Chapter 7
COVID-19 Pandemic and Vaccines

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Introduction

Viral diseases regularly pose threats to the human health, says the World Health Organization (WHO). The mankind has witnessed some serious epidemics, viz. severe acute respiratory syndrome (SARS), H1N1 flu and MERS within the past 20 years. As the year 2019 was coming to its conclusion, a few cases of pneumonia with intense respiratory problems emerged in Wuhan City, Hubei Province, China. The doctors were unable to identify the aetiology of the disease [1]. As the cases spread, the Centers for Disease Control and Prevention (CDC) intervened and discovered that infection was being caused by a new type of coronavirus that was named as the 2019 novel coronavirus disease (2019-nCoV). It was also referred as SARS-CoV-2 and human coronavirus disease 19 (hCoV-19) at the time [2]. Later on, the official name of the disease was announced as coronavirus disease 2019 (COVID-19) by the WHO. As stated above, coronaviruses are already accountable for causing two recent epidemics, namely, SARS-CoV and

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MERS-CoV. SARS-CoV epidemic emerged from China and spread to 24 nations infecting 8000 individuals with a fatality rate of 9.6% [3]. The MERS-CoV initiated in Saudi Arabia and is accountable for 2500 cases with a much higher fatality rate of 35%. It is also causing periodic cases even in the present [4]. COVID-19 is being transmitted chiefly by respiratory droplets, while person-to-person transmission is also taking place through close contacts since mid-December 2019 [5]. COVID-19 was given the status of pandemic by the WHO on 11 March 2020.

COVID-19 Vaccines: A Need of Time

The CoVs have become the significant microbes of emerging respiratory ailments. They are wrapped non-fragmented positive-sense RNA viruses and have a place with the family Coronaviridae. They are comprehensively circulated in people and other animals [6]. Beforehand, six COVID types have been found to cause human illnesses (Fig. 7.1) [7]. The first four species resulted in minor indications; however, the last two SARS-Co and MERS-CoV created havoc with death rates of 10% and 34% for SARS-Co and MERS-CoV, respectively. These two viruses have resulted in death of more than 10 thousand people in the past 20 years [7–9]. SARS-CoV-2 is number 7 in the list of COVID that can affect people [10]. In spite of the fact that the death rate of SARS-COV-2 is lesser compared to MERS-CoV and SARS-CoV, it is much more contagious than MERS-CoV or SARS-CoV (Table 7.1).

Genome sequencing examination of clinical samples obtained from patients exhibited that 88% of the SARS-CoV-2 genome was homologous to two SARS-like COVIDs present in bats: bat-SL-CoVZC45 and bat-SLCoVZXC21. It also showed 79% and 50% nucleotide sequence homology to SARS-CoV and MERS-CoV, respectively [11].

The genome also consists of 16 nonstructural proteins (nsp1–16) along with 5–8 accessary proteins [12]. Phylogenetic assessment proposed that bats can be the basic source for SARS-CoV-2 [13]; the middle host is as yet under scrutiny. The illness essentially influences the respiratory organs, and sickness seriousness can extend from minor rhinorrhoea to acute respiratory syndrome followed by patient death [14–16]. Other documented symptoms include diarrhoea, rash, anosmia, thromboembolic issues, vasculitis and myocarditis [1, 16–22]. The median incubation time frame is assessed to be 5 days with a greater part creating side effects by 11.5 days [23]. The patients of COVID-19 are believed to shed highest levels of viral load at the beginning of symptoms [24]. This and other epidemiological information proposes the risk of transmission starting from indistinct presymptomatic

Fig. 7.1 Types of coronaviruses infecting humans

Table 7.1 Contagiousness of coronaviruses

Coronavirus type	Contagiousness
SARS-COV-2	Middle R0: 5.7
MERS-CoV	R0: <1
SARS-CoV	R0: 3

period [25]. The clinical deterioration is deferred till the second week from the infection onset and appears to be the manifestation of cytokine-mediated immune response, accountable for severe inflammation and dispersed intravascular coagulation, often with low-intensity viraemia [26, 27]. Death rate is highest among the elderly people (older than 70 years); however, other factors can also account for the death of the patient [1, 16, 28] which include gender (higher fatality rate in males than females), obesity, hypertension and diabetes. The announced case casualty rates (CFR) have been somewhere in the range of 0.82% and 9.64%, with changeability in CFR likely because of the testing recurrence and access along with numerous other health framework limiting factors in various areas of the world [29]. The infection fatality rate (IFT), death count of all individuals infected, asymptomatic and not tested, is a superior gauge of populace mortality and is demonstrated to be somewhere in the range of 0.1% and 0.41% [29]. The countries of the world have worked out strict measure to counter the effects of COVID-19 that includes shutdowns which alone are believed to save an estimated three million lives in 11 European countries [30]. These measures have been taken on the expense of slowing down of economies and recession. A great many jobs in all economic sectors have been lost because of COVID-19 restrictions like social distancing, travel bans and self-isolation. There have been a closure of education institutions and a sharp decline in consumer spending. Two sectors to have thrived in strict shutdowns have been pharmaceuticals and food sector – as there was a high demand of food due to the ongoing panic in shutdowns to stock pile the food products.

Coronavirus Vaccine Development: Challenges of the Past

The 30 + kb genome of RNA coronaviruses is enveloped by a helical nucleocapsid (N) which is surrounded by an outer envelope consisting of matrix protein (M), envelope protein (E) and spike proteins (S) [31]. The naturally occurring trimeric form of S protein holds the receptor-binding domain (RBD) accounting for the association with the angiotensin-converting enzyme 2 (ACE2) and facilitates entry into the cell. This S protein, among other structural proteins of SARS-CoV, has been found to cause the production of neutralizing antibodies, therefore a chief candidate antigen for vaccine development [32, 33]. Coronavirus vaccine development has met with some major challenges in the past. So far, not a single coronavirus vaccine has been licensed for human use to combat respiratory infections. As for the animals, only infectious bronchitis virus (IBV) vaccines are approved for upper

Table 7.2 Immunopathology associated with coronavirus vaccines

Sr No	Animal model	Vaccine	Complications postinoculation with the virus	Reference
I	Mice	Inactivated whole virus vaccines Recombinant DNA spike protein vaccine Virus-like particle vaccine	Lung eosinophilic infiltration	[36]
II	Mice	SARS-CoV N protein	Severe pneumonia Lung eosinophilic infiltration	[37]
III	Mice	Viral replicon particles expressing glycoprotein	No complications	[37]
IV	Mice	Inactivated MERS-CoV vaccine	Severe pneumonia Lung eosinophilic infiltration	[38]

respiratory CoV infections in chickens. Generally, the coronavirus vaccines mimicking human disease have been shown to be immunogenic when injected in animal models but do not effectually avoid disease acquisition [34]. Additionally, there is a worry that immunization, just like normal coronaviral infection, may not instigate enduring insusceptibility and reinfection might be conceivable [35]. Additionally, concerning has been the immunization-related disease enhancement. Past utilization of COVID immunizations (SARS-CoV and MERS-CoV) in some animal models has shown safety issues with respect to Th2 interceded immunopathology (Table 7.2).

Vaccine Development Strategies

Vaccine development is an efficient measure to counteract infectious diseases. With knowledge gained from the previous vaccine models considered for MERS and SARS, several vaccine development strategies, i.e. DNA, mRNA, recombinant protein, inactivated/attenuated virus and adenoviral vector, are being explored (Fig. 7.2). In this regard, S protein and its fragments (S1, S2, RBD and N protein) are being considered as potential candidates for COVID-19 vaccine development (Fig. 7.3) since they have also been used in case of MERS and SARS vaccine [39, 40].

As soon as the genetic information of SARS-CoV-2 was made public on 11 January 2020, around 40 pharmaceutical establishments and academic organizations from several countries are actively involved in vaccine development to win the fight against SARS-CoV-2, and many of them have reached the clinical trial stages. Various vaccine platforms being investigated have specific salient points (Table 7.3). The route to successful vaccine development can be abetted by taking an elaborate look into pathological process of SARS-CoV-2 in humans and the coordinates and duration of immunity. A comprehensive look into the way the virus affects the target organs and its spread within the body will aid in developing vaccines which can prevent virus dissemination to the organs and abate its infection. It is an important

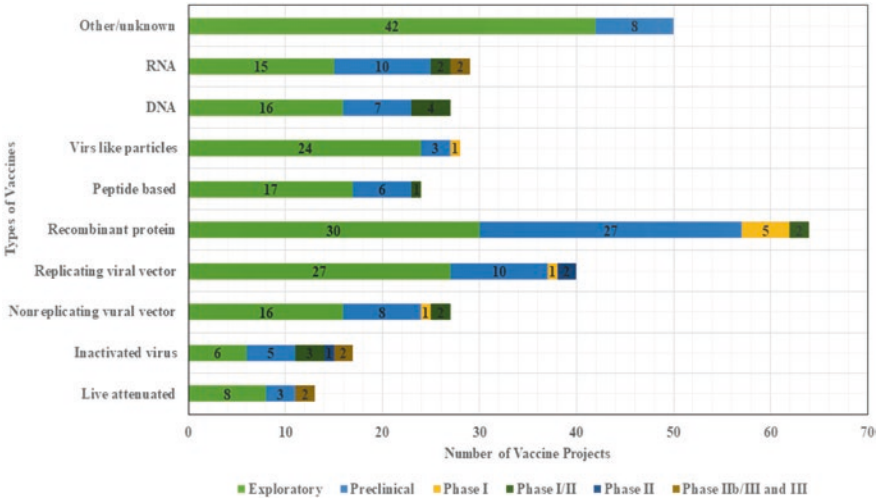


Fig. 7.2 Current standing of COVID-19 vaccine candidates by developmental stage and vaccine type

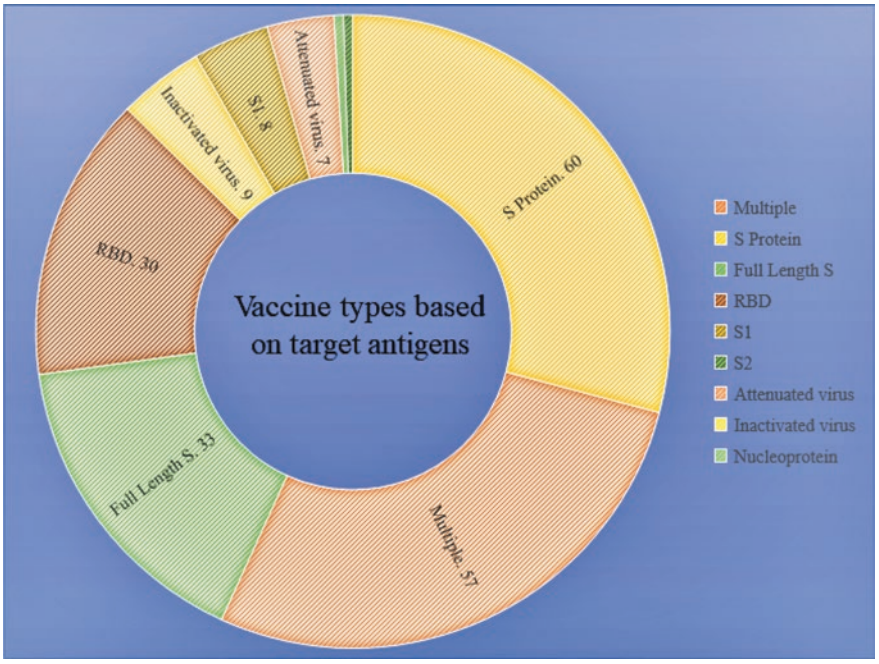
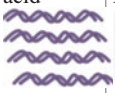


Fig. 7.3 COVID-19 vaccine candidates with respect to target antigen

Table 7.3 A comparison of COVID-19 candidate vaccine platforms

Vaccine types	Nucleic acid 	Live-attenuated virus 	Inactivated virus 	Subunit 	Viral vector 
Working	DNA/RNA expresses viral proteins which trigger the immune responses	Weakened version of the virus	Virus killed by heat or chemical treatment acts as inactivated vaccine	Viral surface proteins utilized which are targeted by immune system	Nonpathogenic viral vectors used to deliver viral genes to confer immunity
Pros	Simple and quick to engineer	Elicits a strong immune response without causing serious infection	Simple to engineer and safe because of the dead nature of the virus	Directs the immune response towards the significant entity of virus without any infection	Live viruses stimulate robust immune reactions
Cons	No approved DNA/RNA vaccines exist so far	Not a viable option for immunocompromised persons	Not as effectual as a live virus, may escalate the infection	Do not elicit a sturdy immune response, may be used in combination with adjuvants to confer long-lasting immunity	Choice of a safe viral vector is crucial; immune response directed at the vector may weaken the vaccine effectiveness
Existing vaccines	None	Measles, mumps, rubella (MMR) Chicken pox	Polio	Hepatitis B Pertussis Papillomaviruses	Ebola Veterinary medicines

point to consider that as SARS-CoV-2 affects the lungs, it will result in pneumonia by viraemia or after the infection of upper respiratory tract. In case of the latter, vaccine systems based on live replicating vectors or attenuated viruses would be effective as they bring out localized mucosal immunity and shelter the upper and lower respiratory tracts and diminish adenoidal emissions. The use of live-attenuated influenza virus vaccine is a good example since it essentially produces localized IgA antibodies and scarcer systemic antibodies and causes prompt defence [41].

On the contrary, if lungs and other organs are being infected via viraemia, then parenteral (intramuscular) vaccines would serve better as they result in the production of substantial amount of virus neutralizing (VN) antibodies in serum blocking viraemia. Upon reaching the lungs and other target organs, VN antibodies could also efficiently wedge the infection. Furthermore, when COVID-19 recovered patients are exposed to the virus again (like seasonal influenza), an annual booster

dose of a parental vaccine alone, such as a subunit S or RBD protein, would suffice. This would result in strong memory B and T cell responses and enable long-term immunity preventing subsequent virus infections. There have been reports of virus shedding in diarrhoea and faeces in few COVID-19 patients, so oronasal vaccines may also be effectual [42]. Therefore, the recipients of COVID-19 vaccines could be divided into three groups, viz. susceptible persons without immunity, healed individuals with varying level of immunity and individuals having immunity (exposed and recovered) against SARS and MERS. Consequently, the immunogenic response, level of protection and lethal effects of candidate vaccines will be different from person to person depending on the above categories. Evaluation of pre-existing levels of immunity with respect to population type and age will be imperative to corroborate the vaccine efficacy and safety.

Vaccine development and its scale-up in the middle of a worldwide pandemic is a demanding and tedious task since it entails the coordination of various activities taking place side by side in comparison to the decennium long, step-by-step vaccine production which includes preclinical trials, clinical trials, scale-up studies and distribution. This situation creates a blend of invested resources and increased financial risk having high stakes [43]. Previous disease outbreaks have witnessed high mortality and morbidity rates due to unorganized and delayed vaccine distribution as in the case of West African Ebola epidemic (2013/2014) which claimed 11,000 casualties [44] causing enormous economic and social losses of an estimated 53 billion dollars [45]. Unfortunately, an effective vaccine developed subsequently might have cut down the social and economic losses at the time controlling the outbreak [46, 47]. Appallingly, the SARS 2003 outbreak finished before an effective vaccine was developed. Disappointingly, funding organizations at that point reallocated reserves that had been focused on vaccine development, leaving producers with monetary misfortune and slowing down other vaccine advancement programmes [43]. In 2017, the Coalition of Epidemic Preparedness Innovation (CEPI) was shaped to answer these previous disappointments with an undertaking to build up a planned reaction to rising irresistible sickness dangers to guarantee robust immunization programmes and early deployment in light of pestilences [48].

Nucleic Acid Vaccines

This strategy involves the use of nucleic acid sequences to express viral antigens. As the cell uptakes the nucleic acid fragments, viral protein expression is initiated which further elicits immune responses (humoral and cellular) similar to the ones taking place in course of natural infections. Such type of vaccines has been tested for animal ailments and exhibited immune protection, for instance, for foot and mouth ailment, deer powassan infection and rabies infection [49, 50]. Nucleic acid vaccines intended for Ebola, influenza and Zika virus have already entered phase I of the clinical trials [51]. The advantage of this platform resides in its ease of

antigen control, rapid synthesis and cell-free engineering which evades the requirement of biosafety level 2 laboratory. The major hindrances are the delicate nature of nucleic acids such as mRNA which require continuous cold conditions during handling and capacity [52]. Stage I clinical studies have been carried on SARS-CoV and MERS-CoV DNA vaccine candidates. A recombinant SARS DNA vaccine synthesizing the SARS-CoV N protein, created by the National Institute of Allergy and Infectious Diseases (NIAID), was explored in ten adult individuals [53]. Clinical trial undertaken by GeneOne Life Science/Inovio for a MERS-CoV DNA vaccine (GLS-5300) encoding full-length S protein genome contained a high number of participants (n = 75) [52]. Both indicated satisfactory safety profiles and initiated humoral and cellular reactions; the MERS-CoV DNA vaccine has progressed into a stage 2 of clinical trials [29]. The only SARS vaccine to have entered a phase I trial is an inactivated immunization (ISCV) created by Sinovac Biotech [54]. There were no reports of human investigations wherein inoculated subjects were tested by the characteristic infection.

A few significant biotechnology organizations are working on advanced models of nucleic acid vaccines for COVID-19. The Innovation and Value Initiative (IVI), Inovio and the Korea National Institute of Health (KNIH) are working together with the Coalition for Epidemic Preparedness Innovations (CEPI) to safety evaluation and immunogenicity of a DNA vaccine named INO-4800 in phase I/II clinical trial in South Korea. Both Moderna/NIH and CureVac are zeroing in on mRNA vaccine improvement, and a safety evaluation trial of Moderna's mRNA-1273 vaccine was conducted on 45 individuals in March 2020 [55].

Protein Subunit Vaccines

Subunit-based immunizations dependent on recombinant S or S1 protein of SARS-CoV and MERS-CoV have been exhibited to be viable in numerous investigations [56] [57–59]. Clover Biopharmaceuticals is building up a vaccine comprising a trimerized SARS-CoV-2 S protein utilizing their patented Trimer-Tag innovation [59]. The receptor-binding domain (RBD) in SARS-CoV-2 S protein was distinguished, and it was additionally shown that SARS-CoV-2 RBD displayed fundamentally higher binding affinity to ACE2 receptor contrasted with interaction between SARS-CoV RBD and ACE2 [60], recommending that the RBD-based SARS-CoV immunizations can possibly be created for counteraction of SARS-CoV-2 infections. RBD-based vaccines are currently being worked on by a few associations through worldwide coordinated efforts [61]. The pneumonic surfactant-biomimetic nanoparticles used to potentiate heterosubtypic flu resistance can be utilized as adjuvant to improve the immunogenicity of SARS-CoV-2 subunit vaccines [62].

Inactivated or Live-Attenuated Virus Vaccines

Whole inactivated or live-attenuated virus-based immunizations speak to a customary vaccine technique. Scientists at the University of Hong Kong have built up a live influenza vaccine that produces SARS-CoV-2 proteins [63]. Codagenix has built up a "codon deoptimization" innovation to weakened viruses, and the organization is investigating COVID-19 vaccine systems [64].

Virus Vector-Based Vaccines

Immunizations dependent on viral vectors offer a significant level of protein expression and long haul dependability and elicit strong immune reactions [65]. Johnson & Johnson is building up an adenovirus-vectored antibody utilizing AdVac®/PER.C6® immunization technique [55]. The first COVID-19 vaccine candidate dependent on adenovirus-vectored immunization created by Chen Wei bunch entered human clinical testing (NCT04313127) with extraordinary quickness right off the bat on 16 March 2020. Another stage I safety evaluation study of a recombinant adenovirus vaccine candidate (Cansino Biologics Inc., Tianjin, China), Ad5-nCoV, selected 108 adult volunteers in Wuhan, China, in March 2020. *IVI, INOVIO and KNIH cooperate with CEPI in stage 1/2 clinical studies of INOVIO's COVID-19 DNA immunization in South Korea, 2020 [38]. Aside from adenovirus vector-based immunization, two lentivirus vector-based vaccine candidates, COVID-19/aAPC and LVSMENP-DC, have been created by Shenzhen Geno-Immune Medical Institute. The COVID-19/aAPC immunization was created by applying lentivirus alteration including the SARS-CoV-2 minigenes and immune modulatory genes, to the counterfeit antigen presenting cells (aAPCs). The phase I clinical study comprising of 100 members began on 15 February 2020, and the assessed study completion date was 31 December 2024 (NCT04299724). The LVSMENP DC vaccine was created by changing DC with lentivirus vectors synthesizing SARS-CoV-2 minigene SMENP and immune modulatory genes.

We as a whole realize that adjuvants assume a basic part by improving immunogenicity of the immunization candidates and aid in the selection of appropriate dose in some vaccine types. Up until this point, there are around ten developers engineering adjuvanted COVID-19 immunizations. Vaccine developers Dynavax, Seqirus and GlaxoSmithKline have focused on making some licensed adjuvants including MF59, AS03 and CpG 1018 accessible for use [65]. Regardless of which strategy we take to build up the COVID-19 immunizations, analysts need to painstakingly assess the viability and safety of the candidate vaccine at each progression. Under these circumstances, SARS-CoV-2-specific animal models appear to be an important requisite. As of not long ago, some extraordinary animal models are in development stages, including hamsters, ferrets, ACE2-transgenic mice and nonhuman primates [65].

Repurposed Vaccines for COVID-19 and Off-Target Effects of Other Vaccines

Authorized immunizations, for example, BCG and oral polio vaccine, are able to induce vague, immune reactions and have modulatory effect insurance against different infectious ailments [66]. This may hint towards the recommendation that they can be exploited as potential vaccine candidates in the avoidance of COVID-19. Some clinical trials involving BCG vaccines are already underway to determine their capacity to combat COVID-19 in Australia [66], the Netherlands [67] and South Africa [68]. A measles vaccine clinical trial to forestall COVID-19 in medical care labourers in Egypt has been enrolled [58], and oral polio immunizations are being examined in the USA [69].

Plant-Based Vaccines

Presently, a number of bacterial, yeast and mammalian expression systems are available for recombinant protein production including subunit vaccines, but they all have certain shortcomings. They are expensive, have safety concerns and do not guarantee target integrity. In comparison, plant expression systems provide a robust solution to these problems since they are cheap with better yield and offer no risk of pathogenic contamination. Hence, plant-based vaccines can prove to be a convalescent approach towards vaccine development. The expressed protein could adopt the desired conformation with expected post-translational modifications while maintaining its functional integrity in a plant-based system [67].

Since the last three decades, plants have been used as a machinery to manufacture a wide range of biopharmaceuticals such as monoclonal antibodies, edible vaccines, human growth factors and cytokines [68]. Fortunately, the production of a recombinant enzyme named β-glucocerebrosidase in carrot cells for Gaucher's disease treatment has also been licensed by the FDA [69]. The usefulness of plant-based systems resides in their incapacity to allow the growth of human pathogens making the purification process less tedious and contamination-free [70]. So far, various clinical trials are underway for plant-based synthesis of vaccine for swine influenza, rabies and hepatitis B [71]. An eminent example would be that of vector-based influenza vaccine (Medicago Inc.) expressing hemagglutinin (HA) encoded by viral regulatory elements in *Nicotiana benthamiana* [72, 73].

The presently existing plant-based foreign protein expression approaches hold the potential to synthesize many vaccine candidates against COVID-19 in short stretch of time. The chosen target antigen will regulate the choice of expression strategy and targeted organelle. The vaccine antigens may be native viral proteins or fusion proteins premeditated to act as multiepitopic vaccines. This is structured by T cell and B cell epitope selection using the relevant bioinformatics tools and experimental evidence. Presently available genetic engineering tools enable the stable/

transient expression of target antigens which give way to explore diverse immunization approaches. In case of transient expression, the antigens need to be purified and are subsequently formulated as injectable vaccines. Stable expression is useful for edible crops and permits executing oral administration of vaccines shorn of purification. In this regard, already existing vaccine models for MERS and SARS-CoV-1 are crucial leads. The SARS-CoV-1 S1 protein (N-terminal fragment) has been expressed in tomato and tobacco by gene integration into the plant's nuclear genome via *Agrobacterium tumefaciens*-assisted plant transformation. Mice models orally administered with this edible tomato vaccine showed high levels of IgA antibody production, specific to SARS-CoV-1. Mice serum in reaction to transgenic tobacco confirmed the presence of anti-SARS-CoV-1-IgG [74]. In another experiment, a fusion protein consisting of green fluorescent protein and 1-658 amino acid residues of SARS-CoV-1 has been transiently expressed in tobacco leaves. Microscopic studies confirmed the presence of fusion protein in cytosol and nuclear periphery [75].

Another study involving the transient expression of SARS-CoV-1 nucleocapsid (rN) protein (423-aa) *N. benthamiana* documented a substantial yield, i.e. almost 1% of the total soluble protein (TSP). The subsequent inoculation of three doses in mice confirmed the effective immune responses with high levels of IgG1 and IgG2a [75]. The transient expression of nucleocapsid protein (N) and the membrane protein (M) in *N. benthamiana* led to the recovery of 3–4 μg/g fresh leaf weight for the N protein and 0.1–0.15% TSP for M protein. The recovered N protein was able to react with N-specific antibodies in human sera. However, immunization assays were not carried out [76]. These findings can direct the scientist to choose the optimum expression systems for specific SARS-CoV-2 antigens.

Animal Models for SARS-CoV-2 Studies

Appropriate animal models for assessing vaccines for SARS-and MERS-CoV are missing or profoundly restricted, making the vaccine advancement exceptionally challenging [77]. Engineered animal model that imitates the clinical infection can illuminate on pathogenesis just as to create vaccines and therapeutics against these CoVs (Fig. 7.4). A few animal models have been assessed for SARS-and MERS CoVs including mouse, guinea pigs, hamsters, ferrets, bunnies, rhesus macaques, marmosets and felines (Table 7.4) [78, 79].

Early focus was on the development of animal models for SARS-CoV, yet the specificity of the virus to ACE2 (receptor of SARS-CoV) was a significant prevention to such endeavours. Afterwards, a SARS-CoV transgenic mouse model was created by integrating hACE2 gene into the mouse genome. The animal model utilized for building up a MERS-CoV vaccine was rhesus macaques. Challenged animals demonstrated clinical side effects, for example, expanded internal heat level, piloerection, cough, slouched pose and decreased food admission [80]. Another often utilized animal model for MERS-CoV is the regular marmoset, wherein the virus caused deadly pneumonia.

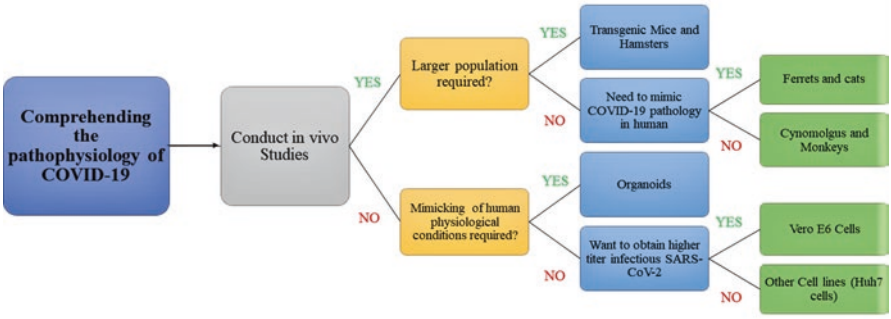


Fig. 7.4 Understanding COVID-19 pathophysiology using animal models, organoids and cell lines

Table 7.4 Animal models being utilized in SARS-CoV-2 research

Sr. No	Animal model	Salient points	Reference
1	Mice (wild-type and human ACE2 transgenic mice)	SARS-CoV-2 could not gain entry into mouse ACE 2 receptors in case of wild-type mice After challenged with the virus, the mice experienced weight reduction, viral replication in lungs and deteriorated alveoli with widespread apoptosis in case of human ACE2 transgenic mice model	[20, 84]
2	Syrian hamster	After infection, animal exhibited weight loss, viral load in lungs and damaged alveoli with apoptosis	[85]
3	Ferrets	Acute bronchitis developed in the ferrets after infection	[86]
4	Cats	After SARS-CoV-2 infection, following complications were observed: Intra-alveolar oedema Congestion in the inter-alveolar septa Abnormal arrangement of the epithelium with loss of cilia Lymphocytic infiltration into the lamina propria	[87]
5	Cynomolgus macaques	Both type I and II pneumocytes were damaged by infection. The animal gathered oedema fluid in alveolar lumina along with pneumonia and pulmonary consolidation postinfection	[88]
6	Rhesus macaques	High viral titre in respiratory tract, humoral and cellular immune reactions and pneumonia were observed in the animal model. The findings suggest that therapeutic responses of adenovirus-vectored vaccine and DNA vaccine candidates expressing S protein could be evaluated	[89–91]

Humoral and cell-mediated immune response could be identified in both rhesus macaques and basic marmoset when challenged with MERS-CoV virus [81]. Roberts et al. engineered golden Syrian hamsters (strain LVG) to examine

immunization protection against various SARS-CoV strains [82]. These hamsters are useful model for contemplating CoV pathology and pathogenesis and vaccine viability. The weakened NSP16 CoV vaccine was inoculated in mice [83].

Endeavours to create animal models for MERS-CoV, for example, mice, hamsters and ferrets, face constraints since MERS-CoV cannot multiply inside the respiratory organs of these species. Animals such as mice or hamsters naturally insusceptible to MERS-CoVs but prone to SARS-CoV have been genetically altered to become more humanized, e.g. hDPP4 human, hDPP4-transduced and hDPP4-Tg mice (transgenic for expressing hDPP4), and liable to MERS-CoV [92]. Modification in the mouse genome utilizing the CRISPR-Cas9 system could make the animals vulnerable to CoV viruses and infection [93]. Genetically engineered 288–330+/+ MERS-CoV mouse model has been designed for the assessment of novel MERS-CoV vaccines and medications [94]. Contrasted with the larger animals, small animal models, for example, mice and bunnies, are favoured because of lower cost, simplicity of alteration and promptly accessible adequacy strategies [95]. Further investigations are expected to perceive reasonable models for rising SARS-CoV-2 by distinguishing receptor specificity of SARS-CoV-2 and examining disease indications and pathologies/viral pathogenesis related with exploratory immunization of the virus in mice, rodents and different models, along with inspection of virus specific immune responses and immunity. This would encourage preclinical assessments of comer COVID-19 vaccine runners and medications.

Cell Culture Systems for SARS-CoV-2 Studies

A few permissive cell lines to hCoVs such as monkey epithelial cell lines (LLC-MK2 and Vero-B4) have been utilized in neutralization tests for evaluating the required titres of neutralization antibodies (Table 7.5). Goat lung cells, alpaca kidney cells and dromedary umbilical line cells have been discovered to allow MERS-CoV replication [96]. Progressed ex vivo 3D tracheobronchial tissue (mirroring epithelium of conductive aviation route) has been utilized for human CoVs [97]. In addition, VLPs showing SARS-CoV S protein were discovered skilful for passage to permissive cells or transfected cells that overexpress infection receptors [98]. SARS-CoV-2 cultures have been purified in Vero and the Huh-7 cells (human liver malignancy cells) [99]. Pseudotyped virions/VLPs expressing reporter genes, for example, GFP or luciferase, can be utilized for measurement and assessment of the viability of mAbs and medications in hindering the entry of CoVs into the cells [100].

Clinical and Immunological Endpoints

The essential endpoints for characterizing the adequacy of a COVID vaccine likewise require conversation (Fig. 7.5). The two most regularly referenced are:

Table 7.5 Cell lines being utilized in SARS-CoV-2 research

Sr. No	Cell lines	Source	Salient points	Reference
1	Human airway epithelial cells	Commercially supplied by Lonza, Pomocell	Allows culturing of SARS-CoV-2 Mimicking of infected human cell lungs Cytopathetic effects observed after infection	[10]
2	Vero E6 cells (wild-type and TMPRSS-2 overexpressing cells)	Epithelial cells isolated from the kidneys of African green monkey	Most popular cell line for culturing SARS-CoV-2 (E6 cells) Allows 100 times higher replicating RNA copies than the wild type (TMPRSS-2 overexpressing cells)	[20, 101]
3	Caco-2 cells	Human colon adenocarcinoma cells	Replication of SARS-CoV-2 possible	[102]
4	Calu-3 cells	Non-small cells from lung cancer	SARS-CoV-2 S pseudovirions exhibited 500 times greater luciferase activity when cultured in Calu-3 cells in comparison to the controls	[103]
5	HEK293T cells	In vitro cultures of human embryonic kidney cells (HEK)	Moderate SARS-CoV-2 replication	[104]
6	Huh7 cells	Hepatocyte originated cellular carcinoma cells	Allows tenfold increase in luciferase activity when transduced by SARS-CoV-2 S pseudovirions	[103]

(i) Safekeeping from infection as manifested in case of seroconversion
(ii) Avoidance of clinically symptomatic illness, particularly enhancement of disease seriousness, including the recurrence of malady requiring high-force clinical consideration and hospitalization.

This requires the deep assessment of the impact of vaccination on the seriousness of COVID-19 illness in a wide assortment of epidemiological and clinical settings among both more youthful and old people and also underserved minorities. Initial efficacy trials are to be evaluated based on these parameters. Accomplishing these endpoints will also lead to a diminished transmission rate of COVID-19 among the population.

Primary endpoints attributed to reduction of the disease require a higher number of people enrolled for clinical trials, given that asymptomatic infection accounts for 20% to 40% of the total instances of COVID-19 [105]. Initial efficacy trials will hence require a large number of persons with monitoring of both clinical and serologic endpoints. Lack of data of incidence rates will pose a major challenge that will add to complications in formulating clinical trial procedures for serological

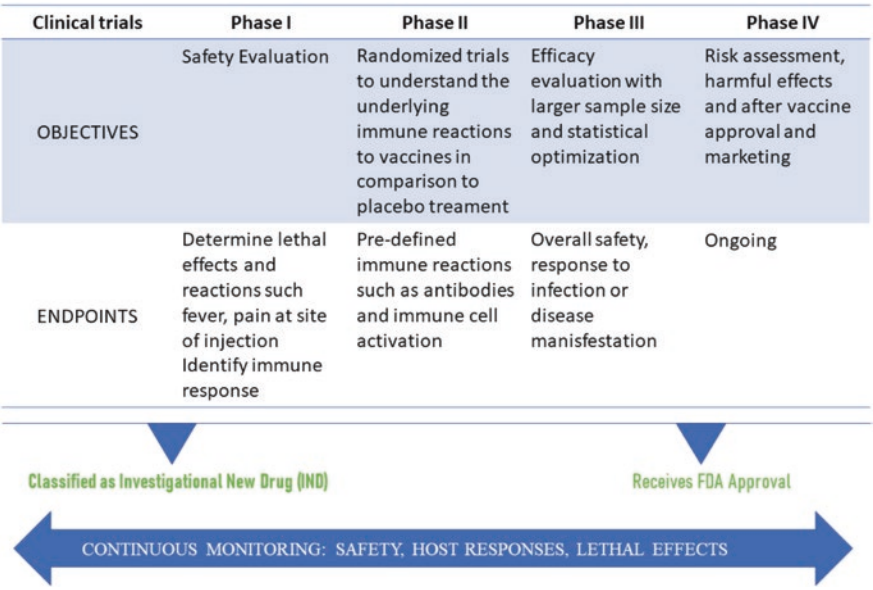


Fig. 7.5 Endpoints of clinical vaccine trials

endpoints [106]. An important requirement for this multi-trial strategy that provides a binding relationship between various vaccinations and vaccine efficacy studies is to establish laboratories with identical validated serologic assays. These laboratories should provide clinical trials and dispersion of crucial samples from a trial. Variables defining the immune reaction coming about because of immunization and from the actual infection are under substantial examination, and it's important to figure measures to manage this issue.

Efficacy studies should be assessed for both positive and negative effects. The probability of SARS-CoV-2 re-exposure is a lot higher than that of SARS-CoV-1, which has vanished from network course, and consequently longer-term assessment of likely upgrade with re-exposure is required. This necessity doesn't block licensure dependent on the endpoints mentioned here; nonetheless, it demonstrates that a more detailed follow-through of the initial immunization entities ought to be embraced. The efficacy of clinical and serologic endpoints will likewise be investigated, as fading of immunity is normal with human COVID diseases [107].

COVIDs have a high transformation rate in their RNA genome. Despite the fact that there has been some hereditary float during the development of the SARS-CoV-2 epidemic, there are no major changes in the spike protein up till now, especially in the parts believed to be significant for balance; this creates a hope that the vaccine to be developed currently will be effective for the coming 6–12 months against the strains [108]. The chance of performing controlled human challenge trials, in which few volunteers are vaccinated and thusly tested with SARS-CoV-2, has been proposed. Such experiments may prove to be useful when performed to define

potential immune correlates or filtering out weaker vaccine strategies. In any case, this methodology has deficiencies regarding pathophysiology and safety [109]. The possibility of performing controlled human test studies, in which not many participants are inoculated and subsequently tried with SARS-CoV-2, is being proposed. These tests may be useful when performed to characterize possible immune correlates or filter out weaker immunization approaches. In spite of the fact that the danger of serious sickness or demise in youthful healthy people from COVID-19 is extremely low, it isn't nil, and yet we have not demonstrated successful treatments of the disease to safeguard volunteers having problems from such a test. Almost certainly, a 2019-nCoV test strain will, by configuration, result in gentle sickness in many volunteers and in this way may not sum up the aspiratory pathophysiology found in some patients. Additionally, limited viability in youthful people doesn't foresee comparable viability among older adults with significant cofactors related with COVID-19 malady, nor would it reveal decrease of contagiousness to significant susceptible groups. Regardless of whether such examinations might be deserving of interest or would beneficially affect timetables for immunization improvement needs cautious assessment by an autonomous board of professionals on vaccine progress.

Vaccine Development Landscape

Vaccine developments usually take 5–10 years, and the procedure includes exceptionally controlled preclinical and clinical studies before the vaccine is approved for the masses. Amazingly within just a couple of weeks of the publication of viral genetic information, COVID-19 vaccines had been prepared for trials on patients. Global COVID-19 vaccine R&D landscape as of 28 August includes 176 vaccine candidates, 35 of which have entered clinical evaluation including phase II and III studies (Table 7.6), whereas 143 candidates are in preclinical stages. Existing and new vaccine production technologies are being deployed for the creation of COVID-19 vaccine candidates and the pace of development.

Cutting-edge immunization advancement can be assisted by using sequence data alone instead of depending on in vitro cultures of viruses. Nucleic acid-based vaccines that utilize this cutting-edge approach have been the leaders for vaccine hunt. One such model is an mRNA vaccine (mRNA-1273, encoding for the viral spike protein which locks onto human host cells) created by the National Institutes of Health and Moderna Therapeutics and has just given encouraging indications and has proceeded to phase III of the clinical studies. A new time record has been set by this vaccine by reaching the preliminaries (NCT04283461) in such a brief period of time after the verification of the SARS-CoV-2 as the agent causing the present pandemic [110]. Another leader in stage IIB/III clinical preparation, ChAdOX1 nCoV19, created by Oxford University and AstraZeneca, is a vaccine programme dependent on an adenovirus vector likewise encoding for SARS-CoV-2 spike protein.

Table 7.6 Candidate vaccines under clinical evaluation

	Vaccine platform	Type of candidate vaccine	COVID-19 vaccine developer	Number of doses	Timing of doses	Clinical stage
1.	Non-replicating viral vector	ChAdOx1-S	University of Oxford/ AstraZeneca	1		Phase 3
2.	Non-replicating viral vector	Adenovirus Type 5 vector	CanSino Biological Inc./ Beijing Institute of Biotechnology	1		Phase 3
3.	Non-replicating viral vector	Adeno-based (rAd26-S+rAd5-S)	Gamaleya Research Institute	2	0, 21 days	Phase 3
4.	Non-replicating viral vector	Ad26COVS1	Janssen Pharmaceutical Companies	2	0, 56 days	Phase 3
5.	Inactivated	Inactivated	Sinovac	2	0, 14 days	Phase 3
6.	Inactivated	Inactivated	Wuhan Institute of Biological Products/ Sinopharm	2	0, 14 or 0, 21 days	Phase 3
7.	Inactivated	Inactivated	Beijing Institute of Biological Products/ Sinopharm	2	0, 14 or 0, 21 days	Phase 3
8.	RNA	LNP-encapsulated mRNA	Moderna/NIAID	2	0, 28 days	Phase 3
9.	RNA	3 LNP-mRNAs	BioNTech/Fosun Pharma/Pfizer	2	0, 28 days	Phase 3
10.	Protein subunit	Full-length recombinant SARS CoV-2 glycoprotein nanoparticle vaccine adjuvanted with Matrix M	Novavax	2	0, 21 days	Phase 1/2
11.	Protein subunit	Adjuvanted recombinant protein (RBD-Dimer)	Anhui Zhifei Longcom Biopharmaceutical/ Institute of Microbiology, Chinese Academy of Sciences	2 or 3	0, 28 or 0, 28, 56 days	Phase 2
12.	RNA	mRNA	Curevac	2	0, 28 days	Phase 2
13.	Inactivated	Inactivated	Institute of Medical Biology, Chinese Academy of Medical Sciences	2	0, 28 days	Phase 1/2
14.	Inactivated	Inactivated	Research Institute for Biological Safety Problems, Rep of Kazakhstan	2	0, 21 days	Phase 1/2

(continued)

Table 7.6 (continued)

	Vaccine platform	Type of candidate vaccine	COVID-19 vaccine developer	Number of doses	Timing of doses	Clinical stage
15.	DNA^	DNA plasmid vaccine with electroporation	Inovio Pharmaceuticals/ International Vaccine Institute	2	0, 28 days	Phase 1/2
16.	DNA	DNA plasmid vaccine + Adjuvant	Osaka University/ AnGes/ Takara Bio	2	0, 14 days	Phase 1/2
17.	DNA^	DNA plasmid vaccine	Cadila Healthcare Limited	3	0, 28, 56 days	Phase 1/2
18.	DNA	DNA vaccine (GX-19)	Genexine Consortium	2	0, 28 days	Phase 1/2
19.	Inactivated	Whole-Virion Inactivated	Bharat Biotech	2	0, 14 days	Phase 1/2
20.	Protein subunit	RBD-based	Kentucky Bioprocessing, Inc	2	0, 21 days	Phase 1/2
21.	Protein subunit	S protein (baculovirus production)	Sanofi Pasteur/GSK	2	0, 21 days	Phase 1/2
22.	RNA	mRNA	Arcturus/Duke-NUS			Phase 1/2
23.	Non-replicating viral vector	Replication defective Simian Adenovirus (GRAd) encoding S	ReiThera/ LEUKOCARE/ Univercells	1		Phase 1
24.	Protein subunit	Native like Trimeric subunit Spike Protein vaccine	Clover Biopharmaceuticals Inc./ GSK/Dynavax	2	0, 21 days	Phase 1
25.	Protein subunit	Recombinant spike protein with Advax™ adjuvant	Vaxine Pty Ltd/Medytox	1		Phase 1
26.	Protein subunit	Molecular clamp stabilized spike protein with MF59 adjuvant	University of Queensland/CSL/ Seqirus	2	0, 28 days	Phase 1
27.	Protein subunit	S-2P protein + CpG 1018	Medigen Vaccine Biologics Corporation/ NIAID/Dynavax	2	0, 28 days	Phase 1
28.	Protein subunit	RBD + adjuvant	Instituto Finlay de Vacunas, Cuba	2	0, 28 days	Phase 1
29.	Protein subunit	Peptide	FBRI SRC VB VECTOR, Rospotrebnadzor, Koltsovo	2	0, 21 days	Phase 1
30.	Protein subunit	RBD (baculovirus production expressed in Sf9 cells)	West China Hospital, Sichuan University	2	0, 28 days	Phase 1

(continued)

Table 7.6 (continued)

	Vaccine platform	Type of candidate vaccine	COVID-19 vaccine developer	Number of doses	Timing of doses	Clinical stage
31.	Replicating viral vector	Measles-vector based	Institute Pasteur/Themis/ Univ. of Pittsburgh CVR/Merck Sharp & Dohme	1 or 2	0, 28 days	Phase 1
32.	Replicating viral vector	Intranasal flu-based-RBD	Beijing Wantai Biological Pharmacy/ Xiamen University	1		Phase 1
33.	RNA	LNP-nCoVsaRNA	Imperial College London	2		Phase 1
34.	RNA	mRNA	People's Liberation Army (PLA) Academy of Military Sciences/ Walvax Biotech.	2	0, 14 or 0, 28 Days	Phase 1
35.	VLP	Plant-derived VLP adjuvanted with GSK or Dynavax adjs.	Medicago Inc.	2	0, 21 days	Phase 1

The other progressed competitors moved into clinical development incorporates:

(i) CoronaVac from Sinovac
(ii) Ad5-nCoV from CanSino Biologics
(iii) BNT162 from Pfizer
(iv) BioNtech
(v) BCG live-attenuated vaccine from Radboud University Medical Center, University of Melbourne and Murdoch Children's Research Institute and Faustman Lab at Massachusetts General Hospital.

The movement of advancement is dramatic when contrasted with new ailments causing serious epidemic pronounced by the WHO (Fig. 7.6). The extraordinary larger part of authorized immunizations is situated in inactivation pathogens which stretch the development, finances and creation of the vaccine. Recombinant viral vectored, DNA/RNA and protein innovations are establishing the quickest precedents in vaccine advancement. Only a few have been authorized so far for veterinary utilization, since, for people, a few immunizations have not met some administrative necessities for endorsement and commercialization. The global crises like the current COVID-19 could give a last push towards acquiring licensure.

This features the capability of vaccinology to gain quick ground when fitting worldwide help exists, demonstrating that when there is a will, there is a way. A comparison has been made between the proposed COVID-19 vaccine development timeline and that of the classical one in Fig. 7.7.

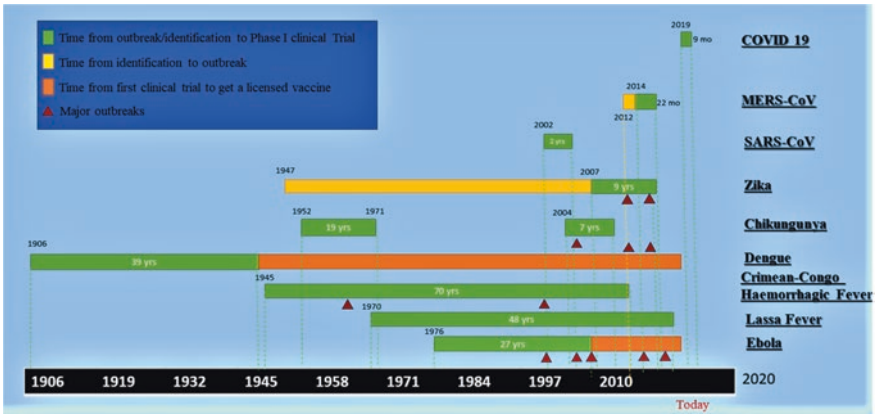


Fig. 7.6 Vaccine development timeline of recently emerged outbreaks

Global and Equitable Distribution of Vaccines

The pandemic has just caused the loss of a huge number of lives and affected the lives of billions of people worldwide. Just as lessening the heartbreaking death toll and assisting with getting the pandemic levelled out, the presentation of a vaccine will forestall the loss of US$ 375 billion to the worldwide economy per month. Worldwide evenhanded admittance to a vaccine, especially securing medical services labourers and those most in danger, is the best way to relieve the general wellbeing and monetary effect of the pandemic. The events and broad supply of COVID-19 clinical medicines are a typical worldwide intrigue. An effective remedy for COVID-19 will be there in a few months' time, and it will take at least a year for vaccine to be developed. To meet the outstanding demand worldwide, once COVID-19 therapeutic and vaccines are approved, they need to be manufactured on a large scale.

This is the ideal time to calculate the production limits, funding and circulation system important to create adequate amounts to address worldwide demand in a reasonable way beneficial for the public health. Countries of the world are currently collaborating with each other, but there is curiosity that which nation will succeed first in the development of therapeutics and vaccines. Governments as of now have the best impetus to team up while vulnerability stays with regard to which countries' immunizations and therapeutics will succeed. In the course of the most recent decade, technological research and production capabilities are widely spread globally. Technological advancements mean that the best cures and vaccines for COVID-19 will be developed in new ways outside the customary drug development centres.

Rich nations cannot depend on outbidding contesters if vaccine and therapeutic supplies are not shared by the countries manufacturing them. For there to be a uniform distribution of therapies for COVID-19, the countries of the world must

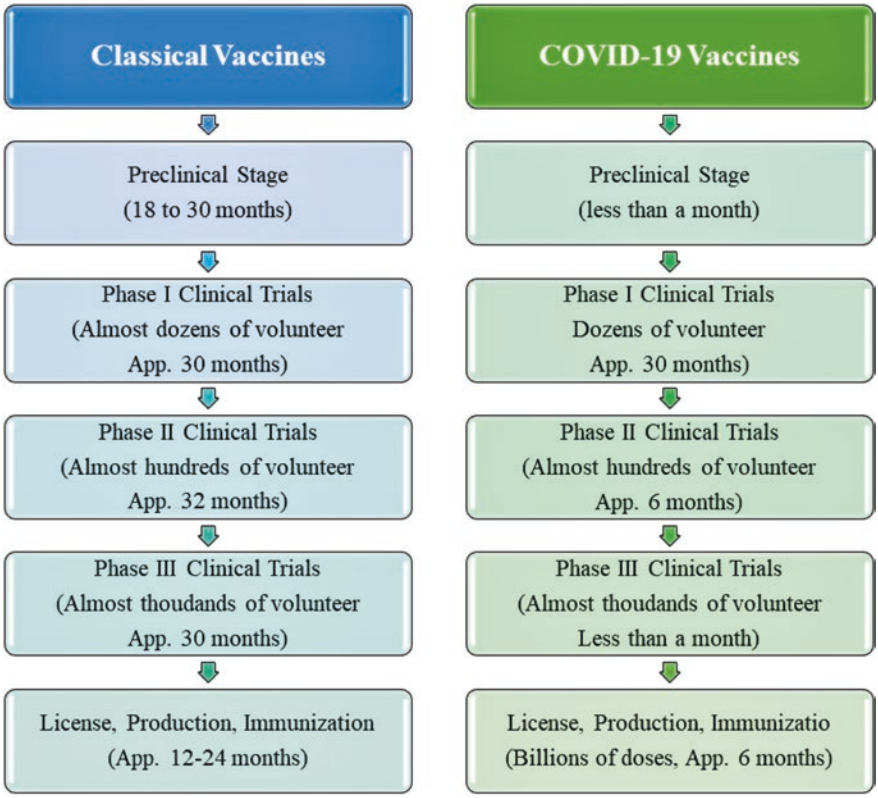


Fig. 7.7 Comparison between the classical vaccine and COVID-19 vaccine development Challenge studies are likely to expedite the development of vaccines and their availability in the presence of well-coordinated collaboration among the scientists, developers and regulators (Fig. 7.8). Whatsoever, these kinds of studies must be included into extensive research plans which involves large-scale studies to establish accurate safety and efficiency. SARS-CoV-2 challenge studies may also be helpful to other types of vaccine experimentation by providing precise evaluation of infections with no apparent symptoms and swift and standardized testing of various vaccine candidates. WHO has issued eight ethical criteria which should be adhered to for conduction challenge trials (Fig. 7.9)

cooperate with each other. However, this seems to be a difficult task. In the midst of rising populism, governments have opposed multilateral organizations and international accords. Numerous nations have reacted to this pandemic by turning internal: shutting borders, accumulating clinical assets and scapegoating outsiders. Building up a structure for reasonable and evenhanded distribution of COVID-19 vaccines and therapeutics is unquestionably more unpredictable and will require coordination of numerous foundations, funders, governments and drug organizations (Fig. 7.10).

European Commission helped start a programme, Access to COVID-19 Tool (ACT) Accelerator, which will focus on fast development and fair supply of

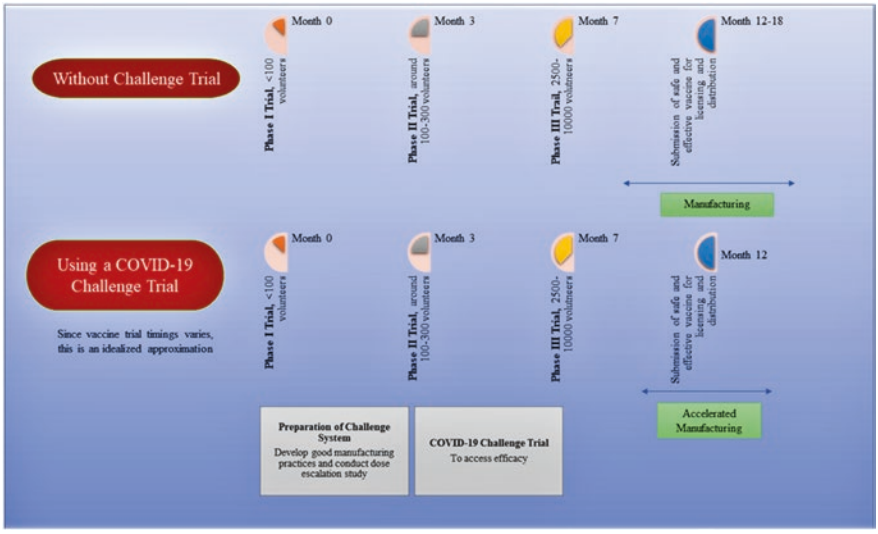


Fig. 7.8 Acceleration of COVID-19 vaccine development using challenge trials

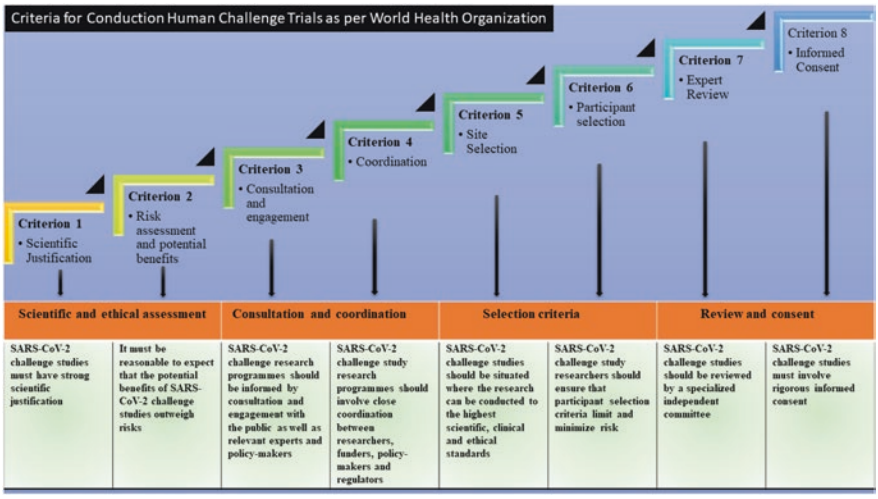


Fig. 7.9 Criteria listed by the WHO for conduction of challenge trials

vaccines, therapeutics and diagnostics. COVAX, co-driven by Gavi, the Coalition for Epidemic Preparedness Innovations (CEPI) and WHO, is the vaccine backbone of the Access to COVID-19 Tools (ACT) Accelerator. The purpose of COVAX is to speed up the process for the development and production of the vaccines for COVID-19 and to ensure their just, fair and equal distribution in all parts of the world (Fig. 7.11).

Currently, a total of 172 countries are working to participate in the COVAX initiative, which has the biggest and most spread COVID-19 vaccine portfolio. This

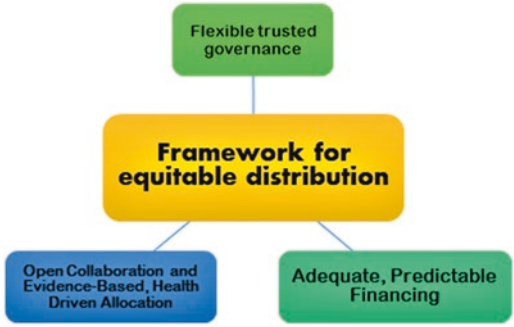


Fig. 7.10 A general framework for the equitable distribution of vaccines

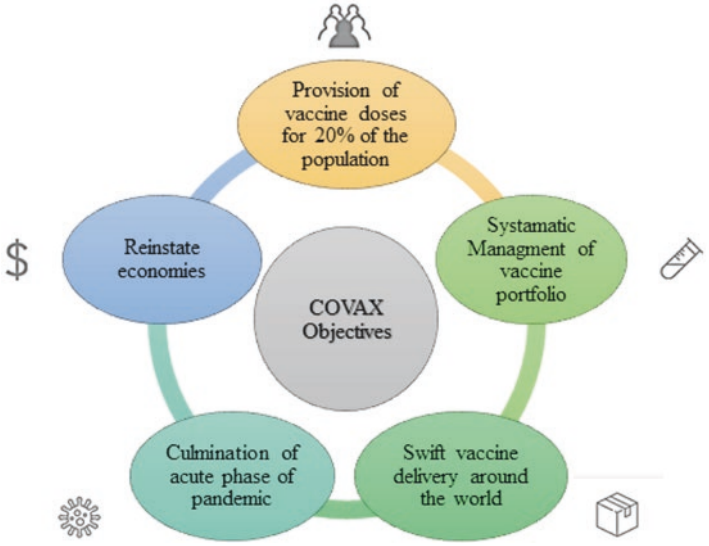


Fig. 7.11 Objectives of COVAX ensuring equitable distribution of vaccines

incorporates nine candidate vaccines, with a further nine under assessment, and discussions are in progress for additional vaccines (Table 7.7). The plan entails the cooperation at worldwide scale, working with governments and manufacturers to guarantee that COVID-19 vaccines are accessible worldwide to both higher-salary and lower-pay nations. So as to tie down equivalent access to COVID-19 vaccines for nations across the globe, the subsequent stage is to affirm self-financing members by 18 September 2020, with the main forthright instalments to be made no later than 9 October 2020. 80 higher-salary economies, which would back the vaccines from their own public money financial plans, have submitted Expressions of Interest in front of the 31 August 2020 cutoff time for affirmation of aim to partake. They will cooperate with 92 low- and middle-salary nations that will be upheld by the AMC on the basis if its funding targets are met. Together, this gathering of 172 nations represents to over 70% of the total population of the world. CEPI has allocated a budget of $ 2 billion for the rapid COVID-19 vaccine development

Table 7.7 Vaccine developers supported by CEPI

Sr. No	Organization	Country	Vaccine clinical stage
1.	Inovio	USA	Phase I/II
2.	Moderna	USA	Phase III
3.	CureVac	Germany	Phase I
4.	Astra Zeneca/University of Oxford	UK and Northern Ireland	Phase III
5.	Institute of Pasteur/Merck/Themis	France/USA/Austria	Preclinical
6.	University of Hong Kong	China	Preclinical
7.	Novavax	USA	Phase I/II
8.	Clover Biopharmaceuticals	China	Phase I
9.	University of Queensland/CSL	Australia	Phase I

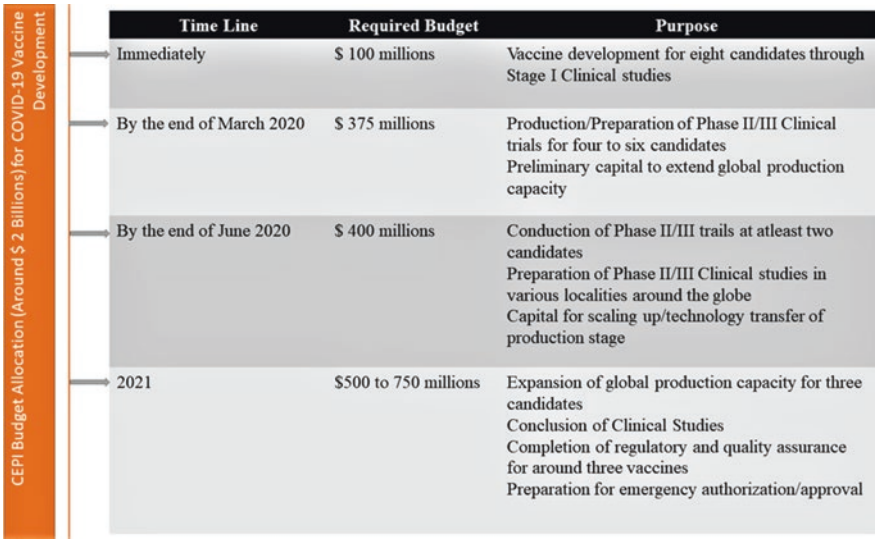


Fig. 7.12 CEPI budget allocation for COVID-19 vaccine development

(Fig. 7.12). The world, in any case, is more ready to react than at any other time. The accompanying proposed structure uses existing global discussions to encourage evenhanded circulation of COVID-19 vaccines and therapeutics.

Vaccine Developers and Geographical Distribution

Greater commitment by large multinational companies has brought many great changes in the general outlook of COVID-19 vaccine development since April. Of the vaccine candidates in the clinic, 11 are being created by Chinese associations, and seven are being upheld by the US Operation Warp Speed programme, which plans to convey 300 million vaccine portions for COVID-19 by January 2021 and

has so far reported subsidizing of more than US$10 billion to propel vaccine advancement. Eight of the clinical applicants have gotten subsidizing from the Coalition for Epidemic Preparedness Innovations (CEPI) and are presently a part of the COVAX, a joint effort drove by CEPI, Gavi and the WHO that plans to provide 2 billion vaccine dosages for worldwide distribution before the end of 2021.

Conclusions

In this time of a pandemic, fast research, manufacture and organization of first-generation vaccines are crucial. For this, the main applicants are the nucleic acid (DNA, mRNA) and subunit (S and N protein) vaccines for artificial immunity induction to confer strong resistance against the actual infection. A methodology used to speed up immunizations during epidemics is to give restrictive licenses; for COVID-19, they could be based on human clinical trial data affirming wellbeing and satisfactory degrees of assurance to lessen death toll in the most vulnerable people (old people and patients having comorbidities, medical services staff). Meanwhile, second-generation, more powerful or effective immunizations to forestall infection and deaths and decrease shedding, as examined, ought to be created in parallel for future organization.

The presence of virus neutralizing antibodies and cell-mediated immune reactions in response to vaccine inoculations in animal models can serve as the pointers of safeguard, yet the coordinates of immunity to COVID-19 in people are obscure. A point of concern is the effectiveness of the vaccine to combat extreme infections and death rates but its inadequacy to limit the nasal shedding, permitting continuity in the spread of the disease. Immunity at the mucosal level may also lead to diminished viral shedding through nasal secretions. Achieving the mucosal immunity is a key argument to break off/recede the transmission chain; however, this may require booster vaccine doses. Aged individuals having chronic illnesses are prone to acquiring severe infections; however, the symptoms can be relieved by the administration of other vaccines such as those existing for flu, etc. Other ways may include the use of adjuvants and varying vaccine dosages in weak groups to uplift their guard. Animal models additionally need to emulate these criteria.

The way the COVID-19 causes infection is not fully understood yet, and immunization systems might require modifications if the infection taints both the respiratory and intestinal tracts. Oral administration through mouth and nose and the subsequent dose of parenteral vaccine might be ideal to forestall both intestinal and respiratory complications as well as viral shedding. Future overflow of coronavirus transmission from animal repositories is likely. New insights to develop vaccines against beta-CoV lineage that are effective equally in both animal models and humans are the dire need of time. Presently, in the absence of an efficient vaccine, the course of battling the COVID-19 shifts towards passive immunization as means of both the prophylactic measures and clinical therapy. The best approach so far is the convalescent plasma therapy which entails the use of plasma recovered from the

recuperated patients. This strategy helps in speedy recovery and lessens the hospitalization ultimately reducing mortality rates. The clinical trials also corroborate its beneficial outcomes when compared to placebo treatment. The establishment of plasma banks having plasma donated by healed patients of COVID-19 would also be effective in suppressing the annihilation caused by this pandemic. Monoclonal antibodies designed against the surface viral proteins which are involved in eliciting immune response can also be engineered and tested in animal models challenged with SARS-CoV-2. These MAB can programme the immune responses and serve as prophylactic therapy. The development of respiratory syncytial virus monoclonal antibody by the name palivizumab is worth mentioning in this regard. This can be inhaled as aerosols through the nose.

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