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Review Article

EVALUATION OF ADENOVIRAL VECTOR-BASED VACCINES FOR PREVENTION OF COVID-19 – AN OVERVIEW

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ABSTRACT

The novel coronavirus and its emerging variants have continued to affect 50.4 million people worldwide, increasing the need for safe and effective vaccines. According to the World Health Organization guidelines, the efficacy of a vaccine should be at least 30% in all age groups and protect for a longer duration without any life-threatening adverse effects. At present, there are 319 vaccines in various stages of development, of which 16 are authorized for emergency use. Of these 16 vaccines, five vaccines are based on adenoviral vectors. This review is focused on understanding the safety and efficacy of the approved adenoviral vector vaccines for COVID-19, particularly highlighting the interim analysis of phase 3 clinical trials of AZD1222, Gam-Covid-Vac, Ad26.COV2.S, and AD5-nCOV vaccine. The efficacy of AZD1222, Gam-Covid-Vac, Ad26.COV2.S, and AD5-nCOV vaccine were found to be 70.4%, 95%, 66%, and 65.7%, respectively. Some serious adverse events such as deep vein thrombosis and thrombosis with thrombocytopenia syndrome were observed among AZD1222 and Ad26.COV2.S vaccinated individuals. Meanwhile, Gam-Covid-Vac and AD5-nCOV vaccines did not report any significant adverse events. In addition, we have also focused on the efficacy of these vaccines against SARS-CoV-2 variants such as B.1.1.7, B.1.351, and P.1. Although the efficacy of these approved vaccines against novel SARS-CoV-2 variants, pediatric and geriatric population and long-term efficacy remains uncertain, they are reasonably efficient in preventing mortality due to COVID-19.

Keywords: COVID-19 vaccine, viral vector, AZD1222, Gam-Covid-Vac, Ad26.COV2.S, AD5-nCov.

Key points

- The safety and efficacy of approved adenoviral vector vaccines
- Emerging variants of SARS-CoV2 and its efficacy against adenoviral vector vaccine
- Real-world evidence against approved vaccines.

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INTRODUCTION

Novel coronavirus disease (COVID- 19) is caused by a single, sense-stranded RNA virus known as SARS-CoV-2 that belongs to the Coronaviridae family [1]. This virus, although identical to SARS-CoV and MERS-CoV, is novel due to the mutations in the receptor-binding domain (RBD) of the spike (S) protein and the difference in their transmission and diagnosis. The first case of SARS-CoV-2 infection was reported in Wuhan, Hubei Province, China, in late December 2019, and since then, several new variants of the virus have emerged worldwide [2]. The discovery and development of coronavirus disease are described in Fig. 1. The virus rapidly disseminated worldwide in a short period, and hence, the World Health Organization (WHO) declared COVID-19 as the sixth public health emergency of global concern on March 11, 2020 [3]. The incidence and case fatality rate of SARS-COV-2 infection has progressed across more than 220 countries and territories, with approximately 50.4 million laboratory-confirmed cases and 6.2 million deaths as of 20, April 2022 [4]. Abdullahi *et al*. reported high fatality rates of covid-19 in patients belonging to >60years of age. However, the new variants seem to be affecting people irrespective of age. Although substantial improvement in clinical research has led to a better understanding of the novel SAR-CoV-2 infection and the management of COVID-19 disease, limiting the virus's continuing spread has become an issue due to the virus's rapid mutation. It has been proven beyond doubt that timely vaccines can prevent several life-threatening diseases, and the average lifespan in most countries has substantially improved owing to these developments [5,6]. Hence, successful vaccines are in urgent need to battle against COVID-19 disease. Researchers have employed various vaccine techniques such as nucleic acid (DNA and RNA), peptides, virus-like particles, recombinant proteins, live attenuated viruses, viral vectors (replicating and non-replicating), and inactivated virus among which nucleic acid, viral vectors, and protein-based vaccines are currently in use. Viral vectors are proven to be efficient vehicles for transporting vaccines to the host. Moreover, the positive results of the viral vector vaccine against MERS/SARS infection had given a fillip to vaccine development, and the other prominent example was the "Ebola vaccine." Hence, viral vector-based vaccine candidates against COVID-19 may provide good results [7]. This review aims to provide a detailed understanding of the efficacy and safety of the various adenoviral vector vaccines against COVID-19 and an overview of its effectiveness against the new variants of the SARS-CoV-2 virus.

THE WHO GUIDELINES FOR THE DEVELOPMENT OF VACCINES

The COVID-19 pandemic scenario has led to the remarkable speeding of vaccine development. The most common target against COVID-19 is the spike(S) protein since it facilitates the binding of the virus to the host cell. The development of a vaccine against the S-protein prevents this interaction by inducing neutralizing antibodies [8]. WHO guidelines recommend that all vaccines should have an efficacy of at least above 30% and above 50% are highly recommended. In addition, the estimated risk reduction should be 50% to avoid vaccine-induced immunopathology such as acute autoimmune disease, vaccine-induced disease enhancement, T-cell immunopathology, and antibody-dependent enhancement [9]. The vaccine's efficacy in the geriatric and pediatric population is critical due to its vulnerability to respiratory diseases, reducing its effectiveness. Thus, vaccine development aims to improve immunogenicity in all age groups and clinical protection against the COVID-19 disease for a longer duration without any life-threatening adverse effects. According to the W.H.O vaccine tracker, 319 vaccine candidates are in various stages of development, and

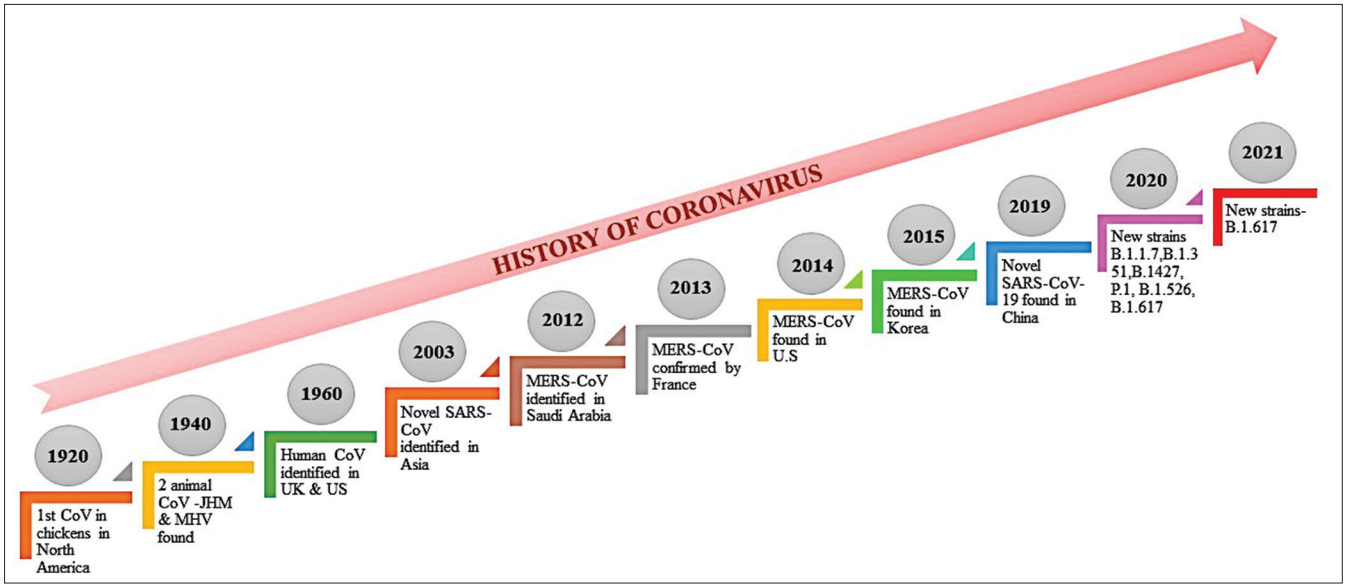

Fig. 1: Timeline of the development of SARS-CoV-2 infection

96 vaccines are in clinical testing. In April 2021, 16 vaccines were authorized by national regulatory authorities for emergency use, including two mRNA vaccines (Moderna and Pfizer-BioNTech), seven conventional virus-inactivated vaccines (Covaxin, CoronaVac, BBIBP-CorV, WIBP-CorV, Covivac, Minhai-Kangtai, and QazVac), five viral vector vaccines (Sputnik Light, Sputnik V, Oxford-AstraZeneca, Convidecia and Johnson and Johnson), and two protein subunit vaccines (EpiVacCorona and RBD-Dimer), as shown in Fig. 2 [10]. In this review, we have discussed the approved viral vector vaccines against COVID-19.

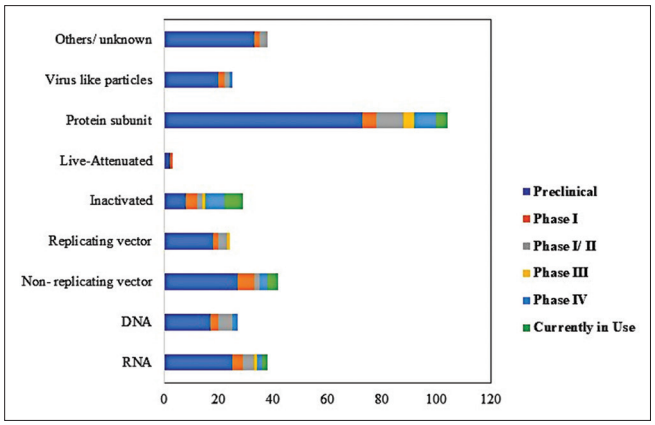

Fig. 2: Types of vaccine platforms under various phases of trials

VIRAL VECTOR VACCINES AGAINST COVID-19

The concept of the viral vector was first introduced in 1972 and was mainly used for gene therapy which was later utilized for vaccine preparation [11]. The advantages of utilizing viral vectors as vaccines are that it does not require any adjuvant to achieve high immunogenicity can stimulate the innate immune response leading to the production of interferon and inflammatory cytokines, precisely targeted gene delivery, improved gene transduction efficiency, enhanced safety, and efficacy and easy large-scale manufacturing [12,13]. The most commonly used viral vectors are adenovirus, poxvirus, measles virus, and adeno-associated virus. Among these, adenovirus is widely utilized for COVID-19 vaccine production [7]. Adenoviruses are non-enveloped, double-stranded DNA viruses of

about 30–40 kb in size contained in an icosahedral capsid with 240 hexon proteins, known to cause upper respiratory tract infections and other life-threatening multi-organ diseases. There are 57 serotypes of adenoviruses found in humans, grouped into seven species (A-G) based on their antibody cross-neutralization properties. The serotypes HAd1, HAd2, and HAd5, cause mild respiratory diseases, and ≤80% of the human population have previously encountered infections caused by HAd5, thereby containing neutralizing antibodies against this serotype.

Similarly, HAd4 and HAd7 cause severe pneumonia. The structure of the viral genome is comprised of inverted terminal repeats (ITRs), early transcription regions (E1A, E1B, E2A, E2B, E3, and E4), and late transcription regions (L1, L2, L3, and L4 aL5), as shown in Fig. 3a. The ITRs are 90-140bp regions flanking the genome, and their *cis* form is essential for viral replication. E1A and E1B are the most critical regions among the early transcription regions, regulating viral replication. E3 is the primary regulator of the viral evasion of host immunosurveillance. E2A and E2B also are responsible for viral replication. E4, apart from replication, is also involved in manipulating several cellular processes. On the contrary, all the late transcription regions encode viral structural components [14].

CONSTRUCTION OF ADENOVIRUS VECTOR FOR VACCINATION

The primary method of adenoviral vector construction is through the insertion of a transgene cassette into the backbone of the virus. These transgene cassettes are employed to express the antigen-of-interest, which is achieved with the help of a promoter, such as a cytomegalovirus, that provides robust and consistent transgene expression. The promoters are chosen based on their size, complexity, and expression pattern. The transgene cassette is inserted into the viral genome by deleting the E1 transcription region, thereby improving the vectors' safety and accommodating a transgene cassette of up to 5000 bp in size. These replication-deficient vectors are known as first-generation vectors due to their absence of E1 unit and most of these vectors also are deficient in E3, increasing the size of the cassette to about 7500 bp [15,16]. Deletions of these multiple early transcription regions also prevent anti-vector immunity by reducing the number of viral-encoded antigens. These recombinant vectors can be developed by two main approaches: Homologous recombination and direct molecular cloning of the adenoviral genome.

The former method employs homologous recombination between a shuttle plasmid comprising a transgene cassette and the adenoviral

backbone [15]. Transfection of this shuttle plasmid into the mammalian system is performed along with a rescue plasmid DNA that contains an E1-defective genome slightly overlapping the 3' end of the shuttle plasmid or a wild-type adenoviral genome that splits in the E1 region. Since they are replication-deficient, amplifying these vectors requires cell lines that can produce E1 in the "*trans*" form, such as HEK-293, a modified human embryonic kidney cell line, expressing E1 from AdHu5 serotype. It has several demerits, such as lack of recovery of all adenoviruses other than AdHu5 type and cross-contamination with replication-competent adenovirus [17]. To avoid these complications, Per.C6 cell lines can be utilized to propagate adenovirus vectors. Major limitations of the homologous recombination approach are that it is not highly efficient as it can generate recombinant vectors-without the transgene cassette and requires multiple rounds of viral purification. An alternative strategy to homologous recombination is the molecular cloning of the entire adenovirus genome into a plasmid vector. This is a complex and challenging approach where the transcription units are replaced with linker regions containing restriction sites such as PI-Sce I and I-Ceu I, which are then used to insert transgene cassettes. A shuttle vector is then constructed to express the transgene cassette, which contains restriction sites similar to the linker region. Following amplification of the shuttle vector, the transgene cassette is excised from the vector and fused with the adenoviral plasmid by direct cloning technique, thereby forming the recombinant adenoviral plasmid is then purified, recovered, and transfected into E1-expressing cell lines described in Fig. 3b. The main advantage of this technique is that multiple transgene cassettes of different sizes can be accommodated in these vectors. Moreover, this strategy prevents infectious contaminants instead of the homologous recombination approach [18-20].

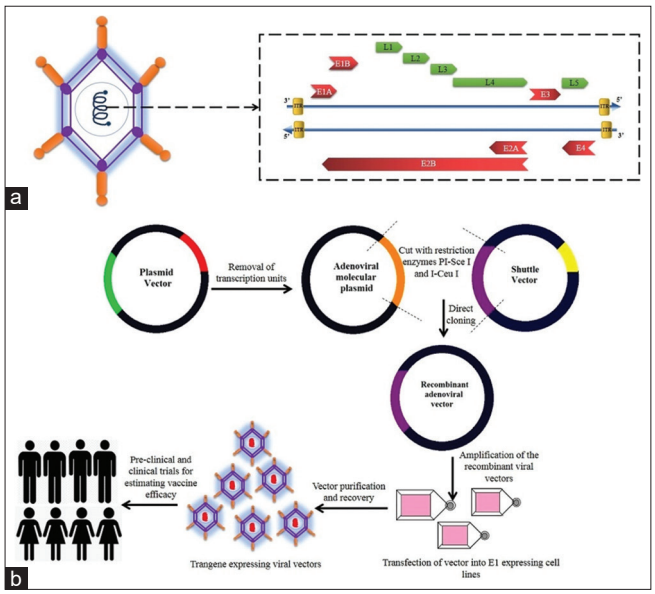

Fig. 3: (a) The structure of the adeno-viral genome; (b) construction of adenovirus vector for vaccination

MECHANISM OF ACTION OF ADENOVIRUS VECTOR VACCINES AGAINST COVID-19

Adenovirus vectors have emerged as promising vaccine candidates as they possess distinct immunogenicity properties and efficiency in inducing host protection [21]. The RNA from SARS-CoV-2 infected cells is isolated; the spike sequence is amplified and cloned into the shuttle vector. The expression cassette is then cloned into a recombinant adenoviral vector via molecular cloning technique. Further, the adenoviral molecular clones are transfected into the E1 helper cell line to recover the vaccines, later tested in pre-clinical studies and clinical trials [22]. When the vaccine is administered, it enters the host cell. The gene (RNA) encodes S protein, undergoes translation, and produces the S proteins, which trigger the production of antibody-secreting plasma cells and memory B cells. The S protein is then taken up by antigen-presenting cells that stimulate CD4+ and CD8+ T cells. On future exposure to SARS-CoV-2 infection, the T-cells will recognize the antigen and neutralize the virus before the infection becomes uncontrollable [22]. Fig. 4 provides a schematic representation of the mechanism of action of adenoviral vector vaccines (AZD1222, Gam-Covid-VacLyo and Ad26.COV2.S) for COVID-19. The findings of phase 3 trials of the approved vaccines are discussed in this review. The adenoviral vector vaccines under various phases of clinical trials and those awaiting approval are listed in Table 1.

AZD1222 (ChAdOx1-S)

The ChAdOx1-S or AZD1222 vaccine (also known as the Oxford/ AstraZeneca vaccine) was first approved in the UK for emergency

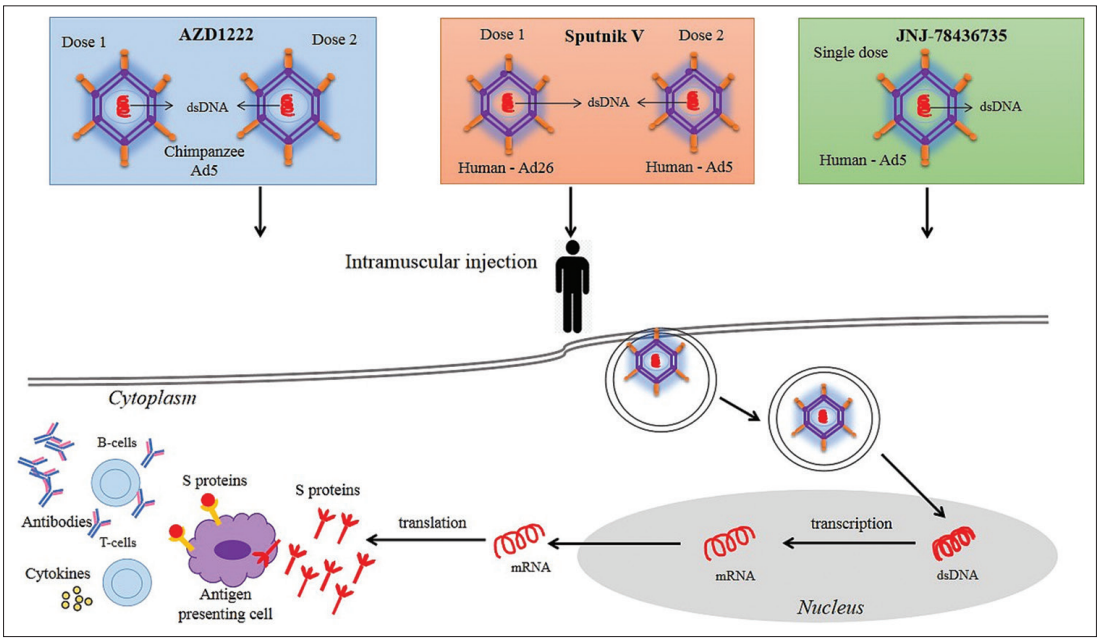

Fig. 4: Schematic representation of the mechanism of action of the adenoviral vector vaccine

Table 1: List of adenoviral vector vaccine for COVID-19 in different phases of clinical trial

Vaccine/ Developer	Vector/ antigen	Delivery and dosage	Number of participants (age range)	Phase trial and description	Outcomes	Price/storage
AZD1222 University of Oxford/ AstraZeneca	ChAdOx1 Antigen: S protein	IM 5×10^{10} vp	1077 (18–55)	Phase I/II COV001 (UK)	• No SAEs • T-Cell response peaked on day 14 • Neutralising antibody increased after booster dose	• $2.15 (U.S.) in the EU • $3-4 (U.S.) in the UK and U.S • Stable in refrigerator for at least 6 months.
		IM 2.2×10^{10} vp (LD), $3.5–6.5\times10^{10}$ vp (SD)	160 (18–55) 160 (56–69) 240 (≥70)	Phase II/ III COV002 (UK). Other Phase II/III trials are ongoing in the U.K and India.	• 13 SAEs not related to vaccine. • Reduced reactogenicity in older adults (≥56). • T-cell response peaked on day 14 after. • High level of Nabs observed inn booster groups	
		IM 2.2×10^{10} vp (LD), 5×10^{10}vp (SD)	23,848 (≥18)	Phase I/II, II/ III, III Safety: COV001, COV002, COV003 (Brazil), COV005 (South Africa).	• Vaccine related neurological events observed • 62.1% efficacy in the SD/SD cohort • 90.0% in the LD/SD cohort • Overall vaccine efficacy 70.4%. • Efficacy was 58.9% and 3.8% in the LD/SD and SD/SD against asymptomatic infection	
GamCOVID-Vac (frozen); GamCOVID-Vac Lyo (lyophilized) Gamaleya Research Institute	Ad26, Ad5 Antigen: S protein	IM 10^{11} vp	76 (18–60)	Phase I: single-dose (rAd26-S or rAd5-S). Phase II: heterologous rAd26-S on day 1 and rAd5-S on day 21.	• No severe adverse effects • RBD-specific IgG responses on day 28 and 42 • Antigen-specific CD4+ and CD8+ T-cells and increased on Dy 28 • NAbs observed on day 42 in all participants	• $10/dose • Freezer storage. • Alternative formulation can be refrigerated.
Ad26Cov2-S Janssen Pharmaceutical Companies	Ad26 Antigen: S protein	IM 5×10^{10} vp	1045 (18–55 and ≥ 65)	Phase I/IIa Two-dose schedules 28, 56, 84 days or 1 year after the first vaccination	• 90% of participants had sufficient antibodies to neutralize the virus and it reached one hundred on 57 day.	• $10/dose • Up to 2 years frozen at −4°F (−20°C), and up to 4.5 months refrigerated at 36–46° F (2–8° C).
			1210 (12–17, 18– ≥65)			
			6000 (≥18)	Phase III single-dose vaccine.	• 66% protection against moderate and severe • 0 Mortality rate in the vaccine group • antibody titre values remained stable at least until day 71 • 100% seroconversion rate • 15cases of thrombosis with thrombocytopenia syndrome observed	
Ad5-nCoV CanSino Biologics/ Beijing Institute of Biotechnology	Ad5 Antigen: S protein	IM 5×10^{10}, 1×10^{11}, 15×10^{11} vp	108 (18–60)	Phase I (Two other ongoing in China)	• 9% participant showed dose-dependent reactogenicity. • T-cell responses detected on day 14. • Specific humoral responses peaked on day 28	US ($58). 2°C and 8°C
			508 (≥18)	Phase II	• Reduced reactogenicity in older adults (≥55). • Specific T-cell responses in 90% (HD) and 88% (LD) of participants. • Seroconversion of NAbs in 59% (HD) and 47% (LD) of participants. • RBD-specific Ab seroconversion in 96% (HD) and 97% (LD) of participants by day 28.	

(Contd...)

Table 1: ***(Continued)***

Vaccine/ Developer	Vector/ antigen	Delivery and dosage	Number of participants (age range)	Phase trial and description	Outcomes	Price/storage
		IM 5×10^{10} vp 10×10^{10} vp	40,000 (≥18) 500 (18–85)	Phase III (Ongoing in multiple countries)	• 65.7% efficacy in moderate cases • 90.98% efficacy at preventing severe cases • 74.8% efficacy against symptomatic (Pakistan population) • 100% efficacy in preventing severe disease (Pakistan population)	
GRAd-CoV2 ReiThera/ LEUKOCARE/ Univercells	Simian Ad (GRAd) Antigen: S protein	IM 5×10^{10} 1×10^{11} 2×10^{11} vp	90 (18–85)	Phase I (Ongoing)	• Well tolerate and all three doses showed clear immune response in 45 healthy volunteers	-
Ad5-nCoV Institute of Biotechnology, Academy of Military Medical Sciences, PLA of China	Ad5 Antigen: n on-specified	IM/mucosal 5×10^{10} vp (IM) 2×10^{10} vp (mucosal), 1×10^{10} vp (mucosal)	149 (18 and ≥60)	Phase I	Ongoing	-
VXA-CoV2-1 Vaxart	rAd5 Antigen: n on-specified	Oral 1×10^{10} I.U 1×11^{10} I.U	35 (18–54)	Phase I Single-dose or two-dose (day 29) with dsRNA adjuvant	Ongoing	-
hAd5 S+ N ImmunityBio, Inc./NantKwest Inc.	Ad5 (E1/ E2b/E3 deletions). Antigens: S + N- (ETSD) proteins	SC, SL and Oral	65 (18–55)	Phase I	Ongoing	-
AdCLD-CoV19 Cellid Co., Ltd.	RAd 5/35 -S proteins	IM (2.5×10^{10} 5.0×10^{10} 1.0×10^{10}VP	150 (19–64)	Phase I	Ongoing	-
AdCOVID AltImmune	RD-Ad5	2.5×10^{8} ifu (0.5 ml) Single dose intra Nasal spray	-	Phase I	Ongoing	-
BBV154 Vaccine Bharat Biotech	RD-Ad-stabilized spike protein	1.0×10^{10} VP	175 (18–60)	Phase I	Ongoing	-

use in December 2020, under the brand name Vaxzevria. It is a monovalent, single recombinant, replication-deficient chimpanzee adenovirus (ChAdOx1) vector derived from Y25 isolate, encoding the SARS-CoV-2 S-protein modified in HEK-293 cells. The pre-clinical study conducted by Van Doremalen *et al*. investigated the immunogenicity of the AZD1222 vaccine in mice and rhesus macaques, which reported robust humoral and type 1 helper cell-mediated response in mice. In contrast, type 2 T-helper cell responses and decreased viral load were observed in rhesus macaques [23]. Based on these findings, the phase I/II clinical trials were performed, which provided additional evidence for the vaccine's safety, immunogenicity, and tolerability [24]. The first interim analysis of phase III clinical trial showed an overall efficacy of 70.4% against symptomatic COVID-19. The efficacy of a single standard dose of the vaccine was found to be 64.1%. The study participants were classified into two groups, where the first group received a low dose + standard dose of the vaccine and the second group received two standard doses. The efficacy of these groups was found to be 90% and 62%, respectively. The interval between the 1st and 2nd dose of the vaccine was delayed to achieve the extended benefits of the vaccine rapidly. Among participants who were injected with two standard doses showed an efficacy of 63.1% (51.8–71.7), whereas those who received a low dose + standard dose showed 80.7% (62.1–90.2) efficacy [25]. Another study reported an efficacy of approximately 80% when the two doses were administered at an interval of ≥12 weeks. In addition, the study also suggested that a single standard dose of the vaccine was sufficient to reduce viral transmission and provide protection against both symptomatic and asymptomatic infection [26]. A third dose of the ChAdOx1 nCoV-19 vaccine was found to significantly elevate immune response to the viral protein by increasing the neutralizing antibody titers and also greatly improved the antibody activity against SARS-CoV-2 variants alpha, beta and delta. Elevated levels of the neutralizing antibodies against these variants were observed 28 days after a third dose of vaccine administration [27].

SAFETY OF AZD1222

The vaccine was found to have a good safety profile with some serious adverse events, which occurred in 168 participants, of which 79

belonged to the vaccine group and 89 belonged to the control group. Three cases of transverse myelitis were reported 14 days post 2nd dose vaccination (two in the vaccine group and one in the control group). But they were ruled out by the neurological committee as being unrelated to the vaccine. In March 2021, 30 cases of thromboembolic events, 15 cases of deep vein thrombosis, and 22 events of pulmonary embolism were reported among the vaccine groups across the EU and U.K. AstraZeneca claimed this to be unrelated to the vaccine as the frequency was lesser than that seen in the general population. However, the German Paul-Ehrlich Institute reported seven cases of cerebral vein thrombosis with thrombocytopenia, which is significantly higher than the actual incidence rate, suggesting that it may be related to the vaccination. Due to this, the vaccination program was withheld for a brief period [28,29]. As of October 2021, EMA reported 214,528 cases of side effects among 68.8 million doses of vaccine. In that, fatal outcomes were observed in 1,259 patients. However, the WHO reported that, since the benefit of the vaccine was higher than its potential risk, the vaccinations could be resumed with precaution to the patient who already has got diagnosed for disseminated intravascular coagulation, cerebral venous sinus thrombosis, capillary leak syndrome, Guillain- Barre syndrome, immune thrombocytopenia, acute macular neuro-retinopathy, and anaphylaxis, other hypersensitivity reactions such as urticaria and angioedema. EMA stated that rare events. Nevertheless, the above events have led to the widespread reluctance of many European nations to use AZV for COVID-19 immunization [28,30,31]. Following a third/booster dose of ChAdOx1 nCoV-19, the vaccine was found to be well tolerated and safe in older adults compared to young adult population and also reported comparable immunogenicity among individuals across all ages. However, the efficacy of the vaccine booster dose needs to be evaluated in individuals across all age groups and those having co-morbid conditions [32]. Another study reported that the booster dose administration facilitated an enhanced immunogenicity among end stage kidney disease (ESKD) patients undergoing dialysis. The findings of the study emphasized the importance of booster dose administration in patients with ESKD and kidney transplant due to their increased susceptibility to COVID-19-related diseases and mortality [33].

GAM-COVID-VAC

Gam-Covid-Vac was the world's first registered vaccine for COVID-19, developed by the Gamaleya National Research Institute. Initially, the vaccine was administered as a single dose of rAd26 (Sputniklight) by the Russian Ministry of Health, mainly used in areas with acute outbreaks with an efficacy of 79.4% after the 28th day of vaccination. The concept of two doses was introduced to maintain long-term immunity. Sputnik V is a heterologous human adenovirus vaccine designed using two different serotypes, namely, Ad26 and Ad5. The pre-clinical data (unpublished) suggested an increased response in humoral and cellular immunity in non-human primates and 100% protection against SARS-CoV-2in immuno-suppressed hamsters.

Moreover, antibody-dependent enhancement was also not observed in any of the vaccinated animals. The phase I/II trials assessed the safety and immunogenicity of Sputnik V in two formulations, namely, lyophilized and frozen. Both these formulations were reported to be safe and well-tolerated. The frozen formulation was found to exhibit enhanced neutralizing antibody production and cell-mediated immune response compared to the lyophilized form. However, the frozen liquid form requires storage temperature conditions of <−18°C, as opposed to the lyophilized form, which can be stored at above freezing temperatures (2–8°C) in a normal home refrigerator. Hence, the lyophilized form of the vaccine is more convenient to store and transport to remote locations without any risk of spoilage [34]. In the phase 3 trial, 21977 participants were chosen at random in a 3:1 ratio, where 16,501 participants received the vaccine intra-muscularly (0.5 mL/dose), and 5476 received placebo. The participants in the vaccine group received rAd26 in the first dose and rAd5 in the second dose of vaccination. The presence of confirmed COVID-19 disease 21 days post first dose administration was reported as the primary outcome of the study. The interim analysis revealed that 16 out of 14964 participants in the vaccine group and 62 out of 4902 participants in the placebo group were found to develop the primary outcome, thus showing the efficacy of 91.6% (95% CI 85·6–95·2). Sputnik is the only vaccine other than the mRNA vaccines to report efficacy of above 90% [35].

SAFETY OF GAM-COVID-VAC

The safety data were reported for 12296 participants who received both doses, among which 7966 participants exhibited adverse effects that were classified as grade 1–3, where 93.9% participants had grade 1, 5.6% had grade 2, and 0.3% had grade 3 adverse effects. Ninety-one participants in the vaccine group were found to have rare adverse effects such as deep vein thrombosis, hemorrhagic stroke, and hypertension. Mortality was observed in three participants in the vaccine group. One participant reported the cause of death as thoracic vertebra fracture, and the other two deaths were due to comorbidities. The independent data monitoring committee ruled out all rare effects unrelated to the vaccine [35,36].

Ad26.COV2.S

The Ad26.COV2.S vaccine was approved for emergency use by US-FDA and EMA. Johnson and Johnson developed it in partnership with Beth Israel Deaconess Medical Center in the Netherlands [37]. In pre-clinical studies, the vaccine showed a high titer of neutralizing antibodies and T-cell mediated immune response against SARS-CoV-2 in Syrian golden hamsters and rhesus macaques [38,39]. The interim results of phase I/IIa reported that 90% of participants produced sufficient antibodies required to neutralize the virus, and on the 57th day of vaccination, it reached 100% [40]. The phase III trial (ENSEMBLE) showed 66% protection against moderate and severe COVID-19 with no mortality in the vaccine group. In cohort 1 (18–55 years), 92% and 96% of participants produced antibodies against the S protein on day 29 and day 57, respectively. The antibody titers remained stable until day 71, and a 100% seroconversion rate was observed among cohort 3 (>65years). About 76–83% of participants in cohort 1and 60–67% in cohort 3 exhibited CD4+ T-cell response, predominantly belonging to type 1 T-helper cells. Moreover, CD8+ T-cell response was found to be higher among younger cohorts when compared to the older cohort [41]. Simultaneously, Janssen launched ENSEMBLE 2 trials in collaboration with the U.K National Institute for Health Research on 15 November 2020. The study has recruited 31,836 adult participants from 125 study sites who have received the JNJ78436735 vaccine (0.5×10^{11} or 1×10^{11} viral particles) or placebo in a 57-day interval. The purpose of the trial is to evaluate the efficacy of Ad26.CoV2.S vaccine in preventing lab-confirmed moderate to severe COVID-19. Since the trial is ongoing, there are no results from the trial for discussion [42]. The US-FDA had recently authorized the use of JNJ78436735 vaccine as a heterologous single booster dose, that is, mix and match after completing the first dose vaccination with a different vaccine type (such as Pfizer -BioNTech or Moderna COVID-19 vaccine) [43].

SAFETY OF Ad26.COV2.S

The vaccine adverse event reporting system and the national vaccine safety monitoring system observed 15 cases of thrombosis with thrombocytopenia syndrome (TTS) and clots located in the cerebral venous sinuses, portal vein, splenic vein, and a combination of venous and arterial thrombosis. All 15 cases were women, among which 13 were aged 18–49 years and 2 were ≥50 years. Further age stratification analysis revealed a high incidence of TTS among women aged 30–39 years. Some of them had risk factors such as obesity, combined oral contraceptive use, hypothyroidism, and hypertension. Still, none of them had any history of thrombotic events and were treated with heparin. All patients were hospitalized with three deaths. After a detailed risk-benefit assessment of the TTS events, the Advisory Committee on Immunization Practices recommended the Janssen COVID-19 vaccine for individuals' ≥18 years, under a warning sign for rare clotting events. US-FDA approved the vaccine for emergency use

Table 2: CDC classification of novel variants of SARS-CoV-2 virus and the efficacy of adenoviral vector vaccines against the variants

Name	Origin	S protein substitutions	Attributes
Variants of concern			
B.1.1.7 (alpha)	UK October 2020	69del, 70del, 144del, E484K, S494P, N501Y, A570D, D614G, P681H, T716I, S982A, D1118H, K1191N	• 70.4–81.5% efficacy to Astrazeneca • 40–80% increased transmissibility • Detected in 120 countries
B.1.351 (Beta)	South Africa December 2020	D80A, D215G, 241del, 242del, 243del, K417N, E484K, N501Y, D614G, A701V	• 50% more rapid in transmission • Spread to 68 countries • J&J vaccine showed 74.4% efficacy in U.S and 52% efficacy in South Africa population • 10% efficacy to AstraZeneca vaccine
B.1.427 (Epsilon)	California	L452R, D614G	• 20% increase in transmission
B.1.429	June 2020	S13I, W152C, L452R, D614G	• Vaccine efficacy- data unavailable
P.1 (Gamma)	Japan/ Brazil Dec 2020	L18F, T20N, P26S, D138Y, R190S, K417T, E484K, N501Y, D614G, H655Y, T1027I	• 161% higher in transmission • 37 countries have reported • 85.5% efficacy to Sputnik-V vaccine
B.1.617.2(delta)	India December 2020	D614G, L452R, P681R, T478K	• Higher transmissibility and reduced neutralisation • This variant responsible for second wave in India
Variants of Interest			
B.1.525 (Eta)	United Kingdom/ Nigeria – December 2020	A67V, 69del, 70del, 144del, E484K, D614G, Q677H, F888L	• Variant under investigation • 23 countries have reported • Vaccine efficacy-data unavailable
B.1.526 (Lota)	New York November 2020	L5F, T95I, D253G, S477N, E484K, D614G, A701V	• Found in 18 countries • Vaccine efficacy-data unavailable
B.1.526.1	October 2020	D80G, 144del, F157S, L452R, D614G, T791I, T859N, D950H	
B.1.617	India February 2021	L452R, E484Q, D614G T95I, G142D, E154K, L452R, E484Q, D614G, P681R, Q1071H	
B.1.617.1	December 2020		
B.1.617.3	October 2020	T19R, G142D, L452R, E484Q, D614G, P681R, D950N	
P.2 (Zeta)	Brazil April 2020	E484K, F565L, D614G, V1176F	• Johnson and Johnson showed efficacy

because they should sensitize potential recipients about the risk of TTS in women aged <50 years of age and provide the individuals with the option of an alternate vaccine for COVID-19 [44-46]. In addition, multisystem inflammatory syndrome and menstrual disorder have been reported. Since October 28, 2021, 16.3 million doses of Ad26.COV2.S were administered, among these 28,244 suspected side effects were observed and 198 fatal outcomes were reported by EudraVigilance from EU/EEA countries [47].

AD5-nCOV

The AD5-nCOV vaccine was developed by Cansino biological INC and Beijing institute of Biotechnology under the brand name Convidecia. Pre-clinical studies reported complete protection against SARS-CoV-2 infection in mice and ferrets after a single dose [48]. Phase I trial assessed the vaccine's safety among 108 individuals aged 18–60 years at Wuhan, Hubei province. Elevated COVID-19 antibodies were observed in the participants after six months of first dose vaccination, indicating prolonged immunity [49]. Based on these dose-escalating findings, a Phase II trial was performed on 508 participants randomly administered with the vaccine (1×10^{11} or 5×10^{10} viral particles) or placebo. Both doses of the vaccine produced neutralizing antibodies against the virus, and the high dose group showed higher immunogenicity and reactogenicity. Since the high dose (5×10^{10} viral particles) induced a significant immune response in almost all of the participants after a single dose administration and exhibited no serious adverse events, the vaccine was reported to be effective and safe [50]. The interim analysis of the Phase III trial showed 65.7% and 90.98% efficacy at preventing moderate and severe COVID-19 disease, respectively. Meanwhile, the trial performed among the Pakistan population showed 74.8% efficacy against symptomatic cases and 100% efficacy in preventing severe disease [51]. Furthermore, Phase III trials are being conducted in various parts of the world, including Saudi Arabia, Mexico, Russia, Chile, and Argentina [52,53]. The non-peer review of these studies concluded the safety and immunogenicity was significant [54]. Convidecia also provided better protection against the novel variant Omicron. Moreover, when administered as a heterologous booster dose, the vaccine induced significantly higher cellular immune response, especially among the individuals who inhaled the vaccine resulting in 100% (95% CI, 92.6–100.0%) and 95.7% (95% CI, 85.2–99.5%) stronger interferon-γ response at day 14 and 28 post-booster dose administration, respectively. This indicates that, irrespective of the route of administration, the heterologous booster dose of Convidecia elicited stronger cellular and humoral immunity than recombinant and inactivated vaccines [55].

SAFETY OF AD5-nCOV

The safety data were reported for 108 participants, of which 81% reported at least one adverse event within a week after immunization. The adverse events were found to be mild to moderate, such as fever (46%), fatigue (44%), headache (39%), and muscle pain (17%). No serious adverse events were observed until 28 days after vaccination [50].

THE REGULATORY STATUS OF THE APPROVED ADENOVIRAL VECTOR VACCINES

There are 219 countries involved in the vaccination program. As of April 17, 2022, 11.32 billion doses of vaccines have been administered, and half of the global population has received at least one dose of vaccine [4,52].

In April 2020, the WHO launched a joint-purchasing framework known as COVAX, which collaborated with multiple manufacturers for

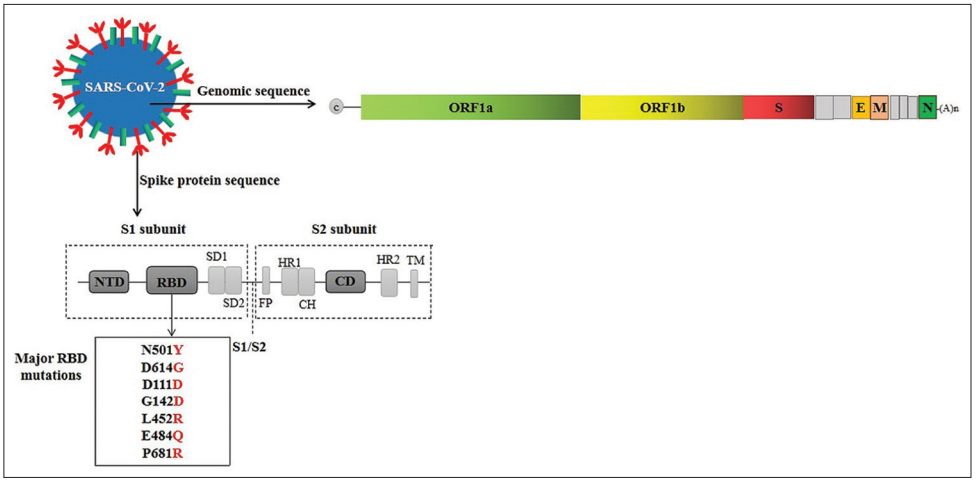

Fig. 5: SARS-CoV-2 genomic sequence and major mutations

equal distribution of the vaccines to all middle and lower economic countries [56], through which 5% of the population benefited in low-income countries [52].

By the end of 2021, 19 billion doses of vaccines are expected to be produced by all the pharmaceutical companies, of which 29.4% consist of viral vector vaccines. As of November 2021, two billion doses of AstraZeneca were delivered to more than 170 countries. Through the COVAX supply program 175 million doses were delivered to 130 countries [57]. Among those, Accra, Ghana, was the first African country to have benefited from the COVAX. AstraZeneca established 25 sites in 15 countries, including Serum Institute of India, Halix B.V of Netherlands, Emergent Bio-solutions, Beximco pharma of Bangladesh, and SK Bioscience of South Korea. In the meantime, the South African government canceled their agreement with AstraZeneca due to discrepancies in the vaccine's efficacy in the African population [58,59]. In addition, 18 countries have joined in suspension of AstraZeneca due to the suspected side effects [60].

The Russian government will manufacture approximately 620 million doses of sputnik V and sputnik light vaccines by June 2023. In addition, they had opened a Moscow based R-pharm for manufacturing 10 million doses/month and initiated eight pharmaceutical companies belonging to GL Rapha to manufacture and distribute the vaccine globally. In India, Dr. Reddy's Laboratories signed an agreement to produce and distribute the vaccine to other countries like Uzbekistan, Mexico, Nepal, and Egypt. On May 14, 2021, India initiated the first dose administration of the Sputnik V vaccine in Hyderabad [56,61]. In addition, 70 countries were authorized for emergency use of sputnik V as of November 2021 [62].

Johnson and Johnson have collaborated with Sanofi, Aspen Pharmacare, and Merck and Co to increase vaccine production and distribution. They have also associated with the vaccine Alliance programs, namely, COVAX and Gavi, to ensure equal vaccine distribution to all the lower-income countries, particularly Africa [63].

Convidecia also applied for the COVAX scheme's approval and decided to provide 10 million doses to the initiative. Chen Wei announced that their annual production of vaccines could be 500 million doses in 2021.

CHALLENGES OF VACCINE DEVELOPMENT

There are several serotypes of adenovirus, and some of these are prevalent pathogens affecting humans. One such serotype is the Ad5. The major challenge in utilizing this serotype of adenovirus for vector construction is the possibility that several individuals may have already been exposed to this virus and would have developed pre-existing immunity. Hence, it might cause a hindrance to the vaccines and thereby reduce their efficacy in those who have developed immunity against this virus [14]. AstraZeneca has reported significant challenges in vaccine manufacturing. They were expected to supply 90 million doses of the vaccine during the first quarter and about 180 million doses in the second quarter of the European Union. However, they could only manage to deliver 40 million doses in the first quarter and maybe even deliver only less than half of 180 million doses in the second quarter. Although the Serum Institute in India has been licensed to produce the AZD1222 vaccine, the roll-out is gradually decreasing [65]. As of May 12, 2021, Russia had manufactured only 33 million Sputnik V doses and exported even less than 15 million doses of the vaccine. Russia is in discussion with other manufacturers to help speed up the production of the vaccine [66]. After an inspection at Pharmstandard-Ufa Vitamin plant, Russia conducted between May 31 and June 4, 2021. The UN health agency reported issues regarding data integrity, testing results during manufacturing and quality control, traceability and identification of the vaccine batches, and risk of cross-contamination [67]. All vaccine manufacturers take extensive measures for rapid and large-scale manufacturing of the vaccines to resolve the current pandemic. One of the significant challenges is the need for redesigning the vaccine and its platform due to the continuous emergence of new variants of the virus. Moreover, the mechanism of action of these vaccines against the novel SARS-CoV-2 variants is still unexplored.

NOVEL VARIANTS IN SARS-CoV-2 ARE AN EMERGING CONCERN

SARS-CoV-2 is reported to have high mutation rates with enhanced virulence and evolvability, due to which these mutation hotspots have become the prime focus of many researchers. With the continued emergence of multiple variants, the CDC and the WHO have independently established a classification system for distinguishing the emerging variants of SARS-CoV-2 into variants of concern (VOCs), variants of interest (VOIs), and variants of high consequences, as shown in Table 2 [68]. Mutation at the D614G region was reported to be the most predominant hotspot in both VoI and VoC, indicating their high transmission rate than its original form [69,70]. The VOCs, namely, B.1.1.7, B.1.351, and P.1, possess several mutations in the RBD and the N-terminal domain (NTD), as shown in Fig. 5. Among these mutations, N501Y was found to be the most predominant mutation of the RBD common to all variants. In addition, the mutation leads to enhanced binding of the S protein to ACE 2 receptors, thereby improving the attachment of the virus and its entry into the host. Both RBD and NTD are exclusive targets for virus neutralization and enable the production of antibodies in response to vaccines. Recently, studies have reported that the N501Y mutation is sufficient to cause an increased affinity between RBD and ACE2, almost ten times more than that of the parental strain (N501-RBD). Fig. 5 illustrates the structure of the SARS-CoV-2 genome and the site of mutations of the new variants [6]. The WHO had

announced new nomenclature for the variants under the categories of VOC and VOI, using Greek alphabets. The variants B.1.1.7, B.1.351, P.1, B.1.617.1, and B.1.617.2 are now named alpha, beta, gamma, kappa, and delta, respectively [68].

ADENOVIRAL VECTOR VACCINES EFFICACY AGAINST NEW STRAINS OF SARS-CoV-2

The evolution of these new strains with various mutations is alarming since it questions the efficacy of our immune system to prevent re-infection and the efficacy of the vaccines. The randomized controlled trial by Emary *et al*., 2021, investigated the efficacy of the AZD1222 vaccine against a novel variant, B.1.1.7, which was the primary cause of the outbreak in the U.K. Findings of the study revealed that the efficacy of the vaccine against symptomatic SARS-CoV-2 infection was 70·4% and 81·5% for B.1.1.7 and non-B.1.1.7 lineages (Victoria), respectively. Therefore, the AZD1222 vaccine was proven to be effective against the variant B.1.1.7 and the Victoria lineage, which has just a single mutation (Ser247Arg) in the spike protein and has phylogenetic similarities with the original strain reported in the Wuhan outbreak [71]. In addition, an interim analysis of the multicenter phase 1b-2 trial conducted in South Africa studied the safety and efficacy of the AZD1222 vaccine against the B.1.351 variant, which reported an efficacy of only about 10.4% (95% CI, −76.8–54.8) [72]. The study also reported that it did not provide any protection against the infection in mild and moderate cases even after administering two doses of the vaccine. The P1 variant caused the severe outbreak of COVID-19 in Latin America, and the Sputnik V vaccine was found to be effective against the variant after a single dose administration which further improved after the second dose [73]. The WHO Strategic Advisory Group of Experts found J&J vaccine was effective against both B1.351 and P.2 variants. The vaccine's efficacy was found to be 74.4% in the U.S and only 52% in South Africa, which was dominated by the B.1.351 variant [74].

FUTURE DIRECTIONS

AstraZeneca has committed to supply about 3 million doses of their vaccine globally by the end of this year. The distribution of vaccines to low and middle-income countries will be done with the help of COVAX, the Coalition for Epidemic Preparedness Innovations, and WHO [75]. The University of Oxford had announced on March 25, 2021, the initiation of Phase I clinical trial to evaluate their vaccine efficacy when used as an intranasal spray [76]. Generium and Biocad, significant producers of the Sputnik V vaccine, plan to make 200–300 million doses/year. Furthermore, Russia and AstraZeneca have decided to develop a mix-and-match vaccine against COVID-19, for which human trials are under process in various countries [66]. A combination vaccine comprising one dose of Convidecia followed by ZF2001 as a second dose administered 28 or 56 days later is under clinical trial, registered in Jiangsu by CansinoBio in April 2021 [77]. Further, a two-dose regimen of the Convidecia vaccine is concurrently being tested in 6–17 years and older than 55 years individuals [78].

REAL-WORLD EVIDENCE

Real-world evidence of the vaccine is essential to improve awareness, knowledge, and understanding that these vaccines will help prevent hospitalizations and protect lives. It provides information regarding the ability of the vaccine to manage the pandemic by studying its effectiveness against different severities, ages, ethnicity, and co-morbidities. The Public Health England, Italian Institute of Public Health and UK surveillance project has reported that the AZD1222 vaccine reduced the risk of mortality, hospitalization, and likelihood of household transmission [79]. According to the Ministry of Health of Russia data, the new efficacy of Gam-covid-vac was 97.6%, obtained from vaccinating 3.8 million Russians between December 5, 2020 and March 31, 2021 [80]. The real-world evidence for Ad26.COV2-S and AD5-nCOV are not available yet. These data will help improve the acceptance of vaccines among non-vaccinated individuals globally.

CONCLUSION

The adenovirus vector vaccines were beneficial among different vaccines due to their cost-effectiveness, ease in manufacturing, and storage conditions. The interim analysis of phase 3 clinical trials of these vaccines has provided promising results within a short duration. Although the AZD1222 vaccine has shown extensive real-world evidence for its effectiveness against COVID-19, 18 countries have suspended its usage due to its serious adverse events. The Ad26.COV2.S, Gam-Covid-Vac, and Convidecia vaccines lack real-world evidence and hence require further scrutiny. In addition, the long-term efficacy of these vaccines and efficacy against variants affecting vulnerable populations (pediatric and geriatric) needs to be explored.

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Consent to participate
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