



## REVIEW

# Vaccine development and technology for SARS-CoV-2: Current insight

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## Abstract

Severe acute respiratory syndrome coronavirus 2 is associated with a severe respiratory disease in China, that rapidly spread across continents. Since the beginning of the pandemic, available data suggested the asymptomatic transmission and patients were treated with specific drugs with efficacy and safety data not always satisfactory. The aim of this review is to describe the vaccines developed by three companies, Pfizer-BioNTech, Moderna, and University of Oxford/AstraZeneca, in terms of both technological and pharmaceutical formulation, safety, efficacy, and immunogenicity. A critical analysis of Phases 1, 2, and 3 clinical trial results available was conducted, comparing the three vaccine candidates, underlining their similarities and differences. All candidates showed consistent efficacy and tolerability; although some differences can be noted, such as their technological formulation, temperature storage, which will be related to logistics and costs. Further studies will be necessary to evaluate long-term effects and to assess the vaccine safety and efficacy in the general population.

## KEYWORDS

lipid nanoparticles, pandemic, pneumonia, SARS-CoV-2, vaccine, viral vector

## 1 | BACKGROUND

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pandemic continues to spread at alarming rates and there appears to be no end in sight due to the long viral incubation period and lack of effective treatment or vaccines.<sup>1,2</sup> As of October 2021, over 243 million confirmed cases and up to 4.9 million deaths had been reported globally.<sup>3</sup> The development of a coronavirus disease-2019 (COVID-19) vaccine is crucial given that available data indicate asymptomatic transmission of the causative virus.<sup>4</sup>

According to the World Health Organization, in August 2021, 294 companies and academic institutes worldwide were developing COVID-19 vaccines. Among these, most had conducted clinical trials with 110 identified vaccine candidates.<sup>5,6</sup>

The SARS-CoV-2 virus is an enveloped single-stranded RNA virus with a spike-like glycoprotein protruding from its outer membrane surface and each spike forms a “corona.”<sup>7,8</sup> The spike (S), envelope (E), membrane (M), and nucleocapsid (N) proteins are the four structural proteins of viruses of the *Betacoronavirus* genus. In particular, the S protein is a focal point for the design of vaccines since it facilitates virus entry into host cells.<sup>8–10</sup> S protein mutations in

**Abbreviations:** COVID-19, coronavirus disease-2019; EMA, European Medicines Agency; EUA, Emergency Use Authorization; FDA, Food and Drug Administration; LNPs, lipid nanoparticles; MenACWY, meningococcal conjugate vaccine; MHC, major histocompatibility complex; RBD, receptor-binding domain; SAEs, systemic adverse effects; VITT, vaccine-induced immune thrombotic thrombocytopenia.

SARS-CoV-2 variants can impact vaccine efficacy and the risk of reinfection. Many variants have spread rapidly in the UK (alpha variant, also called variant B.1.1.7 and 20I/501Y.V1), South Africa (beta variant, also called variant B.1.351 and 20H/501Y.V2), Brazil (gamma variant, also called variant B.1.1.248 and P.1 and 20J/501Y.V3) and California (epsilon variant, also called variant B.1.429 and Cal.20C and 452R.V1).<sup>11–15</sup> The alpha variant contains 17 nonsynonymous mutations and, in particular, 8 principal mutations in the S protein.

The alpha and beta variants share a mutation (N501Y) in the S protein receptor-binding domain (RBD) which contributes to increased transmission (between 40% and 70%) through the angiotensin-converting enzyme-2 cellular receptor. The beta variant has two additional mutations (E484K and K417N) in the S protein that further potentiate antibody avoidance.<sup>16</sup> Another series of mutations (N501Y, E484K, and K417T) has been identified in the S protein of the gamma variant.<sup>17</sup>

Today the vaccines which have received Emergency Use Authorization (EUA) have been developed by Pfizer/BioNTech (sold under the brand name *Comirnaty*), Moderna (*Spikevax*), University of Oxford/AstraZeneca (*Covishield* or *Vaxzevria*), Johnson & Johnson (J&J; also known as Ad26.CoV2.S), Gamaleya National Research Center for Epidemiology and Microbiology (Sputnik V), Sinovac Biotech (CoronaVac), and Sinopharm 1/2 (BBIBP-CorV).<sup>18</sup> Furthermore, worthy to be mentioned is Novavax (NVX-CoV2373), which is in the process of submitting the EUA application. For the purpose of the study, which is to discuss vaccine technology and innovation, the review focuses on vaccines developed by Pfizer/BioNTech, Moderna, and University of Oxford/AstraZeneca.<sup>19–21</sup>

## 2 | LIPID NANOTECHNOLOGY STUDIES

Adenoviral and adeno-associated viral vectors lead to highly efficient transfection; however, viral elements can have drawbacks, such as recombination with the wild-type virus, immune or toxic reactions, and insertional mutagenesis.<sup>22</sup> Therefore, many synthetic nonviral transfer systems based on cationic nanoparticles have been developed.<sup>22–24</sup> Viruses and nanoparticles operate at the same nanoscale, so lipid nanoparticles (LNPs) that mimic viruses' structural features have been employed to encapsulate and deliver nucleic acid-based vaccines. Nanolipids incorporating RNA-based vaccines operate by 1) neutralizing negatively charged messenger RNA (mRNA), condensing the full-length RNA into a nanoscale range, and allowing LNPs to penetrate the host cell membrane (usually negatively charged); 2) escaping destruction by endosomal enzymes inside the host cell cytoplasm; and 3) discharging their mRNA cargo into the cytoplasm, allowing it to reach the ribosomes in the endoplasmic reticulum.

After incorporation, S protein mRNA transcripts are produced. The protein is processed by antigen-presenting cells, and the epitopes are presented by major histocompatibility complex (MHC)-1 and MHC-2. This induces the activation of CD8+ cytotoxic T cells or CD4+ T helper cells, which are essential for antiviral antibody production (Figure 2B). Therefore, LNPs have multiple roles; they act as a

synthetic virus vector and stabilize the mRNA and prevent its destruction by RNase.

The Pfizer/BioNTech vaccine encapsulates the S protein mRNA within LNPs provided by a partnership with Acuitas Therapeutics. Although the composition has not been fully disclosed, previous publications from Acuitas Therapeutics<sup>25</sup> reported that their LNPs (70–100 nm in size) are made of ionizable cationic lipids, phosphatidylcholine, cholesterol, and polyethylene glycol (PEG)-lipids, and deliver mRNA *in vivo*.<sup>25</sup> LNPs with ionizable cationic lipids are one of the most advanced technological systems, similar in composition to those used for small interfering RNA delivery.<sup>26,27</sup> Further optimization of the LNP formulation enabled rapid elimination *in vivo* while maintaining efficacy.<sup>27</sup> The exact Moderna formulation has not been publicly described, but it is known that previous LNP formulations from Moderna used ionizable lipids, 1,2-distearoyl-sn-glycero-3-phosphocholine, cholesterol, and PEG-lipid.<sup>28–30</sup> Phospholipids with phosphatidylcholine are usually present in liposome formulations, as they are in the Moderna and Pfizer/BioNTech vaccine formulations (Figure 2A).

Many cationic lipids,<sup>31,32</sup> such as lipofectin, DOTAP, DOPE, DOTMA, DMRIE, and other analogs,<sup>33–35</sup> have been described. In recent years, several ionizable aminolipids (probably used by Moderna) have been designed for systemic administration, such as DLin-KC2-DMA.<sup>26</sup> These are characterized by the presence of a dilinoleyl group in which the unsaturated alkyl chain (*cis*-double bond) is optimal for the activity and ionization (at pH = 5.5 inside the endosomes) of the dimethylamino head group.<sup>26,28–30,36</sup>

The cationic lipid ((4-hydroxybutyl)azanediyl)bis(hexane-6,1-diyl)bis(2-hexyldecanoate) (ALC-0315) (Figure 1C) was used by Pfizer/BioNTech as a component of the lipid mixture of BNT162b2 to form LNPs. ALC-0315 is a physiological pH cationic (pKa 5.5) synthetic lipid that can be used together with other lipids to form LNPs.<sup>37,38</sup>

Ionizable aminolipids play a dual role in the delivery process. First, they promote the self-assembly of the components into LNPs, encapsulating the RNA through electrostatic interactions with polyanionic nucleic acids. Second, the subsequent endocytosis of LNPs by targeted cells enables RNA to exit from the endosomal compartment and enter the cell cytoplasm. This mechanism is similar to that used by pH-sensitive phospholipids, such as DOPE, in liposomes. At pH 5.5 (present in endosomes), DOPE pegylated liposomes trigger a transition phase (from lamellar to inverse hexagonal phase) that disrupts the liposomal membrane, discharging the encapsulated mRNA into the cell cytosol.<sup>39</sup> It is possible that, through a similar mechanism, LNPs discharge the mRNA cargo into the cell cytoplasm, allowing it to reach the ribosomes in the endoplasmic reticulum. A possible explanation for the activity of ionizable aminolipids is that the proximity of the opposing surface of the endosomal lumen allows the formation of an ion pair that prefers to adopt inverted non-bilayer configurations; this disrupts the endosome membrane integrity, leading to the release of the RNA into the cytoplasm.

PEG-lipid conjugates are present in both the Moderna and Pfizer formulations. Many PEG-lipids are described in the literature. For example, DMG-PEG-2000, 1,2-dimyristoyl-rac-glycero-3-methoxy-PEG-2000, and 2-[(polyethylene glycol)-2000]-N,N-di(tetradecyl)

acetamide (ALC-0159) are used in the Pfizer/BioNTech formulation.<sup>40</sup> PEG is inserted at the surface of nanoparticles close to the aqueous phase and the lipid/phospholipid segments point to the inner hydrophobic moiety. Thus, the nanolipid surface appears to be covered in very hydrophilic PEG "hairs"; this makes the nanoparticles very stable in serum. PEG-coated liposomes<sup>41</sup> circulate for a remarkably long time after intravenous administration (24–30 h). The term "stealth" was used to describe these nanoparticles because of their ability to evade the host immune system.<sup>42</sup> Cholesterol is also present in the Moderna and Pfizer nanolipid formulations as it confers high stability in vivo.

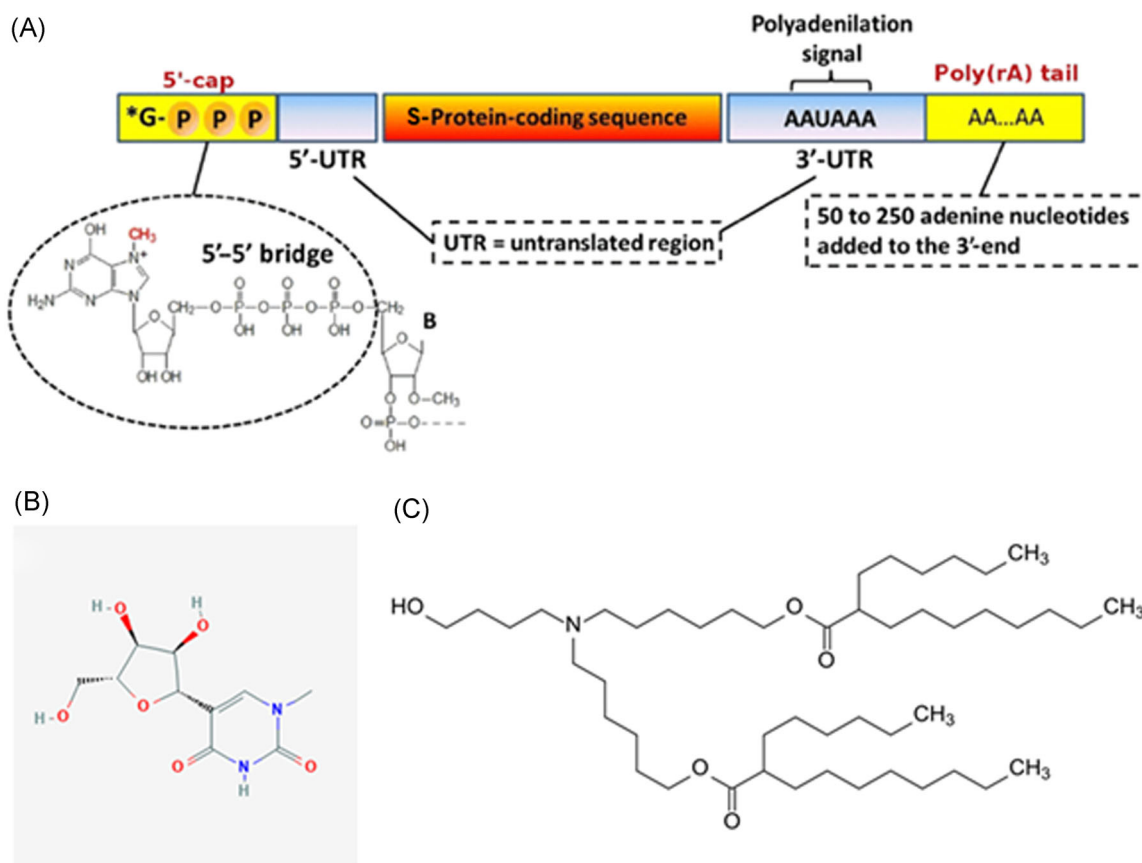
A more attractive feature of pegylated nanoparticles is their ability to preferentially access the lymphatic system. Due to their nanoscale size and high stability, LNPs may cross the interstitial space and access nearby lymph nodes, and this may be a highly beneficial process in the race to develop COVID-19 vaccines.<sup>43,44</sup>

The involvement of an adjuvant can also increase the immunostimulatory properties of mRNA. In both nanolipid formulations, there is no indication of the use of an adjuvant, although the LNP itself may be an adjuvant, like other lipids.<sup>45–47</sup> In addition, other components may be present in the formulations, some of which may require low-temperature storage. For example, Pfizer/BioNTech stipulates vaccine storage at a low temperature (–80°C) because of

mRNA instability, whereas Moderna affirms that a higher storage temperature (–20°C) is sufficient to maintain vaccine activity. The nature of these components is unknown, but what is known and supported in the literature is that several classes of emulsifiers (concerning charge and molecular weight) or antioxidants have been used to stabilize lipid dispersion and prevent particle agglomeration.<sup>48</sup>

### 3 | PFIZER/BIONTECH

The Pfizer/BioNTech vaccine technology is based on mRNA that encodes the S protein of SARS-CoV-2.<sup>49</sup> Given the structural variability of the prefusion form of the S protein due to its intrinsic thermodynamically metastable state, generating a stabilized mutant conformation (nucleoside-modified RNA [modRNA]) that mimics the prefusion conformation is critical for vaccine development.<sup>50</sup> modRNA (4284 nucleotides) (Figure 1A) includes a 5' cap and an untranslated region derived from a human alpha-globin sequence that has a profound effect on mRNA stability and translation. In addition, it consists of a signal peptide-coding region (bases 55–102), which encodes the S2P mutated version of the S protein. This version contains two proline substitutions (K986P and V987P, referred to as



**FIGURE 1** (A) modRNA including a 5' cap and two untranslated regions (UTR) and the S protein-coding sequence. (B) 1-methyl-5'-pseudouridine. (C) Pfizer-BioNTech cationic lipid ALC-0315. modRNA, nucleoside-modified RNA

"2P") that stimulate neutralizing antibodies (bases 103–3879).<sup>51–53</sup> In this sequence, uridine is replaced by 1-methyl-5'-pseudouridine (Figure 1B), which increases mRNA translational capacity.<sup>51,52</sup>

### 3.1 | Clinical studies

Clinical studies began with an initial phase 1 trial conducted in Germany in which two LNP-formulated, modRNA vaccine candidates were tested against SARS-CoV-2, BNT162b1, and BNT162b2.<sup>25–52</sup> BNT162b1 encodes SARS-CoV-2 RBD, which is trimerized by the addition of a T4 fibrin foldon domain that guides protein folding to produce the native trimeric state, thus increasing immunogenicity.<sup>54–57</sup> The T4-mediated trimerization also augments immunogenicity by generating a multivalent display of antigens. BNT162b2 encodes the SARS-CoV-2 full-length S protein. The use of nonimmunogenic mRNA is crucial because a series of innate immunity receptors, including Toll-like receptors (TRL3, TLR7, and TLR8), can recognize RNA, resulting in the release of type I interferons and the inhibition of translation.<sup>52–59</sup>

BNT162b1 and BNT162b2 safety data from younger and older adults supported the selection of BNT162b2 for advancement to Phase 1/2 trials to evaluate safety and efficacy and to the final Phase 2/3 clinical trial.<sup>60</sup> The immune responses elicited by BNT162b1 and BNT162b2 were similar, but BNT162b2 showed milder reactogenicity, particularly in older adults. Given the similarities in the modRNA platform and LNP formulation of these two candidates, it has been suggested that the different RNA nucleotide compositions may be the source of their immune stimulatory activity and reactogenicity profile.<sup>61</sup>

In Phase 1 placebo-controlled, observer-blinded, dose-escalation trial conducted in the USA, adults 18–55 and 65–85 years old were randomly administered either a placebo or one of two mRNA vaccine candidates. The principal outcome was safety (i.e., local and systemic reactions and adverse effects [AEs]) and the secondary outcome was immunogenicity (Table 1).<sup>60</sup> Each trial group received two doses of a vaccine (10, 20, 30, or 100 µg) with a 3-week interval between doses, except for one group (100 µg of BNT162b1) that only received one dose. After the first dose, systemic events reported by participants aged 65–85 who received BNT162b2 were similar to those reported by the placebo group participants. Subsequently, after the second dose of BNT162b2 (30 µg), only 17% of participants aged 18–55% and 8% of participants aged 65–85 reported fever, compared to 75% of participants aged 18–55% and 33% of participants aged 65–85 who were administered the second dose of BNT162b1. Severe transient and manageable systemic events were observed in a limited number of younger BNT162b2 recipients, while older BNT162b2 recipients did not report any severe systemic events. These safety and immunogenicity data supported the selection of BNT162b2 at a 30 µg dosage in a two-dose regimen for progression to Phase 2/3 safety and efficacy assessment.

Between July and November 2020, a Phase 3 multicentric, randomized 1:1 clinical trial was conducted.<sup>19</sup> This efficacy study

involved 43 448 participants aged 16–85 or older: 21 720 volunteers received BNT162b2 (30 µg per dose), and 21 728 received a placebo on Days 0 and 21 (Table 2). The principal endpoints were vaccine efficacy and safety. BNT162b2 had a 95% effectiveness in preventing COVID-19, and similar efficacy was reported across subgroups defined by age, sex, race, ethnicity, baseline body index, and the presence of concomitant conditions. After the first dose, COVID-19 occurred in 39 participants in the BNT162b2 group and 2 participants in the placebo group (52.4% efficacy). Within 7 days after the second dose, COVID-19 resulted in 2 participants in the BNT162b2 group and 21 in the placebo group (efficacy 90.5%); beyond 7 days after the second dose, 9 COVID-19 cases were found in the BNT162b2 group and 172 in the placebo group (94.8%). These Phase 3 trial findings confirmed the favorable profile of the BNT162b2 vaccine.

The most common systemic effects reported after BNT162b2 administration were short-term, mild-to-moderate pain in the area of injection, fatigue, and headache. A limited number of participants in each group experienced severe side effects, profound effects, or AEs, which led to their resignation from the trial. Two BNT162b2 recipients and four placebo recipients died; however, investigators determined that no deaths were caused by the vaccination in the BNT162b2 group.

This study's limitation is that the number of participants was not large enough to observe uncommon and rare side effects. Also, the period of protection remains to be determined, and data do not specify whether vaccination prevents asymptomatic infection. Long-term safety and efficacy assessment for the vaccine is recommended, and additional studies are necessary for other populations (i.e., adolescents 12–15 years old, children younger than 12 years old, pregnant women, and immunocompromised individuals).

The efficacy of the Pfizer/BioNTech vaccine against the SARS-CoV-2 alpha variant was compared to its efficacy against the Wuhan reference strain in a preliminary study.<sup>62</sup> The data showed a slightly reduced neutralization susceptibility of the BNT162b2 vaccine. Other studies<sup>63–65</sup> have suggested that the Pfizer/BioNTech vaccine is less effective against a pseudovirion of the alpha variant (two times less effective) and less efficient against the gamma variant (4–6 times less effective). Variable results have been reported about its efficacy against the beta variant (1–35 times less effective).<sup>64–66</sup>

## 4 | MODERNA

The mRNA-1273 vaccine developed by Moderna encodes the prefusion form of the S (named S2-P) protein and includes a transmembrane anchor and an entire S1–S2 cleavage site.<sup>67</sup> Two proline substitutions in the S2 subunit at amino acids 986 and 987, within the central helix, keep the protein stable in its prefusion conformation.<sup>68</sup> Prefusion-stabilized S protein variants are superior immunogens compared to wild-type S protein ectodomains. Importantly, these successful substitutions in the SARS-CoV-2 S protein (SARS-CoV-2S-2P) allow for rapid structural

TABLE 1 Phase 1 efficacy results from Pfizer-BioNTech, Moderna, and Oxford-AstraZeneca

Authors, journal, year	Vaccine name	Design and population	Outcome measurement	Results
Walsh et al., N Engl J Med, 2020	BNT162b2 (Pfizer-/BioNTech)	Placebo-controlled, observer-blinded, dose-escalation; 195 healthy adults 18–55 or 65–85 years of age randomized to receive placebo or one of two vaccines (BNT162b1 or BNT162b2), two administration doses of 10, 20, and 30 µg, 21 days apart or one single 100 µg dose	Geometric mean concentrations of recombinant antigen (S1)-binding IgG (U/ml) at Day 35  50% SARS-CoV-2-neutralizing geometric mean titers at Day 35	Placebo: 0.9 BNT162b1 <sup>a</sup> – 10 µg: 5120 and 1527 – 20 µg: 7480 and 6399 – 30 µg: 13 940 and 4798 BNT162b2 <sup>a</sup> – 10 µg: 4717 and 3560 – 20 µg: 7367 and 2656 – 30 µg: 8147 and 6014 HCS <sup>b</sup> : 631 Placebo: 0 BNT162b1 <sup>a</sup> – 10 µg: 180 and 33 – 20 µg: 203 and 105 – 30 µg: 437 and 105 BNT162b2 <sup>a</sup> – 10 µg: 97 and 111 – 20 µg: 292 and 81 – 30 µg: 163 and 206 HCS <sup>b</sup> : 94
Jackson et al., N Engl J Med, 2020	mRNA-1273 (Moderna)	Dose-escalation, open-label; 45 healthy adults 18–55 years of age receiving two doses of 25, 100, or 250 µg, 28 days apart	Geometric mean humoral immunogenicity titer (ELISA) anti-S-2P at Day 36  Geometric mean humoral immunogenicity titer (ELISA) antireceptor binding domain at Day 36  PsVNA ID <sub>50</sub> geometric mean response at Day 36  Live virus PRNT <sub>80</sub> geometric mean response at Day 43	mRNA-1273 – 25 µg: 391 018 – 100 µg: 781 399 – 250 µg: 1 261 975 HCS: 142 140 mRNA-1273 – 25 µg: 208 652 – 100 µg: 499 539 – 250 µg: 720 907 HCS: 37 857 mRNA-1273 – 25 µg: 105.8 – 100 µg: 256.3 – 250 µg: 373.5 HCS: 109.2 mRNA-1273 – 25 µg: 339.7 – 100 µg: 654.3 – 250 µg: NA HCS: 158.3
Anderson et al., N Engl J Med, 2020	mRNA-1273 (Moderna)	Dose-escalation, open-label. Extension of the study by Jackson et al. Including 40 participants (56–70 and ≥71 years of age) receiving two doses of 25 or 100 µg, 28 days apart	IgG titers on RBD (ELISA) at Day 36  PsVNA ID <sub>50</sub> geometric mean response at Day 36  FRNT-mNG <sup>d</sup> ID <sub>50</sub> at Day 43  Live virus PRNT <sub>80</sub> at Day 43	mRNA-1273 <sup>c</sup> – 25 µg: 198 643 and 160 591 – 100 µg: 1 471 882 and 711 752 HCS: 37 244 mRNA-1273 <sup>c</sup> – 25 µg: 79 and 121 – 100 µg: 289 and 310 HCS: 106 mRNA-1273 <sup>c</sup> – 25 µg: 550 and 448 – 100 µg: 1425 and 900 HCS: NA mRNA-1273 <sup>c</sup> – 25 µg: NA – 100 µg: 878 and 317

TABLE 1 (Continued)

Authors, journal, year	Vaccine name	Design and population	Outcome measurement	Results
			Response in Th1 cells at Day 43 (mean percentages of CD4 T-cells that produced the cytokines) <sup>e</sup>	mRNA-1273 <sup>c</sup> – 25 µg: 0.264 and 0.095 – 100 µg: 0.336 and 0.317
			Response in Th2 cells at Day 43 (mean percentages of CD4 T-cells that produced the cytokines)	mRNA-1273 <sup>c</sup> – 25 µg: 0.022 and 0.015 – 100 µg: 0.029 and 0.023
Folegatti et al., Lancet, 2020	ChAdOx1 nCoV-19 (University of Oxford/AstraZeneca)	Participant-blinded, multicentre, randomized controlled trial; 1077 healthy adults (18–55 years of age) assigned to receive 5 × 10 <sup>8</sup> viral particles of ChAdOx1 nCoV-19 or 0.5 ml MenACWY (placebo)	Antispike IgG using standardized ELISA at Day 28 (median)	ChAdOx1 nCoV-19 – Prime: 157.1 – Prime-boost: 210.7 – Prime-boost at Day 35: 821.1 MenACWY: 1
			Multiplex MSD– antispike IgG (AU/ml) at Day 28 (median)	ChAdOx1 nCoV-19 – Prime: 10 471.8 – Prime-boost: NA – Prime-boost at Day 42: 33 830.8 MenACWY: 43.9
			Multiplex MSD–RBD (AU/ml) at Day 28 (median)	ChAdOx1 nCoV-19 – Prime: 3182.5 – Prime-boost: NA – Prime-boost at Day 42: 16 825.4 MenACWY: 15.8
			Marburg VN at Day 28 (median)	ChAdOx1 nCoV-19 – Prime: 1 – Prime-boost: 3.2 – Prime-boost at Day 42: 32 MenACWY: 1
			PHE PRNT <sub>50</sub> <sup>1f</sup> at Day 28 (median)	ChAdOx1 nCoV-19 – Prime: 218 – Prime-boost: NA MenACWY: 36.5
			PseudoNA at Day 28 (median)	ChAdOx1 nCoV-19 – Prime: 87.9 – Prime-boost: 162.9 – Prime-boost at Day 42: 450.9 MenACWY: 40
			IFN $\gamma$ ELISpot response against SARS-CoV-2 spike peptides at Day 28 (median)	ChAdOx1 nCoV-19 – Prime: 554.3 – Prime-boost: 528.7 MenACWY: 61.3

Abbreviations: ELISA, enzyme-linked immunosorbent assay; HCS, human convalescent sample; IFN $\gamma$ , interferon- $\gamma$ ; IgG, immunoglobulin G; Marburg VN, Marburg SARS-CoV-2 virus neutralization; MenACWY, meningococcal conjugate vaccine; mRNA, messenger RNA; MSD, mesoscale discovery; nCoV, novel coronavirus; PRNT<sub>80</sub>, plaque-reduction neutralization testing assay; PseudoNA, monogram biosciences pseudotype neutralization assay; PsVNA ID<sub>50</sub>, pseudotype lentivirus reporter neutralization assay 50% inhibitory dilution; RBD, receptor-binding domain; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

<sup>a</sup>In the 18–55 and 65–85 years of age groups, respectively.

<sup>b</sup>SARS-CoV-2 infection convalescent serum samples (HCS).

<sup>c</sup>In the group of 56–70 and  $\geq$ 71 years of age, respectively.

<sup>d</sup>Focus reduction neutralization test mNeonGreen assay.

<sup>e</sup>Simulation with the SARS-CoV-2 S1 peptide pool.

<sup>f</sup>Public Health England Plaque Reduction Neutralization Test.



identification using cryogenic electron microscopy and hastened the development of vaccine candidates.<sup>69,70</sup> In fact, several studies focused on increasing the stability of the S protein ectodomain in the prefusion conformation, using multiple proline variants, disulfide bonds, and cavity-filling substitutions to increase S protein expression and stability.<sup>50–71</sup>

#### 4.1 | Clinical studies

Eligible participants (healthy adults, aged 18–55,  $n = 45$ ) in a Phase 1 trial received two injections of the mRNA-1273 vaccine 28 days apart at a dose of 25, 100, or 250  $\mu\text{g}$  (Table 1).<sup>72,73</sup> After the first dose, systemic adverse effects (SAEs) with mild or moderate severity appeared in 5 (33%) participants in the 25- $\mu\text{g}$  group, 10 (67%) in the 100- $\mu\text{g}$  group, and 8 (53%) in the 250- $\mu\text{g}$  group. SAEs were more common after the second dose, reported by: 7 of the 13 participants (54%) in the 25- $\mu\text{g}$  group, all 15 (100%) in the 100- $\mu\text{g}$  group, and all 14 (100%) in the 250- $\mu\text{g}$  group; 21% reported  $\geq 1$  severe event. There were no febrile episodes after the first dose. After the second injection, no participants showed severe events in the 25- $\mu\text{g}$  group, whereas six participants (40%) in the 100  $\mu\text{g}$ -group and eight (57%) in the 250- $\mu\text{g}$  group reported fever.

The 25 and 100  $\mu\text{g}$  doses generated CD4 T-cell responses stimulated by S protein-specific peptide pools; the responses were strongly biased towards Th1 cytokine expression with minimal Th2 involvement. CD8 T-cell responses to S-2P were equal to or greater than the levels reported after the second administration in the 100  $\mu\text{g}$  dose group. In this clinical trial, the duration of the immune response could not be assessed (participants were followed up until Day 57). In summary, this Phase 1 clinical trial showed how, after the first dose, the mRNA-1273 vaccine was immunogenic; it caused strong antibody responses to full-length S-2P and RBD and exhibited dose-dependent activity. Within 2 weeks after the first vaccination, seroconversion occurred, but the pseudovirus neutralizing activity was lower after a single administration, indicating the need for a two-dose scheme.

In a Phase 2 trial, Moderna evaluated the safety, reactogenicity, and immunogenicity of two mRNA-1273 doses administered 28 days apart. Participants ( $n = 600$ ) were divided into two cohorts and administered either two doses of a placebo ( $n = 300$ ) or 50 or 100  $\mu\text{g}$  of vaccine (same quantity two times,  $n = 300$ ). Another Phase 1 study is underway, led by the National Institutes of Health. That study focuses on older adults and has completed enrollment (adults aged 56–70,  $n = 300$ , and adults  $\geq 71$ ,  $n = 300$ ). The follow-up period will last for a year after the second administration.<sup>73</sup>

A Phase 3 clinical trial (coronavirus efficacy) began on July 27, 2020 (Table 2).<sup>20</sup> This was a randomized, placebo-controlled trial, including approximately 30 000 participants enrolled in the US, to assess the efficacy, safety, and immunogenicity of the mRNA-1273 vaccine compared to placebo. Participants were adults  $\geq 18$  years old who presented with an unknown history of SARS-CoV-2 infection at

risk of COVID-19. The enrollment was concluded on October 22, 2020.

On the basis of Phase 1 trial results, the 100  $\mu\text{g}$  dose was chosen as the optimal dose to maximize immune response and minimize adverse reactions. Participants were randomized 1:1 to receive 100  $\mu\text{g}$  of mRNA-1273 or placebo, stratified by age and comorbidities. The primary endpoint was the prevention of symptomatic COVID-19; secondary endpoints included prevention of COVID-19 severe symptoms, defined as the need for hospitalization, and of SARS-CoV-2 infection. Primary efficacy was determined by performing an event-driven analysis based on the number of symptomatic participants with confirmed COVID-19 2 weeks after the second dose. The first analysis (November 16, 2020) was conducted on 95 cases in the placebo group and 5 cases in the vaccine group; the vaccine efficacy was estimated to be 94.5% ( $p < 0.0001$ ) and steady across age, gender, race, and ethnicity.

The primary efficacy analysis was extended to 196 cases: 185 COVID-19 cases were observed in the placebo group and 11 in the vaccine group, indicating a 94.1% efficacy. In a secondary analysis, starting 2 weeks after the first injection, vaccine efficacy was found to be 95.2% (225 cases in the placebo group and 11 in the vaccine group) and 93.6% within SARS-CoV-2 positive participants at baseline (187 cases in the placebo group and 12 in the vaccine group).

The severe COVID-19 cases were analyzed, including 30 participants from the placebo group. One COVID-19-related death reported in the study was in the placebo group. The most common adverse reactions included injection site pain/erythema/redness, fatigue, myalgia, arthralgia, and headache; these were reported more frequently by younger adults. After the second dose, the frequency and severity of solicited adverse reactions increased in the vaccine group. Participants in the vaccine and placebo groups reported hypersensitivity reactions (1.5% and 1.1%, respectively) and Bell's palsy (3% and 1%, respectively). The relative incidence of these AEs in the vaccine group was not influenced by age.

In a lentivirus-based pseudovirus assay, the susceptibility of the alpha variant to convalescent serum and Moderna vaccine recipient serum was investigated.<sup>74</sup> This variant exhibited only a modest reduction in neutralization susceptibility to the Moderna vaccine (twofold average after two doses). The preliminary study that was conducted to assess Pfizer/BioNTech vaccine efficacy against SARS-CoV-2 variants<sup>63</sup> was also performed using the Moderna vaccine, and the results were the same. The Pfizer/BioNTech and Moderna vaccines have been shown to induce a 10-fold increase in neutralizing antibodies after the second dose, suggesting that a twofold reduction in neutralization susceptibility will have a minimal impact on vaccine efficacy. Moreover, it has been suggested that the Moderna vaccine is less efficient against a pseudovirion of the alpha variant (1–2 times less effective) and less efficient against the gamma variant (4–5 times less effective).<sup>56</sup> There are two reports of variable efficacy (3–20 times less effective) against the beta variant.<sup>64–75</sup>

**TABLE 2** Phases 2–3 efficacy results from Pfizer–BioNTech, Moderna, and Oxford–AstraZeneca

Authors, journal, year	Vaccine name	Design and population	Outcome measurement	Results
Polack et al., N Engl J Med, 2020	BNT162b2 (Pfizer/BioNTech)	Multinational, randomized, placebo-controlled, observer-blinded, pivotal efficacy trial. Healthy adults and adults with stable chronic medical conditions, 16 years of age or older were eligible; 43 448 participants were randomly assigned in a 1:1 ratio to receive two doses, 21 days apart, of 30 µg of the BNT162B2 vaccine (n = 21 720) or the placebo (n = 21 728)	Efficacy against confirmed Covid-19 with onset at least 7 days after the second dose in participants without prior evidence of infection (CI 95%) <sup>a</sup>  Efficacy against confirmed Covid-19 with onset at least 7 days after the second dose in participants with and those without prior evidence of infection (CI 95%) <sup>a</sup>	BNT162b2 group Number of cases: 8/18 198 Placebo group Number of cases: 162/18 325 Efficacy: 95.0% (90.3%–97.6%)  BNT162b2 group Number of cases: 9/18 198 Placebo group Number of cases: 169/18 325 Efficacy: 94.6% (89.9%–97.3%)
			Efficacy against confirmed Covid-19 with onset at any time after the first dose in participants with and those without prior evidence of infection (CI 95%) <sup>b</sup>	a) Occurrence after the first dose: BNT162b2 group Number of cases: 39/21 669 Placebo group Number of cases: 2/21 686 Efficacy: 52.4% (29.5%–68.4%)  b) Occurrence between second dose and 7 days after: BNT162b2 group Number of cases: 2/21 669 Placebo group Number of cases: 21/21 686 Efficacy: 90.5% (61.0%–98.9%)  c) Occurrence at least after 7 days after the second dose: BNT162b2 group Number of cases: 9/21 669 Placebo group

(Continues)



TABLE 2 (Continued)

Authors, journal, year	Vaccine name	Design and population	Outcome measurement	Results
Baden et al., N Engl J Med, 2020	mRNA-1273 (Moderna)	Multicentric, randomized, placebo-controlled, observer-blinded trial, 30 420 healthy adults ( $\geq 18$ years of age) with high risk for SARS-CoV-2 infection or its complications were randomly assigned in a 1:1 ratio to receive two doses, 28 days apart, of 100 $\mu$ g of the mRNA-1273 vaccine ( $n = 15\ 210$ ) or the placebo ( $n = 15\ 210$ )	Efficacy against confirmed Covid-19 with onset at least 14 days after the second dose in participants without prior evidence of infection (CI 95%) <sup>a</sup>  Efficacy against confirmed Covid-19 with onset at any time after the first dose (CI 95%) <sup>a</sup>  Efficacy against confirmed severe Covid-19 with onset at least 14 days after the second dose (CI 95%) <sup>a</sup>  Efficacy against confirmed Covid-19 with onset at least 14 days after the first dose (CI 95%) <sup>a</sup>  Efficacy against confirmed secondary definition Covid-19 <sup>b</sup> with onset at least 14 days after the first dose (CI 95%) <sup>a</sup>	Number of cases: 172/21 686 Efficacy: 94.8% (89.8%–97.6%)  mRNA-1273 group Number of cases: 11/14 134 Placebo group Number of cases: 185/14 073 Efficacy: 94.1% (89.3%–96.8%)  mRNA-1273 group Number of cases: 7/14 550 Placebo group Number of cases: 65/14 598 Efficacy: 93.0% (88.9%–95.6%)  mRNA-1273 group Number of cases: 0/14 073 Placebo group Number of cases: 30/14 073 Efficacy: 100.0% (N.E.–100.0%)  mRNA-1273 group Number of cases: 11/14 073 Placebo group Number of cases: 225/14 073 Efficacy: 95.2% (91.2%–97.4%)  mRNA-1273 group Number of cases: 11/14 073 Placebo group Number of cases: 221/14 073 Efficacy: 95.1% (91.1%–97.3%)

TABLE 2 (Continued)

Authors, journal, year	Vaccine name	Design and population	Outcome measurement	Results
Ramasamy et al., Lancet, 2020	ChAdOx1 nCoV-19 (University of Oxford/AstraZeneca)	Multicentric, randomized, controlled, single-blind trial; 560 healthy adults aged 18 years or older were enrolled in an age-escalation (18–55 years, 56–69 years, and ≥70 years) subgroups; 300 participants were randomly assigned to receive a low dose (LD) of ChAdOx1 nCoV-19 vaccine ( $2.2 \times 10^{10}$ viral particles) or the MenACWY vaccine as control; 260 volunteers received a standard dose (SD) of ChAdOx1 nCoV-19 vaccine ( $3.5-6.5 \times 10^{10}$ viral particles) or the MenACWY vaccine	Antispike IgG using standardized ELISA at Day 28 (median)	ChAdOx1 nCoV-19 - 18–55 years LD/LD: 149 - 55–69 years LD: 78 - ≥70 years LD: 89 - 55–69 years LD/LD: 74 - ≥70 years LD/LD: 77 - 18–55 years SD/SD: 174 - 55–69 years SD: 145 - ≥70 years SD: 90 - 55–69 years SD/SD: 119 - ≥70 years SD/SD: 149 MenACWY: - One-dose regimen: 10 Two-dose regimen: 1
		Participants randomization: a) 18–55 years group: - LD regimen: 1:1 ratio to receive two doses - SD regimen: 5:1 ratio to receive two doses b) 55–69 years group: - LD regimen and SD regimen: 3:1:3:1 ratio to receive one dose of vaccine, one dose of control, two doses of vaccine, and two doses of control, respectively c) ≥70 years group: LD regimen and SD regimen: 5:1:5:1 ratio to receive one dose of vaccine, one dose of control, two doses of vaccine, and two doses of control, respectively	Multiplex immunoassay – antispike IgG (AU/ml) at Day 28 (median)	ChAdOx1 nCoV-19 - 18–55 years LD/LD: 6439 - 55–69 years LD: 5032 - ≥70 years LD: 4103 - 55–69 years LD/LD: 4040 - ≥70 years LD/LD: 3168 - 18–55 years SD/SD: 9807 - 55–69 years SD: 6693 - ≥70 years SD: 3454 - 55–69 years SD/SD: 4474 - ≥70 years SD/SD: 4603 MenACWY: - 18–55 years LD/LD: 50 - 55–69 years LD: 27 - ≥70 years LD: 37 - 55–69 years LD/LD: 73 - ≥70 years LD/LD: 55 - 18–55 years SD/SD: 31 - 55–69 years SD: 42 - ≥70 years SD: 37 - 55–69 years SD/SD: 35 - ≥70 years SD/SD: 48

(Continues)

TABLE 2 (Continued)

Authors, journal, year	Vaccine name	Design and population	Outcome measurement	Results
Voysey et al., Lancet, 2021	ChAdOx1 nCoV-19 (University of Oxford/AstraZeneca)	Multicentric, randomized, controlled, single-blind trial. Phase 3 of the study by Ramasamy et al., <i>The Lancet</i> , 2020; 23 848 adults ( $\geq$ 18 years old) were randomly assigned in a 1:1 ratio to receive two doses of ChAdOx1 nCoV-19 vaccine or the MenACWY vaccine or saline as control. Participants assigned to the vaccine group received two SD/SD of $5 \times 10^{10}$ viral particles or a half dose as their first dose and an SD as their second dose (LD/SD)	PHE $MNA_{80}$ (normalized $IC_{80}$ values) at Day 28 (median)	ChAdOx1 nCoV-19 <ul style="list-style-type: none"> <li>- 18-55 years LD/LD: 79</li> <li>- 55-69 years LD: 64</li> <li>- <math>\geq</math>70 years LD: 21</li> <li>- 55-69 years LD/LD: 55</li> <li>- <math>\geq</math>70 years LD/LD: 33</li> <li>- 18-55 years SD/SD: 47</li> <li>- 55-69 years SD: 9</li> <li>- <math>\geq</math>70 years SD: 49</li> <li>- 55-69 years SD/SD: 72 <math>\geq</math>70 years SD/SD:48</li> </ul>
			IFN $\gamma$ ELISpot response against SARS-CoV-2 spike peptides at Day 28 (median)	ChAdOx1 nCoV-19 <ul style="list-style-type: none"> <li>- 55-69 years LD: 511</li> <li>- <math>\geq</math>70 years LD:420</li> <li>- 55-69 years LD/LD: 488</li> <li>- <math>\geq</math>70 years LD/LD: 397</li> <li>- 18-55 years SD/SD: 292</li> <li>- 55-69 years SD: 29</li> <li>- <math>\geq</math>70 years SD: 47</li> <li>- 55-69 years SD/SD: 591</li> <li>- <math>\geq</math>70 years SD/SD:300</li> </ul>
Voysey et al., Lancet, 2021	ChAdOx1 nCoV-19 (University of Oxford/AstraZeneca)	Multicentric, randomized, controlled, single-blind trial. Phase 3 of the study by Ramasamy et al., <i>The Lancet</i> , 2020; 23 848 adults ( $\geq$ 18 years old) were randomly assigned in a 1:1 ratio to receive two doses of ChAdOx1 nCoV-19 vaccine or the MenACWY vaccine or saline as control. Participants assigned to the vaccine group received two SD/SD of $5 \times 10^{10}$ viral particles or a half dose as their first dose and an SD as their second dose (LD/SD)	Efficacy against confirmed Covid-19 with onset at least 14 days after second dose in all LD/SD and SD/SD recipients without prior evidence of infection (CI 95%) <sup>a</sup>	ChAdOx1 nCoV-19 group Number of cases: 30/5807 Control group Number of cases: 101/5829 Efficacy: 70.4% (54.8%-80.6%)
			Efficacy against confirmed Covid-19 with onset at least 14 days after the second dose in all LD/SD recipients without prior evidence of infection (CI 95%) <sup>a</sup>	ChAdOx1 nCoV-19 group Number of cases: 3/1367 Control group Number of cases: 30/1374 Efficacy: 90.0% (67.4%-97.0%)
Voysey et al., Lancet, 2021	ChAdOx1 nCoV-19 (University of Oxford/AstraZeneca)	Multicentric, randomized, controlled, single-blind trial. Phase 3 of the study by Ramasamy et al., <i>The Lancet</i> , 2020; 23 848 adults ( $\geq$ 18 years old) were randomly assigned in a 1:1 ratio to receive two doses of ChAdOx1 nCoV-19 vaccine or the MenACWY vaccine or saline as control. Participants assigned to the vaccine group received two SD/SD of $5 \times 10^{10}$ viral particles or a half dose as their first dose and an SD as their second dose (LD/SD)	Efficacy against confirmed Covid-19 with onset at least 14 days after the second dose in all LD/SD recipients without prior evidence of infection (CI 95%) <sup>a</sup>	ChAdOx1 nCoV-19 group Number of cases: 27/4440
			Efficacy against confirmed Covid-19 with onset at least 14 days after the second dose in all LD/SD recipients without prior evidence of infection (CI 95%) <sup>a</sup>	ChAdOx1 nCoV-19 group Number of cases: 27/4440

TABLE 2 (Continued)

Authors, journal, year	Vaccine name	Design and population	Outcome measurement	Results
			dose in all SD/SD recipients without prior evidence of infection (CI 95%) <sup>a</sup>	Control group Number of cases: 71/4455 Efficacy: 62.1% (41.0%–75.7%)
			Efficacy against confirmed other non-primary symptomatic Covid-19 disease <sup>c</sup> with onset at least 14 days after second dose in all LD/SD and SD/SD recipients without prior evidence of infection (CI 95%) <sup>b</sup>	ChAdOx1 nCoV-19 group Number of cases: 7/5807 Control group Number of cases: 11/5829 Efficacy: 36.4% (–63.4%–75.3%)

Abbreviations: CI, confidence interval; Covid, coronavirus disease-2019; IRR, incidence rate ratio; MenACWY vaccine, meningococcal group A, C, W, and Y conjugate vaccine; mRNA, messenger RNA; PHE MNA<sub>80</sub>, Public Health England Microneutralization Assay; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

<sup>a</sup>Calculated as  $100 \times (1 - IRR)$ ; IRR is the calculated ratio of confirmed cases of Covid-19 per 1000 person-years of follow-up in the vaccine group to the corresponding illness rate in the placebo group.

<sup>b</sup>Secondary definition of Covid-19 disease was defined as including systemic symptoms and a positive nasopharyngeal swab, nasal swab or saliva sample for SARS-CoV-2.

<sup>c</sup>Other nonprimary symptomatic Covid-19 disease includes cases who have other symptoms than fever  $\geq 37.8^\circ\text{C}$ , cough, shortness of breath, or anosmia or ageusia.

## 5 | UNIVERSITY OF OXFORD/ASTRAZENECA

The University of Oxford/AstraZeneca vaccine uses a modified adenovirus, a double-stranded DNA virus. In particular, the University of Oxford/AstraZeneca employs the chimpanzee adenovirus vector ChAdOx1 (Figure 2A).<sup>76</sup> A challenge associated with using these vectors is that there can be pre-existing immunity in humans, which can lessen their efficacy. For this reason, using a chimpanzee adenovirus minimizes possible interactions with prevalent antiadenovirus antibodies.<sup>77,78</sup>

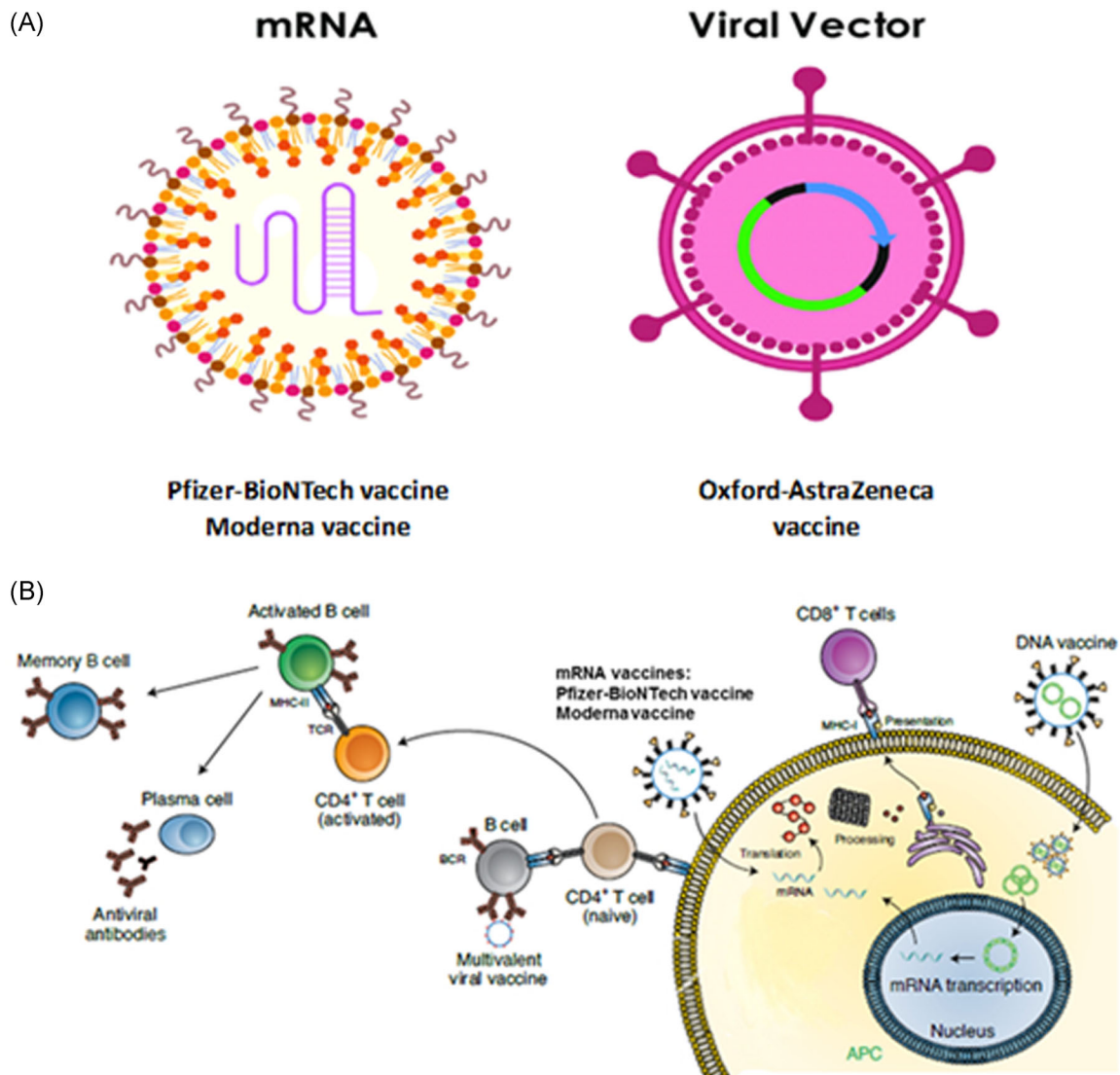
After deleting E1 and E3, the SARS-CoV-2 Wuhan-Hu-1 gene was cloned into a viral vector.<sup>79</sup> E1 deletion inactivates the vaccine's potential replication, whereas E3 deletion allows stable incorporation of larger gene sections (up to 8 kb) into the viral vector. The additional sequence encodes the full-length S protein and was optimized with a tissue plasminogen activator leader sequence. The addition of an adjuvant has not been mentioned, so it is more likely that the efficacy of this vaccine depends on immune recognition of the non-replicating virus, which, perhaps through its DNA, can activate TLRs within endosomes.<sup>80</sup>

The in situ cellular production of the protein avoids post-translational modifications, especially in the S protein case, which can have up to 22 glycosylation sites.<sup>40</sup> The presence of glycosylation sites in the S protein can reduce antibody-mediated neutralization, rendering the vaccines ineffective.<sup>40–81</sup> The in situ production of a protein with few or no glycosylation sites makes these RNA vaccines very attractive.<sup>81</sup>

### 5.1 | Clinical studies

The University of Oxford/AstraZeneca published the results of a Phase 1/2 single-blind, multicentric (five sites) randomized controlled clinical trial<sup>76</sup> that tested a chimpanzee adenovirus-vectored vaccine (ChAdOx1 novel coronavirus-2019 [nCoV-19], which expresses the SARS-CoV-2 S protein) and a control meningococcal conjugate vaccine (MenACWY) (Table 1). Healthy adults (18–55 years old), without a history of SARS-CoV-2 infection or COVID-19-like symptoms, were randomly chosen (1:1) to receive ChAdOx1 nCoV-19 at a dose of  $5 \times 10^{10}$  viral particles or a single dose of MenACWY.

From April 23 to May 21, 2020, 1077 volunteers were enrolled to receive either ChAdOx1 nCoV-19 ( $n = 543$ ) or MenACWY ( $n = 534$ ). In the ChAdOx1 nCoV-19 boost group, local and systemic reactions were more common, including pain, malaise, mild fever, muscle ache, chills, and headache (all  $p < 0.05$ ), which were reduced by preventive paracetamol. No serious effects related to ChAdOx1 nCoV-19 were reported. In the ChAdOx1 nCoV-19 group, S protein-specific T-cell responses peaked on Day 14, and anti-S protein immunoglobulin G (IgG) responses peaked by Day 28. Neutralizing antibody response was detected (after the first dose) in 91% and 100% of the participants when measured with the MNA80 assay and PRNT50, respectively. After the boost dose, participants showed



**FIGURE 2** (A) The nanolipid vector (80–100 nm) of Pfizer-BioNTech and Moderna and the viral vector of Oxford-AstraZeneca. (B) The nanolipid carrying the mRNA blends the cell membrane entering the cytoplasm through endosomal vesicles. From the endosomes, nanolipids spill out their cargo (mRNA) into the cytoplasm, reaching the ribosomes where mRNA is translated into a spike protein to be processed and presented to MHC-1, activating CD8+ T cells. Analogously, CD4+ (naive) T-cells are activated and, by the MHC-2 activation, the B cells are operative, producing whether memory T cells or plasma cells, which can produce antiviral antibodies. MHC, major histocompatibility complex; mRNA, messenger RNA

neutralizing activity linked with antibody levels, which were measured by enzyme-linked immunosorbent assay ( $R^2 = 0.67$ ;  $p < 0.001$ ).

To summarize, ChAdOx1 nCoV-19 demonstrated a good safety profile and increased antibody response following the boost dose (Table 2). The University of Oxford/AstraZeneca vaccine phase 2 study<sup>82</sup> enrolled 560 volunteers, divided into three groups: adults aged 18–55, 56–69, and over 70. The study aimed to evaluate the immune response and detect any variations in older people (over 70) or adults (18–55 and 56–69). Adult volunteers were randomly assigned to receive one or two doses of either the ChAdOx1 nCoV-19 or the MENACWY vaccine. First, participants designated to receive a low dose ( $2.2 \times 10^{10}$  virus particles) were randomized to receive either ChAdOx1 nCoV-19 or MENACWY; block randomization was

used and participants were stratified by age, dose group, and study site. The booster vaccine dose was given 28 days after the first dose. The remaining participants were designated to receive the standard dose ( $3.5\text{--}6.5 \times 10^{10}$  virus particles) and the same randomization protocol was used, except that the 18–55-year-old group was randomized to a 5:1 ratio (two doses of ChAdOx1 nCoV-19 or MENACWY). Participants and investigators were masked to vaccine allocation; only the administering staff knew the randomization. Between May 30 and August 8, 2020, 560 participants were enrolled: 300 in the low-dose cohort (100 in the 18–55 group, 80 in the 56–69 group, and 120 in the over 70 group) and 260 in the standard-dose group (60 in the 18–55 group, 80 in the 55–69 group, and 120 in the over 70 group).

Within the groups that received two standard doses of ChAdOx1 nCoV-19, after the first dose, local reactions were reported in 88% of younger adult participants ( $n = 49$ ), in 73% of the 56–69 group ( $n = 30$ ), and in 61% of the over 70 group ( $n = 49$ ). Seven days after the first dose of ChAdOx1 nCoV-19, the incidence of fever was lower in the 18–55 group (24%), whereas in the other groups, no fever was recorded.

Participants across the three age cohorts who received two doses showed similar median anti-S protein IgG responses and neutralizing antibody titers after 28 days. Moreover, 2 weeks after the second dose, more than 99% of the participants showed neutralizing antibody responses. On Day 14, a T-cell response peak was observed, but after the boost vaccination, there was not a significant increase. Participants who received two standard doses of the vaccine showed a remarkable difference across age groups, with the 56–69 group displaying higher responses at Day 42 than the other groups. Limitations of this study refer to the single-blind design used to assess vaccine efficacy and safety.

In August 2020, a multicentric randomized Phase 3 trial was initiated to evaluate the University of Oxford/AstraZeneca vaccine, which is also known as AZD1222.<sup>21</sup> The trial enrolled over 30 000 adults at 80 sites. The primary outcomes investigated were: efficacy of two intramuscular doses of vaccine compared to placebo for safety and tolerability (timeframe: one year); incidence of AEs and SAEs (timeframe: from Days 1 to 730); and reactogenicity of the vaccine compared to placebo.

Protection was reported as 90% ( $n = 2741$ ) in an analysis performed on 3000 people in a single-dose regimen, where ChAdOx1 nCoV-19 was given as a half dose followed by a full dose after 1 month. Another dosing regimen was tested on 8895 participants, where AZD1222 was given in two full doses after 1 month; this showed 62% efficacy. The analysis of both dosing schemes ( $n = 11\,636$ ) showed an average efficacy of 70% ( $p < 0.0001$ ). The higher protection afforded by the half/full dose regimen represented a considerable complication because it was an undesigned error. Thus, the US FDA suggested that the University of Oxford/AstraZeneca undertake supplementary studies to validate these results. No serious AEs were reported, and ChAdOx1 nCoV-19 was tolerated in both dosing schemes. Moreover, the Phase 3 study was paused on September 6, 2020, after a UK participant presented with a suspected serious adverse reaction (neurological reaction). Trials restarted in late October 2020.

On January 29, 2021, the European Medicines Agency (EMA) recommended the University Oxford/AstraZeneca vaccine for a conditional marketing authorization to prevent the spread of SARS-CoV-2 in people  $\geq 18$  years old. The vaccine's safety has been demonstrated across four clinical trials in the UK, Brazil, and South Africa. However, the EMA based its calculation of how well the vaccine worked on the COV002 study (conducted in the UK) and COV003 study (conducted in Brazil) results. The remaining two studies had a limited number of COVID-19 cases (fewer than six in each), which was not enough to measure the vaccine's efficacy. In participants who received two standard doses, a 59.5% reduction in

the number of symptomatic COVID-19 cases was shown (64 cases out of 5258 participants in the vaccine group and 154 cases out of 5210 participants in the control group). The majority of the participants were aged 18–55 years old. To date, no sufficient results are available to show how well the vaccine works in older participants (over 55 years old). However, based on the results of trials with other vaccines, protection in older participants is expected. There is also reliable information on safety in this population. Therefore, the EMA determined that vaccines could be used in older adults.

The COVID-19 Genomics United Kingdom Consortium, the AMPHEUS Project, and the Oxford COVID-19 Vaccine Trial Group released the results of their exploratory analysis of the Phase 2/3 randomized controlled trial.<sup>83</sup> This study explored the efficacy of the University of Oxford/AstraZeneca vaccine against the alpha variant. The data indicate that the vaccine will not need modifications to protect against the variant (71% efficacy). Another study<sup>40</sup> showed poor efficacy of the vaccine against the beta variant (22% efficacy); for this reason, South Africa (where the 501Y.V2 variant dominates) suspended the use of the University of Oxford/AstraZeneca vaccine.<sup>84</sup> Results about vaccine efficacy against the gamma variant have not yet been published.

## 6 | STORAGE

The three vaccines detailed in this review have different storage requirements because of differences in RNA and DNA stability and in their specific formulations. Due to the instability of mRNA, both of the mRNA vaccines need to be stored at low temperatures; indeed, mRNA is more susceptible to degradation than DNA because it is single-stranded. In contrast, the adenovirus-based University of Oxford/AstraZeneca vaccine allows the use of simple storage conditions with a temperature range of 2–8°C. The advantages of this vaccine storage temperature have multiple repercussions on production, stock, distribution, and administration.

The Pfizer/BioNTech vaccine has been shown to be stable at –80°C for 6 months, ensuring the possibility of stocking the product for long periods.

Moderna affirmed that its vaccine is stable at 2–8°C for up to 30 days; this has been supported by further tests that extended the previous estimate of 7 days. The Moderna vaccine storage temperature of –20°C is sufficient to maintain its vaccine activity, perhaps because some components in the LNPs stabilize lipid dispersion and prevent particle agglomeration.<sup>48</sup> The possibility of stocking the vaccine at a common freezer temperature permits favorable management of worldwide vaccination.

## 7 | FURTHER OPPORTUNITIES

Even if the review focuses on the first three vaccines approved, others could be available and distributed to the population as the cited vaccines in the background section. In fact, the Ad26.CoV2.S by



J&J is a viral vector vaccine deriving from a human adenovirus serotype 26, encoding full-length S-protein, which shows the great advantage of a single-dose regimen.

A single dose of Ad26.COV2.S elicited a strong humoral response in a majority of vaccine recipients, with the presence of S-binding and neutralizing antibodies in more than 90% of the participants, regardless of either age group or vaccine dose. Other Ad26-based vaccines, including an approved Ebola vaccine, are safe and have induced durable immune responses.<sup>85</sup>

Sadoff et al.<sup>85</sup> reported an efficacy rate of 66.1% in 28 days in South Africa and it should be stressed that the low percentage of efficacy was due to the wide diffusion of South Africa variant in the study population.

The Sputnik vaccine is a viral vector too, but carrying a full-length S protein by two adenoviral vectors, Ad26 and Ad5, using Ad26 in the first dose and Ad5 in the second one to get a 91.6% efficacy against SARS-CoV-2 infection.<sup>86</sup> NVX-CoV2373 is a protein subunit vaccine instead, including a recombinant full-length prefusion S protein, needing 2 doses to get an 89.7% efficacy (after 7 days from the second dose).<sup>87</sup>

From a storage point of view, all vaccines listed are stable for 6 months at a fridge temperature (2–8°C), apart from the J&J's which is stable for 3 months. Vaccines produced in China, such as CoronaVac and BBIBP-CorV, do not show Phase 3 studies published yet; but, from the clinical studies available, it is possible to understand their differences in terms of technology (they are inactivated virus from CN02 and HB02 strain of SARS-CoV-2, respectively). Both vaccines need a second-dose regimen to reach the preliminary efficacy values disseminated, which are still ongoing.<sup>88,89</sup>

## 8 | VACCINE-INDUCED THROMBOTIC THROMBOCYTOPENIA

Vaccine-induced immune thrombotic thrombocytopenia (VITT, also known as thrombosis with thrombocytopenia syndrome) emerged in February 2021, initially described as occurring sporadically in populations vaccinated with the University of Oxford/AstraZeneca COVID-19 vaccine.<sup>90</sup> Reports indicated that after receiving the vaccine, normally healthy patients developed thrombocytopenia and thrombosis in unusual sites (cerebral and/or splanchnic veins). Two months later, similar complications were described in patients administered with the J&J adenoviral vaccine.<sup>91</sup> From March 2021 besides, cases of mRNA vaccine-induced VITT have started to be published<sup>92,93</sup>

Case reports and case series are rare, and research is restricted, but the understanding of VITT's epidemiology, pathophysiology, diagnosis, and treatment is growing.<sup>94</sup> The estimated incidences of VITT with the University of Oxford/AstraZeneca and J&J vaccines were 7–10 cases per million individuals and 3.2 cases per million individuals, respectively.<sup>95</sup> However, because of the limited availability of the J&J vaccine and delays in reporting, the stated rates are likely an underestimation of true incidence levels.<sup>94,95</sup>

The pathogenesis of VITT has been somewhat elucidated. It is described in published studies as an immunological disease, similar to

autoimmunity-induced thrombocytopenia, unlikely to be the result of COVID-19 infection and independent of anti-SARS-CoV2 protective immunity.<sup>94</sup> Nevertheless, VITT is a morbid condition with a high death rate. Twenty-six percent to eighty percent of published case series include cerebral hemorrhage as the primary cause of death.<sup>90–97</sup> Twenty percent of patients with VITT die, likely because of the delayed detection of clinical symptoms and signs by individuals with VITT and/or healthcare professionals.<sup>90–97</sup> Future instances should help clarify clinical understanding and enhance clinical outcomes given the greater awareness and recognition of this illness syndrome by both doctors and the public.

## 9 | CONCLUSION

Pfizer–BioNTech, Moderna, and the University of Oxford/AstraZeneca have now completed their Phase 3 clinical studies and received authorization from the FDA in the US and the EMA in Europe (with some exceptions, e.g., the University of Oxford/AstraZeneca vaccine has not been approved in Switzerland and in the USA). From the initial Phase 1 Study (April 2020) to the end of the Phase 3 Studies (December 2020), comprehensive clinical studies have been completed in just 8–9 months. This rapid development of new COVID-19 vaccines has been possible because in the last 20 years there have been rapid improvements in vaccine development in different scientific areas, such as molecular biology, genetic engineering, nanomaterials, and lipid nanotechnology.

SARS-CoV-2 variants could rapidly spread, impacting the vaccines' efficacy and the risk of massive reinfection. Multiple studies have investigated the efficacy of the vaccines against variants circulating in the UK, South Africa, and Brazil. The results indicate that the Pfizer/BioNTech and Moderna vaccines are efficient against the variant in the UK and less efficient against the variant in Brazil. The data on the efficacy of these two vaccines against a variant circulating in South Africa is variable, whereas the University of Oxford/AstraZeneca vaccine is not effective against the variant in South Africa. Furthermore, the results of the efficacy of the University of Oxford/AstraZeneca vaccine against Brazilian variants have not yet been published.

Pfizer/BioNTech and Moderna used analogous procedures to produce stable prefusion state mRNA. The mRNA stabilization prolongs the alternatively short half-life of RNA and thereby boosts the S protein expression level. Moreover, mRNA-based vaccines have an advantage over DNA vaccines, because mRNA does not integrate and poses no risk of insertional mutagenesis. A further advantage of using mRNA-based vaccines is that the mRNA is translated directly in the cytoplasm (i.e., endoplasmic reticulum) on the ribosomes once inside the cell. Technological and scientific advances now allow the encapsulation of mRNA into custom-designed LNPs (instead of a modified adenovirus) that mimic the structural features of a viral vector. Previously, the use of a nanolipid carrier for delivering genetic material (DNA or RNA) did not achieve high efficiency because it was difficult to mimic the complex machinery evolved by viral vectors.

The nanoparticles containing mRNA travel a short distance within the cell, easily reaching the endoplasmic reticulum outside the nucleus and avoiding the difficulty of penetrating the nuclear membrane. For these reasons, Pfizer/BioNTech and Moderna used the lipid nanovector with high efficiency.

The trial resulting from the conditional marketing authorization for the University of Oxford/AstraZeneca vaccine showed a 59.5% reduction in the number of symptomatic COVID-19 cases (64 cases out of 5258 participants in the vaccine group vs. 154 cases out of 5210 participants in the control group). There are not enough results yet to show how well the vaccine works in older participants (over 55 years old); however, when the experience in the UK and the immune response seen in older participants are taken into account, protection in this age group is expected. This is why the EMA also approved the use of the vaccine in older adults.

A EUA is a mechanism to facilitate the availability and use of medical countermeasures, including vaccines, during public health emergencies, such as the current COVID-19 pandemic.<sup>98</sup>

Among the different vaccine candidates developed by many companies and universities, the Pfizer/BioNTech and Moderna vaccines emerged as favorable candidates for the prevention of COVID-19. BNT162b2 vaccine was approved by FDA in August 2021. In the UK, the Pfizer/BioNTech vaccine was approved for commercialization by the Medicines and Healthcare Products Regulatory Agency on December 2, 2020.<sup>99</sup> The vaccine received a EUA by the FDA on December 11,<sup>100</sup> and by the EMA on December 21, 2020.<sup>101</sup> The Moderna vaccine was the second vaccine candidate to receive a EUA from the FDA on December 18, 2020,<sup>102</sup> and by the EMA on January 15, 2021.<sup>103</sup> The University of Oxford/AstraZeneca vaccine received a EUA on January 29, 2021, by the EMA, with some limitations.<sup>104</sup> The three companies have predicted the production of billions of vaccines during 2021. Therefore, with this in mind, it is fundamental that these effective vaccines be delivered and administered globally to achieve global herd immunity.

## CONFLICT OF INTERESTS

The authors declare that there are no conflict of interests.

## AUTHOR CONTRIBUTIONS

The search and the selection of articles were performed by Luigi Cattel. The first draft of the manuscript was written by Luigi Cattel. Susanna Giordano, Sara Traina, and Tommaso Lupia revised the manuscript and all authors commented on subsequent versions of the manuscript. All authors read and approved the final manuscript.

## DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no datasets were generated or analyzed during the current study.

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## REFERENCES

1. Wang C, Horby PW, Hayden FG, Gao GF. A novel coronavirus outbreak of global health concern. *Lancet*. 2020;395(10223):470-473. doi:10.1016/S0140-6736(20)30185-9
2. Lupia T, Scabini S, Mornese Pinna S, Di Perri G, De Rosa FG, Corcione S. 2019 novel coronavirus (2019-nCoV) outbreak: a new challenge. *J Glob Antimicrob Resist*. 2020;21:22-27. doi:10.1016/j.jgar.2020.02.021
3. COVID-19 dashboard. Accessed August 6, 2021. <https://covid19.who.int/>
4. Astuti I, Ysrafil Y. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2): an overview of viral structure and host response. *Diabetes Metab Syndr*. 2020;14(4):407-412. doi:10.1016/j.dsx.2020.04.020
5. Draft landscape of COVID-19 candidate vaccines. Accessed August 6, 2021. <https://www.who.int/publications/m/item/draft-landscape-of-covid-19-candidate-vaccines>
6. Milken Institute's COVID-19 treatment and vaccine tracker tracks the development of treatments and vaccines for COVID-19. Accessed August 6, 2021. <https://covid-19tracker.milkeninstitute.org/>
7. Schoeman D, Fielding BC. Coronavirus envelope protein: current knowledge. *Viol J*. 2019;16(1):69. doi:10.1186/s12985-019-1182-0
8. Xia S, Zhu Y, Liu M, et al. Fusion mechanism of 2019-nCoV and fusion inhibitors targeting HR1 domain in spike protein. *Cell Mollmmunol*. 2020;17(7):765-767. doi:10.1038/s41423-020-0374-2
9. Hofmann H, Hattermann K, Marzi A, et al. S protein of severe acute respiratory syndrome-associated coronavirus mediates entry into hepatoma cell lines and is targeted by neutralizing antibodies in infected patients. *J Virol*. 2004;78(12):6134-6142. doi:10.1128/JVI.78.12.6134-6142.2004
10. Bosch BJ, van der Zee R, de Haan CA, Rottier PJ. The coronavirus spike protein is a class I virus fusion core: structural and functional characterization of the fusion core complex. *J Virol*. 2003;77(16):8801-8811. doi:10.1128/jvi.77.16.8801-8811.2003
11. Fontanet A, Autran B, Lina B, Kieny MP, Karim S, Sridhar D. SARS-CoV-2 variants and ending the COVID-19 pandemic. *Lancet*. 2021;397(10278):952-954. doi:10.1016/S0140-6736(21)00370-6
12. Tegally H, Wilkinson E, Giovanetti M, et al. Emergence and rapid spread of a new severe acute respiratory syndrome-related coronavirus 2 (SARS-CoV-2) lineage with multiple spike mutations in South Africa. *MedRxiv*. 2020. doi:10.1101/2020.12.21.20248640
13. Zhang W, Davis BD, Chen SS, Sincuir Martinez JM, Plummer JT, Vail E. Emergence of a Novel SARS-CoV-2 Variant in Southern California. *JAMA*. 2021;325(13):1324-1326. doi:10.1001/jama.2021.1612
14. Naveca F, Nascimento V, Souza V, et al. Phylogenetic relationship of SARS-CoV-2 sequences from Amazonas with emerging Brazilian variants harboring mutations E484K and N501Y in the spike protein. 2021. doi:10.21203/rs.3.rs-275494/v1. <https://virological.org/t/phylogenetic-relationship-of-sars-cov-2-sequences-from-amazonas-with-emerging-brazilian-variants-harboring-mutations-e484k-and-n501y-in-the-spike-protein/585>
15. Rambaut A, Loman N, Pybus O, et al. Preliminary genomic characterisation of an emergent SARS-CoV-2 lineage in the UK defined by a novel set of spike mutations. 2020. <https://virological.org/t/preliminary-genomic-characterisation-of-an-emergent-sars-cov-2-lineage-in-the-uk-defined-by-a-novel-set-of-spike-mutations/563>
16. Volz E, Mishra S, Chand M, et al. Transmission of SARS-CoV-2 lineage B.1.1.7 in England: insights from linking epidemiological and genetic data. *medRxiv*. 2021. doi:10.1101/2020.12.30.20249034
17. Faria NR, Claro IM, Candido D. Genomic characterisation of an emergent SARS-CoV-2 lineage in Manaus: preliminary

- findings. *Virological*. 2021. <https://virological.org/t/genomic-characterisation-of-an-emergent-sars-cov-2-lineage-in-manaus-preliminary-findings/586>
18. Creech CB, Walker SC, Samuels RJ. SARS-CoV-2 vaccines. *JAMA*. 2021;325(13):1318-1320. doi:10.1001/jama.2021.3199
  19. 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. doi:10.1056/NEJMoa2034577
  20. Baden LR, El Sahly HM, Essink B, et al. Efficacy and safety of the mRNA-1273 SARS-CoV-2 vaccine. *N Engl J Med*. 2020;384:403-416. doi:10.1056/NEJMoa2035389
  21. 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(10269):99-111. doi:10.1016/S0140-6736(20)32661-1
  22. Miller Andrew D. Cationic liposomes for gene therapy. *Angewandte Chemie Int Ed*. 1998;37:1768-1785.
  23. Pati R, Shevtsov M, Sonawane A. Nanoparticle vaccines against infectious diseases. *Front Immunol*. 2018;9:2224. doi:10.3389/fimmu.2018.02224
  24. Chattopadhyay S, Chen JY, Chen HW, Hu CJ. Nanoparticle vaccines adopting virus-like features for enhanced immune potentiation. *Nanotheranostics*. 2017;1(3):244-260. doi:10.7150/ntno
  25. Pardi N, Tuyishime S, Muramatsu H, et al. Expression kinetics of nucleoside-modified mRNA delivered in lipid nanoparticles to mice by various routes. *J Control Release*. 2015;217:345-351. doi:10.1016/j.jconrel.2015.08.007
  26. Jayaraman M, Ansell SM, Mui BL, et al. Maximizing the potency of siRNA lipid nanoparticles for hepatic gene silencing in vivo. *Angew Chem Int Ed Engl*. 2012;51(34):8529-8533. doi:10.1002/anie.201203263
  27. Maier MA, Jayaraman M, Matsuda S, et al. Biodegradable lipids enabling rapidly eliminated lipid nanoparticles for systemic delivery of RNAi therapeutics. *Mol Ther*. 2013;21(8):1570-1578. doi:10.1038/mt.2013.124
  28. Richner JM, Himansu S, Dowd KA, et al. Modified mRNA vaccines protect against Zika virus infection. *Cell*. 2017;168(6):1114-1125.e10. doi:10.1016/j.cell.2017.02.017
  29. Chen S, Tam YYC, Lin PJC, Sung M, Tam YK, Cullis PR. Influence of particle size on the in vivo potency of lipid nanoparticle formulations of siRNA. *J Control Release*. 2016;235:236-244. doi:10.1016/j.jconrel.2016.05.059
  30. Chen S, Tam YY, Lin PJ, Leung AK, Tam YK, Cullis PR. Development of lipid nanoparticle formulations of siRNA for hepatocyte gene silencing following subcutaneous administration. *J Control Release*. 2014;196:106-112. doi:10.1016/j.jconrel.2014.09.025
  31. Felgner PL, Ringold GM. Cationic liposome-mediated transfection. *Nature*. 1989;337(6205):387-388. doi:10.1038/337387a0
  32. Cai S, Yang Q, Bagby TR, Forrest ML. Lymphatic drug delivery using engineered liposomes and solid lipid nanoparticles. *Adv Drug Deliv Rev*. 2011;63(10-11):901-908. doi:10.1016/j.addr.2011.05.017
  33. Behr JP, Demeneix B, Loeffler JP, Perez-Mutul J. Efficient gene transfer into mammalian primary endocrine cells with lipopolyamine-coated DNA. *Proc Natl Acad Sci USA*. 1989;86(18):6982-6986. doi:10.1073/pnas.86.18.6982
  34. Lee RJ, Huang L. Lipidic vector systems for gene transfer. *Crit Rev Ther Drug Carrier Syst*. 1997;14(2):173-206.
  35. Arpicco S, Canevari S, Ceruti M, Galmozzi E, Rocco F, Cattel L. Synthesis, characterization and transfection activity of new saturated and unsaturated cationic lipids. *Farmaco*. 2004;59(11):869-878. doi:10.1016/j.farmac.2004.06.007
  36. Basha G, Novobrantseva TI, Rosin N, et al. Influence of cationic lipid composition on gene silencing properties of lipid nanoparticle formulations of siRNA in antigen-presenting cells. *Mol Ther*. 2011;19(12):2186-2200. doi:10.1038/mt.2011.190
  37. Sahin U, Karikó K, Türeci Ö. mRNA-based therapeutics—developing a new class of drugs. *Nat Rev Drug Discov*. 2014;13(10):759-780. doi:10.1038/nrd4278
  38. Pardi N, Hogan MJ, Porter FW, Weissman D. mRNA vaccines—a new era in vaccinology. *Nat Rev Drug Discov*. 2018;17(4):261-279. doi:10.1038/nrd.2017.243
  39. Collins D, Litzinger DC, Huang L. Structural and functional comparisons of pH-sensitive liposomes composed of phosphatidylethanolamine and three different diacylsuccinylglycerols. *Biochim Biophys Acta*. 1990;1025(2):234-242. doi:10.1016/0005-2736(90)90102-t
  40. Watanabe Y, Allen JD, Wrapp D, McLellan JS, Crispin M. Site-specific glycan analysis of the SARS-CoV-2 spike. *Science*. 2020;369(6501):330-333. doi:10.1126/science.abb9983
  41. Shen C, Wang X, Zheng Z, et al. 1-PEGylated “stealth” nanoparticles and liposomes (Woodhead Publishing Series in Biomaterials). *Engineering of Biomaterials for Drug Delivery Systems*. Woodhead Publishing; 2018:1-26 doi:10.1016/B978-0-08-101750-0-00001-5
  42. Cattel L, Ceruti M, Dosio F. From conventional to stealth liposomes: a new frontier in cancer chemotherapy. *Tumori*. 2003;89(3):237-249.
  43. Cueni LN, Detmar M. The lymphatic system in health and disease. *Lymphat Res Biol*. 2008;6(3-4):109-122. doi:10.1089/lrb.2008.1008
  44. eTheRNA launches an international consortium and starts development of cross-strain protective CoV-2 mRNA vaccine for high-risk populations. March 24, 2020. Accessed February 15, 2021. <https://www.biospace.com/article/releases/etherna-launches-an-international-consortium-and-starts-development-of-cross-strain-protective-cov-2-mrna-vaccine-for-high-risk-populations/>
  45. Dalpke A, Helm M. RNA mediated Toll-like receptor stimulation in health and disease. *RNA Biol*. 2012;9(6):828-842. doi:10.4161/rna.20206
  46. Wang ZB, Xu J. Better adjuvants for better vaccines: progress in adjuvant delivery systems, modifications, and adjuvant-antigen codelivery. *Vaccines (Basel)*. 2020;8(1):128. doi:10.3390/vaccines8010128
  47. Kreuzt M, Giquel B, Hu Q, et al. Antibody-antigen-adjuvant conjugates enable co-delivery of antigen and adjuvant to dendritic cells in cis but only have partial targeting specificity. *PLoS One*. 2012;7(7):e40208. doi:10.1371/journal.pone.0040208
  48. Radomska-Soukharev A. Stability of lipid excipients in solid lipid nanoparticles. *Adv Drug Deliv Rev*. 2007;59(6):411-418. doi:10.1016/j.addr.2007.04.004
  49. Sahin U, Muik A, Derhovanessian E, et al. COVID-19 vaccine BNT162b1 elicits human antibody and TH1 T cell responses. *Nature*. 2020;586(7830):594-599. doi:10.1038/s41586-020-2814-7
  50. Hsieh CL, Goldsmith JA, Schaub JM, et al. Structure-based design of prefusion-stabilized SARS-CoV-2 spikes. *Science*. 2020;369(6510):1501-1505. doi:10.1126/science.abd0826
  51. Karikó 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(11):1833-1840. doi:10.1038/mt.2008.200
  52. Heil F, Hemmi H, Hochrein H, et al. Species-specific recognition of single-stranded RNA via toll-like receptor 7 and 8. *Science*. 2004;303(5663):1526-1529. doi:10.1126/science.1093620
  53. Pallesen J, Wang N, Corbett KS, et al. Immunogenicity and structures of a rationally designed prefusion MERS-CoV spike antigen. *Proc Natl Acad Sci USA*. 2017;114(35):E7348-E7357. doi:10.1073/pnas.1707304114

54. Mulligan MJ, Lyke KE, Kitchin N, et al. Phase I/II study of COVID-19 RNA vaccine BNT162b1 in adults. *Nature*. 2020; 586(7830):589-593. doi:10.1038/s41586-020-2639-4
55. 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(2):773-781. doi:10.1016/j.bbrc.2004.09.106
56. Güthe S, Kapinos L, Möglich A, Meier S, Grzesiek S, Kiefhaber T. Very fast folding and association of a trimerization domain from bacteriophage T4 fibrin. *J Mol Biol*. 2004;337(4):905-915. doi:10.1016/j.jmb.2004.02.020
57. Bachmann MF, Zinkernagel RM. Neutralizing antiviral B cell responses. *Annu Rev Immunol*. 1997;15:235-270. doi:10.1146/annurev.immunol.15.1.235
58. Alexopoulou L, Holt AC, Medzhitov R, Flavell RA. Recognition of double-stranded RNA and activation of NF-kappaB by Toll-like receptor 3. *Nature*. 2001;413(6857):732-738. doi:10.1038/35099560
59. Diebold SS, Kaisho T, Hemmi H, Akira S, Reis e Sousa C. Innate antiviral responses by means of TLR7-mediated recognition of single-stranded RNA. *Science*. 2004;303(5663):1529-1531. doi:10.1126/science.1093616
60. 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(25):2439-2450. doi:10.1056/NEJMoa2027906
61. Kondili M, Roux M, Vabret N, Bailly-Bechet M. Innate immune system activation by viral RNA: how to predict it? *Virology*. 2016; 488:169-178. doi:10.1016/j.virol.2015.11.007
62. Muik A, Wallisch AK, Sängler B, et al. Neutralization of SARS-CoV-2 lineage B.1.1.7 pseudovirus by BNT162b2 vaccine-elicited human sera. *Science*. 2021;371(6534):1152-1153. doi:10.1126/science.abg6105
63. Wang Z, Schmidt F, Weisblum Y, et al. mRNA vaccine-elicited antibodies to SARS-CoV-2 and circulating variants. *Nature*. 2021; 592:616-622. doi:10.1038/s41586-021-03324-6
64. Garcia-Beltran WF, Lam EC, St. Denis K, et al. Multiple SARS-CoV-2 variants escape neutralization by vaccine-induced humoral immunity. *Cell*. 2021;184:2372-2383. doi:10.1016/j.cell.2021.03.013
65. Hoffmann M, Arora P, Groß R, et al. SARS-CoV-2 variants B.1.351 and P.1 escape from neutralizing antibodies. *Cell*. 2021;184: 2384-2393. doi:10.1016/j.cell.2021.03.036
66. Zhou D, Dejnirattisai W, Supasa P, et al. Evidence of escape of SARS-CoV-2 variant B.1.351 from natural and vaccine-induced sera. *Cell*. 2021;184:2348-2361. doi:10.1016/j.cell.2021.02.037
67. Crank MC, Ruckwardt TJ, Chen M, et al. A proof of concept for structure-based vaccine design targeting RSV in humans. *Science*. 2019;365(6452):505-509. doi:10.1126/science.aav9033
68. Wrapp D, Wang N, Corbett KS, et al. Cryo-EM structure of the 2019-nCoV spike in the prefusion conformation. *Science*. 2020; 367(6483):1260-1263. doi:10.1126/science.abb2507
69. Ou X, Liu Y, Lei X, et al. Characterization of spike glycoprotein of SARS-CoV-2 on virus entry and its immune cross-reactivity with SARS-CoV. *Nat Commun*. 2020;11(1):1620. doi:10.1038/s41467-020-15562-9
70. Wang N, Rosen O, Wang L, et al. Structural definition of a neutralization-sensitive epitope on the MERS-CoV S1-NTD. *Cell Rep*. 2019;28(13):3395-3405. doi:10.1016/j.celrep.2019.08.052
71. Zhang DY, Wang J, Dokholyan NV. Prefusion spike protein stabilization through computational mutagenesis. *Proteins*. 2021;89: 399-408. doi:10.1002/prot.26025
72. Jackson LA, Anderson EJ, Roupael NG, et al. An mRNA vaccine against SARS-CoV-2—preliminary report. *N Engl J Med*. 2020; 383(20):1920-1931. doi:10.1056/NEJMoa2022483
73. Anderson EJ, Roupael NG, Widge AT, et al. Safety and immunogenicity of SARS-CoV-2 mRNA-1273 vaccine in older adults. *N Engl J Med*. 2020;383(25):2427-2438. doi:10.1056/NEJMoa2028436
74. Shen X, Tang H, McDanal C, et al. SARS-CoV-2 variant B.1.1.7 is susceptible to neutralizing antibodies elicited by ancestral spike vaccines. *Cell Host Microbe*. 2021;29(4):529-539. doi:10.1016/j.chom.2021.03.002
75. Edara VV, Norwood C, Floyd K, et al. Infection- and vaccine-induced antibody binding and neutralization of the B.1.351 SARS-CoV-2 variant. *Cell Host Microbe*. 2021;29(4):516-521. doi:10.1016/j.chom.2021.03.009
76. 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(10249):467-478. doi:10.1016/S0140-6736(20)31604-4
77. Milligan ID, Gibani MM, Sewell R, et al. Safety and immunogenicity of novel adenovirus type 26- and modified vaccinia Ankara-vectored ebola vaccines: a randomized clinical trial. *JAMA*. 2016; 315(15):1610-1623. doi:10.1001/jama.2016.4218
78. Van Doremalen N, Lambe T, Spencer A, et al. ChAdOx1 nCoV-19 vaccine prevents SARS-CoV-2 pneumonia in rhesus macaques. *Nature*. 2020;586(7830):578-582. doi:10.1038/s41586-020-2608-y
79. 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(10240):1845-1854. doi:10.1016/S0140-6736(20)31208-3
80. Dicks MD, Spencer AJ, Edwards NJ, et al. A novel chimpanzee adenovirus vector with low human seroprevalence: improved systems for vector derivation and comparative immunogenicity. *PLoS One*. 2012;7(7):e40385. doi:10.1371/journal.pone.0040385
81. Zhou D, Tian X, Qi R, Peng C, Zhang W. Identification of 22 N-glycosites on spike glycoprotein of SARS-CoV-2 and accessible surface glycopeptide motifs: implications for vaccination and antibody therapeutics. *Glycobiology*. 2021;31:69-80. doi:10.1093/glycob/cwaa052
82. Ramasamy MN, Minassian AM, Ewer KJ, et al. Safety and immunogenicity of ChAdOx1 nCoV-19 vaccine administered in a prime-boost regimen in young and old adults (COV002): a single-blind, randomised, controlled, phase 2/3 trial. *Lancet*. 2020;396: P1979-P1993. doi:10.1016/S0140-6736(20)32466-1
83. Emary KRW, Golubchik T, Aley PK, et al. Efficacy of ChAdOx1 nCoV-19 (AZD1222) vaccine against SARS-CoV-2 variant of concern 202012/01 (B.1.1.7): an exploratory analysis of a randomised controlled trial. *Lancet*. 2021;397(10282):1351-1362. doi:10.1016/S0140-6736(21)00628-0
84. Cohen J. South Africa suspends use of AstraZeneca's COVID-19 vaccine after it fails to clearly stop virus variant. *Science*. 2021. doi:10.1126/science.abg9559
85. Sadoff J, Gray G, Vandebosch A, et al. Safety and efficacy of single-dose Ad26.COV2.S vaccine against Covid-19. *N Engl J Med*. 2021; 384(23):2187-2201.
86. 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(10275): 671-681.
87. Heath PT, Galiza EP, Baxter DN, et al. Safety and efficacy of NVX-CoV2373 Covid-19 Vaccine. *N Engl J Med*. 2021;385:1172-1183.
88. 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*. 2021;21(2): 181-192. doi:10.1016/S1473-3099(20)30843-4



89. Xia S, Zhang Y, Wang Y, et al. Safety and immunogenicity of an inactivated SARS-CoV-2 vaccine, BBIBP-CorV: a randomised, double-blind, placebo-controlled, phase 1/2 trial. *Lancet Infect Dis*. 2021;21(1):39-51. doi:10.1016/S1473-3099(20)30831-8
90. Greinacher A, Thiele T, Warkentin TE, Weisser K, Kyrle PA, Eichinger S. Thrombotic thrombocytopenia after ChAdOx1 nCoV-19 vaccination. *N Engl J Med*. 2021;384:2092-2101. doi:10.1056/NEJMoa2104840
91. Sadoff J, Davis K, Dougouih M. Thrombotic thrombocytopenia after Ad26.COV2.S vaccination—response from the manufacturer. *N Engl J Med*. 2021;384(20):1965-1966.
92. Lee EJ, Cines DB, Gernsheimer T, et al. Thrombocytopenia following Pfizer and Moderna SARS-CoV-2 vaccination. *Am J Hematol*. 2021;96(5):534-537. doi:10.1002/ajh.26132
93. Carli G, Nichele I, Ruggeri M, et al. Deep vein thrombosis (DVT) occurring shortly after the second dose of mRNA SARS-CoV-2 vaccine. *Intern Emerg Med*. 2021;16(3):803-804. doi:10.1007/s11739-021-02685-0
94. Arepally GM, Ortel TL. Vaccine-induced immune thrombotic thrombocytopenia: what we know and do not know. *Blood*. 2021;138(4):293-298. doi:10.1182/blood.2021012152
95. Centers for disease control and prevention emergency preparedness and response. Johnson & Johnson/Janssen COVID-19 vaccine and thrombosis with thrombocytopeniasyndrome (TTS): update for clinicians. Accessed August 3, 2021. [https://emergency.cdc.gov/coca/calls/2021/callinfo\\_042721.asp](https://emergency.cdc.gov/coca/calls/2021/callinfo_042721.asp)
96. Scully M, Singh D, Lown R. Pathologic antibodies to platelet factor 4 after ChAdOx1 nCoV-19 vaccination. *N Engl J Med*. 2021;384:2202-2211. doi:10.1056/NEJMoa2105385
97. See I, Su JR, Lale A, et al. US case reports of cerebral venous sinus thrombosis with thrombocytopenia after Ad26.COV2.S vaccination, March 2 to April 21, 2021. 2021;325:2448-2456. doi:10.1001/jama.2021.7517
98. FDA emergency use authorization for vaccines explained. Accessed August 6, 2021. <https://www.fda.gov/vaccines-blood-biologics/vaccines/emergency-use-authorization-vaccines-explained>
99. MHRA. Vaccine BNT162b2—conditions of authorisation under regulation 174. December 2, 2020. Accessed February 15, 2021. [https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment\\_data/file/956687/Pfizer\\_conditions\\_28Jan2021.pdf](https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/956687/Pfizer_conditions_28Jan2021.pdf)
100. FDA. Vaccine BNT162b2—letter of authorization. December 11, 2020. Accessed February 15, 2021. <https://www.fda.gov/media/144412/download>.
101. EMA. Vaccine BNT162b2. Accessed February 15, 2021. <https://www.ema.europa.eu/en/medicines/human/EPAR/comirnaty#authorisation-details-section>
102. FDA. Moderna vaccine—letter of authorization. December 18, 2020. Accessed February 15, 2021. <https://www.fda.gov/media/144636/download>
103. EMA. Moderna vaccine. Accessed February 15, 2021. <https://www.ema.europa.eu/en/medicines/human/EPAR/covid-19-vaccine-moderna#authorisation-details-section>
104. EMA. University of Oxford-AstraZeneca. Accessed February 15, 2021. <https://www.ema.europa.eu/en/medicines/human/summaries-opinion/covid-19-vaccine-astrazeneca#opinion-section>

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