COVID-19: Clinical status of vaccine development to date

Sunny Kumar | Malini Basu | Pratyasha Ghosh | Aafreen Ansari | Mrinal K. Ghosh

Cancer Biology and Inflammatory Disorder Division, Council of Scientific and Industrial Research-Indian Institute of Chemical Biology (CSIR-IICB), Kolkata, India

Department of Microbiology, Dhruba Chand Halder College, Dakshin Barasat, India

Department of Economics, Bethune College, University of Calcutta, Kolkata, India

Correspondence
Mrinal K. Ghosh, Cancer Biology and Inflammatory Disorder Division, Council of Scientific and Industrial Research-Indian Institute of Chemical Biology (CSIR-IICB), TRUE Campus, CN-6, Sector V, Salt Lake, Kolkata 700091, India.
Email: mrinalghosh@iicb.res.in and mrinal.res@gmail.com

Malini Basu, Department of Microbiology, Dhruba Chand Halder College, South 24 Parganas, Dakshin Barasat 743372, India.
Email: drmalini.basu@gmail.com

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Severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2)-induced COVID-19 is a complicated disease. Clinicians are continuously facing difficulties to treat infected patients using the principle of repurposing of drugs as no specific drugs are available to treat COVID-19. To minimize the severity and mortality, global vaccination is the only hope as a potential preventive measure. After a year-long global research and clinical struggle, 165 vaccine candidates have been developed and some are currently still in the pipeline. A total of 28 candidate vaccines have been approved for use and the remainder are in different phases of clinical trials. In this comprehensive report, the authors aim to demonstrate, classify and provide up-to-date clinical trial status of all the vaccines discovered to date and specifically focus on the approved candidates. Finally, the authors specifically focused on the vaccination of different types of medically distinct populations.

KEYWORDS
clinical research and vaccine efficacy, COVID-19/SARS-CoV-2, immune responses, production, vaccination

1 | INTRODUCTION

COVID-19 escalated into a global crisis within the initial few months of the outbreak. It has spread rapidly throughout the world through human-to-human transmission and was declared a pandemic disease on 11 March 2020 by the World Health Organization (WHO). Many critical patients require intensive care, immediate use of mechanical ventilation and have been diagnosed without any chance of urgent medical care. As of December 2021, globally ~272 million confirmed cases and ~5.5 million deaths have been reported. Achieving overall victory in this invisible “world war” against COVID-19 and preventing the spread and recurrence of disease is one of the major challenges for clinicians, research scientists and leaders of several nations throughout the globe. Coronaviruses are mainly classified into four groups, namely, alpha, beta, gamma and delta, among which alpha and beta coronaviruses are of mammal (specifically bats) origin, whereas gamma and delta variants emerged from birds and swine, respectively. People infected with coronaviruses are at potential risk and the virus evolves significantly with continuous mutations.

Coronavirus variants like MERS-CoV and SARS-CoV-1 were found to have mortality rates of around > 30% and 10–15%, respectively, in the infected hosts. Throughout the world, the pharmaceutical industry and research institutes are working collaboratively to develop a potential preventive strategy. The development of COVID-19 vaccine is an important step to control infection and minimize community spread. At the time of writing, more than a hundred vaccine candidates are in the clinical developmental stages. A few of
them have been approved by regulatory bodies of specific countries for use and still a large number of other vaccine candidates are in different stages of clinical trials.\textsuperscript{9,10} Here, we have compiled comprehensive information regarding all the approved vaccines, candidate vaccines currently in different phases of clinical trials and lastly discuss the vaccination of medically distinct groups.

2 | CLASSIFICATION, DEVELOPMENT AND CLINICAL STATUS OF COVID-19 VACCINES

The frequent mutational changes in the viral structure make it very difficult to treat the virus due to the lack or non-availability of specific anti-SARS-CoV-2 therapeutic agents and change in drug targets. Generally, the antiviral therapeutic approach is “one bug–one drug” and often it is no drugs. Therefore, vaccines are the most valuable approach against COVID-19 and need to be taken in advance by the host for immunization before infection. It will take time and a huge effort for development and validation of vaccines through preclinical and clinical trials.\textsuperscript{11,12} Here, we have divided the vaccines into two major classes – (a) component vaccines and (b) whole virus vaccines – and discuss their clinical development in detail (Figure 1).

2.1 | Component vaccines

As the name indicates, component vaccines are those which utilize the individual components of viral particles to train our immune system and prepare it in case of future viral exposure to keep us safe and healthy.\textsuperscript{13} The component vaccines are classified into six types and are discussed below.

2.1.1 | DNA vaccines

DNA vaccines contain genetic material of pathogens that encodes viral antigenic response expressed from plasmid vectors and finally delivered into the host. It is a precise and flexible strategy to deliver the antigens/plasmid DNAs and additional components through electroporation. After successful inoculation, plasmid DNA-based vaccines are capable of inducing T cell activation which may induce neutralizing antibodies. Recently, a research group has developed a series of DNA vaccines that induce antibodies against different components of S protein. In animal studies, these vaccines were documented to induce both humoral and cellular immunity, with high titre of neutralizing antibodies.\textsuperscript{14-17} It is noticed that different kinds of vaccine candidates are specifically targeted or designed against specific viral epitopes, namely, spike proteins (S1, S2 and S2'), receptor-binding domains, nucleocapsid (N) and membrane (M) proteins that produce specific immunological responses (Table 1).\textsuperscript{35}

\textbf{Production}

DNA vaccines are produced by the incorporation of antigen-containing eukaryotic expression vector into bacterial system. The elements of plasmid backbone help in the propagation and selection of the positive vector colonies in bacteria. Generally, \textit{Escherichia coli} is preferred for the replication because that gives high copy number of a plasmid in the presence of kanamycin as a selectable marker. In the new era of vaccines for human use, regulatory bodies replace the antibiotic resistance markers present in the plasmid backbone. Additionally, the DNA constructs (semi-synthetic minicircle DNA\textsuperscript{27} and fully synthetic DoggyboneTM\textsuperscript{38}) with minimal length are also developed by removing the bacterial backbone. However, eukaryotic expression vector used for the generation of DNA vaccines contains promoter at the 5’ end (CMV), gene of interest (viral DNA) and 3’ poly(A) tail.\textsuperscript{36}
Additionally, the administration of DNA vaccines is tested. Of these, only ZyCoV-D (Zydus Cadila) vaccine alone. Generally, for effective functionality, the DNA vaccine must cross two membranes (namely, plasma and nuclear) to regulate the protein expression, whereas RNA vaccines are immediately translated after crossing the plasma or endosomal membrane. Thus, to increase the chances of successful gene delivery, uptake and immunogenicity of DNA vaccines, multiple gene delivery techniques (namely, gene gun, jet injection and electroporation) have been developed and implemented. Of these, electroporation based on in vivo (preclinical and clinical) gene delivery is well accepted and shows favourable outcomes. Additionally, the administration of DNA vaccines is tested through multiple strategies such as lipid nanoparticles (cationic lipids and cholesterol), polymeric absorption (PEI) and adsorption of biocompatible nanoparticles (PLGA and chitosan), which increases the DNA uptake and antigenic expression. Furthermore, pattern recognition receptors (PRRs) and IL-12 cytokine as molecular adjuvants are also preferably combined with the antigen to guide it directly towards the antigen-presenting cell (APC), to initiate the immunological response. Additionally, these vaccines are documented for use in association with protein or viral vector-based vaccines, which gives a synergistic boost to the immune system.

### Delivery

The investigation and experimentation on DNA vaccines were initiated in the early 1990s. At that time, the intramuscular (IM) and intradermal (ID) route of incorporation was preferred. However, low immunogenicity is observed in clinical administration of the DNA vaccine alone. Generally, for effective functionality, the DNA vaccine must cross two membranes (namely, plasma and nuclear) to regulate the protein expression, whereas RNA vaccines are immediately translated after crossing the plasma or endosomal membrane. Thus, to increase the chances of successful gene delivery, uptake and immunogenicity of DNA vaccines, multiple gene delivery techniques (namely, gene gun, jet injection and electroporation) have been developed and implemented. Of these, electroporation based on in vivo (preclinical and clinical) gene delivery is well accepted and shows favourable outcomes. Additionally, the administration of DNA vaccines is tested through multiple strategies such as lipid nanoparticles (cationic lipids and cholesterol), polymeric absorption (PEI) and adsorption of biocompatible nanoparticles (PLGA and chitosan), which increases the DNA uptake and antigenic expression. Furthermore, pattern recognition receptors (PRRs) and IL-12 cytokine as molecular adjuvants are also preferably combined with the antigen to guide it directly towards the antigen-presenting cell (APC), to initiate the immunological response. Additionally, these vaccines are documented for use in association with protein or viral vector-based vaccines, which gives a synergistic boost to the immune system.

### Mechanism

Several studies report that both innate immunity and humoral immunity are induced through the administration of DNA vaccine. Both CD8+ (cytotoxic) and CD4+ helper T cells get activated, but the exact mechanism has not yet been established. After incorporation, these vaccines are recognized by multiple components of the innate immune system. Moreover, reports show that STING/TBK1/IRF3 pathways and the AIM2 inflammasome are involved and regulate the efficacy of these vaccines. ID administration of these vaccines is taken up and processed by APCs (macrophages, monocytes and dendritic cells [DCs]), which bind to the naïve T cells and lead to the activation of adaptive immunity. However, subcutaneous administration of these vaccines is taken up by fibroblast and keratinocytes, which generates antigenic responses that are recognized by APCs to initiate the immunological responses. Vaccine administered via transdermal delivery is taken by Langerhans cells, which further process and express the antigen. Several studies reported that APCs are primarily responsible for the activation of MHC-I restricted CD8+ T cells in the DNA vaccinated individuals. However, the cross-priming and APC-mediated presentation of both MHC-I and MHC-II restricted antigens are the primary mechanisms involved. Overall, extensive study of the involved mechanism is still needed.

### Clinical status of DNA vaccines against SARS-CoV-2

Globally, pharmaceutical companies and research institutes are making efforts to develop DNA-based vaccines against SARS-CoV-2. Currently, some DNA-based vaccines are in the development stages, namely, AG0302-COVID19 (AnGes), INO-4800 (Inovio), ZyCoV-D (Zydus Cadila) and GX-19 (Genexine) vaccines are in phase III clinical trials; COVID-eVax (Takis), GLS-5310 (GeneOne Life Science Inc.), VB10.2210 (Nykode Therapeutics), VB10.2129 (Nykode Therapeutics), Covigenix VAX-001 (Entos Pharmaceuticals Inc.) and AG0301-COVID19 (AnGes) vaccines are in phase II clinical trials; and CORVax12 (Providence Health & Services), COVIGEN (University of Sydney), COVIDITY (Scancell) and bacTRL-Spike (Symvivo) vaccines are in phase I clinical trials. Of these, only ZyCoV-D (Zydus Cadila) has been approved for emergency use in India and has shown overall 66.6% efficacy. It encodes the genes specific to the spike protein and IgE signal unit. The ID administration of three doses over the course of 56 days has shown robust cellular and humoral immune responses. The ongoing registered clinical trials of ZyCoV-D (Zydus Cadila) vaccines include two phases I and II and one phase III (Table 2).

### Adverse effects

Interim result of 28 000 individuals (≥12 years of age) vaccinated with ZyCoV-D DNA vaccine showed 66.6% efficacy in symptomatic
| S. No. | Vaccine (countries app.) | Type and developer | Target | Booster dose (day) | Efficacy | Immunological features | Ongoing registered clinical trial number | Ref. |
|-------|--------------------------|--------------------|--------|--------------------|----------|------------------------|-----------------------------------------|------|
| 1     | ZF2001 (3)               | • Protein subunit  | RBD    | 28 and 56          | 82% (alpha: 93%, Delta: 78%) | ↑ in Th1, Th2 responses and GMT | Phase I: NCT04445194, NCT04636333, NCT04503051 and NCT04961359  
Phase II: NCT04466085, NCT04813562 and NCT05109598  
Phase III: NCT04646590, ChCTR2100050849, NCT05091411 and NCT05128643 | 50,51 |
| 2     | Covaxin (12)             | • Inactivated      | Whole virion | 28 | Symptomatic: 77.8  
Asymptomatic: 63.6  
Severe: 93.4% | ↑ in GMT, S1 protein, RBD and N protein | Phase I: NCT04471519 and CTRI/2020/09/027674  
Phase II: NCT04918797, NCT04471519 and CTRI/2020/09/027674  
Phase III: NCT04918797, NCT04641481 | 52,53 |
| 3     | Ad5-nCoV (10)            | • NRVVV            | Spike   | Single | Moderate: 65.7%  
Severe: 91% | ↑ in spike receptor and GMT | Phase I: NCT05043259, NCT04313127, NCT04568811 and NCT04840992  
Phase II: NCT05043259, NCT05005156, NCT04840992, NCT04341389 and NCT04566770  
Phase III: NCT04526990, NCT04540419 | 54,55 |
| 4     | CIGB-66 (4)              | • Protein subunit  | RBD    | 14 and 28          | 92.28% | N/A                    | Phases I and II: RPCEC00000345 and RPCEC00000346  
Phase III: RPCEC00000359 | 56,57 |
| 5     | KovIVac (1)              | • Inactivated      | Whole virion | 14 | N/A | N/A | Phases I and II: 502 | 58,59 |
| 6     | EpiVacCorona (2)         | • Protein subunit  | Spike  | 21 | N/A | Seroconversion (≥1:20) | Phases I and II: NCT04527575  
Phase III: NCT04780035 | 60,61 |
| 7     | Sputnik Light (21)       | • NRVVV            | Spike  | Single | Non-hospitalized: 79%  
Hospitalized: 88%  
Death: 85% | ↑ in GMT and neutralizing Ab | Phase I: NCT04713488  
Phase II: NCT04713488 and NCT05027672  
Phase III: NCT04741061 | 62,63 |
| 8     | Sputnik V (74)           | • NRVVV            | Spike  | 21 | 91.6% | ↑ in both cellular and humoral immune responses | Phase I: NCT04760730, NCT04684446, NCT04436471, 241 and NCT04387875  
Phase II: NCT05027672, NCT04988048, NCT04760730, NCT04954092, NCT04962906, NCT04983537, | 64,65 |
| S. No. | Vaccine (countries app.) | Type and developer | Target | Booster dose (day) | Efficacy | Immunological features | Ongoing registered clinical trial number | Ref. |
|-------|--------------------------|--------------------|--------|--------------------|---------|------------------------|-------------------------------------------|------|
| 9     | Soberana 02 (3)          | • Protein subunit  | RBD    | 21                 | Two doses: 62% Booster: 91.2% | ↑ in IFN-γ, IL-4, seroconversion and anti-RBD Ab | NCT04684446, NCT04686773, NCT04436471, 241, NCT04437875, NCT04587219 and NCT04640233 | 66,67 |
|       |                          | • IFVC             |        |                    |         |                        | Phase III: NCT04954092, NCT04640233, NCT04564716, NCT04530396, NCT04642339 and NCT04656613 |      |
| 10    | Soberana Plus (1)        | • Protein subunit  | Dimeric-RBD | Single             | 91.2%   | ↑ in ACE2 interaction and GMT | Phase I: IFV/COR/05 and IFV/COR/15, Phase II: IFV/COR/11 and IFV/COR/15, Phase III: IFV/COR/09 | 67,68 |
|       |                          | • Instituto Finlay de Vacunas Cuba |        |                    |         |                        |                                           |      |
| 11    | Ad26.COV2.S (85)         | • NRVVV           | Spike  | 56                 | 66.9%   | ↑ in S protein and GMT | Phase I: NCT04889209, NCT05109559, NCT04509947, NCT04894305 and NCT04436276 | 69,70 |
|       |                          | • Johnson & Johnson |        |                    |         |                        | Phase II: NCT04889209, NCT05109559, NCT04436276, NCT04525453 and NCT04765384 |      |
|       |                          |                     |        |                    |         |                        | Phase III: NCT05048940, NCT05047640, NCT04505722, NCT04614948, NCT04838795 and NCT05091307 |      |
| 12    | QazVac (2)               | • Inactivated      | Whole virion | 21             | N/A     | ↑ in Th1 response and GMT | Phase I and II: NCT04530357, Phase III: NCT04691908 | 71,72 |
|       |                          | • RIBSP            |        |                    |         |                        |                                           |      |
| 13    | MVC-COV1901 (1)          | • Protein subunit  | Spike (S2) | 29             | N/A     | ↑ in seroconversion and GMT | Phase I: NCT04487210, Phase II: NCT04695652, NCT04822025, NCT04951388, NCT05038618, NCT05048849 and NCT05054621 | 73,74 |
|       |                          | • Medigen          |        |                    |         |                        | Phase III: NCT05011526 |      |
| 14    | SARS-CoV-2 Vaccine (2)   | • Inactivated      | Whole virion | 14 or 28         | N/A     | ↑ in IFN-γ and T cell responses, GMT and RBD Ab | Phase I: NCT04758273 and NCT05003479, Phase II: NCT04756323 and NCT05003466, Phase III: NCT04852705 | 75,76 |
| S. No. | Vaccine (countries app.) Type and developer | Target | Booster dose (day) | Efficacy | Immunological features | Ongoing registered clinical trial number | Ref. |
|--------|------------------------------------------|--------|-------------------|----------|-----------------------|-----------------------------------------|------|
| 15     | mRNA-1273 (79) • RNA • Moderna            | Modified RNA of spike | 28             | 94.1%     | ↑ in CD4⁺ T cell response | Phase I: NCT04785144, NCT04813796, NCT04889209, NCT04839315, NL9275 and NCT04283461<br>Phase II: NCT05027672, ISRCTN73765130, NCT04889209, NCT04894435, NCT04761822, NCT04847050, NCT04796896, NCT04930770, NCT04969263, NCT04988048, NL9275, NCT05022329, NCT05077254, NCT04405076, NCT04748471, NCT04649151 and EUCTR2021-002348-57<br>Phase III: NCT05119855, NCT04805125, NCT04796896, NCT04811664, NCT04806113, NCT04860297, NCT05022329, NCT05048940 and NCT04649151 | 77,78 |

| 16     | FAKHRAVAC (MIVAC) (1) • Inactivated • ODIR | Whole virion | 21             | N/A       | N/A                   | Phase I: IRCT20210206050259N1<br>Phase II: IRCT20210206050259N2<br>Phase III: IRCT20210206050259N3 | 79,80 |

| 17     | AZD1222 (127) • NRVVV • Oxford and AstraZeneca | 28 and 84 | 70.4% (Std. dose: 62.1%; low dose: 90%) | N/A       | Phase I: NCT04760730, NCT04684446, TCTR20211102006, NCT05133609, NCT04446474, PACTR202005681895696, NCT04816019, NCT04324606 and NCT04568031<br>Phase II: NCT04973449, NCT05027672, ISRCTN73765130, CTRI/2020/08/027170, NCT05087368, NCT05054621, NCT04894435, NCT04988048, NCT04885764, EUCTR2021-002348-57, NCT04760730, NCT04962906, NCT04983537, NCT04684446, | 81,82 |

(Continues)
| S. No. | Vaccine (countries app.) | Type and developer | Target | Booster dose (day) | Efficacy | Immunological features | Ongoing registered clinical trial number | Ref. |
|-------|--------------------------|---------------------|--------|-------------------|---------|------------------------|----------------------------------------|------|
|       | BNT162b2 (112)           | RNA Pfizer/BioNTech | RBD    | 21                | 95%     | | Phases I: NCT04380701, 83,84 NCT04889209, NCT04839315, NCT04969601, TCTR20211102006, NCT04816643, NCT04588480, NCT04936997 and EUCTR2020-005442-42 |}

|       |                           |                     |        |                   |         | Phase II: NCT04368728, NCT04949490, ISRCTN73765130, NCT04380701, NCT05077254, NCT04889209, NCT04969601, NCT04761822, NCT04969263, NCT05022329, EUCTR2021-002348-57, NCT04992182, NCT04969263 and ISRCTN69254139, NCT04444674, PACTR202013895696, NCT05059106, TCTR20211004005, NCT04324606, NCT04568031, ISRCTN15638344 and NCT04400838 |
| S. No. | Vaccine (countries app.) Type and developer | Target | Booster dose (day) | Efficacy | Immunological features | Ongoing registered clinical trial number | Ref. |
|--------|---------------------------------------------|--------|-------------------|----------|------------------------|-----------------------------------------|------|
| 19     | * Razi Cov Pars (1) Protein subunit RVSRI   | Spike  | 21 and 51         | N/A      | N/A                    | NCT04860739, NCT04907331, NCT04588480, NCT04649021, NCT04824638, NCT04895982, NCT04754594 and EUCTR2020-005442-42 | 85,86 |
| 20     | Covishield (47) NRVVV Serum Institute       | Spike  | 56 and 96         | 61%      | Responses similar to ADZ1222 | Phases II and III: CTRI/2020/08/027170 | 82,87 |
| 21     | COVOVAX (2) Protein subunit Serum Institute | Spike  | 21                | 61% (symptomatic: 76%) | ▲ in GMT, IFN-γ, IL-2 TNF-α and CD4⁺ T cell response | Phases II and III: CTRI/2021/02/031554 | 88,89 |
| 22     | COVID-19 Inactivated Vaccine (1) Inactivated Shifa Pharmed | Whole virion | 28              | N/A      | 93.5% response in Ab production | Phase I: IRT20201202049567N1, IRT20201202049567N2 and IRT20171122037571N3 | 90,91 |
| 23     | BBIBP-CorV (72) Inactivated Sinopharm       | Whole virion | 21              | 79%      | ▲ in neutralizing Ab production | Phase I: IRT20171122037571N3, NCT05109559 and ChiCTR2000032459 | 92,93 |

(Continues)
| S. No. | Vaccine (countries app.) | Target | Booster dose (day) | Efficacy | Immunological features | Ongoing registered clinical trial number | Ref. |
|--------|--------------------------|--------|--------------------|----------|------------------------|----------------------------------------|------|
|        | Vaccine Type and developer |        |                    |          |                        |                                        |      |
| 24     | Inactivated (Vero cells) (2) • Inactivated • Sinopharm | Whole virion | 21 | Symptomatic: 72.8% Severe: 100% | Seroconversion rate: 99.3% and GMT: 94.5 | NCT04998240 and TCTR20210920005 Phase III: IRCT20210206050259N3, IRCT20210214049709N3, NCT04612972, ChiCTR2000034780, NCT04510207, NCT04560881, NCT04917523 and NCT04984408 Phase II: ChiCTR2000034780 Phase III: NCT04885764 and ChiCTR2000031809 Phase III: NCT04612972, ChiCTR2000034780, NCT04510207 and ChiCTR2000039000 | 92,94 |
| 25     | CoronaVac (47) • Inactivated • Sinovac | Whole virion | 14 | 84% (symptomatic: 50.7%; mild cases: 83.7%) | Seroconversion: 95.6% (adults) and 87.5% (elderly) S1-RBD Ab: 96% (adults) and 100% (elderly) | Phase I: NCT05043259, NCT05109595, NCT04352608, NCT04383574 and NCT04551547 Phase II: NCT049979949, NCT05087368, NCT04884685, PHRR210210-003308, NCT04992182, NCT05049226, NCT05043259, NCT05109559, NCT04352608, NCT04383574 and NCT04551547 Phase III: NCT05137418, NCT05077176, NCT04942405, NCT04800133, PHRR210210-003308, NCT04617483, NCT04651790, NCT04992260, NCT04456595, NCT04508075 and NCT04582344 | 95–97 |
| 26     | TAK-919 (1) • RNA • Takeda | Modified RNA of spike | 28 | N/A | ↑ in neutralizing Ab and CD4+ T cell response | Phases I and II: NCT04677660 | 98,99 |
| 27     | COVAX-19 (1) • Protein subunit • Vaxine/ CinnaGen | Spike | 21 | N/A | ↑ in neutralizing Ab and T cells response | Phase I: NCT04453852 Phase II: IRCT20150303021315N23 and NCT04994368 Phase III: NCT05005559 | 100,101 |
individuals and 100% in moderate disease after a third dose. In the phase I trial, 14.58% and 12.5% individuals showed at least one solicited and one unsolicited adverse event. However, no serious complications were reported.

**Merits and demerits**

These vaccines offer a number of advantages in terms of their production and development. Their development and production platform is a rapid process in comparison to other types. They are attractive, reliable and time-saving, in cases like the spread of viral diseases including life-threatening SARS-CoV-2-like pandemics where a huge amount of vaccine production is required for mass vaccination. They are safe, well tolerated, highly adaptable to new pathogens like SARS-CoV-2 and are stable at room temperature.

DNA vaccines have a few disadvantages like low immunogenicity and difficulty in administration inside the body. However, genomic incorporation is a bigger risk factor or clinical challenge in DNA-vaccinated individuals. The long-term existence of DNA plasmids in the host cells after injection may lead to several problems. Preclinical studies show that after IM administration, plasmid was present in the relevant animal models be determined. As a safety measure, the WHO has recommended that the persistence time of cytokine-expressing plasmids in the relevant animal models be determined.

**Storage**

The storage temperature is 2°C to 8°C.

### 2.1.2 | RNA vaccines

RNA vaccines contain genetic material that encodes the viral mRNA and are capable of translating antigenic proteins in humans, resulting in the stimulation of our immune system to fight against viral diseases. mRNA acts as an intermediary biomolecule between DNA and proteins. These vaccines deliver the antigen after translation of mRNA on ribosome. It also activates CD8+ T cells after vaccination via MHC-I and MHC-II pathways. These vaccines are simple, cheap and can fulfil the requirement of a huge number of vaccines to mass vaccinate the human population throughout the world in this SARS-CoV-2 pandemic.

**Production**

These vaccines that are designed against specific pathogens may contain either non-replicating or self-amplifying mRNA. Non-replicating mRNA consists of an antigen coding sequence with 5′ and 3′ untranscribed leader sequences (UTRs). Generally, they are produced by the transcription of cDNA which is obtained from the plasmid DNA of E. coli. Transcription of cDNA into mRNA is achieved via recombinant phage (T7 or T3 or Sp6 phage) DNA-dependent RNA polymerase and nucleoside triphosphates (NTPs). In order to remove the reaction impurities and get pure forms of mRNA, liquid chromatography techniques (fast protein liquid chromatography [FPLC] and high-performance liquid chromatography [HPLC]) are implemented. This transcribed mRNA product consists of protein-encoding open reading frame, UTRs at both the 5′ and 3′ end and 3′ poly(A) tail. On the other
hand, the self-amplifying mRNA vaccines are generally based upon the alpha viral genome where the genes that encode the structural and functional proteins are replaced by the antigen (mRNA). Here, the viral RNA polymerase plays a crucial role in the transcription and replication of mRNA. The full-length mRNA used in this type of vaccine is 9–10 kb, which is generally greater than in non-replicating mRNA vaccines. However, it contains UTRs at the 5’ and 3’ ends, a cap and poly(A) tail. Generally, the production of self-amplifying mRNA vaccines is very low due to the larger size of the mRNA coding sequence than the non-replicating mRNA vaccines. Generally, the encoded mRNAs are supposed to contain sub-genomic promoter and open reading frame (ORF) for the transcription of several non-structural protein (NSPs) through the utilization of RdRP enzyme. It leads to the generation of negative strand copies which act as template for the synthesis of two positive strands and lead to the amplification of mRNA that encodes the antigen.

**Delivery**

In order to achieve the protein expression, the mRNA vaccine must be delivered in cytosol. Once it crosses the plasma and endosomal membrane, it has the capability to stimulate the innate immune cells. The ability of mRNA vaccines to stimulate cellular immunity and delivery may be enhanced in several ways. Generally, this type of vaccination is achieved through direct injection via ID and intranal route to the APCs. Furthermore, gene gun and electroporation methods are also preferred to enhance the administration of mRNA vaccine to cytosol. However, the IM administration of lipid-based nanoparticle (LNP)-formulated mRNA is more effective in inducing both innate as well as humoral immunity than the naked mRNA. Recently, novel strategies like use of mRNA with other adjuvant molecules have been developed and found to work efficiently. Lipid or polymer-based nanoparticles of mRNA vaccine have been studied and documented to improve the efficacy, cellular uptake and delivery to the cytosol.

For the cell-based delivery of mRNA, various polymers (e.g., lipofectamine) and cationic lipids are preferred, while their use is not preferred for in vivo delivery due to cytotoxicity and less transfectability. For in vivo delivery of mRNA and si-RNAs, lipid-based nanoparticles are effective and well tolerated. Generally, LNP-based formulations consist of ionizable amino lipids, phospholipids, cholesterol and polyethylene glycol (PEG). Additionally, the knowledge of route of administration along with the formulation is also important to improve the efficacy and safety of mRNA vaccines. The IV administration of lipid-based nanoparticle formulation targets the liver while the administration of similar formulations through the ID and IM routes promotes the durable expression of the antigen of interest at the site. Thus, the selection of the right route of administration matters for the generation of desired outcome. However, the IM route is the most common, easy to deliver and preferred route during the COVID-19 pandemic for the mRNA-based vaccines. IM injection of mRNA-based vaccines has been studied for the strong induction of immune cells at the site of injection.

**Mechanism**

Exogenous mRNA in the form of vaccines is recognized by several PRRs present in endosome and cytosol. mRNA vaccine-mediated activation of PRRs leads to the generation of cytokines (IL12 and TNF) and chemokines at the site of injection, which act as innate immune factors for the induction of adaptive immunity. ID administration of mRNA vaccines is documented to enhance the expression of multiple chemokines (CXCL9, CXCL10 and CXCL11), which promotes the recruitment of dendritic cells and macrophages to the site of administration. Furthermore, the injection of protamine-based non-replicating mRNA vaccines is reported to be taken by both leukocytic and non-leukocytic cells and later on presented by APCs. Then, these mRNAs are transported by migratory DCs to lymph nodes (iLNs) which further leads to the activation of adaptive immune responses after the proliferation of T cells and innate immune cells. Notably, the activation of immune cells is primarily noticed at the site of administration and lymphoid system in the preclinical mice model. Moreover, the IM injection of self-amplifying mRNA vaccine is documented to restrict the CD8+ T cell priming and helps in the antigen transfer from myocytes to APCs in mice. Recently, the higher expression of non-leukocytic cells, neutrophils and professional APCs were also observed at the site of IM injection of lipid nanoparticle-based mRNA vaccines. Similarly, the recruitment of various immune cells such as neutrophils, monocytes and DCs to the site of injection were also observed in non-human primates upon lipid nanoparticle-based non-replicating mRNA vaccination. After internalization, translation of mRNA is followed by the upregulation of CD80 and CD86 co-stimulatory receptor molecules. Simultaneously, the upregulated expression of type-1 interferon-stimulated gene (ISGs) was also observed. Therefore, the transiently expressed innate immune cells lead to the T cell priming to cause the activation of B cells to produce antigen-specific antibodies.

**Clinical status of RNA vaccines against SARS-CoV-2**

In the last two decades, RNA vaccines have been documented for SARS or MERS-CoV. But, in this SARS-CoV-2 pandemic, a number of RNA vaccines are under study and some of them are in developmental stages: LNP-nCoV saRNA-02 Vaccine (MRC/UVRI and LSHTM Uganda Research Unit), HDT-301 (SENAl CIMATEC), PTX-COV19-B (Providence Therapeutics Holdings Inc.), mRNA-1283 (Moderna), mRNACOVID-19 Vaccine (Stemima Therapeutics Co. Ltd), mRNA-1273.351 (Moderna) and CoV2 SAM (LNP) (GlaxoSmithKline) are in phase I clinical trials; EXG-5003 (Elixirgen Therapeutics Inc.), TAK-919 (Takeda), DS-5670a (Daiichi Sankyo Co. Ltd), ARCT-165 (Arcturus Therapeutics Inc.), BNT162b3 (Pfizer/BioNTech), BNT162c2 (Pfizer/BioNTech), HGCO19 (Gennova Biopharmaceuticals Ltd), LUNAR-COV19/ARCT-021 (Arcturus Therapeutics Inc.), BNT162a1 (Pfizer/BioNTech) and ChulaCov19 (Chulalongkorn University) are in phase II clinical trials; and BNT162b2 (Pfizer/BioNTech), BNT162b1 (Pfizer/BioNTech), mRNA-1273 (Moderna), mRNA-1273.617.2 (Moderna), mRNA-1273.211 (Moderna), ARCT-154 (Arcturus Therapeutics Inc.), mRNA (Walvax) and BNT162b2s01 (Pfizer/BioNTech) are in phase III trial (see Table 2).
Of these, mRNA-1273 (Moderna) carries the nucleoside-modified mRNA of the spike protein and has been approved in 79 countries. Two doses of the vaccine administered intramuscularly over 28 days show 94.1% overall efficacy and enhanced CD4+ T cell responses against Th1 cytokine. Registered clinical trials are phase I (6), phase II (18) and phase III (10). Dual doses of mRNA-1273 vaccine showed 98.4% effectiveness in alpha, 95.5% in gamma and 86.7% in delta variants. A Qatar-based study showed 100% effectiveness against alpha and 96.4% effectiveness against beta variants. Additionally, mRNA-1273 vaccine also showed 93% efficacy against omicron. Another vaccine containing the same targeted sequence, TAK-919 (Takeda), has been approved by Japan for emergency use. Two IM doses resulted in elevated neutralizing antibody titres and also induced CD4+ T cells. As the vaccine is still under trial, the efficacy percentage has not been established, and it is currently registered for phase I and phase II clinical trials. The most widely accepted RNA vaccine, approved in 115 countries, is BNT162b2 (Pfizer/BioNTech) which showed 95% overall efficacy and enhanced level of SARS-CoV-2 neutralizing antibody and prominent antigen-targeted CD8+ and Th1-specific CD4+ T cell responses when administered intramuscularly. Registered clinical trials are phase I (9), phase II (26) and phase III (11). BNT162b2 had 88% efficacy against the delta variant in comparison to 93.7% against the alpha variant. Another study reported that BNT162b2 vaccine had 89%, 100%, 63% and 92% efficacy against the alpha, beta, gamma and delta variants, respectively. Moreover, another study showed it had 70% efficacy against the omicron variant. In addition, vaccines LPN-nCoVsaRNA (Imperial), CVnCoV (Curevac) and MRT5500 (Sanofi Pasteur) are no longer progressing in trials. Development of RNA-based vaccines is still in progress, hopefully resulting in safer and more effective outcomes.

Clinical trials are often affected by the confines and nature of investigations, which may influence expected results in real-life scenarios. Determination of vaccine efficacy and associated adverse effects in post-public vaccination are being estimated worldwide through real-life interim analysis. In Israel, BNT162b2 mRNA vaccine efficacy was estimated in 596,618 individuals, vaccinated between 20 December 2020 and 1 February 2021. Ninety-two per cent overall vaccine efficacy, 94% against the symptomatic group, 87% against hospitalization and 92% against severe disease were estimated. Meta-analysis of 19 large-scale observational studies showed 95% overall efficacy of BNT162b2 vaccine. In a study where 352,878 individuals who received two doses of mRNA-1273 vaccine were matched with a similar number of unvaccinated candidates, 87.4% effectiveness against infection, 95.8% against hospitalization and 97.9% against death were found. Moreover, VOC-specific effectiveness was found to be 100% against the alpha variant and 96.4% against the beta variant. A meta-analysis of 23 real-life studies of BNT162b2 mRNA vaccine showed 91.2% efficacy against infection, 97.6% against hospitalization and 98.1% against associated death. Similarly, analysis of five articles related to mRNA-1273 (Moderna) mass vaccination showed 98.1% effectiveness. Commonly reported side effects of RNA vaccine included tenderness, weakness, fever, palpitations, headache, joint/muscle pain, nausea, anorexia, insomnia, local swelling, tingling/itching, diarrhoea and nasal congestion.

**Immune response persistence, rapid waning and booster vaccination**

A study on ~600,000 dual-dose vaccinated individuals with RNA-based vaccines (viz., BNT162b2 [33%], ChAdOx1 nCoV-19 [65.3%] and mRNA-1273 [1.7%]) has been performed to detect the immunity persistence, waning and their risk factors. Subsequently, in this study SARS-CoV-2 diagnostic tests were performed up to 6 months in certain time gaps immediately after the vaccination with dual doses of the respective vaccines. After 6 months, it was found that 10% of vaccinated individuals tested positive. The effectiveness of all these vaccines was noticed to wane after 5 months: the effectiveness of BNT162b2 was 82.1%, ChAdOx1 nCoV-19 was 75.7% and mRNA-1273 was 84.3%. In this study, vaccine effectiveness was also studied in various age groups and found to wane more in older aged persons (age ≥ 55) and in persons with co-morbidities. It also found that the effectiveness of the BNT162b2 primary dose was boosted to more than 92.5% by receiving a booster dose after 3 months. Similarly, the effectiveness of ChAdOx1 nCoV-19 was boosted to more than 88.8% after receiving the booster dose after a gap of 3 months. Additionally, adverse effects were observed in 10.1% participants. Muscle tenderness was the most common adverse effect in 59.2% of participants. However, it was also observed that the heterologous booster doses result in more systemic adverse effects than the homologous schedules of vaccines. Similarly, the effectiveness and safety of BNT162b2 waned to 53.4% and 16.5% after 1 and 3 months of post vaccination, respectively. This study was performed during the period of the omicron variant and the persistence of immunity waned to a much larger extent. Thus, it can be concluded that the RNA-based vaccines prolong immunity for more than 6 months in young individuals and booster doses at regular intervals are capable of boosting the safety, effectiveness and immunity against SARS-CoV-2.

**Hybrid immunity**

Immunity against SARS-CoV-2 can be induced naturally (infection-mediated) and passively (vaccine-mediated). However, the term ‘hybrid immunity’ is used for immunity acquired in individuals who had received one or more doses of vaccines and had experienced SARS-CoV-2 infections at least once before and after immunization. Recently, hybrid immunity has been shown to provide more protection in comparison to both natural and passive immunity. It was also observed during the period of delta variant that people with hybrid immunity had superior immune protection in comparison to uninfected persons vaccinated with dual doses or infected persons not vaccinated at all. Additionally, a reduction in the efficacy of hybrid immunity was observed during the period of omicron variants but its magnitude and duration is not well studied yet. Thus, much validation and precise measurement of hybrid immunity is still required.
Adverse effects

Occurrences of pericarditis/myocarditis events in adolescent/young adult males have been reported as an unusual complication of COVID-19-associated mRNA vaccines. According to Centers for Disease Control and Prevention (CDC), the rate of myocarditis cases/million mRNA vaccine second doses was found to be 62.8 and 50.5 among 12–17- and 18–24-year-old males, respectively.\(^{154}\) Two to three days after the second dose, vaccine-induced myocarditis patients had symptomatic chest pain in association with elevated ST segment and levels of cardiac troponin.\(^{155}\) In Israel, among 5.1 million individuals vaccinated with dual doses of BNT162b2 mRNA vaccine, 136 recipients showed definitive myocarditis symptoms post vaccination with an incidence ratio of 5.34 in males aged 16–19 years.\(^{156}\) Moreover, 23 male US military personnel (20–51 years old) were diagnosed with myocarditis symptomatic chest pain within 4 days of vaccination with BNT162b2 or mRNA-1273 vaccine.\(^{157}\) This risk was further estimated to increase 18.28-fold in vaccinated individuals previously infected with SARS-CoV-2.\(^{158}\)

Arrhythmia is another rare adverse event associated with COVID-19 vaccination. This could be a result of impaired vasoconstriction due to autoimmunity-induced damage of cardiovascular adrenergic receptors.\(^{159}\) Among BNT162b2-vaccinated candidates, three individuals who had a history of SARS-CoV-2 infection developed tachycardia.\(^{160}\) A similar postural orthostatic tachycardia event was observed in a patient 6 days after receiving the first dose of BNT162b2.\(^{161}\)

Myocardial infarction has also been reported as a rare complication of the AstraZeneca, Pfizer and Sinovac vaccines and the occurrence post-vaccination varied between 15 minutes and 2 days.\(^{162–165}\) The risk ratio was determined to be 4.47 in Pfizer vaccine recipients with COVID-19 infection history and 1.07 without.\(^{158}\)

Stage III hypertension was presented minutes after vaccination with the Pfizer/BioNTech vaccine in eight patients and the Moderna vaccine in one patient.\(^{166}\) In another group, among 113 individuals, six had average elevation of systolic/diastolic blood pressure by \(\geq 10\) mmHg.\(^{167}\) Rare cases of Takotsubo cardiomyopathy have also been reported after vaccination with Moderna and AstraZeneca vaccines.\(^{168,169}\)

Merits and demerits

The non-replicating mRNA vaccines are much preferred over the self-amplifying mRNA vaccines due to their small and simple construct which promotes the generation of immune-specific responses. Furthermore, the ID and intranodal administration of naked mRNA is shown to induce the immune responses but they are highly unprotected due to the presence of ribonucleases in the extracellular matrix. Also, they are highly unstable in the biological environment because of their negative charge and ‘water-loving’ nature. However, the lipid nanoparticle-based complexation of mRNA is preferred in order to overcome this stability issue.\(^{166–169}\) Generally, the mRNA vaccine-based activation of PPRs leads to the activation of innate immunity followed by activation of acquired immunity. Along with this, activated PPRs are also reported to activate type-1 interferons, followed by the phosphorylation of eIF2α, which results in reduction and inhibition of protein synthesis. Thus, it is concluded that mRNA-based vaccines works like a double-edged sword.\(^{170}\) Furthermore, self-amplifying mRNA-based vaccines induced rapid inflammatory immune responses through the activation of several ISGs. It is suggesting that the potency of these vaccines can be improved by reducing the early type I interferon responses. Furthermore, the basic production platform of these vaccines is quite similar to the DNA-based vaccines. However, the production of these vaccines does not require a microbial system for amplification. Its production is quite simple and easier to handle than the DNA vaccines. Also, there is no chance of genomic integration in RNA vaccines because they do not interact with host DNA. Furthermore, the chances of the generation of anti-vector immune responses are very much lower than in the viral vector-based vaccines. So these vaccines can be administered a number of times because there is little or no chance of development of pre-existing immunity. These vaccines do not require any special device such as a gene gun or electroporation; instead of these methods they can be injected through multiple routes by normal injection syringes. Thus, the utilization of these vaccines is much preferred than other vaccine strategies in pandemic situations such SARS-CoV-2.\(^{25,171–174}\)

These vaccines are also safe, well tolerated and flexible to new pathogens and their efficacy is also enhanced by lipid, protamine and polymer-based nanoparticles.\(^{12,175}\) Furthermore, RNA vaccines do not need host cell genome integration. This is the major advantage of RNA vaccines over the DNA vaccines. The chances of genomic mutations are lower in RNA-vaccinated individuals than in DNA-vaccinated individuals. RNA vaccines can be injected into the host via the intravenous route, while DNA-based vaccines need electroerosion or gene gun for their administration. However, these vaccines also have disadvantages like possible degradation of the mRNA and interferon’s mediated antiviral immune response resulting in suppression of the efficacy of RNA vaccines.\(^{176}\) Furthermore, activation of interferon signalling is documented for their association with inflammation and autoimmunity.\(^{23}\) However, no report has been documented for the RNA vaccine-based induction of autoimmune diseases. Hence, further study is needed for verification of any adverse effects caused by RNA vaccines.

Storage

mRNA molecules are more unstable than DNA molecules. So long-term storage of mRNA vaccines must be between \(-70^\circ C\) and \(-20^\circ C\) while short-term storage up to 6 months is at temperatures between 2°C and 8°C.\(^{23}\)

2.1.3 | Subunit vaccines (protein and virus-like particles)

Administration of whole pathogen is not an essential prerequisite of vaccine-based stimulation of the immune system. Purified antigenic fragment of virus particle can act as an adequate inducer. Antigenic fragment can be a protein, polysaccharide or even a virus-like particle.
Moreover, combination of antigens can be used as conjugated vaccine. Major classes of subunit vaccines are discussed below.

**Protein-based subunit vaccines**

Protein subunit vaccines are developed by using the protein components of the viral particles like spike proteins of the virus. They are also safer and well tolerated, although they have disadvantages too. These vaccines are documented to have low immunogenicity and require adjuvant/conjugates to enhance their immunogenicity.\(^{177-183}\) Recently, one group has reported that disulphide-associated dimeric variant of MERS-RBD has improved the immunological activity of protein-based vaccines and consecutively this protocol was explored for SARS-CoV-2, resulting in a 10–100-fold increment in neutralizing antibody titres.\(^{184}\) Hopefully, this platform will help in increasing the immunogenicity of protein subunit vaccines globally in this pandemic situation and into the future.

Previously, viral S protein subunit vaccines were well known for SARS-CoV as well as for MERS-CoV. The S protein segment of MERS-CoV is documented to contain non-neutralizing antibodies, which may hinder the generation of neutralizing antibodies as well as the immunogenic response against MERS-CoV. Therefore, it is mandatory to search for novel neutralizing epitopes for the development of protein subunit-based vaccines. Spike proteins expressed on SARS-CoV/MERS-CoV viral surfaces are a type I trimeric membrane protein that binds to DPP4 on the target cells. At present, neutralizing epitopes and operational channels of monoclonal antibodies have been studied at molecular level by structural and functional methods. They include 2E6, 4C2, m336, MCA1, 7D10, D12, MERS-27, CDC-C2, JC57-14, MERS-GD27 and MERS-4. These antibodies target the RBD sub-domain of SARS-CoV/MERS-CoV and overlap with the binding surface of DPP4.\(^{18,19,185-188}\) These documented studies are crucial keys for the development of protein subunit vaccines against SARS-CoV-2.

**Production.** The antigenic protein part of pathogen can be generated either by culturing a large quantity of pathogen or synthesis of recombinant protein. Protein subunit vaccines are produced in live microbial systems like yeast. The antigenic gene is inserted into the yeast cells and grown in large fermentation bioreactors to produce recombinant protein subunits. After purification, the subunits are combined with preservatives for maintenance of shelf-life and adjuvant. Currently, nine protein-based subunit vaccines against SARS-CoV-2 have been approved.\(^{50,60,61,189}\)

**Clinical status of protein subunit vaccines against SARS-CoV-2.** Up to now, clinically approved protein subunit-based vaccines are available against pertussis, influenza, Streptococcus pneumoniae and Haemophilus influenzae type B.\(^{51}\) SARS-CoV-2 protein subunit-based vaccines are also being developed, among which some are in the developmental stages. Some of the SARS-CoV-2 protein subunit vaccines are PIKA COVID-19 (Yisheng Biopharma), CoV2-OGEN1 (VaxForm), PepGNP-SARSCoV2 (Emergex Vaccines Holding Ltd), SpFN COVID-19 (US Army Medical Research and Development Command), CoVepiT (OSE Immunotherapeutics), IN-B009 (HK inno. N Corporation), NBP2001 (SK Bioscience Co. Ltd) and SARS-CoV-2 Vax 1 (Baiya Phytopharm Co Ltd Baiya) in phase I clinical trials; AdimRmSc-2f (Adimmune Corporation), EuCorVac-19 (EuBiologics Co. Ltd), AKS-452X (University Medical Center Groningen), 202-CoV (Shanghai Zerun Biotechnology, Walvax Biotechnology), QazCoVac-P (Research Institute for Biological Safety Problems) and SARS-CoV-2 Protein Subunit Recombinant Vaccine (PT Bio Farma); Sii Bivalent (Novavax), Sii B.1.617.2 (Novavax), Sii B.1.351 (Novavax), KBP-201 (Kentucky Bioprocessing), ICC (Novavax), Soberana 01 (Instituto Finlay de Vacunas Cuba), IVX-411 (Icosavax), HIPRA (Laboratorios Hipra SA), SCB-2020s (Clover), TAK-019 (Takeda), COVAC-2 (University of Saskatchewan), COVAC-1 (University of Saskatchewan), Recombinant RBD Protein Vaccine (Bagheiat-allah University of Medical Sciences), CIGB-669 (Center for Genetic Engineering and Biotechnology [CIGB]), BECOV2D (Biological E Ltd), BECOV2C (Biological E Ltd), BECOV2B (Biological E Ltd) and BECOV2A (Biological E Ltd) are in phase II clinical trials; and Recombinant Protein vaccine (Sanofi/GSK), SCB-2019 (Clover), UB-612 (COVAXX), FINLAY-FR-2 (Instituto Finlay de Vacunas Cuba), NVX-CoV2373 (Novavax), Nanocovax (Nanogen), SP/GSK subunit D614 (Sanofi/GSK), V-01 (Livzon Mabpharm Inc.). ZF2001 (Anhui Zhifei Longcom), Recombinant SARS-CoV-2 (CHO Cell) (National Vaccine and Serum Institute), ReCOV (Jiangsu Rec-Biotechnology Co. Ltd), Razi Cov Pars (Razi Vaccine and Serum Research Institute), Soberana 02 (Instituto Finlay de Vacunas Cuba), Soberana Plus (Instituto Finlay de Vacunas Cuba), NVX-CoV2373 (Novavax), Nanocovax (Nanogen), SP/GSK subunit D614 (Sanofi/GSK), V-01 (Livzon Mabpharm Inc.). ZF2001 (Anhui Zhifei Longcom), Recombinant SARS-CoV-2 (CHO Cell) (National Vaccine and Serum Institute), ReCOV (Jiangsu Rec-Biotechnology Co. Ltd), Razi Cov Pars (Razi Vaccine and Serum Research Institute), Soberana 02 (Instituto Finlay de Vacunas Cuba), Soberana Plus (Instituto Finlay de Vacunas Cuba), SP/GSK subunit B.1.351 vaccine (Sanofi/GSK), COVOVAX (Novavax formulation) (Serum Institute of India), MCV-COV1901 (Medigen), S-268019 (Shionogi), EpiVacCorona (FBRI), SCTV01C (Sinocelltech), CIGB-66 (CIGB), GBP510 (SK Bioscience Co. Ltd), AKS-452 (University Medical Center Groningen), COVAX-19 (Vaccine/CinnaGen Co.) and Recombinant (Si9) cell (West China Hospital) are in clinical trial phase III. Of these, EpiVacCorona (FBRI), ZF2001 (Anhui Zhifei Longcom), CIGB-66 (CIGB), Soberana Plus (Instituto Finlay de Vacunas Cuba), MCV-COV1901 (Medigen), Razi Cov Pars (Razi Vaccine and Serum Research Institute), COVOVAX (Novavax formulation) (Serum Institute of India), COVAX-19 (Vaccine/CinnaGen Co.) and Soberana 02 (Instituto Finlay de Vacunas Cuba) have been approved; and Sclamp (Queensland) vaccines are no longer progressing in clinical trials\(^{47}\) (Figure 2).
EpiVacCorona developed by FBRI has been approved for emergency use by the Russian Federation and Turkmenistan. This spike protein-based vaccine is administered intramuscularly twice over 28 days and showed seroconversion ≥1:20 in 100% vaccinated individuals. Registered clinical trials are phase I and phase II (1) and phase III (1).

ZF2001 (Anhui Zhifei Longcom) containing the RBD peptide is approved in China, Indonesia and Uzbekistan. Two/three doses over 4-week intervals results in 82% (overall), 93% (alpha) and 78% (delta) efficacies. Notable humoral responses (Th1 and Th2) and two-fold increase in geometric mean titres (GMTs) were observed as compared to convalescent serum. Ongoing clinical trials are taking place in five countries: phase I (4), phase II (3) and phase III (4).

CIGB-66 (Genetic Engineering and Biotechnology) is a RBD protein-based dual-dose vaccine, approved in four countries (registered: phase I and phase II (2) and phase III (1)) and has shown 92.28% overall efficacy.

Soberana 02 (dual dose) and Soberana Plus (single dose) developed by Instituto Finlay de Vacunas Cuba are based on RBD and dimeric-RBD protein, respectively. Soberana Plus used as a booster dose for Soberana 02-vaccinated people showed 62% overall efficacy with fourfold seroconversion and 91.2% after Soberana Plus booster. Soberana Plus increases ACE2 interaction by 60.9% to 89.2%; increases mVNT50 GMT by 94.5 to 340 and increases viral neutralization by 24.2% to 65.6%. Registered clinical trials for Soberana 02 are: one in each phase I, phase II and phase III (1); Soberana Plus phase I (2), phase II (2) and phase III (1).

MVC-COV1901 (Medigen) vaccine targeting the S2 subunit of the spike protein is approved by Taiwan for emergency use. It increases GMT from 163.2 to 662.3 and the seroconversion rate to 99.8%. Clinical trials for Soberana 02 are: one in each phase I, phase II and phase III (1) and Soberana Plus phase I (2), phase II (2) and phase III (1).

NVX-CoV2373, a protein subunit vaccine in phase I trial, creates common side effects like soreness, pain at injection site, headache, fatigue and muscle pain. In the MVC-COV1901-vaccinated group, 76.2% of individuals had pain at injection site, 36% had malaise/fatigue and 0.7% had fever. In phase I, 40% out of 40 enrolees, and in phase IIa, 32% out of 100 enrolees reported at least one adverse effect within 28 days of Soberana 02 administration. Moreover, in phase IIa, one recipient acquired serious, grade 3 erythema and induration.

Vaccines containing virus-like particles

Self-assembled VLP or virus-like particles are structural proteins of a virus that imitates the orientation of parent virus, and it also lacks a viral genome. VLP-based vaccines use the epitopes in conformation which is similar to the parent virus, resulting in superior immune responses. Like whole virus vaccines, VLP-based vaccines do not incorporate live or inactivated viruses, which makes them a safer candidate. VLP-based vaccines mediate the induction of high antibody expression due to cross-linking with B cell receptors. These vaccines
have disadvantages like other types of vaccines. They have low immunogenicity and their manufacturing process is too irregular. 195–197

Production. Viral vaccines are based on artificial non-viral molecules that closely mimic viruses but do not cause infection due to the lack of genetic material. Generally, the vaccines are made up of structural parts that assemble to form empty virus. The production method mainly comprises different expression platforms like bacteria (E. coli), yeast, insect cells, mammalian cells and plants (e.g., tobacco mosaic virus [TMV]) and the choice of these systems depends on the required post-translation modifications and protein-folding mechanism. VLP-based vaccines have previously been designed against hepatitis B, malaria, human papilloma viruses, influenza virus A and human immunodeficiency virus (HIV) and currently clinical trials of vaccine against SARS-CoV-2 are in process. 62,198–202

Mechanism. VLP cannot replicate but presents dense and repetitive conformation of structural protein epitopes which can stimulate potent cellular and humoral responses without adjuvant. Moreover, VLP size ranges from 20 to 200 nm which can easily be transferred to draining lymph nodes. After the transfer, it enters the subcapsular sinus where it is caught by macrophages. 203

Delivery. These vaccines are usually administered intramuscularly to avoid local adverse effects. 204

Clinical status of VLP vaccines against SARS-CoV-2. Until now, the clinically accepted VLP vaccines are against human papillomavirus and hepatitis B virus. 195–197 However, in this COVID-19 scenario, VLP-based vaccines are also in the developmental stages to combat the SARS-CoV-2 virus. VBI-2902a (VBI Vaccines Inc.), RBD SARS-CoV-2 HBsAg VLP (SpyBiotech), ABNCoV2 (Radboud University), SARS-CoV-2 VLP Vaccine Alpha Variant (The Scientific and Technological Research Council of Turkey) and SARS-CoV-2 VLP Vaccine (The Scientific and Technological Research Council of Turkey) are in phase II; LYB001 (Yantai Patronus Biotech Co. Ltd) is in phase I; and plant-based VLP (Medicago) vaccine is currently in phase III clinical trial. 47 These VLP-based vaccine candidates are not yet approved.

Merits and demerits. Use of targeted antigen induces sensitive and specific immunological response. The absence of viral genome makes the vaccines a safe choice for vaccination of elderly, children, pregnant/nursing women and patients with underlying diseases or immune system disorder. Moreover, production technology is well established and time efficient. However, isolated proteins slowly getting partial denaturation may stimulate non-specific antibody production. The lack of PAMPs makes these vaccines less immunogenic and requires the aid of adjuvant and additional booster doses to achieve the required immune responses. Pre-development identification of suitable antigen is a necessity, and this process may take extensive time. 195,205,206

Storage. Subunit vaccines are temperature sensitive so require refrigeration at 2–8°C for storage. 206

2.1.4 Viral vector vaccines (replicating and non-replicating)

This vaccine platform is highly versatile and can deliver one or more antigens, forming a multi-vaccine system that has several advantages over other vaccines. Additionally, it can include both replicating and non-replicating viral vectors for vaccine development. Since the 1980s, a wide variety of viral vectors has been engineered to encode the antigen in humans. After successful delivery and antigen expression, the host system automatically induces immunological responses against the antigen. 207

Viral vectors

Some of the common viruses used for vector-based vaccine development are adenovirus (Ad), measles virus (MV) and vesicular stomatitis virus (VSV). Brief details of each system is given below.

Adenovirus (Ad)

These vaccines are commonly reported for the application of Ad vectors in wide array of preclinical and clinical research. These vectors can give stable expression up to 8 kb of antigenic gene sequence. It operates in two distinct modes, namely, as replication competent and defective vector. The early transcript 1A and 1B (E1A, E1B) gene is usually replaced by a target gene for encoding the antigen and disables the replication ability. Moreover, the E3 gene is deleted to protect the Ad-infected host cells from the immune system. Additionally, deletion of the E4 gene from the Ad vector prevents any leaky expression of the gene of interest. 208–210

Measles virus (MV)

It is a negative-sense ssRNA (~16 kb) based non-segmented enveloped virus that is used to develop these vaccines by sequential passing of MV in various cell lines. This allows integration of various mutations in the virus, generating a live-attenuated viral vector, which cannot replicate in humans and is non-pathogenic. MV vectors can allow insertion of a gene sequence up to 6 kb abiding by the rule of six. This vector system allows development of multivalent vaccines which can be generated in Vero/MRC-5 cell lines or in chick embryonic fibroblasts. These vectors are usually generated in mammalian cell lines like HEK293. 209,211–213

Vesicular stomatitis virus (VSV)

A negative-sense ssRNA virus of ~11 kb genome size has been widely utilized as an attenuated vector for translation of a 4–5 kb gene of interest. The viral attenuation can be attained by mutating the viral matrix protein, reorganizing the viral protein order, integrating non-viral protein and deleting viral glycoproteins which measures infection potential. Commonly, the glycoprotein gene is replaced by transgene,
subsequently changing viral tissue tropism. VSV can be generated in insect and mammalian cell lines.\textsuperscript{214-217}

**Mechanism**

Based on the serotype employed, adenoviral vectors can induce varied levels of T cell responses and antibody production. Ad5, a replication-deficient vector can induce remarkably efficient antibody production and CD8\(^+\) T cell responses. Nonetheless, humans having pre-existing immunity against this virus may inactivate the vector and restrain expression of transgene. To overcome this limitation, non-human AV vectors like ChAd63, chimpanzee virus-derived vector and unusual serotypes like Ad26 or Ad35 having low human prevalence can be used. As compared to Ad5, use of such serotypes can enhance memory and multi-functionality of CD8\(^+\) T cells.\textsuperscript{218-222}

Recombinant MV can potently stimulate the levels of cellular and humoral immune responses against antigenic transgene. MV can directly deliver transgene into APCs by infecting dendritic cells and macrophages. Moreover, they activate CD4\(^+\) T cell-mediated responses. In some countries, children vaccination programmes used MV-based vaccines to induce immunity against MV. However, clinical studies of MV-based vaccines for CHIKV have shown that such pre-acquired immunity did not affect vaccine efficacy.\textsuperscript{211,219,223,224} VSVs have reported to induce both CD4\(^+\) and CD8\(^+\) T cell responses.\textsuperscript{219}

**Delivery**

Clinical studies of viral vector-based vaccines can use multiple routes of delivery, namely, IM, intranasal, oral and ID. The type of immunological response is dependent on the mode of vaccination and the selection of route of administration. However, delivery of the vaccine should ideally be reliable, easy and requires no such special training in the pandemic situation of SARS-CoV-2.\textsuperscript{225-227} Furthermore, these vaccines do not require adjuvants for supra-addition in their immunological responses as they induce strong immune responses themselves. Recently, these vaccines were clinically tested in association with immune-stimulating agents, but no significant increment in immune responses was noted. However, adjuvant-based changes in the immunological compartments is documented, but still the mechanism behind this is unknown.\textsuperscript{20,21}

**Clinical status of replicating viral vector vaccines against SARS-CoV-2**

These vaccines contain SARS-CoV-2 gene(s) in a replicating viral vector. The chances of genomic integration are also high with the use of these vaccines. They can potentially produce strong immunogenic responses.\textsuperscript{64,65,228} Such SARS-CoV-2 vaccines are MVA-SARS-2-S (Universitätsklinikum Hamburg-Eppendorf), VXA-CoV2-1 (Vaxart), SC-Ad5-6 (Tetherex Pharmaceuticals Corporation), Ad5-nCoV (AMMS), ChAdV68-S (NIAID), ChAd-triCoV-Mac (McMaster University), Ad5-triCoV-Mac (McMaster University), COVID-19-EDV (EnGeneIC), CVXGA1 (Cyvac LLC), AdCLD-CoV19-1 (Cellid Co.), BBV154 (Bharat Biotech) and SAM-LNP-S (NIAID) in phase I clinical trials; hAd5-Covid-19 (Immunity Bio Inc.), MVA-SARS-2-ST (Universitätsklinikum Hamburg-Eppendorf), VXA-CoV2-1.1-S (Vaxart), COVI-VAC (Institute of Vaccines and Medical Biologicals), BCD-250 (Biocad), NDV-HXP-S (Mahidol University) and LV-SMENP (Shenzhen Geno-Immune Medical Institute) in phase II clinical trials; and vaccines GRAd-COV2 (ReiThera), Covishield (Serum Institute of India), Ad26.COV2.S (Janssen [Johnson & Johnson]), AZD1222 (Oxford/AstraZeneca), Sputnik V (Gamaleya), Ad5-nCoV (CanSino), Ad5-nCoV-H (CanSino), AZD2816 (Oxford/AstraZeneca) and Sputnik Light (Gamaleya) in phase III clinical trials. Six non-replicating viral vector-based vaccines, Ad5-nCoV (CanSino), Sputnik V (Gamaleya), Ad26.COV2.S (Janssen [Johnson & Johnson]), AZD1222 (Oxford/AstraZeneca) and Sputnik Light (Gamaleya) are approved for use, but trials of AdCOVID (Altimmune Inc.) are no longer in progress due to its lower effectiveness.\textsuperscript{47} Still, some of these vaccines are in the developmental stages.

All the approved vaccines are designed against the SARS-CoV-2 spike protein among which Ad5-nCoV showed the presence of anti-SARS-CoV-2 spike receptor IgG and neutralizing antibodies after 28 days of vaccination. Owing to its 65.7% efficacy against moderate and 91% against severe symptoms, it was approved for use in 10 countries. Presently, 11 trials (phase I (4), phase II (5) and phase III (2)) are in progress in six countries.\textsuperscript{63,230} The Russian vaccines Sputnik V (dual dose) and Sputnik Light (single dose), developed by Gamaleya, are popular vaccines, being widely used in 74 and 21 countries, respectively. Additionally, Sputnik Light could also be used as a third booster dose of Sputnik V. Robust cellular and humoral immune responses and 91.6% overall efficacy were observed in Sputnik V-vaccinated people whereas quicker humoral response, 100% binding, 81.7% neutralizing antibody responses and 13.1-fold increments in
GMTs of seropositive participants with 79% efficacy against infection, 88% against hospitalization and 85% against death were observed in the case of Sputnik Light. Ongoing registered trials of Sputnik V include phase I (4), phase II (12) and phase III (6) and Sputnik Light clinical trials include phase I (1), phase II (2) and phase III (1). The Janssen vaccine, Ad26.COV2.S, currently approved in 85 countries given as either single or double doses (56 days apart) showed an increase of spike protein-specific antibodies and neutralizing antibodies; GMTs from 2432 to 5729 and 242 to 449, respectively. Furthermore, its overall efficacy was found to be 66.9%. Phase I (5), phase II (5) and phase III (6) clinical trials are registered across 18 countries. AZD1222 and Covishield are both based on the Oxford/AstraZeneca formulation. AZD1222 results in an overall efficacy of 70.4% in which two standard doses had 62.1% efficacy and a standard dose followed by a low dose showed 90.0% efficacy. Covishield had 61% overall efficacy and 76% against symptomatic infection. AZD1222 has been approved in 127 countries and has ongoing 52 (phase I (9), phase II (31) and phase III (12)) clinical trials in 23 countries. On the other hand, Covishield is approved in 47 countries and has been registered for phase II and III trials. Covishield showed 81% vaccine effectiveness against delta variant. Dual dose of ChAdOx1 nCoV-19 vaccine leads to 74.5% efficacy against alpha and 67% against delta variant. Another study reported that this vaccine had 91%, 100%, 41% and 95% efficacy against alpha, beta, gamma and delta variants, respectively. Multiple studies have shown that ChAdOx1 nCoV-19 vaccine is not effective against the omicron variant. Detailed data regarding the effectiveness of other viral vector-based vaccines like Ad5-nCoV, Sputnik Light, Sputnik V, Ad26.COV2.S and Covishield are not yet available in the context of variants of concern (VOCs).

Adverse effects
In one study, 20.9% out of 599 vaccinated German healthcare workers received a viral vector vaccine (AstraZeneca). It was reported that: (i) 87.2% suffered overall systemic side effects including fatigue, headache, chills, fever, muscle/joint pain, nausea, malaise and lymphadenopathy; (ii) 24.8% developed at least one non-communicable disease like allergy, asthma, blood/bone/cardiac/bowel/hepatic/neurologic/ophthalmic/otolaryngologic/renal/thyroid diseases, cancer, chronic hypertension, chronic obstructive pulmonary disease, dermatologic disorder, diabetes and rheumatoid arthritis; (iii) 70.4% showed local side effects like pain, swelling and redness at the site of injection; and (iv) 12.4% and 3% developed at least one oral and skin-related side effect, respectively. In a phase III clinical trial of ChAdOx1 nCoV-19, transverse myelitis was reported in two recipients. Adenoviral vector-based vaccines have also been associated with development of rare Guillain–Barre syndrome (GBS). In the United States, 132 reports of GBS surfaced after mass vaccination with 13.2 million doses of Ad26.COV2.S vaccine. The rate was estimated to be 9.8 cases/million doses between 45 and 62 years of age, and the median onset time was stated to be 13 days post vaccination. Among these, 35% were severe and death of one patient was reported. Moreover, bilateral facial weakness was also observed in a few cases.

Vaccine-induced generation of spike protein can interact with ACE2 receptor leading to cardiovascular complications like inflammation, aggregation of platelets and thrombosis. Thrombotic thrombocytopenia has been reported as an adverse effect of adenoviral vector-based COVID-19 vaccines. In the United States, six women (ages 18–48 years) were diagnosed with cerebral venous sinus thrombosis 6–13 days post vaccination with the Janssen vaccine. In comparison to the non-vaccinated population, the ChAdOx1-S recipient cohort showed 1.97-fold higher venous thromboembolic event morbidity and 20.25-fold higher cerebral venous thrombosis. During phase III clinical trial of Sputnik V, a candidate developed deep vein thrombosis.

Merits and demerits
Availability of multiple viral vectors and ease of manipulation makes this platform valuable and flexible for vaccine development. This system has the ability to express any antigen via genome manipulation and can be utilized for the administration of large DNA sequences in the viral genome. These two advantages make it ideal for the production and development of a wide variety of vaccines. Furthermore, the administration of genetic material as antigen is the key advantage of these vaccines, which helps in production and processing of antigen in a suitable manner. The utilization of host antigen from naturally infected patients is mostly preferred in the development of these vaccines as the antigen from other sources (e.g., a bacterial system) may cause difficulties and express differentially in humans. As the antigen belongs to the natural source, these vaccines are administered without any additional components making it an effective platform for pandemic situations like COVID-19 where huge production is required for global vaccination.

In spite of the numerous advantages of viral vector-based vaccines, a number of challenges limit its effective use and several key points need to be examined during the development and production phases. The viral vectors involved are genetically modified organisms, can be hazardous for humans and the environment, and to regulate this, European regulatory agencies and the FDA have published guidelines to evaluate the potential risks to humans and the environment. Additionally, these vaccines have probable threat of genome integration which may lead to genomic mutagenesis or even development of cancer. Such concerns can delay the development of vaccines, although the need is quite urgent in the pandemic situation. Furthermore, every viral system requires distinct manufacturing facilities, resulting in increased production costs. In addition, as the viruses can go through recombination during the manufacturing process, special care towards cell culture must be taken and unintentional contamination with harmful microorganisms must be avoided.

If a person is previously exposed to a similar type of virus or viral vector (naturally or through vaccination), the immunity stored in the individual is in the form of memory immune cells, collectively termed as a pre-existing immunity. This pre-existing immunity against viral vector-based vaccines is a major challenge observed during
vaccination. Post vaccination, these pre-existing memory immune cells recognize the viral components or viral vectors and get activated, which leads to limited effectiveness and immune responses. Human adenovirus serotype 5 (Ad5) is well characterized and greatly studied among all the adenovirus vectors. Recently, most of the global population is reported to have natural pre-existing immunity against Ad5. The vaccination potential was found to be low in mice and non-human primates who had such pre-existing immunity against Ad5. The presence of neutralizing antibodies prevents the vectors for transducing target cells while the memory T cells expel the transduced cells, which leads to the reduction in vaccination potential and efficacy. To overcome this problem, viral vector from other adenovirus serotype (Ad35, Ad11 and Ad26) is selected based upon their low prevalence. They are less immunogenic than Ad5 but effective for vaccine development. Alternatively, Ads from chimpanzee, cattle and pig species are also preferred for vaccination.249,250

Storage

It is recommended to keep these vaccines in lyophilized form for long-term storage and liquid form for short-term storage at 2–8 °C.27

2.2 Whole virus vaccines

The second and most effective class of vaccines in which inactivated or attenuated live virion particles were used, are known as whole virus-based vaccines.13,28 These vaccines are of two types discussed below.

2.2.1 Inactivated vaccines

Inactivated vaccines consist of whole virions inactivated chemically or by radiation, hence inactive in nature. They consist of all immunogenic components of original parent virions but are in an inactive state. They are much safer than live-attenuated vaccines if the inactivation is properly done. These vaccines have a strong immunogenic response. Structures of immunogenic epitopes are completely deformed due to the inactivation of virion particles. SARS-CoV-2 inactivated vaccines are documented to induce lung pathology due to eosinophilic storm. Because of these disadvantages, SARS-CoV-2 inactivated vaccines are not that much of an attractive approach for vaccine development.29,197

The production of inactivated vaccines is relatively easier than for other vaccines and is capable of inducing a strong immunogenic response against the multiple epitopes of the viral surface.30 A strong eosinophilic pro-inflammatory pulmonary response as a major challenge is recently documented, due to the SARS-CoV inactivated vaccines. It is also documented that vaccination of SARS-CoV N protein component increases the pulmonary immune-pathological changes. Hence, it is mandatory to find novel methods through which pathological response of anti-N inactivated vaccines can be minimized and protein S specific immune response can be enhanced. The safety of inactivated vaccines must be investigated before their clinical application.251–254

In preclinical studies, these vaccines against SARS-CoV-2 infection are documented to produce a huge amount of neutralizing antibodies. These neutralizing antibodies are highly specific to viral proteins such as S, N and M. In in vivo experiments in mice, antibodies against proteins N and S were found higher numbers than that of M protein. It may suggest that epitopic polypeptides from protein N/S are key targets for the development of recombinant vaccines against SARS-CoV-2.29,255

Production

Inactivated vaccine, also known as killed vaccine, consists of dead pathogenic particles which have been rendered incapable of producing infection-related illness by chemical treatments, heat or gamma radiation. These vaccines were first introduced to combat cholera, typhoid and plague during the late 1800s and early 1900s. At present, inactivated vaccines have been designed against polio, rabies, influenza, pertussis and hepatitis A. Immunogenicity exerted by these vaccines is generally weak; therefore, the immune response is elicited by aid of immunological adjuvants and the vaccination programme includes multiple booster doses.256–258

Mechanism

Depending upon the coupled adjuvant, inactivated vaccines can stimulate both humoral and cellular immunological responses. Once the vaccine is injected, the APCs recognize and take the antigen, transporting it to draining lymph nodes where T cells are activated leading to specialized adaptive responses along with development of immunological memory against future infection.259–261

Delivery

IM administration is the preferable route for delivery as these vaccines are usually coupled with adjuvant and may lead to local side effects like redness, swelling and pain.257,262

Clinical status of inactivated vaccines against SARS-CoV-2

Clinically approved inactivated vaccines are developed against typhoid,243 cholera,244 hepatitis A virus,265 plague,246 rabies,267 influenza252,53 and polio.92,93 SARS-CoV-2 inactivated vaccines are in various developmental phases: Covi Vax (National Research Centre Egypt), Adjuvanted Inactivated Vaccine (The Scientific and Technological Research Council of Turkey), Recombinant NDV Vectored Vaccine (Laboratorio Avi-Mex) and Koçak-19 Inaktif Adjuvani COVID-19 Vaccine (Kocak Farma) are presently in phase I clinical trial; KoviVac (Chumakov Center) is in phase II; and vaccines QazVac (Kazakhstan RIBSP), Inactivated (Vero Cells) (Chinese Academy of Medical Sciences), Covaxin (Bharat Biotech), COVID-19 Inactivated Vaccine (Shifa Pharmade Industrial Co.), BBIBP-CorV (Sinopharm [Beijing]), Inactivated (Vero Cells) (Sinopharm [Wuhan]), CoronaVac (Sinovac), ERUCOV-VAC (Health Institutes of Turkey), VLA2001 (Valneva), SARS-CoV-2 Vaccine (Vero Cells) (Minhao Biotechnology Co.), KD-414 (KM Biologics Co. Ltd) and FAKHRAVAC (MIVAC) (Organization
of Defensive Innovation and Research) are in phase III clinical trials. Covaxin (Bharat Biotech), BBIBP-CorV (Sinopharm [Beijing]), Vero Cells (Sinopharm [Wuhan]), CoronaVac (Sinovac), KoviVac (Chumakov Center), QazVac (Kazakhstan RIBSP), Co SARS-CoV-2 Vaccine (Vero Cells) (Minhai Biotechnology), FAKHRAVAC (MIVAC) (Organization of Defensive Innovation and Research) and COVID-19 Inactivated Vaccine (Shifa Pharmed Industrial Co.) have already been approved and are in use.47 Still, several other inactivated vaccines are in the pipeline of various development stages.

Covaxin showed significantly high GMTs and increased SARS-CoV-2 lgG seropositive (194.3) than the seronegative ones (118) and increased GMTs for S1 protein (9742), RBD (4124) and N protein (4161) with 77.8%, 63.6% and 93.4% efficacy against symptomatic, asymptomatic and severe COVID-19 cases, respectively. It has been approved for use in 12 countries and clinical trials are registered for phase I (2), phase II (3) and phase III (2).94,95 The Beijing and Wuhan centres of Sinopharm developed two inactivated viral vaccines: BBIBP-CorV and Vero Cells (also called WIBP-CorV). BBIBP-CorV showed prominent humoral response and production of 100% neutralizing antibody titres with an overall efficacy of 79% and has been approved for use in 72 countries,96 whereas WIBP-CorV, approved in China and the Philippines, showed 99.3% seroconversion rate and 94.5 GMT with 72.8% and 100% efficacy against symptomatic and severe cases, respectively.96 BBIBP-CorV clinical trials are underway in 10 countries for phase I (3), phase II (8) and phase III (8).97 WIBP-CorV is registered for phase I (1), phase II (2) and phase III (5) clinical trials.58 Upon CoronaVac vaccination, the seroconversion rate of S1-RBD-lgG and neutralizing anti-S1-RBD in adults (18–59 years old) was found to be 95.6% and 96%, whereas in elderly (60 years and older), it was 87.5% and 100%, respectively, with an overall efficacy of 84%, and 50.7% and 83.7% against symptomatic and mild cases, respectively. This vaccine has been approved for use in 47 countries and clinical trials are registered in phase I (5), phase II (11) and phase III (11).59,71,72 KoviVac is taken twice intramuscularly over 2 weeks and has been approved for emergency use in the Russian Federation with one ongoing trial registered in phases I and II.73,74 QazVac developed by Kazakhstan RIBSP induced Th1 response and showed an increase in neutralizing antibody titres by fourfold. It has been approved in Kazakhstan and Kyrgyzstan and has one trial registered in phase I and II (NCT04530357) and phase III (NCT04691908) trials.79,80 Co SARS-CoV-2 Vaccine (Vero Cells) by Minhai Biotechnology increased T cell and positive IFN-γ immunospot responses. GMTs increased from 29.3 to 49.1 (Days 0–14) and 100.2 to 131.7 (Days 0–28) and RBD-lgG 605.3 to 1169.8 (Days 0–14) and from 1496.8 to 2485.5 (Days 0–28). Co SARS-CoV-2 Vaccine has been approved in China and Indonesia, and two trials have been registered in phase I (2), phase II (2) and phase III (1).90,91 FAKHRAVAC (MIVAC) (Organization of Defensive Innovation and Research) and COVID-19 Inactivated Vaccine (Shifa Pharmed) is approved by Iran as dual-dose vaccine which could be injected intramuscularly 21 and 28 days apart. Both vaccines are still in clinical trials; therefore, the efficacy data have not yet been published. COVID-19 Inactivated Vaccine showed the presence of neutralizing antibodies in 93.5% of the vaccinated individuals. Presently, FAKHRAVAC only one clinical trial has been registered in each of phase I, phase II and phase III.268,269 On the other hand, Shifa COVID-19 Inactivated Vaccine has been registered for phase I (3), phase II (2) and phase III (1).270,271

Adverse effects
Adverse reaction of mild severity was reported in 44.2% and 41.7% participants, who received WIV04 or HB02 vaccine, respectively. The most common side effect was pain at injection site followed by headache.96 Common adverse effects related to vaccination with COVAXIN include pain and swelling at the site of injection, fever, headache, malaise, vomiting, nausea and skin rashes. After vaccination with COVAXIN, Herpes zoster reactivation was reported in a 60-year-old male with underlying hypertension and type II diabetes mellitus.272 A CoronaVac recipient reported urticaria in the phase I clinical trial72 and an 82-year-old female developed petechial rash 1 day after receiving CoronaVac vaccine as a hypersensitivity reaction.273

Merits and demerits
These vaccines have increased stability over live pathogens, which aids in storage and transportation. Also, the loss of pathogenicity avoids the risk of reactivation of the vaccine in virulent form and can be administered in immunocompromised groups. Lack of robust immunological responses is the major disadvantage of these vaccines, and the maintenance of proper defence responses requires extra doses and adjuvants. But over time, antibody titre level in recipients can reduce. Moreover, culturing the pathogen during manufacturing requires a good biosafety facility and makes the whole process of vaccine development lengthy.259–261,274

Storage
Vaccine must not be frozen and should be kept in a sealed set-up at a temperature between 2°C and 8°C.261

2.2.2 | Live-attenuated vaccines
Live-attenuated vaccines are live, weak and deleted or mutated pathogenic components of the viral genome. They consist of full live parent virion but are in the attenuated state. These vaccines have strong immunogenicity and are popular for controlling several types of infections such as mumps, measles, polio (Sabin), yellow fever, rotavirus, varicella, Bacillus Calmette–Guerin (BCG) and rubella. Still, these vaccines have a high risk for possible reversion of virions from attenuated to virulent state. This may create a serious problem in an immune-compromised person. Due to the serious disadvantages, the biosafety of these vaccines should be checked before proceeding to clinical trials.275

Production
Live-attenuated vaccines are developed using live pathogens having extremely little to no virulence. The pathogens are weakened to form harmless and non-virulent forms which are capable of facilitating
quick and potent immunological responses for the long term. Pathogen attenuation can be carried out by introduction of evolutionary mutation and reduced selection pressure via continuous passages in foreign host systems like live animals, cell lines and embryonic eggs. Moreover, the attenuation can also be achieved by reverse genetics. These vaccines stimulate the host immune system to generate antibodies and memory cells against specified pathogens. Today, commonly known live-attenuated vaccines have been designed against influenza, rubella, measles, yellow fever and mumps.31,276,277

**Delivery**
These vaccines can be delivered subcutaneously, intradermally or through nasal or oral passage.32,275

**Mechanism**
Live-attenuated vaccines function through induction of macrophage-based cellular immunity and activation of CD8⁺ cytotoxic T cell responses, followed by development of antibody-based humoral responses. As long as the population of these cells is maintained inside the host, a long-lasting immunity can be archived.32–34

**Clinical status of live-attenuated vaccines against SARS-CoV-2**
These vaccines were produced by weakening the infectious organism which can propagate and be able to produce protective immunogenic responses. These attenuated organisms are not able to cause any disease. These vaccines are known to induce both innate as well as adaptive immunity with long-lasting immunogenic memory. These vaccines can be produced in less time and at a minimum cost. Thus, these vaccines are better and ideal to respond against lethal coronavirus outbreak.278–281 Live-attenuated vaccines COVI-VAC (Codagenix Inc.) are currently in the early stages (phase I) of development. Still, neither of these two live-attenuated vaccines are approved yet even for emergency use.47

**Merits and demerits**
Live-attenuated COVID-19 vaccine can closely mimic the real-life infection and can stimulate long-term humoral and cellular immunity in vaccinated people. Vaccination is cost effective, does not require frequent boosters and can be achieved by single dose. In rare scenarios, attenuated pathogens may revert to virulent form. One such instance was the gain of virulence of poliovirus in oral polio vaccine (OPV). Due to such risks, individuals with immune system disorders and pregnant/breast-feeding women are advised not to take this vaccine. It has been reported that yellow fever and varicella-based live vaccines lead to adverse complications in fetuses and infants. Moreover, maintenance of live pathogens requires high-end facilities.259,282

**Storage**
It is recommended to store the live-attenuated vaccine in a sealed container at 2°C and 8°C. This temperature slows down the pathogen’s metabolism and replication.32,282

3 | ADJUVANTS USED IN ANTI-SARS-COV-2 VACCINE DEVELOPMENT

Most of the vaccine types are under development using various platforms according to their reactivity and specificity to develop immune responses. The addition of adjuvants to vaccines is also a potential platform to improve the efficacy and duration of their immunological responses. Additionally, adjuvants are also preferred to reduce the concentration of antigen used and the number of immunizations required for their protective efficacy. It also makes the vaccines more cost effective and therapeutically potent. Moreover, the incorporation of adjuvants for development of subunit and certain inactivated vaccines is preferred due to the occasional lack of specific immune responses. Incorporation of adjuvants will improve the magnitude, direction and specificity of immune responses.283

Various types of adjuvants such as aluminium salts (2% alhydrogel), STING agonists (CF501), manganese (nanoMn), oil/water emulsions (MF59, AS03), TLR agonists (LR1/2, TLR3, TLR4, TLR7/8), cationic nano-carriers (Chitosan, PEI, DOTAP), matrix-M1 and Advax-SM (Advax™ and CpG55.2) are preferred in the development of anti-SARS-CoV-2 vaccines (Table 3). Two per cent alhydrogel is preferred as an adjuvant in both inactivated and RBD subunit vaccines to activate the pro-inflammatory NLPR3 pathway and T helper 2 cell responses.72,284 MF59 is a STING agonist preferred in RBD-Fc region protein subunit vaccines to activate both cellular and humoral immune responses.285,286 NanoMn is also used in RBD protein vaccines to enhance the production of cGAMP and its binding with STING.287,288 Furthermore, MF59 and AS03 oil-in-water emulsion adjuvants are being studied for use in S protein-based vaccines to enhance the dendritic cell recruitment, CD4⁺ T cell priming and humoral immunity.289,290 TLR agonists are also preferred for use in both subunit and inactivated vaccine development to induce IFN, pro-inflammatory cytokines and chemokines.291–294 Nano-carriers such as chitosan, PEI and DOTAP are also used as adjuvants in RBD subunit vaccines to activate the cytotoxic CD8⁺ and CD4⁺ T cells.295 Adjuvant matrix-M1 is used in trimeric S protein vaccines to activate both cellular and humoral immunity.194,296 Adjuvants Advax™ and CpG55.2 are preferred for use in S protein vaccines that activate CD8⁺ dendritic cells and robustness of T cell-based response.297 Thus, the use of adjuvants is important in the development of various anti-SARS-CoV-2 vaccines to promote immune specificity and durability.

4 | CAN VACCINE ADMINISTRATION VIA THE IM ROUTE PREVENT VIRUS TRANSMISSION?

The majority of the SARS-CoV-2 vaccines available for mass vaccination are administered intramuscularly and provide significant induction of IgG-neutralizing response against viraemia and disease severity in systemic circulation. The prime effects of these vaccines are
It has been documented that translocation of circulatory MHC-I of stromal and dendritic (CD103+ T cell priming and enhances antibody affinity by polymeric IgA. Mucosal IgA can inhibit viral load and lower the chances of disease severity by neutralizing the invading viral particles and protect the uninfected epithelial cells from infection. In addition, IgA facilitates functionality of targeted effector by Fcα-receptor cross-linking and enhances antibody affinity by polymeric IgA. It has been documented that translocation of circulating polymeric or non-polymeric IgG is relatively low in secretions, which might make intramuscularly induced systematic antibodies less dominant in mucosal layers. Circulating IgG and cytotoxic T cells induced by IM delivery stimulates inflammation by phagocytosis and through complement activation which tackles the propagation of severe SARS-CoV-2 pathology in terminal lung airways by removing already infected cells but does not have potent effect on infection prevention. Mucosal IgA can be elevated by protein subunit-based vaccines delivered intranasally, but induction of mucosal CD8+ T RM requires locally produced antigen presented by MHC-I of stromal and dendritic (CD103+) cells. As these T RM stay in close vicinity of viral doorways like respiratory airways and epithelium, upon secondary infection, they can quickly react with humoral and cellular responses. It was reported that Ad26.COV2. S vaccine failed to induce production of IgG and IgA antibodies in recipient saliva. Also, IM delivery of BNT162b2 vaccine enhances the anti-spike immunoglobin A (1 and 2) and G (1, 2 and 3) in serum but only IgG antibody in saliva. However, a week or two after the second dose, the level of salivary IgG and IgA was detected in a few individuals.

Abbreviations: cGAMP, 20,30-cyclic guanosine monophosphate adenosine monophosphate; IFN, type I interferon; N/A, not available; NLPR3, NOD-like protein receptor 3; PAPE, pickering emulsion; PEI, polyethyleneimine; STING, stimulator of interferon genes; Th2, T helper 2; TLR, toll-like receptor.

Table 3: Type of adjuvants used in anti-SARS-CoV-2 vaccines development.

| Classification | Adjuvants | Vaccine type | Route | Mechanism | Technology | Ref. |
|----------------|-----------|--------------|-------|-----------|------------|------|
| Aluminium      | 2% alhydrogel | Inactivated vaccines (BBV1-Coviran, CoronaVac) | IM, ID | Activate pro-inflammatory NLRP3 pathway and stimulate prime Th2 cell response | Alum-stabilized PAPE preparation, alum nano-encapsulation | 72,284 |
| STING agonist | CF501 | Subunit vaccines (RBD-Fc) | IM | Activate STING, induces cellular and humoral immunity | Derivative designing to improve solubility, potency and side effects | 285,286 |
| Manganese      | Nano-manganese | Subunit vaccines (RBD) | IM | Enhance cGAMP production and its binding with STING | NanoMn preparation by chemical engineering | 287,288 |
| O/W emulsion   | MF59, AS03 | Subunit vaccines (S) | IM | Enhances DC recruitment, CD4+ T cell priming and humoral immunity | Surfactant used to make uniform mixture of both oil and water phase | 289,290 |
| TLR agonist    | TLR3, 4, 7/8, LR1/2 agonist | Subunit vaccines (RBD, RBD-Fc, S1, S) | IM | Induces IFN, cytokines, chemokine and humoral immunity | N/A | 291–294 |
| Cationic nano-carriers | Chitosan, PEI, DOTAP | Subunit vaccines (RBD) | IM, IN | Activate cytotoxic CD8+ T lymphocytes and CD4+ Th cells | N/A | 295 |
| Matrix-M1      | N/A | Subunit vaccines (S) | IM | Activate cellular and humoral immunity | N/A | 194,294 |
| Advax-SM       | Advax™ and CpG55.2 | Subunit vaccines (ECD of S protein) | IM | Activate CD8+ DC’s, robust T cell responses | N/A | 297 |

To overcome the severe effects of the SARS-CoV-2 pandemic, all the vaccines available have been authorized for emergency use. The clinical trials of these vaccines focused predominantly on healthy adults, and efficacy drawn does not include cohorts with complicated medical conditions. Children, pregnant/nursing women and individuals with immune system disorders are extremely vulnerable to infection-related serious outcomes; therefore, special attention must be paid to vaccination of these groups.

In the next section, vaccination possibilities for special groups of individuals are discussed.
5 | VACCINATION OF MEDICALLY DISTINCT POPULATIONS

In initial clinical trials of SARS-CoV-2 vaccines, special cohorts like pregnant and breast-feeding women, children and adolescents, immune-compromised and autoimmune populations have not been included. Such groups present higher infection-related morbidity, and the approved vaccines may also cause adverse reactions. Specialized vaccines are essential for achieving significant vaccination rates in these groups.305

5.1 | Pregnant and breast-feeding women

In comparison to the non-pregnant population, pregnant women do not show any major difference in infection transmission rate; however, they are at a higher risk of severe infection-related illness. Occurrence of perinatal complications like placenta thrombosis, placentalitis, premature births, miscarriages and stillbirths were reported, and infants are commonly referred to the neonatal care unit. Maternal gestational diabetes, pre-eclampsia and post-traumatic stress disorder (PTSD) were also observed.304,306 The CDC reported that in the United States, 0.44% of pregnant SARS-CoV-2-infected women were transferred to the intensive care unit (ICU) and 0.11% died.307 Another study showed that infected pregnant women in ICU had a 70% increase in mortality risk, received extracorporeal membrane oxygenation (ECMO) and were on invasive ventilation.308 In vivo, preclinical investigation of COVID-19 vaccines on pregnant and breast-feeding women has not raised any safety alarms, but currently no vaccines have been registered for pre-marketing trial in this cohort.309

BNT162b2 mRNA vaccine is currently under phase III clinical trial (NCT04754594) and Ad26.COV2.S vaccine (registered as NCT04765384) is in phase II trial and is actively recruiting participants. In an observational study in Israel, BNT162b2 showed 96% overall effectiveness, 97% against symptomatic infection and 89% against hospitalization in pregnant women.310 In another study, vaccine effectiveness was found to be 78% and no severe adverse effects were reported. Less than 0.1% of individuals showed fatigue, headache, stomach ache, dizziness and rashes. A major limitation of these studies is the lack of information regarding perinatal effects.311 Development of prominent maternal IgG antibody upon vaccination with mRNA vaccines (Pfizer/BioNTech and Moderna vaccines) and subsequent translocation into the placenta has also been reported.312 In another cohort study, breast milk samples of Pfizer/BioNTech vaccinated individuals (two doses) were analysed for presence of antibodies. Virus-specific IgA and IgG antibody was secreted significantly for 6 weeks post vaccination.313

The WHO recommends vaccination in pregnant women when the associated potential risk is outweighed by vaccination benefits in events like the high risk of COVID-19 infection and presence of pregnancy-related co-morbidities.314 In the United Kingdom, Pfizer/BioNTech or Moderna mRNA vaccines are specially recommended by the Royal College of Obstetricians and Gynaecologists, and no adverse effect was associated in 275 000 vaccinated pregnant women.315

5.2 | Children/adolescents

A cohort-based study showed low SARS-CoV-2 transmission in children as compared to adults. A total of 234 children aged between 0 and 10 belonging to populations showing 71.5% and 85.9% infection were found to be COVID-19 negative even after being in close contact with family members who tested positive.316–318 On event of infection, children were asymptomatic or had mild symptoms like weakness, fever and cough.319,320 In the United States, the preliminary cumulative rates of COVID-19-related hospitalization as of 5 February 2022 were 913.5/100 000 adult population (aged 18 and above), which was significantly higher than 166.3 in the 0–4-year-old age group, 49.4 in the 5–11-year-old age group and 110.3 in the 12–17-year-old age group.320 Moreover, preliminary IgG-SARS-CoV-2 seroprevalence investigation reported only 1.3% in the 0–5-year-old age group.321 In comparison to children/adolescents, neonates and infants are more susceptible to severe induction-related diseases like Kawasaki-like illness, paediatric inflammatory multisystem syndrome temporally associated with SARS-CoV-2 virus (PIMS-TS) and mortal shock.322,323

Currently, 13 trials in phase I, 31 in phase II and 26 in phase III have been registered for clinical investigation of SARS-CoV-2 Vaccine (Vero Cells), SF9 Cells, CHO Cells, BBV152, inactivated vaccine (Sinovac), Ad5-nCoV-1H, 9vHPV, mRNA-1273, MCV-COV1901, BNT162b2, CoronaVac, Gam-COVID-vac M, SCB-2019, LV-SMENP-DC, aAPC and COVAXIN for children and adolescents. FDA approved Pfizer/BioNTech vaccine for 5–11-year-old children based on 90.7% efficacy, which is comparable to adults. Moreover, no adverse effect was observed in 3100 enrolled children.324 Later, the vaccine was approved for 12–15-year-old participants showing 100% efficacy and for those aged 16 years and above, showing 94.6% efficacy. Post-authorization adverse effects included pain at the site of injection, diarrhoea and hypersensitivity reactions like anaphylaxis, rashes, angioedema and pruritus.324

At the time of writing, clinical evaluations of eight trials are registered in phase I, 18 in phase II and 10 in phase III. The vaccines included in these trials are Nanocovax (NCT04683484, NCT04611802), BNT162B2 (NCT04895982, NCT04761822, NCT04368728, NCT04713553, NCT04816643, NCT04800133), VLA2001 and VLA2101 (NCT04956224), COVAXIN (NCT04918797, NCT04175193, NCT04566770, NCT04916886), UB-612 (NCT04773067), Spikevax (NCT04761822, NCT04796896, NCT04649151), Vivo Cells (NCT04917523, NCT04884685, NCT04551547), CHO Cell (NCT04869592), aAPC Vaccine (NCT04299724), MCV-COV1901 (NCT04951388), LV-SMENP (NCT04276896) and Gam-COVID-Vac (NCT04954092) (ClinicalTrials.gov).
### 5.3 Immune system disorder (immunodeficiency and autoimmune/auto-inflammatory diseases)

Observational studies have reported that cohorts with underlying conditions like immunodeficiency and autoimmunity are potentially vulnerable to severe COVID-19-associated diseases. Individuals with haematologic malignancies, solid tumour, organ/cell transplant, HIV/AIDS, allergy, autoimmunity and primary/secondary immunodeficiency are at higher risk. Moreover, therapeutic approaches like chemotherapy under COVID-19 effect have shown increased mortality of 30 days. Metadata analysis of 300,000 autoimmunity–auto-inflammatory patients showed that the intake of steroids considerably increases COVID-19 infection in this group. Individuals with immune system disorder should avoid live virus-based vaccines and must opt for subunit and inactivated vaccines.

In a study, 46% individuals among 658 solid organ transplant enrolees, vaccinated with SARS-CoV-2 mRNA vaccines, showed absence of humoral antibody response. BNT162b2 and ChAdOx1 vaccinated individuals with haematologic malignancies and solid organ tumors or recipient of immunosuppressive treatment had significantly low spike protein antibodies in partially vaccinated (single dose) candidates than in fully vaccinated ones (dual doses), which stresses the efficacy of early administration of a second dose in these groups. The humoral response induced by BNT162 vaccine showed lack of IgG titre production in dialysis and kidney transplant. A similar outcome was observed in the case of lung transplant recipients, where the virus-specific IgG level was studied in 33 patients with history of COVID-19 infection and 48 who were vaccinated with BNT162 vaccine. Eight-five per cent of the participants with prior infection showed presence of humoral antibody response but it was completely absent in the vaccinated group. The lack of humoral immune response from vaccine could be the result of immunosuppressive drugs involved in the treatment of this cohort. Major challenges in vaccination are its effect on other prescribed treatments and adverse effects. In patients with autoimmune/auto-inflammatory disorders, vaccine may lead to elevated stimulation of stabilized illness. Among 491 BNT162b2 vaccinated autoimmune and inflammatory rheumatic disease patients, 1.2% showed Herpes zoster reactivation.

However, the lack of clinical results regarding vaccine efficacy and safety does not outweigh the fatal COVID-19-linked complication of these individuals and makes them top priority in the COVID-19 vaccination drive. The efficacy of BNT162B2 and ChAdOx1 after a second dose increases considerably to 73% and 74.6%, respectively, and efficacy of a third dose is currently being investigated. According to the Australasian Society of Clinical Immunology and Allergy (ASCIA), non-live-attenuated vaccines, namely, mRNA-based COVID-19 vaccines, BNT162B2 (Pfizer/BioNTech) and Spikevax (Moderna) and viral vector COVID-19 vaccine ChAdOx1 (AstraZeneca/Oxford) are safe for individuals with immunodeficiency, autoimmunity, auto-inflammation and allergy.

A total of 4927 immune-deficient individuals are currently enrolled in six clinical trials (one in phase II, two in phases III and IV and one with unknown status). These vaccines include two mRNA vaccines: BNT162B2 (NCT04895982, NCT04780659) and Spikevax (NCT04806113, NCT04805125, NCT04847050), an adenovirus vector-based Covishield vaccine (NCT04794946) and whole virus inactivated vaccine CoronaVac (NCT04754698). All enrolled candidates are ≥18 years of age and associated conditions include primary/secondary immunosuppressive disorders, HIV/AIDS, organ transplantation, solid and haematological malignancies, liver cirrhosis and rheumatic diseases. (ClinicalTrials.gov).

Recently, the cellular and humoral responses of BNT162b2 vaccine has been assessed in patient groups with rheumatoid arthritis receiving treatment with methotrexate (synthetic) and TNF blockers (biologic). The TNF blocker recipient group shows strong antibody responses in more than 90% of patients, while the methotrexate recipient group shows adequate response in 62.2% of patients. It occurs due to the prevention of CD8+ T cell activation in the methotrexate-treated arthritis patients after vaccination. Further, much study is needed to confirm these results and to make vaccination safer for arthritis patients.

However, it is also important to study the treatment of inflammatory bowel disease (IBD) patients along with anti-SARS-CoV-2 vaccination. In a recent study, IBD patient groups were treated with six different drugs (namely, thiopurines, tofacitinib, thiopurine + infliximab, ustekinumab, infliximab and vedolizumab) and two doses of vaccines (namely, AstraZeneca, BNT162b2 and mRNA1273). Immediately, after the second dose of vaccine, the antibody concentration was measured. Vaccinated IBD patient groups treated with different drugs such as infliximab, infliximab + thiopurine and tofacitinib show lower production of anti-SARS-CoV-2 spike protein antibody while IBD patients treated with ustekinumab, thiopurine and vedolizumab show no such significant change in antibody concentration. This study suggests that infliximab, infliximab + thiopurine and tofacitinib recipient IBD patient groups need a third booster dose to maintain the anti-SARS-CoV-2 antibody concentration.

Anti-rheumatic drugs (DMARDs) are one of the possible treatment options for the immune-inflammatory diseases. SARS-CoV-2 infection shows complications in DMARD (biologic) treated individuals. Vaccination of this population may reduce such serious complications. A recent clinical study was performed to evaluate the immune responses in TNF inhibitor (adalimumab) or IL-17A inhibitor (secukinumab) receiving spondyloarthrits patient groups receiving dual doses of BNT162b2 vaccine. A high seroconversion rate was observed in both the test groups. A huge production of CD4+ and CD8+ T cells but no such significant change is observed in the level of reactive T cells between the test groups. Thus, this study concluded that both the biologic DMARDs are not able to affect the cellular or the humoral immune responses of BNT162b2-vaccinated spondyloarthrits patients. Thus, more clinical trial-based study is required to evaluate and assess the vaccine safety and efficacy in these types of immunosuppressive diseases.
### 5.4 Inborn error of immunity

Primary immunodeficiency (PID) or ‘inborn error of immunity’ (IEI), as phrased by the International Union of Immunological Societies (IUIS), are germline disorders which alter optimal performance of immune system making individuals susceptible to infections, allergies, auto-inflammatory disorders, autoimmune diseases and malignancies.339–341

### 5.4.1 IEI patients versus SARS-CoV-2 infection

SARS-CoV-2 infection in immunocompromised patients is associated with elevated severity and ICU admission. Several case studies have reported that mortality and morbidity through COVID-19-related complications like chronic lung/liver diseases and prophylactic antibiotics were raised in young IEI patients in comparison to the general cohort.342–345 Another study on 4718 Iranian PID patients showed an

#### Table 4 Vaccination of patients with inborn errors of immunity (IEI) and their immunological responses

| IEI diagnosis                              | Patient # | Vaccine         | Adaptive immunity | Cellular immunity | Ref.  |
|--------------------------------------------|-----------|-----------------|-------------------|-------------------|-------|
| CVID                                       | 17        | BNT162B2        | 65%               | NR                | 353   |
| CVID, XLA, Ab deficiency                   | 21        | BNT162B2        | 86-95% anti-spike RBD Ab, 76% | 349   |
| CVID                                       | 17        | BNT162B2        | 70.5%             | 82%               | 344   |
| CVID, XLA, CID                             | 78        | BNT162B2        | 73%               | NR                | 342   |
| CVID                                       | 33        | BNT162B2        | 33%               | NR                | 349   |
| CVID                                       | 30        | BNT162b2 booster, ChAdOx1 | 83% after any booster, 80% after mRNA booster | 53% for ChAdOx1, 83% for mRNA | 343   |
| CVID                                       | 58        | BNT162B2        | 34% post vaccination, 100% post-infection | 1/9 post vaccination, 0/3 convalescent | 345   |
| CVID                                       | 5         | BNT162B2, mRNA-1273 | 80%              | NR                | 346   |
| CVID                                       | 28        | Ad26.COV2.S, ChAdOx1 nCoV-19, BNT162b2, mRNA-1273 | 71.4%             | 71%               | 349   |
| CVID                                       | 18        | BNT162B2, mRNA-1273, ChAdOx1 | 83% after any dose, 50% neutralizing Ab | 83% | 347   |
| CVID, SAD                                   | 25        | BNT162B2, mRNA-1273 | 73%               | NR                | 348   |
| CVID, XLA                                  | 47        | BNT162B2        | 20%               | 70% CVID, 83% XLA  | 349   |
| CVID                                       | 1         | BNT162B2        | 100%              | NR                | 350   |
| CVID, PAD, SAD, XLA, CID, thymoma           | 168       | BNT162B2, ChAdOx1 | 55%               | 46.20%             | 346   |
| CVID XLA, WAS                              | 11        | BNT162B2, mRNA-1273 | 91%               | NR                | 349   |
| CVID XLA, CID, PAD, phagocytic defects      | 505       | mRNA-1273       | >80% in all cases | 88% overall, 67% CVID | 352   |
| WAS                                        | 1         | BNT162B2        | 100%              | 100%              | 351   |
| MagT1                                      | 1         | BNT162B2        | 100%              | NR                | 352   |
| Primary antibody deficiency                 | 62        | BNT162B2, mRNA-1273, Ad26, COV2.S | 59.7% after primary dose, 14 × higher after booster 2 | NR | 349   |
| CVID, CID, SAD, XLA, CHH, CTLA4, CGD, WAS, ADA2, IFNGR1, STAT3 LOF | 156 | BNT162B2, mRNA-1273, ChAdOx1 | 67% | NR | 353   |
| SCID, APECED, CID, CVID, RAG, MagT1, RALD, STAT-3 LOF, WAS, WHIM, XLA | 81 | BNT162B2, mRNA-1273, Ad26, COV2.S | 85% | NR | 350   |
| XLA, SAD, CVID, good syndrome, ATP6AP1 and PIK3R1 deficiency | 33 | BNT162B2, mRNA-1273 | 48% anti-RBD Ab, 6% anti-ACE2 receptor activity | 77% T cell-specific Ab | 354   |
| XLA, STAT3 LOF, CVID, NFKB1                 | 26        | BNT162B2        | 69%               | 73%               | 349   |

Abbreviations: APECED, autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy; CID, combined immunodeficiency; CVID, common variable immunodeficiency; IEI, inborn errors of immunity; NR, not reported; PAD, primary antibody deficiency; RBD, receptor-binding domain; SAD, specific antibody deficiency; SCID, severe combined immunodeficiency; WAS, Wiskott–Aldrich syndrome; XLA, X-linked agamaglobulinemia.
increase in COVID-19 infection and mortality rate by 1.23- and 10-fold, respectively. It has been reported that some IEI patients with deficient antibodies had considerably robust humoral and T cell-based responses against SARS-CoV-2 spike and nucleocapsid proteins. Another study showed that SARS-CoV-2 infected IEI individuals had 4 month long viral shedding and T cell activity against the virus.

5.4.2 | Vaccination immune responses

To develop suitable vaccine for IEI individuals, it is important to assess the response of existing COVID-19 vaccines in the PID population. Various studies have been conducted to evaluate the immunological effect of RNA-based vaccines (BNT162B2 and mRNA-1273) and inactive viral vaccines (ChAdOx1 nCoV-19, Ad26.COV2.S and Coronavac) in IEI patients. A study showed that among 81 IEI individuals, 85% generated antibodies against spike protein after primary doses of AdV26.COV2.S and mRNA-based vaccines, whereas lower anti-S IgG level was observed in individuals with a history of rituximab medication and autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED) patients. BNT162B2 vaccination led to seroconversion in 73% of IEI patients, which included monogenic disorder and common variable immunodeficiency (CVID). Salivary anti-S IgG levels were relatively low in the IEI population. A survey of 505 IEI patients vaccinated with mRNA-1273 reported that in comparison to the general population, seroconversion was similar in milder IEIs but was lower in severe IEIs like CID and CVID. Various articles reported vaccination responses associated with diverse B cell dysfunction of IEI s. Seventeen CVID patients vaccinated with BNT162B2 in cohort with normal peripheral and switched B cell population showed usual serologic activity but individuals with deficient switched memory B cells showed lower serologic responses. Moreover, serologic activity was totally absent in patients lacking peripheral B cells (Table 4). Overall, post vaccination, serological responses are apparent in IEI populations but the long-term efficacy of the vaccines is still uncertain. Few studies showed a comparable decrease in antibody titre in IEI and normal populations.

5.4.3 | Vaccination safety

Low immunological efficiency of IEI patients requires extra attention to the safety of COVID-19 vaccines. An article reported that IEI patients vaccinated with mRNA vaccine had higher reactogenicity in comparison to the general population where they observed common symptoms including mild myalgias, fever and fatigue. Among 130 individuals with various auto-inflammatory conditions, vaccinated with BNT162B2 or ChAdOx1-S, no severe adverse reaction was observed without any significant inflammatory flare. To reduce the severity, mortality and hospital admissions, total public vaccination is of utmost importance, and this could not be achieved without the vaccination of individuals with the above-mentioned underlying complications. To date, special clinical investigation of vaccines for such groups is still in progress, but not enough data are available to determine the effectiveness of approved vaccines among these cases. Nevertheless, some vaccines discussed above have been approved, but further examination of these vaccines is still required for safety issues in these special groups.

6 | CONCLUDING REMARKS

The COVID-19 pandemic is a worldwide threat with non-availability of specific treatment. In this review, we have discussed the clinical development of vaccine candidates. Ongoing vaccine development and status of many approved vaccines after successful clinical trials and various classes of vaccines are still in progress. Here, we have highlighted the clinical status of more than 100 vaccines. Most of the vaccines are still in various stages of clinical trials and a few of them have been approved by respective regulatory authorities for their clinical use. So researchers and clinicians will have to put more effort and attention into clinical trial limitations. Moreover, investors and the pharmaceutical industry must come together for the advancement of specific and efficient vaccines for children and adults.

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COMPETING INTERESTS

The authors have no conflicts of interest to declare.

CONTRIBUTORS

S.K., M.B., A.A., P.G. and M.K.G. conceived the idea, review structure and various classes of vaccines are still in progress. Here, we have highlighted the clinical status of more than 100 vaccines. Most of the vaccines are still in various stages of clinical trials and a few of them have been approved by respective regulatory authorities for their clinical use. So researchers and clinicians will have to put more effort and attention into clinical trial limitations. Moreover, investors and the pharmaceutical industry must come together for the advancement of specific and efficient vaccines for children and adults.

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CONTRIBUTORS

S.K., M.B., A.A., P.G. and M.K.G. conceived the idea, review structure and writing. P.G., M.B., S.K. and M.K.G. revised the manuscript. All authors have read and agreed to the final draft of the manuscript.

DATA AVAILABILITY STATEMENT

Data available on request.

ORCID

Mrinal K. Ghosh https://orcid.org/0000-0003-4959-3065

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