-2 RNA virus may eventually overshadow or dimin-

ish the efficacy of the first-generation vaccines.[174] Almost all of the vaccine candidates currently in clinical trials are injected intramuscularly inducing strong IgG responses. Luckily, viral vectors have the benefit of intranasal application with the potential of inducing strong mucosal immune responses in addition to IgG responses.

Long-term data concerning vaccination studies are also imperative to evaluate if there is any risk of vaccine-associated enhanced disease (VAED).[175] VAED is a disorder that would arise when an immunized individual subsequently infected with a virus develops a more severe illness than they would have had if they were not immunized.[176] Intriguingly, instead of providing protection, adenoviral vectors used as HIV vaccines have enhanced the susceptibility to HIV infection due to previous exposure to Ad infection in vaccinated subjects.[177] ADE is another concern that remains to be explored as was observed in previous respiratory syncytial virus and dengue virus vaccine studies, which revealed human clinical safety risks.[178] In addition to ongoing research into SARS-CoV-2-induced immunity (innate, humoral, cellular), the efficacy and longevity of vaccine-induced protection need to be established. In particular, we are not sure how to protect the elderly from lethal variants and heterologous SARS-CoV-2 strains. Since it is more challenging to achieve neutralizing antibody titers against infection in older individuals for protection, different vaccine formulations involving a virus-vectored prime-boost regimen, in particular, might be needed to augment immune responses in individuals from this age group. There is also preexisting cross-reactive immunity issue between CD4+ T cells specific for SARS-CoV-2 and CD4+ T cells specific for human common cold coronaviruses. Adults who had been exposed to common cold coronaviruses may manifest preexisting cross-reactive immunity to SARS-CoV-2 antigens resulting in differing susceptibility to SARS-CoV-2 infection.[179]

Currently, vaccine supply is not sufficient to meet the worldwide demand and encounter the first wave of the pandemic for most countries. Yet, future waves are also anticipated since SARS-CoV-2 is expected to circulate as a seasonal virus around the globe. With the current limitations on vaccine supply, many nations are asking to what degree the recommended dosing regimen of COVID-19 vaccines could be altered (dose-sparing strategies) without impacting effectiveness.[180] For instance, the trials of the Oxford-AstraZeneca vaccine concluded that a longer gap between doses (2–3 months) led to greater immune responses.[115,181] Andrew Pollard, the head of the Oxford Vaccine Group and chief investigator into the trial of this vaccine, claimed: ''a longer gap between vaccine doses leads to a better immune response, with the second dose causing a better boost.'' The Oxford vaccine trials involving dose comparison of the second dose after 4 weeks versus the second dose after 2–4 months, revealed 70% protection after the first dose up to the second dose, and the immune response being three times greater after the second dose when the second dose was delayed.[182] Accordingly, on December 30, 2020, the four United Kingdom chief medical officers recommended the second doses of the covid vaccines should be given toward the end of 12 weeks rather than in the previously recommended 3–4 weeks.[183] Concerning both the Pfizer-BioNTech and Oxford-AstraZeneca vaccines, the Joint Committee on Vaccines and Immunization, along with the United Kingdom Chief Medical Officers have approved a prolonged second dosing interval up to 12 weeks to spread the initial phases of vaccination to a greater number of people.[184,185] Furthermore, pooled analysis of four randomized trials of the ChAdOx1 nCoV-19 (AZD1222) vaccine revealed that, contrary to the expectation, a prolonged dosing interval is linked to superior efficacy and better postdose-2 antibody titers.[186] Last but not least, SARS-CoV-2 may not be the last coronavirus to cause a pandemic requiring constant virus surveillance, as well as ongoing vaccine development.

AUTHORS' CONTRIBUTIONS

A.B. and Y.E.E. drafted the review article. S.S. designed and Y.E.E. composed the figures. A.D.S. and T.S.G. edited and improved the review content. S.S. supervised the study. All authors commented on the article.

ACKNOWLEDGMENTS

This work is supported by the Akdeniz University Scientific Research Administration Division and the Small and Medium Enterprises Development Organization of Turkey (KOSGEB-42MUD).

AUTHOR DISCLOSURE

No competing financial interests exist.

FUNDING INFORMATION

No funding was received for this study.

REFERENCES

1. Hui DS, Azhar EI, Madani TA, et al. The continuing 2019-nCoV epidemic threat of novel coronaviruses to global health—the latest 2019 novel coronavirus outbreak in Wuhan, China. Int J Infect Dis 2020;91:264–266.
2. Zhu N, Zhang D, Wang W, et al. A novel coronavirus from patients with pneumonia in China, 2019. N Engl J Med 2020;382:727–733.
3. Zhou P, Yang XL, Wang XG, et al. A pneumonia outbreak associated with a new coronavirus of probable bat origin. Nature 2020;579:270–273.
4. Sanche S, Lin YT, Xu C, et al. High contagiousness and rapid spread of severe acute respiratory syndrome coronavirus 2. Emerg Infect Dis 2020;26:1470–1477.
5. Huang C, Wang Y, Li X, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. Lancet 2020;395:497–506.
6. Wu C, Chen X, Cai Y, et al. Risk factors associated with acute respiratory distress syndrome and death in patients with coronavirus disease 2019 pneumonia in Wuhan, China. JAMA Intern Med 2020;180:934–943.
7. Song P, Li W, Xie J, et al. Cytokine storm induced by SARS-CoV-2. Clin Chim Acta 2020;509: 280–287.
8. Castelli V, Cimini A, Ferri C. Cytokine storm in COVID-19: "when you come out of the storm, you won't be the same person who walked in". Front Immunol 2020;11:2132.
9. Dong Y, Dai T, Wei Y, et al. A systematic review of SARS-CoV-2 vaccine candidates. Signal Transduct Target Ther 2020;5:237.
10. Poland GA, Ovsyannikova IG, Crooke SN, et al. SARS-CoV-2 vaccine development: current status. Mayo Clin Proc 2020;95:2172–2188.
11. Tang X, Wu C, Li X, et al. On the origin and continuing evolution of SARS-CoV-2. Natl Sci Rev 2020 [Epub ahead of print]; DOI: 10.1093/nsr/nwaa036.
12. Plante JA, Liu Y, Liu J, et al. Spike mutation D614G alters SARS-CoV-2 fitness. Nature 2020; 592:116–121.
13. Shi PY, Plante J, Liu Y, et al. Spike mutation D614G alters SARS-CoV-2 fitness and neutralization susceptibility. Res Sq 2020;rs.3.rs-70482.
14. Korber B, Fischer WM, Gnanakaran S, et al. Tracking changes in SARS-CoV-2 spike: evidence that D614G increases infectivity of the COVID-19 virus. Cell 2020;182:812–827.e9.
15. Galloway SE, Paul P, MacCannell DR, et al. Emergence of SARS-CoV-2 B.1.1.7 lineage-United States, December 29, 2020–January 12, 2021. MMWR Morb Mortal Wkly Rep 2021;70: 95–99.
16. Mwenda M, Saasa N, Sinyange N, et al. Detection of B.1.351 SARS-CoV-2 variant strain-Zambia, December 2020. MMWR Morb Mortal Wkly Rep 2021;70:280–282.
17. Francisco RDS, Jr., Benites LF, Lamarca AP, et al. Pervasive transmission of E484K and emergence of VUI-NP13L with evidence of SARS-CoV-2 co-infection events by two different lineages in Rio Grande do Sul, Brazil. Virus Res 2021;296: 198345.
18. Hoffmann M, Arora P, Gross R, et al. SARS-CoV-2 variants B.1.351 and P.1 escape from neutralizing antibodies. Cell 2021 [Epub ahead of print]; DOI: 10.1016/j.cell.2021.03.036.
19. Zhang J, Zeng H, Gu J, et al. Progress and prospects on vaccine development against SARS-CoV-2. Vaccines (Basel) 2020;8:153.
20. Guo YR, Cao QD, Hong ZS, et al. The origin, transmission and clinical therapies on coronavirus disease 2019 (COVID-19) outbreak—an update on the status. Mil Med Res 2020;7:11.
21. Phan T. Novel coronavirus: from discovery to clinical diagnostics. Infect Genet Evol 2020;79: 104211.
22. Mishra KP, Singh AK, Singh SB. Hyperinflammation and immune response generation in COVID-19. Neuroimmunomodulation 2020;27: 80–86.
23. Shah VK, Firmal P, Alam A, et al. Overview of immune response during SARS-CoV-2 infection: lessons from the past. Front Immunol 2020;11: 1949.
24. Gomez-Rial J, Rivero-Calle I, Salas A, et al. Role of monocytes/macrophages in Covid-19 pathogenesis: implications for therapy. Infect Drug Resist 2020;13:2485–2493.
25. Soy M, Keser G, Atagunduz P, et al. Cytokine storm in COVID-19: pathogenesis and overview of anti-inflammatory agents used in treatment. Clin Rheumatol 2020;39:2085–2094.
26. Tan Y, Tang F. SARS-CoV-2-mediated immune system activation and potential application in immunotherapy. Med Res Rev 2021;41:1167–1194.
27. Barteling SJ. Development and performance of inactivated vaccines against foot and mouth disease. Rev Sci Tech 2002;21:577–588.
28. Minor PD. Live attenuated vaccines: historical successes and current challenges. Virology 2015; 479–480:379–392.
29. Zhou Y, Jiang S, Du L. Prospects for a MERS-CoV spike vaccine. Expert Rev Vaccines 2018;17:677–686.
30. Du L, He Y, Zhou Y, et al. The spike protein of SARS-CoV—a target for vaccine and therapeutic development. Nat Rev Microbiol 2009;7:226–236.
31. Zakhartchouk AN, Sharon C, Satkunarajah M, et al. Immunogenicity of a receptor-binding domain of SARS coronavirus spike protein in mice: implications for a subunit vaccine. Vaccine 2007; 25:136–143.
32. Woo PC, Lau SK, Tsoi HW, et al. SARS coronavirus spike polypeptide DNA vaccine priming with recombinant spike polypeptide from *Escherichia coli* as booster induces high titer of neutralizing antibody against SARS coronavirus. Vaccine 2005;23:4959–4968.
33. He Y, Zhou Y, Liu S, et al. Receptor-binding domain of SARS-CoV spike protein induces highly potent neutralizing antibodies: implication for developing subunit vaccine. Biochem Biophys Res Commun 2004;324:773–781.
34. Wrapp D, Wang N, Corbett KS, et al. Cryo-EM structure of the 2019-nCoV spike in the prefusion conformation. Science 2020;367:1260–1263.
35. Lan J, Ge J, Yu J, et al. Structure of the SARS-CoV-2 spike receptor-binding domain bound to the ACE2 receptor. Nature 2020;581: 215–220.
36. Li F. Structure, function, and evolution of coronavirus spike proteins. Annu Rev Virol 2016;3: 237–261.
37. Yang ZY, Kong WP, Huang Y, et al. A DNA vaccine induces SARS coronavirus neutralization and protective immunity in mice. Nature 2004; 428:561–564.
38. Coleman CM, Liu YV, Mu H, et al. Purified coronavirus spike protein nanoparticles induce coronavirus neutralizing antibodies in mice. Vaccine 2014;32:3169–3174.
39. Bisht H, Roberts A, Vogel L, et al. Severe acute respiratory syndrome coronavirus spike protein expressed by attenuated vaccinia virus protectively immunizes mice. Proc Natl Acad Sci U S A 2004;101:6641–6646.
40. Chen Z, Zhang L, Qin C, et al. Recombinant modified vaccinia virus Ankara expressing the spike glycoprotein of severe acute respiratory syndrome coronavirus induces protective neutralizing antibodies primarily targeting the receptor binding region. J Virol 2005;79:2678–2688.
41. Muthumani K, Falzarano D, Reuschel EL, et al. A synthetic consensus anti-spike protein DNA vaccine induces protective immunity against Middle East respiratory syndrome coronavirus in nonhuman primates. Sci Transl Med 2015;7: 301ra132.
42. Kam YW, Kien F, Roberts A, et al. Antibodies against trimeric S glycoprotein protect hamsters against SARS-CoV challenge despite their capacity to mediate FcgammaRII-dependent entry into B cells in vitro. Vaccine 2007;25:729–740.
43. Luo F, Liao FL, Wang H, et al. Evaluation of antibody-dependent enhancement of SARS-CoV infection in rhesus macaques immunized with an inactivated SARS-CoV vaccine. Virol Sin 2018;33: 201–204.
44. Wang Q, Zhang L, Kuwahara K, et al. Immunodominant SARS coronavirus epitopes in

humans elicited both enhancing and neutralizing effects on infection in non-human primates. ACS Infect Dis 2016;2:361–376.

45. Liu L, Wei Q, Lin Q, et al. Anti-spike IgG causes severe acute lung injury by skewing macrophage responses during acute SARS-CoV infection. JCI Insight 2019;4:e123158.

46. Jiang S, He Y, Liu S. SARS vaccine development. Emerg Infect Dis 2005;11:1016–1020.

47. Zhu X, Liu Q, Du L, et al. Receptor-binding domain as a target for developing SARS vaccines. J Thorac Dis 2013;5(Suppl 2):S142–S148.

48. Lan J, Yao Y, Deng Y, et al. Recombinant receptor binding domain protein induces partial protective immunity in rhesus macaques against middle east respiratory syndrome coronavirus challenge. EBioMedicine 2015;2:1438–1446.

49. Nyon MP, Du L, Tseng CK, et al. Engineering a stable CHO cell line for the expression of a MERS-coronavirus vaccine antigen. Vaccine 2018;36:1853–1862.

50. Du L, Zhao G, Lin Y, et al. Priming with rAAV encoding RBD of SARS-CoV S protein and boosting with RBD-specific peptides for T cell epitopes elevated humoral and cellular immune responses against SARS-CoV infection. Vaccine 2008;26:1644–1651.

51. Du L, Zhao G, Lin Y, et al. Intranasal vaccination of recombinant adeno-associated virus encoding receptor-binding domain of severe acute respiratory syndrome coronavirus (SARS-CoV) spike protein induces strong mucosal immune responses and provides long-term protection against SARS-CoV infection. J Immunol 2008; 180:948–956.

52. Greaney AJ, Starr TN, Gilchuk P, et al. Complete mapping of mutations to the SARS-CoV-2 spike receptor-binding domain that escape antibody recognition. Cell Host Microbe 2021;29:44–57.e9.

53. Bakker WA, Thomassen YE, van't Oever AG, et al. Inactivated polio vaccine development for technology transfer using attenuated Sabin poliovirus strains to shift from Salk-IPV to Sabin-IPV. Vaccine 2011;29:7188–7196.

54. Chumakov K, Ehrenfeld E, Wimmer E, et al. Vaccination against polio should not be stopped. Nat Rev Microbiol 2007;5:952–958.

55. Nathanson N, Langmuir AD. The Cutter incident. Poliomyelitis following formaldehyde-inactivated poliovirus vaccination in the United States during the Spring of 1955. II. Relationship of poliomyelitis to Cutter vaccine. 1963. Am J Epidemiol 1995;142:109–140; discussion 107–108.

56. Horaud F. Albert B. Sabin and the development of oral poliovaccine. Biologicals 1993;21:311–316.

57. Hampton L. Albert Sabin and the coalition to eliminate polio from the Americas. Am J Public Health 2009;99:34–44.

58. Kroiss SJ, Famulare M, Lyons H, et al. Evaluating cessation of the type 2 oral polio vaccine by modeling pre- and post-cessation detection rates. Vaccine 2017;35:5674–5681.

59. Zhao J, Zhao S, Ou J, et al. COVID-19: coronavirus vaccine development updates. Front Immunol 2020;11:602256.

60. Balfour H. First patient dosed with COVI-VAC, an intranasal COVID-19 vaccine candidate. European Pharmaceutical Review. https://www.europeanpharmaceuticalreview.com/news/139089/first-patient-dosed-with-covi-vac-an-intranasal-covid-19-vaccine-candidate/ (last accessed January 12, 2021).

61. Sharma A, Krause A, Worgall S. Recent developments for *Pseudomonas* vaccines. Hum Vaccin 2011;7:999–1011.

62. Zhang Y, Zeng G, Pan H, et al. 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 2020 [Epub ahead of print]; DOI: 10.1016/S1473-3099(20)30987-7.

63. Reuters Staff. China's Sinovac vaccine has "general efficacy" of 50.4% in Brazil trials, says Butantan. Reuters Biotechnology. https://www.reuters.com/article/healthcoronavirus-brazil-coronavirus-idUSE5N2HA01G (last accessed January 12, 2021).

64. Srivastava RK, Ish P, Safdarjung Covid-Vaccination Group. The initial experience of COVID-19 vaccination from a tertiary care centre of India. Monaldi Arch Chest Dis 2021 [Epub ahead of print]; DOI: 10.4081/monaldi.2021.1816.

65. Ella R, Vadrevu KM, Jogdand H, et al. Safety and immunogenicity of an inactivated SARS-CoV-2 vaccine, BBV152: a double-blind, randomised, phase 1 trial. Lancet Infect Dis 2021 [Epub ahead of print]; DOI: 10.1101/2020.12.21.20248643.

66. Ella R, Reddy S, Jogdand H, et al. Safety and immunogenicity clinical trial of an inactivated SARS-CoV-2 vaccine, BBV152 (a phase 2, double-blind, randomised controlled trial) and the persistence of immune responses from a phase 1 follow-up report. medRxiv 2020 [Epub ahead of print]; 10.1101/2020.12.21.20248643.

67. Kumar RN. Coronavirus | Covaxin efficacy is 81%, works against variants. The Hindu. https://www.thehindu.com/sci-tech/health/bharat-biotech-says-covid-19-vaccine-shows-81-interim-efficacy/article33980224.ece (last accessed March 3, 2021).

68. Kariko K, Muramatsu H, Welsh FA, et al. Incorporation of pseudouridine into mRNA yields superior nonimmunogenic vector with increased translational capacity and biological stability. Mol Ther 2008;16:1833–1840.

69. Kauffman KJ, Webber MJ, Anderson DG. Materials for non-viral intracellular delivery of messenger RNA therapeutics. J Control Release 2016;240:227–234.

70. Pardi N, Hogan MJ, Porter FW, et al. mRNA vaccines—a new era in vaccinology. Nat Rev Drug Discov 2018;17:261–279.

71. Jahanafrooz Z, Baradaran B, Mosafer J, et al. Comparison of DNA and mRNA vaccines against cancer. Drug Discov Today 2020;25:552–560.

72. Abbasi J. COVID-19 and mRNA vaccines-first large test for a new approach. JAMA 2020;324:1125–1127.

73. Walsh EE, Frenck RW, Jr., Falsey AR, et al. Safety and immunogenicity of two RNA-based Covid-19 vaccine candidates. N Engl J Med 2020;383:2439–2450.

74. Polack FP, Thomas SJ, Kitchin N, et al. Safety and efficacy of the BNT162b2 mRNA Covid-19 vaccine. N Engl J Med 2020;383:2603–2615.

75. Lewis T. Pfizer-BioNTech COVID vaccine is first to win U.S. authorization. Scientific American. https://www.scientificamerican.com/article/pfizer-biontech-covid-vaccine-is-first-to-win-u-s-authorization/ (last accessed December 11, 2020).

76. Jackson LA, Anderson EJ, Rouphael NG, et al. An mRNA vaccine against SARS-CoV-2—preliminary report. N Engl J Med 2020;383:1920–1931.

77. Baden LR, El Sahly HM, Essink B, et al. Efficacy and safety of the mRNA-1273 SARS-CoV-2 vaccine. N Engl J Med 2021;384:403–416.

78. Dolgin E. COVID-19 vaccines poised for launch, but impact on pandemic unclear. Nat Biotechnol 2020 [Epub ahead of print]; DOI: 10.1038/d41587-020-00022-y.

79. Xu Y, Yuen PW, Lam JK. Intranasal DNA vaccine for protection against respiratory infectious diseases: the delivery perspectives. Pharmaceutics 2014;6:378–415.

80. Liu MA. A comparison of plasmid DNA and mRNA as vaccine technologies. Vaccines (Basel) 2019;7:37.

81. Silveira MM, Moreira G, Mendonca M. DNA vaccines against COVID-19: perspectives and challenges. Life Sci 2020;267:118919.

82. Liu H, Su D, Zhang J, et al. Improvement of pharmacokinetic profile of TRAIL via trimer-tag enhances its antitumor activity in vivo. Sci Rep 2017;7:8953.

83. Takashima Y, Osaki M, Ishimaru Y, et al. Artificial molecular clamp: a novel device for synthetic polymerases. Angew Chem Int Ed Engl 2011;50:7524–7528.

84. Davey M. Australia terminates University of Queensland vaccine deal with CSL after false positives for HIV. The Guardian. https://www.theguardian.com/world/2020/dec/11/australia-terminates-university-of-queensland-vaccine-deal-with-csl-after-false-positives-for-hiv (last accessed December 11, 2020).

85. Azmi F, Ahmad Fuaad AA, Skwarczynski M, et al. Recent progress in adjuvant discovery for peptide-based subunit vaccines. Hum Vaccin Immunother 2014;10:778–796.

86. Di Natale C, La Manna S, De Benedictis I, et al. Perspectives in peptide-based vaccination strategies for syndrome coronavirus 2 pandemic. Front Pharmacol 2020;11:578382.

87. Lundstrom K. RNA viruses as tools in gene therapy and vaccine development. Genes (Basel) 2019;10:189.

88. Lundstrom K. Application of viral vectors for vaccine development with a special emphasis on COVID-19. Viruses 2020;12:1324.

89. Krause A, Worgall S. Delivery of antigens by viral vectors for vaccination. Ther Deliv 2011;2: 51–70.

90. Crotty S, Lohman BL, Lu FX, et al. Mucosal immunization of cynomolgus macaques with two serotypes of live poliovirus vectors expressing simian immunodeficiency virus antigens: stimulation of humoral, mucosal, and cellular immunity. J Virol 1999;73:9485–9495.

91. Chen AY, Fry SR, Forbes-Faulkner J, et al. Evaluation of the immunogenicity of the P97R1 adhesin of *Mycoplasma hyopneumoniae* as a mucosal vaccine in mice. J Med Microbiol 2006; 55:923–929.

92. Wang D, Kandimalla ER, Yu D, et al. Oral administration of second-generation immunomodulatory oligonucleotides induces mucosal Th1 immune responses and adjuvant activity. Vaccine 2005;23:2614–2622.

93. Bulcha JT, Wang Y, Ma H, et al. Viral vector platforms within the gene therapy landscape. Signal Transduct Target Ther 2021;6:53.

94. Wang Q, Finer MH. Second-generation adenovirus vectors. Nat Med 1996;2:714–716.

95. Lee CS, Bishop ES, Zhang R, et al. Adenovirus-mediated gene delivery: potential applications for gene and cell-based therapies in the new era of personalized medicine. Genes Dis 2017;4:43–63.

96. Doerschug K, Sanliogla S, Flaherty DM, et al. First-generation adenovirus vectors shorten survival time in a murine model of sepsis. J Immunol 2002;169:6539–6545.

97. Sanlioglu AD, Aydin C, Bozcuk H, et al. Fundamental principals of tumor necrosis factor-alpha gene therapy approach and implications for patients with lung carcinoma. Lung Cancer 2004; 44:199–211.

98. Sanlioglu AD, Dirice E, Aydin C, et al. Surface TRAIL decoy receptor-4 expression is correlated with TRAIL resistance in MCF7 breast cancer cells. BMC Cancer 2005;5:54.

99. Sanlioglu AD, Karacay B, Koksal IT, et al. DcR2 (TRAIL-R4) siRNA and adenovirus delivery of TRAIL (Ad5hTRAIL) break down in vitro tumorigenic potential of prostate carcinoma cells. Cancer Gene Ther 2007;14:976–984.

100. Sanlioglu AD, Koksal IT, Karacay B, et al. Adenovirus-mediated IKKbetaKA expression sensitizes prostate carcinoma cells to TRAIL-induced apoptosis. Cancer Gene Ther 2006;13: 21–31.

101. Sanlioglu S, Luleci G, Thomas KW. Simultaneous inhibition of Rac1 and IKK pathways sensitizes lung cancer cells to TNFalpha-mediated apoptosis. Cancer Gene Ther 2001;8:897–905.

102. Griffith TS, Anderson RD, Davidson BL, et al. Adenoviral-mediated transfer of the TNF-related apoptosis-inducing ligand/Apo-2 ligand gene induces tumor cell apoptosis. J Immunol 2000;165: 2886–2894.

103. Griffith TS, Broghammer EL. Suppression of tumor growth following intralesional therapy with TRAIL recombinant adenovirus. Mol Ther 2001;4: 257–266.

104. VanOosten RL, Griffith TS. Activation of tumor-specific CD8+ T cells after intratumoral Ad5-TRAIL/CpG oligodeoxynucleotide combination therapy. Cancer Res 2007;67:11980–11990.

105. Norian LA, Kresowik TP, Rosevear HM, et al. Eradication of metastatic renal cell carcinoma after adenovirus-encoded TNF-related apoptosis-inducing ligand (TRAIL)/CpG immunotherapy. PLoS One 2012;7:e31085.

106. Swain SL, Hu H, Huston G. Class II-independent generation of CD4 memory T cells from effectors. Science 1999;286:1381–1383.

107. Coughlan L. Factors which contribute to the immunogenicity of non-replicating adenoviral vectored vaccines. Front Immunol 2020;11:909.

108. Afkhami S, LeClair DA, Haddadi S, et al. Spray dried human and chimpanzee adenoviral-vectored vaccines are thermally stable and immunogenic in vivo. Vaccine 2017;35:2916–2924.

109. Kremer EJ. Pros and cons of adenovirus-based SARS-CoV-2 vaccines. Mol Ther 2020;28:2303–2304.

110. King RG, Silva-Sanchez A, Peel JN, et al. Single-dose intranasal administration of AdCOVID elicits systemic and mucosal immunity against SARS-CoV-2 in mice. bioRxiv 2020 [Epub ahead of print]; DOI: 10.1101/2020.10.10.331348.

111. Zhu FC, Li YH, Guan XH, et al. 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.

112. Zhu FC, Guan XH, Li YH, et al. Immunogenicity and safety of a recombinant adenovirus type-5-vectored COVID-19 vaccine in healthy adults aged 18 years or older: a randomised, double-blind, placebo-controlled, phase 2 trial. Lancet 2020;396:479–488.

113. 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.

114. Folegatti PM, Ewer KJ, Aley PK, et al. 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.

115. Voysey M, Clemens SAC, Madhi SA, et al. Safety and efficacy of the ChAdOx1 nCoV-19 vaccine (AZD1222) against SARS-CoV-2: an interim analysis of four randomised controlled trials in Brazil, South Africa, and the UK. Lancet 2021; 397:99–111.

116. Phillips N, Cyranoski D, Mallapaty S. A leading coronavirus vaccine trial is on hold: scientists react. Nature 2020 [Epub ahead of print]; DOI: 0.1038/d41586-020-02594-w.

117. Rab S, Afjal, Javaid M, et al. An update on the global vaccine development for coronavirus. Diabetes Metab Syndr 2020;14:2053–2055.

118. Logunov DY, Dolzhikova IV, Shcheblyakov DV, et al. Safety and efficacy of an rAd26 and rAd5 vector-based heterologous prime-boost COVID-19 vaccine: an interim analysis of a randomised controlled phase 3 trial in Russia. Lancet 2021; 397:671–681.

119. Tasyurek HM, Altunbas HA, Balci MK, et al. Therapeutic potential of lentivirus-mediated glucagon-like peptide-1 gene therapy for diabetes. Hum Gene Ther 2018;29:802–815.

120. Tasyurek HM, Eksi YE, Sanlioglu AD, et al. HIV-based lentivirus-mediated vasoactive intestinal peptide gene delivery protects against DIO animal model of Type 2 diabetes. Gene Ther 2018; 25:269–283.

121. Kotterman MA, Chalberg TW, Schaffer DV. Viral vectors for gene therapy: translational and clinical outlook. Annu Rev Biomed Eng 2015;17: 63–89.

122. Merten OW, Hebben M, Bovolenta C. Production of lentiviral vectors. Mol Ther Methods Clin Dev 2016;3:16017.

123. Kuate S, Marino MP, Reiser J. Analysis of partial recombinants in lentiviral vector preparations. Hum Gene Ther Methods 2014;25: 126–135.

124. Milone MC, O'Doherty U. Clinical use of lentiviral vectors. Leukemia 2018;32:1529–1541.

125. Dull T, Zufferey R, Kelly M, et al. A third-generation lentivirus vector with a conditional packaging system. J Virol 1998;72:8463–8471.

126. Olgun HB, Tasyurek HM, Sanlioglu AD, et al. High-grade purification of third-generation HIV-based lentiviral vectors by anion exchange chromatography for experimental gene and stem cell therapy applications. Methods Mol Biol 2019;1879:347–365.

127. Olgun HB, Tasyurek HM, Sanlioglu AD, et al. High-titer production of HIV-based lentiviral vectors in roller bottles for gene and cell therapy. Methods Mol Biol 2019;1879:323–345.

128. Miyoshi H, Blomer U, Takahashi M, et al. Development of a self-inactivating lentivirus vector. J Virol 1998;72:8150–8157.

129. Erendor F, Sahin EO, Sanlioglu AD, et al. Lentiviral gene therapy vectors encoding VIP suppressed diabetes-related inflammation and

augmented pancreatic beta-cell proliferation. Gene Ther 2020 [Epub ahead of print]; DOI: 10.1038/s41434-020-0183-3.

130. Erendor F, Eksi YE, Sahin EO, et al. Lentivirus mediated pancreatic beta-cell-specific insulin gene therapy for STZ-induced diabetes. Mol Ther 2021;29:149–161.

131. Wanisch K, Yanez-Munoz RJ. Integration-deficient lentiviral vectors: a slow coming of age. Mol Ther 2009;17:1316–1332.

132. Barouch DH, Nabel GJ. Adenovirus vector-based vaccines for human immunodeficiency virus type 1. Hum Gene Ther 2005;16:149–156.

133. Esslinger C, Chapatte L, Finke D, et al. In vivo administration of a lentiviral vaccine targets DCs and induces efficient CD8(+) T cell responses. J Clin Invest 2003;111:1673–1681.

134. Esslinger C, Romero P, MacDonald HR. Efficient transduction of dendritic cells and induction of a T-cell response by third-generation lentivectors. Hum Gene Ther 2002;13:1091–1100.

135. Gruber A, Kan-Mitchell J, Kuhen KL, et al. Dendritic cells transduced by multiply deleted HIV-1 vectors exhibit normal phenotypes and functions and elicit an HIV-specific cytotoxic T-lymphocyte response in vitro. Blood 2000;96: 1327–1333.

136. Dyall J, Latouche JB, Schnell S, et al. Lentivirus-transduced human monocyte-derived dendritic cells efficiently stimulate antigen-specific cytotoxic T lymphocytes. Blood 2001;97:114–121.

137. Dullaers M, Van Meirvenne S, Heirman C, et al. Induction of effective therapeutic antitumor immunity by direct in vivo administration of lentiviral vectors. Gene Ther 2006;13:630–640.

138. He Y, Zhang J, Donahue C, et al. Skin-derived dendritic cells induce potent CD8(+) T cell immunity in recombinant lentivector-mediated genetic immunization. Immunity 2006;24:643–656.

139. Chapatte L, Colombetti S, Cerottini JC, et al. Efficient induction of tumor antigen-specific CD8+ memory T cells by recombinant lentivectors. Cancer Res 2006;66:1155–1160.

140. Tu YF, Chien CS, Yarmishyn AA, et al. A review of SARS-CoV-2 and the ongoing clinical trials. Int J Mol Sci 2020;21:2657.

141. Ku MW, Bourgine M, Authie P, et al. Intranasal vaccination with a lentiviral vector protects against SARS-CoV-2 in preclinical animal models. Cell Host Microbe 2021;29:236–249.e6.

142. Sanlioglu S, Benson PK, Yang J, et al. Endocytosis and nuclear trafficking of adeno-associated virus type 2 are controlled by rac1 and phosphatidylinositol-3 kinase activation. J Virol 2000;74:9184–9196.

143. Sanlioglu S, Engelhardt JF. Cellular redox state alters recombinant adeno-associated virus transduction through tyrosine phosphatase pathways. Gene Ther 1999;6:1427–1437.

144. Ogden PJ, Kelsic ED, Sinai S, et al. Comprehensive AAV capsid fitness landscape reveals a viral gene and enables machine-guided design. Science 2019;366:1139–1143.

145. Sanlioglu S, Duan D, Engelhardt JF. Two independent molecular pathways for recombinant adeno-associated virus genome conversion occur after UV-C and E4orf6 augmentation of transduction. Hum Gene Ther 1999;10:591–602.

146. Mietzsch M, Jose A, Chipman P, et al. Completion of the AAV structural atlas: serotype capsid structures reveals clade-specific features. Viruses 2021;13:101.

147. Domenger C, Grimm D. Next-generation AAV vectors-do not judge a virus (only) by its cover. Hum Mol Genet 2019;28:R3–R14.

148. Weinmann J, Grimm D. Next-generation AAV vectors for clinical use: an ever-accelerating race. Virus Genes 2017;53:707–713.

149. Georgiadis A, Duran Y, Ribeiro J, et al. Development of an optimized AAV2/5 gene therapy vector for Leber congenital amaurosis owing to defects in RPE65. Gene Ther 2016;23:857–862.

150. Daya S, Berns KI. Gene therapy using adeno-associated virus vectors. Clin Microbiol Rev 2008;21:583–593.

151. Naumer M, Sonntag F, Schmidt K, et al. Properties of the adeno-associated virus assembly-activating protein. J Virol 2012;86:13038–13048.

152. Sanlioglu S, Monick MM, Luleci G, et al. Rate limiting steps of AAV transduction and implications for human gene therapy. Curr Gene Ther 2001;1:137–147.

153. McCarty DM. Self-complementary AAV vectors; advances and applications. Mol Ther 2008;16: 1648–1656.

154. Yan Z, Zhang Y, Duan D, et al. Trans-splicing vectors expand the utility of adeno-associated virus for gene therapy. Proc Natl Acad Sci U S A 2000;97:6716–6721.

155. Du L, He Y, Wang Y, et al. Recombinant adeno-associated virus expressing the receptor-binding domain of severe acute respiratory syndrome coronavirus S protein elicits neutralizing antibodies: implication for developing SARS vaccines. Virology 2006;353:6–16.

156. Zabaleta N, Dai W, Bhatt U, et al. Immunogenicity of an AAV-based, room-temperature stable, single dose COVID-19 vaccine in mice and non-human primates. bioRxiv 2021 [Epub ahead of print]; DOI: 10.1101/2021.01.05.422952.

157. Mikals K, Nam HJ, Van Vliet K, et al. The structure of AAVrh32.33, a novel gene delivery vector. J Struct Biol 2014;186:308–317.

158. The U.S. Food and Drug Administration. Coronavirus (COVID-19) update: FDA authorizes monoclonal antibodies for treatment of COVID-19. https://www.fda.gov/news-events/press-announcements/coronavirus-covid-19-update-fda-authorizes-monoclonal-antibodies-treatment-covid-19 (last accessed November 21, 2020).

159. Moody M. Penn medicine collaborates with regeneron to investigate delivery of COVID-19 antibody cocktail via gene therapy platform. Penn Medicine. https://www.pennmedicine.org/news/news-releases/2020/november/penn-medicine-collaborates-with-regeneron-to-investigate-delivery-of-covid-antibody (last accessed November 30, 2020).

160. Shirley JL, de Jong YP, Terhorst C, et al. Immune responses to viral gene therapy vectors. Mol Ther 2020;28:709–722.

161. Raper SE, Chirmule N, Lee FS, et al. Fatal systemic inflammatory response syndrome in a ornithine transcarbamylase deficient patient following adenoviral gene transfer. Mol Genet Metab 2003;80:148–158.

162. Nieto K, Salvetti A. AAV vectors vaccines against infectious diseases. Front Immunol 2014;5:5.

163. Mingozzi F, Anguela XM, Pavani G, et al. Overcoming preexisting humoral immunity to AAV using capsid decoys. Sci Transl Med 2013;5: 194ra192.

164. Chicoine LG, Montgomery CL, Bremer WG, et al. Plasmapheresis eliminates the negative impact of AAV antibodies on microdystrophin gene expression following vascular delivery. Mol Ther 2014;22:338–347.

165. Kim JT, Liu Y, Kulkarni RP, et al. Dendritic cell-targeted lentiviral vector immunization uses pseudotransduction and DNA-mediated STING and cGAS activation. Sci Immunol 2017;2: eaal1329.

166. Gallinaro A, Borghi M, Bona R, et al. Integrase defective lentiviral vector as a vaccine platform for delivering influenza antigens. Front Immunol 2018;9:171.

167. Toon K, Bentley EM, Mattiuzzo G. More than just gene therapy vectors: lentiviral vector pseudotypes for serological investigation. Viruses 2021; 13:217.

168. Frantz PN, Teeravechyan S, Tangy F. Measles-derived vaccines to prevent emerging viral diseases. Microbes Infect 2018;20:493–500.

169. Horner C, Schurmann C, Auste A, et al. A highly immunogenic and effective measles virus-based Th1-biased COVID-19 vaccine. Proc Natl Acad Sci U S A 2020;117:32657–32666.

170. Frantz PN, Barinov A, Ruffié C, et al. A measles-vectored COVID-19 vaccine induces long-term immunity and protection from SARS-CoV-2 challenge in mice. bioRxiv 2021 [Epub ahead of print]; DOI: 10.1101/2021.02.17.43163

171. Chiuppesi F, Salazar MD, Contreras H, et al. Development of a multi-antigenic SARS-CoV-2 vaccine candidate using a synthetic poxvirus platform. Nat Commun 2020;11:6121.

172. Case JB, Rothlauf PW, Chen RE, et al. Replication-competent vesicular stomatitis virus vaccine vector protects against SARS-CoV-2-mediated pathogenesis in mice. Cell Host Microbe 2020;28:465–474.e4.

173. Yahalom-Ronen Y, Tamir H, Melamed S, et al. A single dose of recombinant VSV-G-spike vaccine provides protection against SARS-CoV-2 challenge. Nat Commun 2020;11:6402.

174. Poland GA. Tortoises, hares, and vaccines: a cautionary note for SARS-CoV-2 vaccine development. Vaccine 2020;38:4219–4220.

175. Huisman W, Martina BE, Rimmelzwaan GF, et al. Vaccine-induced enhancement of viral infections. Vaccine 2009;27:505–512.

176. Cohen J. AIDS research. Did Merck's failed HIV vaccine cause harm? Science 2007;318:1048–1049.

177. Sekaly RP. The failed HIV Merck vaccine study: a step back or a launching point for future vaccine development? J Exp Med 2008;205:7–12.

178. Lee WS, Wheatley AK, Kent SJ, et al. Antibody-dependent enhancement and SARS-CoV-2 vaccines and therapies. Nat Microbiol 2020;5: 1185–1191.

179. Jeyanathan M, Afkhami S, Smaill F, et al. Immunological considerations for COVID-19 vaccine strategies. Nat Rev Immunol 2020;20:615–632.

180. Cobey S, Larremore DB, Grad YH, et al. Concerns about SARS-CoV-2 evolution should not hold back efforts to expand vaccination. Nat Rev Immunol 2021 [Epub ahead of print]; DOI: 10.1038/s41577-021-00544-9.

181. Knoll MD, Wonodi C. Oxford-AstraZeneca COVID-19 vaccine efficacy. Lancet 2021;397: 72–74.

182. Medicines and Healthcare Products Regulatory Agency. Information for healthcare professionals on covid-19 vaccine AstraZeneca. https://www.gov.uk/government/publications/regulatory-approval-of-covid-19-vaccine-astrazeneca/information-for-healthcare-professionals-on-covid-19-vaccine-astrazeneca (last accessed January 5, 2020).

183. Iacobucci G, Mahase E. Covid-19 vaccination: what's the evidence for extending the dosing interval? BMJ 2021;372:n18.

184. UK Joint Committee on Vaccination and Immunisation. Advice on priority groups for COVID-19 vaccination. https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/950113/jcvi-advice-on-priority-groups-for-covid-19-vaccination-30-dec-2020-revised.pdf (last accessed December 30, 2020).

185. Joint Committee on Vaccination and Immunisation (UK). Optimising the COVID-19 vaccination programme for maximum short-term impact. Short statement from the Joint Committee on Vaccination and Immunisation (JCVI). https://www.app.box.com/s/uwwn2dv4o2d0ena726gf4403f3p2acnu (last accessed December 31, 2020).

186. Voysey M, Costa Clemens SA, Madhi SA, et al. Single-dose administration and the influence of the timing of the booster dose on immunogenicity and efficacy of ChAdOx1 nCoV-19 (AZD1222) vaccine: a pooled analysis of four randomised trials. Lancet 2021;397:881–891.

187. Abduljalil JM, Abduljalil BM. Epidemiology, genome, and clinical features of the pandemic SARS-CoV-2: a recent view. New Microbes New Infect 2020;35:100672.

188. Wu A, Peng Y, Huang B, et al. Genome composition and divergence of the novel coronavirus (2019-nCoV) originating in China. Cell Host Microbe 2020;27:325–328.

189. Sharon D, Kamen A. Advancements in the design and scalable production of viral gene transfer vectors. Biotechnol Bioeng 2018;115: 25–40.

190. Santiago-Ortiz JL, Schaffer DV. Adeno-associated virus (AAV) vectors in cancer gene therapy. J Control Release 2016;240:287–301.

191. Ellis BL, Hirsch ML, Barker JC, et al. A survey of ex vivo/in vitro transduction efficiency of mammalian primary cells and cell lines with Nine natural adeno-associated virus (AAV1–AAV9) and one engineered adeno-associated virus serotype. Virol J 2013;10:74.

192. Yang J, Zhou W, Zhang Y, et al. Concatamerization of adeno-associated virus circular genomes occurs through intermolecular recombination. J Virol 1999;73:9468–9477.

Received for publication March 8, 2021;
accepted after revision April 12, 2021.

Published online: April 13, 2021.