Human Vaccines & Immunotherapeutics

ISSN: (Print) (Online) Journal homepage: https://www.tandfonline.com/loi/khvi20

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To cite this article: Idris Nasir Abdullahi, Anthony Uchenna Emeribe, Hafeez Aderinsayo Adekola, Sharafudeen Dahiru Abubakar, Amos Dangana, Halima Ali Shuwa, Sunday Theophilus Nwoba, Jelili Olaide Mustapha, Muyideen Titilope Haruna, Kafayat Adepeju Olowookere, Olawale Sunday Animasaun, Charles Egede Ugwu, Solomon Oloche Onoja, Abdullahi Sani Gadama, Musa Mohammed, Isa Muhammad Daneji, Dele Ohinoyi Amadu, Peter Elisha Ghamba, Nkechi Blessing Onukegbe, Muhammad Sagir Shehu, Chiladi Isomah, Adamu Babayo & Abdurrahman El-Fulaty Ahmad (2021) Leveraging on the genomics and immunopathology of SARS-CoV-2 for vaccines development: prospects and challenges, Human Vaccines & Immunotherapeutics, 17:3, 620-637, DOI: 10.1080/21645515.2020.1812313

To link to this article: https://doi.org/10.1080/21645515.2020.1812313

HUMAN VACCINES & IMMUNOTHERAPEUTICS
2021, VOL. 17, NO. 3, 620–637
https://doi.org/10.1080/21645515.2020.1812313

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REVIEW

Leveraging on the genomics and immunopathology of SARS-CoV-2 for vaccines development: prospects and challenges

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ABSTRACT

The incidence and case-fatality rates (CFRs) of Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) infection, the etiological agent for Coronavirus Disease 2019 (COVID-19), have been rising unabated. Even though the entire world has been implementing infection prevention and control measures, the pandemic continues to spread. It has been widely accepted that preventive vaccination strategies are the public health measures for countering this pandemic. This study critically reviews the latest scientific advancement in genomics, replication pattern, pathogenesis, and immunopathology of SARS-CoV-2 infection and how these concepts could be used in the development of vaccines. We also offer a detailed discussion on the anticipated potency, efficacy, safety, and pharmaco-economic issues that are and will be associated with candidate COVID-19 vaccines.

ARTICLE HISTORY
Received 21 May 2020
Revised 10 August 2020
Accepted 11 August 2020

Keywords
COVID-19; Vaccine discovery; SARS-CoV-2; Host-virus interaction

Introduction

Severe Acute Respiratory Syndrome Coronavirus −2 (SARS-CoV-2) is the third highly pathogenic coronavirus that has affected the human race in the 21st century. As the incidence and case fatality rate (CFR) of SARS-CoV-2 infection, the etiological agent of Coronavirus Disease 2019 (COVID-19), continues to rise across more than 210 countries and territories, several preventive and control measures have been adopted to halt the spread of SARS-CoV-2 and minimize COVID-19 associated deaths. Essentially, all countries in the world have had SARS-CoV-2 infections, with over 30 million infected and about one million COVID-19-related deaths.[1] Several observers have attributed the low reported incidence rate of COVID-19 in Sub-Saharan Africa to under-diagnosis, probably due to inadequate molecular diagnostic capacity.[2] Although it may seem that both European and American countries have the worst CFRs associated with COVID-19, the relatively low CFR reported in Africa is not a true reflection of the reality. This is because the majority of African countries have limited or weak epidemiological system that can account for accurate COVID-19 data. This consequently reflects the grossly under-reported COVID-19 cases and associated mortality in Africa .[2]

Besides the apparent scenarios of underreporting of COVID-19 data in Africa, another possible reason for this variation may be due to the differences in the proportions of elderly population in these continents, as age is a significant risk factor for developing symptomatic and severe form of COVID-19 .[3] High COVID-19 fatality rates have been observed in patients of > 60 years, especially in countries with high average life expectancy .[4] Average life expectancy in most African countries ranges from 56.75 to 69.90 and 58.78 to 73.68 years for males and females, respectively .[5] In

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other continents, higher life expectancy is obtainable in America with 70.65 to 76.49 and 75.56 to 81.41 years for males and females, respectively; in Asia with 68.50 to 75.17 and 71.05 to 79.89 years for males and females, respectively; in Europe with 69.37 to 79.68 and 78.56 to 84.45 years for males and females, respectively .[5] This implies that countries in the Europe, America, and Asia with higher life expectancy have a relatively higher proportion of the elderly population compared to most African countries. Consequently, it will be expected that these countries with a comparatively higher proportion of elderly citizens to have a higher rate of COVID-19-related mortality due to the vulnerability of the elderly individuals to severe COVID-19 .[3]

Specifically, age alongside other risk factors such as metabolic, immunologic, respiratory, and oncogenic disorders are determinants of the severity of SARS-CoV-2 infection .[6] Aside from these, the genetic makeup of SARS-CoV-2 and host immune response are other vital determinants that contribute to SARS-CoV-2 virulence .[7] Hence, understanding these features are crucial to predict future transmission dynamics of SARS-CoV-2 infection, immune protection against re-infection, antiviral, and vaccines development .[8]

There are ongoing scientific investigations, which seek to elucidate the structural basis of SARS-CoV-2 pathology and attempt to predict its transmission dynamics .[9] Mathematical models have been used to determine the transmission rate of SARS-CoV-2. The transmission rate can be referred to as the R_0, a measure of the transmissibility of SARS-CoV-2. R_0 predicts how many people an infected person can transmit SARS-CoV-2 in a population with no prior immunity to the pathogen. Generally, the higher the R_0, the more contagious the pathogen. An R_0 of < 1 means that the outbreak dies out, while $R_0 > 1$ means the infection will continue to spread .[9]

Genetic analysis suggests that SARS-CoV-2 is related to SARS-CoV-1 .[10] Early mathematical modeling reports revealed R_0 value range of 2.0 to 3.9 for SARS-CoV-2 as compared to the R_0 values for SARS-CoV-1 (1.7-1.9) and MERS (<1) .[11-16] This explains why SARS-CoV-2 is more contagious than SARS-CoV -1 and MERS-CoV. Unlike the R_0, the effective reproduction number (R_E) of SARS-CoV-2 is influenced by the level of an individual's immunity and behavioral changes in the absence of vaccines, anti-viral agents or public health interventions .[9]

Currently, the only available means of controlling the spread of SARS-CoV-2 is through consistent adherence to physical distancing, personal hygiene, strict quarantine with monitored self-isolation of infected persons, and case contacts. Despite all these measures, the world continues to experience a sustained rise in new cases of SARS-CoV-2. Hence, most scientists arguably believe that vaccination will be the best way to overcome COVID-19 pandemic .[9,16] For now, there is no available preventive vaccine, although, many have been approved for clinical trials. Furthermore, Remdesivir, a licensed coronavirus-specific antiviral drug produced by Gilead is now available in developed countries. However, it's too expensive for use in developing countries .[16] For a COVID-19 vaccination program to be successfully accepted and implemented in every nation, it must be safe, potent, accessible, affordable, and equity in its distribution must be ensured. Hence, this study critically reviewed the latest scientific advancement in genomics, replication pattern, pathogenesis and immunopathology of SARS-CoV-2 infection and how these concepts could be used in the development of vaccine models. Also, we proffer a detailed narrative on the anticipated potency, efficacy, safety, and pharmaco-economic issues that are and will be associated with candidate COVID-19 vaccines.

Genomic Organization of SARS-CoV-2

Coronaviruses are enveloped viruses possessing positive-sense RNA genome that ranges between 26 and 32 kb in size .[17] They can be categorized into four genera, namely Alpha, Beta, Gamma, and Delta Coronaviruses .[18] SARS-CoV-2 belongs to the Beta genera of coronaviruses. It has a genome of about 29.9 kb in size and contains 15 genes, including the S gene which codes for the S-protein found on the surface of the envelope .[19] The RNA genome of SARS-CoV-2 encodes a large non-structural polyprotein (ORF1 a/b) (Figure 1) .[18] The first Open Reading Frame (ORF1 a/b), which is about two-third of the RNA genome, is then translated into ppla and pplab polyproteins .[19] These polyproteins are cleaved by proteolytic enzymes to produce 16 non-structural proteins (NSPs), which form the viral replicase transcriptase complex .[18] The NSPs of SARS-CoV-2 functions by forming double-membrane vesicles where viral transcription and replication occur using membranes originating from the rough endoplasmic reticulum.[18] The remaining one-third of the RNA genome, ORF 3A, ORF 6, ORF 7, ORF 8, ORF 9 code for four structural proteins and five accessory proteins .[20,21] The four structural proteins which play significant roles in SARS-CoV-2 virion assembly and infection consist of the surface spike glycoprotein (S), the membrane protein (M), an envelope protein (E), and the nucleocapsid protein (N) [20,21] (Figure 1). The spike surface glycoprotein (S) which is the primary inducer of the immune response, primarily mediates invasion of the host cell by binding to a receptor referred to as angiotensin-converting enzyme 2 (ACE-2) which is located on the surface of the host cell membrane .[22] Apart from ACE2, CD147 has also been described as another route of viral entry .[23]

Indeed, RNA viruses, including SARS-CoV-2, have high mutation rates which are significantly correlated with enhanced virulence and evolvability .[24] At proteomic level, amino acid substitutions have been reported in NSP2, NSP3 and S protein .[21]

Various researchers have investigated mutation hotspots on the genomic sequence of SARS-CoV-2. Pachetti *et al* evaluated 220 complete genome sequences from four geographic areas, including Asia, Oceania, Europe, North America. Mutations were reportedly found on sequences belonging ORF1ab (1397 nsp2, 2891 nsp3, 14408 RdRp, 17746 and 17857 NSP143, 18060 NSP14), S (23403 spike protein) and ORF9a (28881,

Figure 1. Genomic organization of SARS-CoV-2. UTR = Untranslated Region: ORF = Open Reading frame: M = Membrane protein: N = Nucleocapsid: E = Envelop protein: S= Spike protein

nucleocapsid phosphoprotein) .[25] It was also observed that genomes from Europe and North America experienced the highest mutation frequency with a distinct mutation hotspot pattern when compared to other geographic areas .[25] In another comparative study by Yin [26] on 558 SARS-CoV-2 genome sequences from 33 countries and territories. They used single nucleotide polymorphisms that recognized mutations on the SARS-CoV-2 genomes due to the error-prone RNA-dependent RNA polymerase to reveal major mutations occurring in the S protein, RNA polymerase, RNA primase, and nucleoprotein which are critical proteins in the virus genome .[26]

The first common single nucleotide polymorphism mutation in the viral genome was reportedly observed in the leader sequence (241C > U), this mutation coevolved with three other vital mutations (3037C > U, 14408C > U, 23403A > G) and consequentially caused amino acid mutations in NSP3, RNA primase, and spike glycoprotein, respectively .[26] Genetic analyses have also revealed that there are two major prevalent types: the Single Nucleotide Polymorphism (SNP) mutation in the nsp8 (28144 U > C) which resulted from mutation of amino acid leucine (L) to serine (S) that is responsible for the difference in the two prevalent types of the SARS-CoV-2 virus, the S type (8782C > U) and the L type (28144U > C) .[26,27]

Besides these, SNP mutation (23403A > G) also occurred in the genes encoding for spike protein (S protein: D614G). However, the function of this mutation is still under extensively study, although it could be significant in the affinity and binding strength of the virus to the ACE-2 receptor. Besides, it has been speculated that D614G strains could be more virulent, thus increasing the infectiousness of SARS-CoV-2 and severity of infected individuals, especially in places where this mutation is prominent .[28]

Mutations in the RNA-dependent RNA polymerase (28144U > C, 28881G > A, 28882G > A, 28883G > C) were also reported. This could improve the fidelity of the virus concerning its lethality. Based on the findings of Yin *et al*, [26] the highest frequency of SNPs was concentrated in the S-glycoprotein (23403A > G) followed by NSP3 (3037C > U) and least in M-protein (27046C > U) .[26] Interestingly, another study suggested that NSP2 and NSP3 mutations play a significant role in virulence and differentiation mechanism of SARS-CoV-2 (Table 1) .[29]

Of interest, is the mutation in S-protein, which is significant in binding of the host cell (tropism) and also serves as a target of neutralizing antibodies. Mutation in the S-protein induces conformational changes, which probably led to the changes of the antigenicity of SARS-CoV-2 .[18] This has made scientists explore the possible differences between the host tropism and transmission rate of SARS-CoV-2. Also, genomic surveillance of SARS-CoV-2 has been used to determine the correlation of these mutations to virulence diversity and their implications on reinfection, immunity, and vaccines development .[34]

SARS-CoV-2 mutation data might provide valuable insights for the development of efficient vaccines, antivirals and laboratory test assays and protocols. Efforts are being made all over the world to control the spread of COVID-19 and most importantly, to develop a potent vaccine. Understanding the genetic evolution and mutation course of SARS-CoV-2 would significantly aid in the development of efficient diagnostic assays, therapeutic drugs, and vaccines. As global efforts are being made to contain and decrease the incidence of COVID-19, it is essential to consider all the features of the circulating strains

Table 1. SARS-CoV-2 Genetic Mutations and their Implications on Vaccine development.

Citation	Study Design	Observed Mutations	Key Findings and Significance in Vaccine Intervention
Tang *et al.*[27]	Population genetic analyses of 103 SARS-CoV-2 genomes	Receptor binding the domain of the S protein	In the discovery of the L and S types of SARS-CoV-2, the L type was reportedly evolutionarily aggressive and more contagious than the S type. This biotype variation might consequently affect the efficacy of candidate vaccine if a monovalent vaccine against either is produced. Therefore, polyvalent vaccines should be produced to act against both virus biotypes.
Angelleti *et al.*[29]	Fast-unconstrained Bayesian approximation and Homology modelling	NSP2 and NSP3	Mutations occurring on NSP2 and NSP3 could have significantly influenced the pathogenesis and transmission of the virus. Thus, structural analogues of the NSPs could be used for vaccines that elicit neutralizing antibodies which can effectively bind to the SARS-CoV-2 NSPs.
Yao *et al.*[30]	Functional characterizations of 11 patient-derived viral isolates.	Intrapersonal variation and 6 different mutations in S protein	A mutation on the S protein could substantially change the pathogenicity of the virus. Therefore, production multiepitope based peptide vaccines against the S protein of SARS-CoV-2 should be considered.
Jin *et al.*[31]	Phylogenetic analysis and Heat mapping of 788 confirmed COVID-19 patients.	Furin cleavage site mutation on S protein	FCS mutation may represent an essential SARSCoV-2 evolution site. Vaccines targeted at the binding of Furin could reduce pathogenesis of SARS-CoV-2 in lungs of infected humans.
Holland *et al.*[32]	Genomic characterization of a 27 amino acid in -frame deletion in accessory protein	An 81- nucleotide deletion in SARS-CoV-2 ORF7a	The occurrence of phylogenetically distinct mutants indicating independent transmissions patterns. Since mutation is occurring slowly, vaccines made from original strains could still significantly confer immunity against distinct mutants.
Van Dorp *et al.*[33]	Curation of dataset of 7666 public genome and genomic diversity analysis	NSP6, NSP11, NSP13, Spike protein	There is a chance of continuous adaptation measures in the SARS-COV-2 genome. However, vaccines would be used prophylactically while targeting different sites on the viral structure so that significant immune response could be elicited against SARS-COV-2.
Pachetti *et al.*[25]	220 genomic sequences analysis from database derived from COVID-19 patients	8 novel recurrent mutations of SARS-CoV-2 RdRp	Development of multi-epitope vaccines would significantly inhibit SARS-CoV-2 viruses with recurrent RdRp mutations reported in this study. Furthermore, similar structural analogues which could act as polyvalent RdRp inhibitors can be considered.
Happi *et al.*[155]	Genome annotation and Mutation Analysis	D614G in S protein	The occurrence of D614G mutation inS protein has been linked to increased transmission rate and pathogenicity as well as immuno-evasion. Hence, vaccine platforms that elicit broadly neutralizing antibodies against both D614G and D614 should be considered.

during the development of a polyvalent vaccine which would be worthy in the fight against the disease .[35]

It is worthy of note that the sequences of 30,983 complete genomes from 80 countries situated in Africa, Asia, America, and Oceania, were analyzed by Alouane *et al* .[36] In comparison to the reference genome Wuhan-Hu-1 genome, the analysis revealed the occurrence of 3,206 variant sites with uniform distribution in all genome sequences analyzed .[36] A low frequency of mutations of about 182 mutations, with a prevalence of 5.67% was reportedly observed .[36] Nevertheless, hotspot mutations with a prevalence higher than 10% were observed on 14 different locations on the genome sequence, seven regions on ORF1ab gene (nsp2, nsp3, nsp6, nsp12, nsp13, nsp14 and nsp 15), 3 in the nucleocapsid protein and one each in the spike protein, RNA primase, ORF3a, and ORF8 respectively .[36] Also, 35 non-synonymous mutations (<1%) were identified in the receptor-binding domain (RBD) of the spike protein. In contrast, only four of the mutations could potentially enhance the binding affinity of the spike protein to the human ACE-2 receptor.

The corroborative findings of the phylogenetic analysis and mutation observations demonstrated that proteomic divergence of analyzed strains in comparison to the reference is insignificant. Therefore, the dawdling mutation rate in SARS-CoV-2 genomes makes the possibility of developing an effective global vaccine .[36]

Pathology and Immunopathological Response to SARS-CoV-2 Infection

Despite the controversies about the origin of SARS-CoV-1, several studies were able to trace its source to a zoonotic origin where COVID-19 patients were exposed to an animal market in Wuhan; a where live animals are sold. Subsequently, efforts have been made to search for a reservoir and/or intermediate hosts of SARS-CoV-2 from which the infection might have spread to humans. Initially, two snake species were identified as the possible reservoir hosts of SARS-CoV-2. However, the only consistently identified SARS-CoV-2 reservoirs are mammals and bats .[37] Notably, genomic sequencing of some SARS-CoV -2 isolates showed 88% nucleotide homology with two bat-derived-SARS-like Coronaviruses .[38] Thus, indicating bats as the most likely reservoir of SARS-CoV-2 .[38]

Although SARS-CoV-2 may affect many organs of the human body, the respiratory tract is the primary site of infection .[9] Following coughing, sneezing, or talking by an infected person, the virus in the respiratory droplets could come in contact with the mucous membrane (especially the mucosa of nasal cavity and pharynx) of a susceptible host leading to respiratory tract colonization and/or infection .[39]

A complex interaction occurs between the receptor-binding domain (RBD) of the S-protein on the virus surface and ACE-2 receptor on the host cell leading to the internalization of the SARS-CoV-2 through clathrin-mediated endocytosis .[39,40] These ACE-2 receptors are expressed on the cell surfaces of the respiratory tract, heart, gastrointestinal tract, kidneys and testicles .[41,42] The virus moves from the upper respiratory tract to the lungs where it multiplies, translocate into the blood causing viremia, and induces its hematogenous spread to organs that express ACE-2 receptors on their cell surfaces. This host–virus interaction represents the beginning of SARS-CoV-2 replication which could ultimately lead to multi-organ disorders .[43-45]

The pathogenesis of SARS-CoV-2 is believed to be closely similar to that of SARS-CoV .[46] These pathological changes include; diffuse alveolar damage, pneumocytes desquamation, pulmonary edema with hyaline membrane formation, and cytomegaly of pneumocytes .[42,47] The incubation period of the SARS-CoV-2 is between 6 and 14 days and 5.2 days on average .[48] Infected persons commonly develop fever, non-productive cough, difficulty breathing, myalgia, confusion, headaches, rhinorrhea, and less common chest pain, diarrhoea, nausea and vomiting .[49] Blood glucose level could be elevated in diabetic patients due to the virus-induced release of stress hormones .[50]

There is currently limited information on the innate immune status of patients diagnosed with SARS-CoV-2 infection. Some studies show an early increase in serum levels of pro-inflammatory cytokines in SARS-CoV-2 infection, indicating a possible cytokine storm-mediated syndrome .[51,52] Active innate immune response to viral infection relies heavily on type – I interferon (IFN) responses and their downstream cascade, resulting in viral replication that eventually leads to adaptive immune response. SARS-CoV-1 and SARS-CoV-2 have similar entry receptor known as ACE-2 receptor that is expressed predominantly in small subsets of lung cells called type – 2 alveolar cells .[53] SARS-CoVs infects macrophages and T cells, a central feature of SARS-CoVs mediated pathogenesis. There are a limited number of macrophages in the lungs that express ACE-2 .[53] If ACE-2 is minimally expressed by the possible target immune cells, other receptors may be utilized, or other modes of cell entry such as antibody-dependent enhancement may be utilized .[9]

Innate immune cells identify invasion of the virus to mount an antiviral response, often by pathogen-associated molecular patterns (PAMPs). For RNA viruses like coronavirus, PAMPs are recognized by either the endosomal RNA receptors – TLR3 and TLR7, or the cytosolic RNA sensor – RIG-I/MDA5, in the form of viral genomic RNA or their intermediates during viral replication like dsRNA. This recognition event triggers downstream signaling cascade, i.e., NF- κB and IRF3, followed by their nuclear translocation. These transcription factors induce type-I IFN expression and other pro-inflammatory cytokines in the nuclei, and these initial responses comprise the first-line defense against viral infection at the entry site .[54]

Type-I IFN via IFNAR, in turn, activates the JAK-STAT pathway, where JAK1 and TYK2 kinases phosphorylate STAT1 and STAT2. STAT1/2 form an IRF9 complex and travel together into the nucleus to initiate transcription of IFN-stimulated genes (ISGs) under the influence of IFN-stimulated response element (ISRE) containing promoters .[54] The successful engagement of type-I IFN response should be able to suppress viral replication and dissemination at an early stage. Coronaviruses use several strategies to interfere with the signals that lead to Type-I IFN production and/or downstream IFNAR signals. This damping technique is closely correlated with the severity of the disease .[55] At the induction stage of Type-I IFN, SARS-CoV interferes directly or indirectly with downstream signalling of RNA sensors

such as the ubiquitination and degradation of MAVS and TRAF3/6 RNA sensor adapter molecules and the inhibition of IRF3 nuclear translocation .[56]

In severe or lethal cases of SARS-CoV infection, an increased influx of neutrophils and monocyte-macrophages is consistently observed .[57,58] Dysregulated type-I IFN and inflammatory monocyte-macrophages are the leading cause of lethal pneumonia seen in a mouse model of SARS-CoV infection .[55] Infiltrated myeloid cells with excessive type-I IFN are, therefore, the primary cause of lung dysfunction that adversely affect the outcome of the infection. Delayed type-I IFN is thought to compromise new viral regulation upon SARS-CoV infection leading to the influx of hyperinflammatory neutrophils and monocyte-macrophages. The rise in these innate immune cells contributes to the worsening consequences for infected the person that manifested in lung immunopathology .[9] Similar scenario with varying degrees of immune involvement is expected in SARS-CoV-2 infection. Ironically, virus transmission is reported to occur even in people diagnosed with asymptomatic conditions. This may be an indication of the innate immune response being suppressed during the early phase of SARS-CoV-2 infection .[9]

Based on the cumulative evidence from the previous infection with coronavirus, innate immune response plays a critical role in pro- or anti-inflammatory responses, this can be used as an approach for immune intervention. Later, successful viral replication results in type-I IFN hyper-production and the release of neutrophils and macrophages, which are the critical sources of pro-inflammatory cytokines. During COVID-19, with related changes in total neutrophils and lymphocytes, SARS-CoV-2 is likely to cause delayed type-I IFN and viral control loss in an early infection .[9] Individuals vulnerable to COVID-19 are those with underlying diseases, including diabetes, hypertension, and cardiovascular disease .[59]

The immune response by type 1 helper T cell (Th1) typically plays a dominant role in adaptive immunity to viral infections. Cytokine microenvironment produced by antigen-presenting cells dictates the direction of T cell responses. Generally, helper T cells orchestrate the overall adaptive response. In contrast, cytotoxic T cells are necessary to destroy cells infected with viruses. Humoral immune response, particularly the development of neutralizing antibodies, plays a protective role by limiting delayed-phase infection and will prevent potential reinfection. In SARS-CoV, the structural proteins, S, N, M, and E proteins were extensively mapped for both T and B cell epitopes (Table 2) .[59]

SARS-CoV infection caused seroconversion as early as day four after disease onset and was discovered in most patients by day 14 .[80] A few serological descriptions of SARS-CoV-2 have been published .[81] In a preliminary analysis, one patient showed peak-specific Immunoglobulin M (IgM) on day nine after the onset of disease and then switched to IgG by week 2 .[81] Interestingly, sera from confirmed COVID-19 patients showed some cross-reactivity with SARS-CoV, but not other coronaviruses. The T-cell response has been investigated extensively in SARS-CoV infections.

Studies have shown that responses to CD8 + T cells were more frequent with greater magnitude than responses to $CD4^+$ T cells .[82] Strong T-cell responses were significantly associated with higher neutralizing antibodies, with more serum Th2 cytokines (IL-4, IL-5, IL-10) in more severe infections .[83]

Available clinical data strongly suggested that the response to Th1 is key to effective control of SARS-CoV, [83] and possibly also valid for SARS-CoV-2 in particular .[84] Cautiously so, $CD8^+$ T cell response needs to be well regulated so as not to cause lung pathology. Since most epitopes found for SARS-CoV are focused on the viral structural proteins, comparing those epitopes associated with SARS-CoV with those of SARS-CoV-2 would be informative. If one can distinguish overlapping epitopes among these coronaviruses, using convalescent serum from recovered SARS patients would be useful for application in passive immunization .[9]

Pharmaco-Economics of SARS-COV-2 Vaccination

The current global economic crisis and recession due to the COVID-19 pandemic have led to decreased budgetary allocations to the health sector. This results in tremendous consequences on healthcare systems and the health of human lives, most especially geriatric individuals. Recently, the pandemic is rapidly transmitting at the community level, and there is yet to be an effective means to prevent the disease. Vaccines are, no doubt, highly pharmacological and economical means of controlling infections and preventing death .[85] The development of effective and cost-efficient vaccines against SARS-CoV-2 is urgently needed to diminish the global COVID-19 pandemic. Despite the slow processes in the vaccines, they are still essential and indispensable. One of such reasons is that the COVID-19 pandemic is still evolving globally with an increase in confirmed cases and casualties, and the level of infection is yet to be attained. Furthermore, the duration of the impact of COVID-19 on global health may assume a seasonal pattern which can persist for an extended period .[86]

Over 40 pharmaceutical companies and intellectual institutions across several countries have disclosed their initiatives through ongoing programs as well as the level of progress based on the development of vaccines targeted towards SARS-CoV-2 .[87] Several vaccines are currently undergoing design and preparation. At the same time, efforts are being made to commence efficacy evaluation for some other candidate vaccines in animals and phase one clinical trials. The measurement of the impact of vaccination is not only restricted to public health terms, but also based on economic values: decreasing healthcare spending, reducing lost of labor force, and contributing to the development of the society and the economy .[88] Several developed countries have taken into consideration the pivotal role of vaccination in their respective preventive healthcare programs. The economic benefits of vaccination reveal preventive healthcare as one of the best measures to identify efficiency and profits .[89]

Vaccination is an essential part of public health; thus, there is an urgent need to reserve an adequate percentage of the budget to guarantee vaccine accessibility and affordability to the populace. The economic and pharmacological values of SARS-CoV-2 needs to be effectively communicated to the government, policymakers, healthcare workers and the populace to instill political will and public acceptance. There is also the need to evaluate further not just the effect of SARS-CoV-2

Table 2. Immunogenic protective epitopes of Coronaviruses, their Sequences and Gene Location.

Position[a]	MERS-CoV	SARS-CoV[b]	HCoV-OC43	HKU1	MHC restriction	Identification[c]	Vaccine evaluation[d]
S411-420	KQSFSNPTCL	KLPDDFMGCV	RIDTTATSCQ	KIDTTSSSCQ	HLA-A*0201	60	60
S787-795	LEPVSISTG	ILPDPLKPT	INFSPVLGC	INFKSLVGC	HLA-A*0201	61	61
S1042-1050	LYFMHVGYY	VVFLHVTYV	LYFIHFNYV	LLFMHFSYK	HLA-A2		
S958-966	SIGDIIQRL	VLNDILSRL	SLQEILSRL	SLQEILSRL	HLA-A*0201	62	62
S978-986[e]	LINGRLTTL	LITGRLQSL	LINGRLTAL	LINGRLTAL	HLA-A2	63	64
S1203-1211	FIAGLVALA	FIAGLIAIV	ICLAGVAML	ISFSFIIFL	HLA-A2		
S1167-1175	SLQQVVKAL	RLNEVAKNL	RLQEAIKVL	LIQESIKSL	HLA-A*0201	63	63
	ALNESYIDL	NLNESLIDL	VLNHSYINL	SLNNSYINL	HLA-A*0201	65	
S1174-1182 S365-374	TCSQISPAAI	KCYGVSATKL	TCNNIDAAKI	SCNNFDESKI	H-2[d]	66	66,67
S436-443	LKYSYINK	YNYKYRYL	WNKRFGFI	WNRRYGFN	H-2[b], H-2[b]	68	69
S525-532	VEYSLYGV	VNFNFNGL	VNYDLYGI	VDYDLYGI	H-2[d]		
S366-374	CSQISPAAI	CYGVSATKL	CNNIDAAKI	CNNFDESKI	H-2[d]		
S884-891	IFYRLNGV	MAYRFNGI	VQYRINGL	VQYRINGL	H-2K[b]	70	70
S1116-1123	STNLPPPL	NNTVYDPL	PYVMLNTS	PLVYLNHS	H-2K[b]		
N216-224	GAVGGDLLY	GETALALLL	VTPDMADQI	VKPDMADEI	HLA-B*4001	71	
N323-332	DDHGNPVYFL	MEVTPSGTWL	DEPQKDVYEL	DSPVKDVFEL	HLA-B*4001		
N223-231	LYLDLLNRL	LLLDRLNQL	QIASLVLAK	EIANLVLAK	HLA-A*0201	61	61,62
N227-235	LLNRLQALE	RLNQLESKV	LVLAKLGKD	LVLAKLGKE	HLA-A*0201		
N317-325	GMSQFKLTH	GMSRIGMEV	FGSKLELAK	FGSKLDLVK	HLA-A*0201		
N220-228	GDLLYLDLL	LALLLLDRL	MADQIASLV	MADEIANLV	HLA-A*0201	72	72
N216-225	GAVGGDLLYL	GETALALLLL	VTPDMADQIA	VKPDMADEIA	HLA-B*4001	73	73
N222-231	LLYLDLLNRL	LLLLDRLNQL	DQIASLVLAK	DEIANLVLAK	HLA-A*0201	74	74
N266-275	TKSFNMVQAF	TKQYNVTQAF	NKQCTVQQCF	NKHCNVQQCF	HLA-B*1525	75	
N346-354	NYNKWLELL	QFKDNVILL	GFETIMKVL	GFETIMKVL	HLA-A*2402	76	
N362-370	KTFPKKEKK	KTFPPTEPK	QQQDGMMNM	VNSNQNTDS	HLA-A*1101	63	
M60-69	SMALSIFSAV	TLACFVLAAV	TIILTTFNCV	TITLTIFNCF	HLA-A*0201	76	76
M88-96	AMMWISYFV	GLMWLSYFV	IIMWIVYFV	IVIWILYFV	HLA-A*0201		
M147-155	HLKMAGMHF	HLRMAGHSL	HLYIQGIKL	HLYIQGVKL	HLA-B*1502	75	
PP1a3709-3717	AYLVFVTTL	SMWALVISV	LLMLASLFG	LLFITAFLG	HLA-A*0201	77	77
PP1a1775-1787	VEHTTPWLL	VQQESSFVM	VRFDVPFLI	TKLNVPFLI	HLA-B*1501	78	
N353-365	LLEQNIDAYKTFP LLNKHIDAYKTFP VLSENLNAYQQQD VLEENLNAYVNSN H-2[d],HLA-DR2,DR3					79	79

SARS-CoV-derived T-cell epitopes and their conservation in human coronaviruses. [a] The position information is based on the SARS-CoV (strain TJF; GenBank accession no. AY654624).[b] The sequences of the corresponding peptides in SARS-CoV, HCoV-OC43 (strain HK04-01; GenBank accession no. JN129834), and HKU1 (strain BJ01-p3; GenBank accession no. KT779555). The variable residues in the peptides compared to MERS-CoV are underlined in bold. [c] The references that identified the peptides. [d] The references that used the peptides as vaccines or used the peptides to evaluate SARS-related vaccines.[e] The positions of HLA-restricted peptides with three or less variable residues between MERS-CoV and any of the three coronaviruses SARS-CoV, HCoV-OC43, and HKU1.

vaccination on health and medical expenses, but also its broader economic impact on society.

Private pharmaceutical companies, foundations, national governments and donor funding agencies are financial drivers for vaccine research, development, and production .[90] Pandemic products are high-risk investments for pharmaceutical industries, thereby requiring public funding from governments, industries, and individuals .[91] Processes from vaccine discovery to approval for use cost billions of dollars and several years to complete with an average success rate of 60% .[92,93]

For instance, the coalition for epidemic preparedness innovations (CEPI) was established in 2016 to oversee stages of vaccine research and development . Since the start of COVID-19 pandemic, CEPI has committed $23 million for vaccine development, the United States is channeling $3 billion for coronavirus vaccine research and development (R & D), and the Canadian government has made available $2.7 million for vaccine research .[94] In another vein, the Prime Minister of Canada promised to invest $192 million into the development and production of SARS-CoV-2 vaccine and therapeutic interventions. This will be achieved through a partnership with indigenous pharmaceutical companies and research institutes .[95]

It was projected that vaccine R & D will cost between $200 million and $1.5 billion. It will take many years for the vaccine to pass through stages of clinical trials to ascertain its safety and effectiveness .[96] Is this in response to the extended timeline projection of the CEPI vaccine development, different vaccine candidates in different stages of R & D are expected to be available for use within one year. Using different technologies such as modified virus-like particles, messenger RNA (mRNA), nanoparticles and DNA-based technology, there are currently 26 candidate vaccines in clinical evaluation and 139 in preclinical evaluations pharmaceutical companies and research institutes globally .[97] Of these, there are two major vaccine candidates developed by Moderna Therapeutics and Inovio Pharmaceuticals, which are mRNA based and DNA-based vaccines, respectively .[97]

There are commitments from several national governments and donor funding agencies to finance the SARS-CoV-2 vaccine, the anticipated profit of $30 billion in 2019 and expected turnover projection of $1.4 trillion by the end of 2020. Furthermore, key Biopharmaceutical companies have indeed committed between US$100 million to some billion US$ in research and development of COVID-19 vaccines .[98] For instance, Pfizer is starting a Phase 3 trial, Merck is well along in COVID-19 vaccine development. GSK-Sanofi have partnered, are well along in development, and have negotiated a US$2 billion contract with the US government for vaccine supply .[99]

Recently, Moderna Therapeutics received $483 million from biomedical advanced research and development agency (BARDA) to develop the mRNA-based vaccine .[99] This vaccine may not be affordable for low- and middle-income countries (LMICs) due to high cost involved in different development stages [100] and little financial strength to acquire this vaccine, amongst other government priorities for their citizens. Equitably, however, vaccines should be made accessible and affordable to all countries because LMICs have the potential of becoming disease epicenter of current and future outbreaks .[81,101] In this vein, part of the funds contributed by Bill and Melinda Gates targeted towards combating COVID-19 have been set aside for LMICs .[81]

Currently, there is no global agency charged with financing vaccine production. However, global stakeholders are considering large-scale vaccine manufacturing and procurement .[81,100]

Overview of Ongoing Vaccine Development

Vaccine development is typically a long, arduous process sometimes extending from 10 to 15 years [102] before final approval. Newer technologies and improvement in molecular and genetic techniques coupled with previous experiences, have yielded several strategies and platforms for vaccine development .[103] These platforms include – live vaccines with vector viruses, inactivated vaccines with viral proteins and gene-based vaccines .[104] Most of the platforms and strategies are novel or based on existing developing platforms. Consequently, Ain Shams University, Egypt is planning to start Phase 3 of testing the Bacilli Calmette-Guerin BCG vaccine in Egyptian healthcare workers on the frontline of the pandemic .[104,105] The rationale behind this hypothesis is in two ways. Firstly, BCG vaccination was shown to confer long-term changes to the innate immunity dubbed "trained immunity" and has been known to be protective against several viruses such as Vaccinia, Influenza A, Herpes Simplex Virus 2 and respiratory syncytial virus via macrophage-dependent epigenetic changes .[105-108] The heterologous immunomodulatory effect of BCG on nonrelated conditions provide a promising avenue for investigation in relation to the COVID-19 pandemic. Secondly, countries with a BCG vaccination policy experienced a reduction in both COVID-19 cases and deaths worldwide as exemplified by several epidemiological studies in the European hotspot with inadequate or no BCG coverage .[109] This suggests that BCG vaccination may be a protecting factor against COVID-19. However, being observational studies, these data should be interpreted with caution and should be considered only as hypothesis due to the significant differences in collecting data relating to COVID-19 between countries .[108]

The WHO reported about 149 COVID-19 vaccine candidates in its updated continuously list, however, Le *et al.* [110] projected the number to be more as there are some not listed by the WHO. Of these, some vaccine candidates have proceeded into clinical trials, while others are in various stages of animal testing and will be planning to enter clinical trials (Table 3). In collaboration with several countries, CEPI is currently supporting and funding a good number of such trials and vaccine development with proposed support of up to $2 billion .[116,117] Two inactivated vaccines are in phase 1 clinical trials .[110]

Sinovac Biotech reported on 23rd April 2020 – one of their many first COVID-19 vaccines in development conferred protection to rhesus macaques from infection by SARS-CoV-2. The scientists gave two separate doses of the candidate vaccine to eight rhesus macaque monkeys. About 3 weeks after the two separate doses of vaccination, the monkeys' lungs were challenged with SARS-CoV-2 virus, and none developed an infection .[118] On 24th April 2020, a potential coronavirus vaccine – ChAdOx1 developed at the University of Oxford began clinical trial on people .[119]

Need for Thorough Efficacy, Potency and Safety Evaluations of SARS-CoV-2 vaccine

It is a rule of thumb that any vaccine after its design and preparation, must undergo efficacy and safety evaluation as well as a validation of its quality standard before entering clinical trials. Generally, three phases of clinical trials are necessary to evaluate the safety, potency, and efficacy of any vaccine .[19] A few vaccine candidates for SARS-CoV-2 have shown efficacy in *in-vitro* studies, however not many have progressed to randomized animal or human trials, hence may have limited use in tackling COVID-19 infection. Bao *et al* [120] recently reported a demonstration of recovery by SARS-CoV-2 challenged rhesus macaques from prior infection with an associated immunity to re-challenge. Early on, it was not clear if COVID-19 patients are at risk of reinfection during the recovery stage. In the search for an answer, rhesus macaques were re-challenged by Bao *et al* [120] with SARS-CoV-2 while at an early recovery phase from the initial infection. During the infection, some of the clinical observations were loss of weight, interstitial pneumonia and systemic viral dissemination majorly in respiratory and gastrointestinal tracts. Gladly, after the monkeys were re-challenged with an identical SARS-CoV-2 strain, viral dissemination, clinical manifestations and histopathological changes were not detected and were absent. Possibly, the protection of rhesus macaques from the reinfection by SARS-CoV-2 might have been caused by a notably enhanced neutralizing antibody response. The outcome of this experiment depicted that primary SARS-CoV-2 infection protects from subsequent reinfection. Therein, a proof of principle for using vaccines to stimulate immunity in humans was demonstrated .[120]

Because of the extensive sequence diversity of coronavirus, sadly, limited cross-protection offered by most vaccine candidates have remained a clog in the wheel of developing efficacious vaccines against human coronavirus infections in recent decades .[87] Several vaccines candidates have been studied for previous CoVs such as SARS-CoV and MERS-CoV .[87]

In addition to scaling up novel researches, some of these previous options can also be further evaluated for their safety, efficacy, and potency in tackling SARS-CoV-2 .[87] This will increase the chances of coming up with an effective vaccine to stem the COVID-19 tide.

Moreover, there has also been the question of how long the vaccine-induced immunity for SARS-CoV-2 lasts. There still exist several grey areas. For instance, experience from studies of human coronavirus infections causing the common cold suggested that immunity may be short-lived probably just for about 3 months or

Table 3. Overview of Some Ongoing COVID-19 Vaccine Trials.

Developer	Candidate	Vaccine Characteristics	Status	Comments
University of Oxford	ChAdOx1 (AZD1222)	Non-replicating viral vector	ISRCTN Phase III ISRCTN89951424 NIH Phase I/II NCT04324606 EURCTR Phase I/II 2020-001072-15 EUCTR Phase II/III 2020-001228-32	Strategy has shown to provide strong immune responses. Success depends on the choice of vector and immune-inducing protein of interest [111]
Sinovac Biotech	PiCoVacc	Inactivated SARS-COV2 plus alum	NIH Phase III NCT04456595 NIH Phase I/II NCT04352608	Generally safe. Some previous inactivated vaccines suffered from ineffectiveness and worse outcome [112]
CanSino Biologicals/ Beijing Institute of Biotechnology	Ad5-nCoV	Adenovirus type 5 vector that expresses S protein	NIH Phase I NCT04313127 ChiCTR Phase II ChiCTR2000031781 ChiCTR Phase I ChiCTR2000030906	Strategy has shown to provide strong immune responses. Success depends on the choice of vector and immune-inducing protein of interest.[111]
Moderna/NIAID	mRNA-1273	LNP-encapsulated mRNA vaccine encoding S protein	NIH Phase III NCT04470427	Easy and quick to design. Looks promising First of its type and its utility has not yet been determined.[113]
Wuhan Institute of Biological Products/ Sinopharm	Inactivated virus	Inactivated SARS-COV2 in Vero cells	ChiCTR Phase III ChiCTR2000034780	Generally safe Some previous inactivated vaccines suffered from ineffectiveness and worse outcome [112]
Beijing Institute of Biological Products/ Sinopharm	Inactivated virus	Inactivated SARS-COV2 in Vero cells	ChiCTR Phase III ChiCTR2000034780	Generally safe. Some previous inactivated vaccines suffered from ineffectiveness and worse outcome [112]
BioNTech/Fosun Pharma/Pfizer	BNT162	RNA vaccine containing 3 LNP-mRNAs	EUCTR Phase I/II 2020-001038-36 NIH Phase I/II NCT04368728	Easy and quick to design. Looks promising. First of its type and its utility has not yet been determined.[114]
Inovio Pharmaceuticals	INO-4800	DNA plasmid encoding S protein delivered by electroporation	NIH Phase I/II NCT04336410 NCT04447781	Easy and quick to design. Looks promising. First of its type and its utility has not yet been determined [114]
Shenzhen Geno-Immune Medical Institute	LV-SMENP-DC	DCs modified with lentiviral vector expressing synthetic minigene based on domains of selected viral proteins; administered with antigen-specific CTLs	NIH Phase I NCT04276896	Strategy has shown to provide strong immune responses. Success depends on the choice of vector and immune-inducing protein of interest.[111]
Shenzhen Geno-Immune Medical Institute	Pathogen-specific aAPC	aAPCs modified with lentiviral vector expressing synthetic minigene based on domains of selected viral proteins	NIH Phase I NCT04299724	Strategy has shown to provide strong immune responses. Success depends on the choice of vector and immune-inducing protein of interest.[111]
Novavax	NVX-CoV2373	Full length recombinant SARA-CoV-2 glycoprotein nanoparticle vaccine adjuvanted with Matrix M	NIH Phase I/II NCT04368988	Focuses on antigenic part of virus and hence cannot cause infection May not stimulate a strong response. Usually needs an adjuvant [115]
Clover Biopharmaceuticals	SCB-2019	Dead vaccine with genetically engineered Native like Trimeric subunit Spike Protein	NIH Phase I NCT04405908	Generally safe Some previous inactivated vaccines suffered from ineffectiveness and worse outcome.[112] Focuses on antigenic part of virus and hence cannot cause infection May not stimulate a strong immunologic response. Usually needs an adjuvant.[115]

(*Continued*)

Table 3. (Continued).

Developer	Candidate	Vaccine Characteristics	Status	Comments
Imperial College London	LNP-nCoVsaRNA	saRNA gene-based vaccine	[ISRTCN]Phase I ISRCTN17072692	Easy and quick to design. Looks promising First of its type and its utility has not yet been determined.[114]
CureVac	CVnCoV	mRNA-based vaccine	[NIH]Phase I NCT04449276	Easy and quick to design. Looks promising. First of its type and its utility has not yet been determined.[114]
Genexine/Binex/ GenNBio/Int. Vaccine Inst./Korea Advanced Inst. Of Science & Technology (KAIST)/ Pohang Univ. of Science and Technology (POSTECH)	GX-19	DNA-based vaccine	[NIH]Phase I/II NCT04445389	Easy and Quick to design. Looks promising First of its type and its utility has not yet been determined.[114]
Vaxine Pty Ltd/Medytox	COVAX-19	Recombinant spike protein with Advax™ adjuvant	[NIH]Phase I NCT04453852	Focuses on antigenic part of virus and hence cannot cause infection May not stimulate a strong response. Usually needs an adjuvant.[115]
Walvax Biotechnology/ People's Liberation Army (PLA) Academy of Military Sciences/ Suzhou Abogen Biosciences	ARCoV	Gene-based vaccine (mRNA)	[ChiCTR]Phase I ChiCTR2000034112	Easy and quick to design. Looks promising First of its type and its utility has not yet been determined.[114]
Natl. Gamaleya Research Center for Epidemiology and Microbiology (Russia)	-	Vector virus vaccine (adenovirus)	[NIH]Phase I NCT04436471 NCT04437875	Strategy has shown to provide strong immune responses. Success depends on the choice of vector and immune-inducing protein of interest.[111]
Anhui Zhifei Longcom Biopharmaceutical/ Institute of Microbiology, Chinese Academy of Sciences	-	Subunit vaccine (recombinant antigen and adjuvant)	[NIH]Phase II NCT04466085	Focuses on antigenic part of virus and hence cannot cause infection May not stimulate a strong response. Usually needs an adjuvant.[114]
University of Queensland/ GSK/Dynavax	-	Dead vaccine with genetically engineered molecular clamp stabilized spike protein and adjuvant	*Phase I NCT04405908	Generally safe Some previous inactivated vaccines suffered from ineffectiveness and worse outcome.[112]
Arcturus Therapeutics/ Duke-NUS	LUNAR-COV19	mRNA-based vaccine	*Phase I/II NCT04480957	Easy and Quick to design. Looks promising First of its type and its utility has not yet been determined [114]
MIGAL (The Galilee Research Institute) and MigVax	-	Oral *E. coli*-based protein expression system of S and N proteins	*Preclinical: Planned for summer 2020	Focuses on antigenic part of virus and hence cannot cause infection May not stimulate a strong response. Usually needs an adjuvant.[115]
ReiThera/Leukocare/ Univercells (Italy/ Germany/Belgium)	-	Replication defective Simian Adenovirus (GRAd) encoding SARS-CoV-2 S	*Preclinical: Planned for summer 2020 in Italy	Strategy has shown to provide strong immune responses. Success depends on the choice of vector and immune-inducing protein of interest.[111]
Sanofi Pasteur/GSK	-	Dead vaccine with genetically engineered (baculovirus) virus antigen and adjuvant	*Preclinical	Generally safe Some previous inactivated vaccines suffered from ineffectiveness and worse outcome.[112]

(*Continued*)

Table 3. (Continued).

Developer	Candidate	Vaccine Characteristics	Status	Comments
Janssen (Johnson & Johnson)	Ad26	Non-replicating viral vector	*Phase I/II NCT04436276	Strategy has shown to provide strong immune responses. Success depends on the choice of vector and immune-inducing protein of interest.[111]
OpenCorona Consortium (Karolinska Institute, University of Gießen and partners)	-	DNA-based vaccine with electroporation	*Preclinical: Planned for 2021	Easy and Quick to design. Looks promising First of its type and its utility has not yet been determined.[114]

NIHUS National Library of Medicine – clinicaltrials.gov
ChiCTRChinese Clinical Trial Registry – chictr.org.cn
EUCTREU Clinical Trials Register – clinicaltrialsregister.eu
ISRCTNISRCTN Registry – isrctn.com
* Pre-clinical – Data obtained from vfa [104] and WHO [97]
Chinese Clinical Trial Registry (ChiCTR). http://www.chictr.org.cn/abouten.aspx
EU Clinical Trials Register. https://www.clinicaltrialsregister.eu
ISRCTN Registry. http://www.isrctn.com/
Note: Vaccine trial is an ongoing clinical process. Hence, data and phase of evaluations presented in table 3 might change with time.

less and individuals stand a chance of re-infection within a short while after initial exposure .[120] On the flip side, longitudinal studies of antibody-mediated immunity from recovered SARS patients suggests that immunity after infection may last for 1-2 years .[121] Furthermore, cellular immunity is also long-lasting with prompt elicitation of cytotoxic T-cell responses to challenge 1-year post-recovery .[121] Bearing in mind that SARS-CoV-2 is a novel virus, there is a need to follow up the persistence of neutralizing antibody levels over time. Animal models are pre-requisites for the evaluation of a vaccine efficacy at the preclinical level. It is worrisome that just a handful of suitable animal models are currently on the radar for SARS-CoV-2 vaccine, especially given the virus is a new pathogen. Good enough, a human ACE-2 transgenic mouse, as well as primate macaques, both, proved suitable models recently .[87]

A recent study in a bid to elucidate the pathogenicity of SARS-CoV-2 used an hACE-2 transgenic mouse infected with the virus .[120] Weight loss, as well as virus replication in lung tissue, were observed in the hACE-2 transgenic mice model. Also observed was the typical histopathology of interstitial pneumonia. Additionally, the bronchial epithelial cells, alveolar epithelia, as well as alveolar macrophages all showed viral antigens. In view of the above, the mouse model may, therefore, facilitate therapeutic drugs and vaccine development against SARS-CoV-2 .[120] Furthermore, a report from the Institute of Zoology of the Chinese Academy of Sciences shows the observation of similar symptoms with human infections in primate macaques as well as viral load changes and computed tomography (CT) images of the lungs. As a result, the primate macaques' model, which has undergone validation, is already being used in drug trials and functional evaluation .[122]

Another significant limitation to SARS-CoV-2-related research is highly contagious and pathogenic nature of the organism. To bridge the gap caused by the scarcity of animal models in the evaluation of the efficacy of SARS-CoV-2 vaccines, virus-like particles (VLPs) can serve as alternative tools. These virus-like particles (VLPs) are structures of many proteins that resembles in its organization and conformation, authentic native viruses, except that they lack viral genome. Application of these VLPs in similar studies has resulted in safe and effective positive outcomes .[35] The search for a potent SARS-CoV-2 vaccine is one with multiple approaches. Except in the case of live attenuated vaccines and live vector vaccines, adjuvants are pre-requisite for boosting immune response in the development of other types of vaccine.

In order to avoid the potential undesired effects of coronavirus vaccination, attention has also been given to the use of adjuvants. Adjuvants are substances that modify and boosts the efficacy and immunogenicity of the vaccines .[35] A study revealed that by using a chemical adjuvant (a delta inulin-based polysaccharide), lung immunopathology previously observed in mice after SARS challenge experiments were no longer seen .[35] After that, there was a hypothesis that the adjuvant helped to avert an exacerbated Th2-polarized response after challenge causative of the adverse effects. Above, therefore, is a scenario where an adjuvant-enhanced vaccine-induced protection as well as helped to minimize adverse effects from coronavirus vaccination .[35]

In order to fast track the development of a SARS-CoV-2 vaccine, however, there is a need to place premium on adjuvants that have been widely used in other vaccines that have gained market approval .[53] However, suitable adjuvants should be selected based on vaccine design. This is bearing in mind that adjuvants can regulate the type of immune response. Additionally, in order to have a more efficacious and effective vaccine, a combination of different types of adjuvants may be necessary. At the moment, GSK has offered to make her established vaccine adjuvant platform technology available to facilitate the development of an effective vaccine against SARS-CoV -2. In addition, GSK has made this adjuvant available to Sanofi as well in their ongoing development program. To this end, agreements have been reached with the University of Queensland, Australia and Clover Biopharmaceutical, Inc. [87]

Safety is an essential factor that must be prioritized in the course of any vaccine development with SARS-CoV-2 vaccine not being an exemption. There exists a dearth of information on the safety of vaccines against SARS-CoV-2. Currently, the S protein has proven to be the right candidate antigen for SARS-CoV-2 vaccine development. Sadly, apart from receptor binding and membrane fusion, the S protein has also shown other biological activities .[121] There have been increasing concerns about the induction of antigen-dependent enhancement (ADE) and other adverse effects derived from vaccination or natural re-exposure. ADE is a situation that sets in when non-neutralizing antibodies against proteins of a virus facilitate virus entry to host cells, as well as enhancing virus infectivity .[87] ADE has been already seen in cats vaccinated against a species-specific coronavirus (feline infectious peritonitis coronavirus) .[35]

In the case of SARS-CoV-2, many studies have associated full-length S protein with severe liver damage, enhanced infection otherwise known as ADE and chances of viral spread within the central nervous system (CNS) .[19] These adverse effects are linked to S protein-specific antibodies .[87] Currently, there is no clarity on the particular domains and critical amino acids in S protein of SARS-CoV-2 that elicit the lung damage, and this will pose safety issues should the full-length S protein be used as an antigen .[87]

There have been attempts to solve the S glycoprotein-induction of ADE by using truncated versions of the same protein. Researchers believed that the use of the receptor-binding domain (RBD) or the S1 subunit of the S glycoprotein could lead to the induction of neutralizing antibodies while avoiding ADE. The concept is to focus the induction of antibodies to relevant S regions for efficient virus neutralization and, by doing so, to avert the induction of potential non-neutralizing antibodies targeting other regions of the S protein. At the moment, there is seemingly a paucity of direct evidence that justifies this phenomenon .[35] More fundamental researches are needed to understand better the structure and function of the S protein will be of help in considering the selection of mutant residues in antigen design.

Prospects and Possible Challenges Associated With SARS-CoV-2 Vaccine Strategies

Owing to the SARS-CoV-2 pandemic, it is now of importance to explore the science of preventive and therapeutic vaccine as a tool to ameliorate the prevailing condition. It is worthy of note that various strategies have been employed in achieving this goal.

Most candidate vaccines are predicated on the induction of serum virus-neutralizing (VN) antibodies and systemic cell-mediated immune responses in the animal models as indicators of protection, but the correlates of immunity to COVID-19 in humans are unknown .[122]

The pathogenesis of COVID-19 in humans is unclear as more research is underway, and as such vaccine strategies may need to be altered if the virus infects both the respiratory and intestinal tracts (pneumoenteric, like Bovine CoV) and is also shed in faeces. Oro-nasal vaccine prime and parenteral spike (S) vaccine booster may be optimal to prevent both enteric and respiratory infections as faecal and nasal shedding used for some animal CoV vaccines .[123]

Several types of COVID-19 vaccines are proposed and are in various stages of development [124] with emphasis on criteria for safety and efficacy and duration of immunity. However, vaccines for pandemics also required rapid and speedy development with high production capacity .[125]

Despite the novel platforms, SARS-CoV-2 vaccine development poses challenges to researchers. Although the virus's S-protein is a promising immunogen for protection, optimizing antigen design is critical to ensure optimal immune response. Scientists and researchers are left in the middle of thought over the best approach. For instance, targeting the full-length protein or only the receptor-binding domain .[126] More so, correlates of protection may be inferred from experience with SARS and MERS vaccines, but they are not yet established. As with naturally acquired infection, the potential duration of immunity is unknown; similarly, whether single-dose vaccines will confer immunity is uncertain .[126]

A recent study by Kuldeep *et al* [127] revealed the possibility of developing a universal CoV vaccine based on the similarity in T-cell epitopes of SARS and MERS-CoV that confirmed the potential for cross-reactivity among CoVs. It was noted that SARS-CoV-2 shares high genetic similarity with the SARS-CoV such that vaccines developed for SARS-CoV may exhibit cross-reactivity with SARS-CoV-2. However, comparative evaluation performed on full-length S=protein sequences of SARS-CoV-2 and SARS-CoV identified that the most variable residues were located in the S1 subunit of S protein, the critical CoV vaccine target. These findings suggest that the specific neutralizing antibodies that are effective against the SARS-CoV might not be effective against the SARS-CoV-2 .[127]

Vaccine development is a lengthy and expensive process with high attrition, and it typically takes multiple candidates and many years to produce a licensed vaccine .[82] Consequent upon this fact, vaccine developers typically follow a linear algorithm, with multiple pauses for data analysis or manufacturing process checks. Vaccine development at pandemic state involves a fast start and many steps executed in parallel before confirming a successful outcome of another step, hence resulting in elevated financial risk .[92] Although classical inactive and attenuated vaccines are currently under investigation, challenges of speedy production of these virus vaccines in large quantities in cell culture under biosafety level-3 conditions abounds, and this limits their rapid deployment in the face of emerging pandemics .[128]

Recombinant vector vaccines are in various stages of development for SARS or MERS, which might be a promising candidate for SARS-CoV-2 .[129] However, safety remains a significant concern for vaccines, therefore the need to investigate an adverse event or vaccine-induced immunopathology evident during candidate vaccine studies in animal models .[130] Eosinophil-related lung pathology was observed in mice vaccinated with formalin and ultraviolet-inactivated SARS vaccine, [124] or γ-irradiated inactivated MERS-CoV vaccine post-murine challenge. However, adding toll-like receptor agonists to an ultraviolet-inactivated SARS-CoV vaccine reduced the Th2-associated lung pathology .[131] In one ferret study, the MVA-S vaccine was associated with liver pathology, but this was not evident in other studies [131,132]

In tests of a SARS-CoV-2 S-protein candidate vaccine, antibody-dependent enhancement (ADE) of infection was reported post-challenge in hamsters, but not in mice using a spike (S) protein nanoparticle vaccine for MERS .[87] ADE has remained a long-term obstacle to the development of safe vaccines for feline infectious peritonitis, a systemic CoV infection of cats .[87] In feline infectious peritonitis-infected cats, ADE was triggered by antibody-mediated virus entry into macrophages via Ig Fc receptors .[53]

The inconsistencies in these events among animal models necessitate an improved understanding of the biological basis for their occurrence and a better knowledge of human immunology to avoid similar reactions in humans because the animals used in the experiment shares the similar physiologic pattern with humans .[133] More so, experts generally agree that animal experiments and human clinical trials of candidate vaccines for COVID-19 should include a careful assessment of possible immune complications before releasing the vaccine to the public .[134]

There are particular disadvantages of each vaccine strategy. Nucleic acid vaccines can be easier to design. However, DNA vaccines may not be as immunogenic enough to elicit host antibody defensive mechanism. In contrast, mRNA vaccines may be more unstable and challenging to predict their immunologic effect .[135] Additionally, viral vector vaccines and subunit vaccines generally exhibit higher safety profiles. They are more immunogenic, but they may have reduced efficacy due to pre-existing immunity to the vector, and subunit vaccines may be globally inaccessible due to its expensive cost of production .[136]

The non-replicating adenovirus type 5 viral vector vaccine strategies by CanSino Biological, Inc., and Beijing Institute of Biotechnology is designed with a gene encoding the antigenic SARS-CoV-2 S=protein .[137] The viral vector delivers the spike protein gene into human cells, leading to the production of the protein that is designed to trigger an immune response. This robust strategy has scaled through preclinical trials. It has recently begun phase I clinical trials, with more than 100 participants aged 18 to 60 years. Despite this landmark ~~of~~ success, possible limitations exist. The type of adenovirus used in this candidate is a virus associated with the common cold, and, consequently, it is possible that people may already have been exposed to the viral vector, which could hinder human cell uptake of the viral vector or even lead to possible safety concerns if immunization triggers an inappropriate immune response .[138]

The sequence for the vaccine strategy, called mRNA-1273, by Moderna, Inc. and National Institute of Allergy and Infectious Diseases (NIAID) was first identified in mid-January and contains genetic code for the SARS-CoV-2 spike protein such that on administration, the mRNA sequence prompts cells to start producing the antigenic SARS-CoV-2 S-protein, thus initiating an immune response. mRNA vaccines do not contain infectious material and are easier to develop and manufacture; this serves as its advantage .[139] Nevertheless, mRNA vaccine candidates, including those in development by Moderna, have not made it past phase III clinical trials .[140]

DNA vaccine strategy called INO-4800 by Inovio Pharmaceuticals, was designed to be administered intradermally. Administration of this vaccine candidate requires the use of an electroporation device called CELLECTRA, which uses a small electrical current to make the human cells more permeable and thereby enables proper entry and incorporation of the DNA molecule into the cell .[141] This vaccine strategy is in its first clinical trial. It consists of plasmid DNA that, upon administration, prompts human cells to produce the antigenic SARS-CoV-2 S-protein .[142] While DNA vaccines carry certain advantages, including optimal development speeds and thermostability, past trials have shown that producing sufficient immunogenicity can be a challenge, thereby eliciting little immunological effect. Additionally, the administration can often require larger volumes of DNA vaccine compared to more traditional vaccine types, and it requires the use of an electroporation device, which can be inconvenient .[142,143]

Suitable Animal Models of COVID-19 Vaccine Candidates

Rhesus Macaques

Studies proved that humans share the same essential 12 amino acid residues present in ACE-2 with macaques .[143] Therefore, it has been chosen as a closer model system to humans, making it fit for COVID-19 vaccine testing. At a high SARS-CoV-2 viral load, macaques suffered pulmonary infiltrates when observed in radiographs .[144,145] This matches with the milder symptoms seen in SARS-CoV-2 infected humans. Further, it was also observed that SARS-CoV-2 affected older macaques than the young ones, and this is also the same in human .[145] Consequently, rhesus macaques can be a promising animal model for efficacy and safety study of candidate COVID-19 vaccines.

Ferrets (*Mustela putorius furo*)

Ferrets are domesticated forms of European polecat that are commonly used in veterinary research. Ferrets cough, sneeze, and have similar lung morphology as humans .[146] These could support their use as an animal model for SARS-CoV-2. Vasan *et al* [147] have shown the susceptibility of ferrets to SARS-CoV-2. It is also interesting to note that SARS-CoV-2-infected ferrets could spread the virus with high transmission rates .[148] Importantly, since most of the countries are now under community transmission stage, ferrets may be a valuable animal model to study COVID-19 vaccines .[149]

Mice

The use of mice as an animal model has economic benefits as it is cost-effective. However, mice have 11 of the 29 amino acids while for a rat, 13 of 29 amino acids differ in the binding region of ACE-2, respectively, when compared to humans; therefore,

SARS-CoV-2 does not infect mice .[150] Perlman Laboratory developed humanized mouse models for SARS, where they incorporated human ACE-2 in mice .[151] Successful SARS-CoV-2 infection transgenic mice that express human ACE-2 were observed. The development of typical interstitial pneumonia with infiltration of significant macrophages and lymphocytes in the alveolar interstitium and the accumulation of macrophages in alveolar cavities were noted on histopathological examination .[152] This made it another choice animal model to study COVID-19 vaccines safety .[152]

Syrian Hamster (also: Golden Hamster; *Mesocricetus auratus)*

Syrian hamster ACE2 shows maximum similarity to that of human, with differences in only 4 amino acids out of 29. This made this animal a model of choice for SARS-CoV-2 infection. Importantly, Syrian hamsters show similar immune responses as that of humans where IFN-gamma and pro-inflammatory cytokines are induced at day two post-infection. Consequently, there were a decline in type II interferon and IL6 and increase of TGF- β at day 7 post-infection .[150] Besides, Syrian hamsters also transmit infections to other non-infected Syrian hamsters .[150] It was also reported that Syrian hamsters showed similar SARS-CoV-2 pathogenesis and immune responses to humans. These make them suitable animal models for COVID-19 vaccine studies .[149]

Conclusion

There are uncertainties on the extent and time of the decline of the COVID-19 pandemic. Hence, preventive vaccination measures remain the only hope to mitigate the spread of SARS-CoV -2 infections. The various SARS-CoV-2 vaccine development strategies show a promising future as much advances had been made in a minimal time frame. However, there is need for more research funding that will enable an extension of the ongoing clinical trials to all or most parts of the world, in order to ensure unified vaccine trails outcomes, regardless of racial and regional variation. It is highly recommended that comprehensive evaluation of the various vaccine platforms be considered in order to assure their potency, efficacy, safety, and global accessibility.

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Disclosure of potential conflicts of interest

None declared.

Funding

None received

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Contributors' Statement

INA, AUE and HAA conceptualized and planned the study. All the authors conducted a literature search, compilation of data and wrote the first draft manuscript. All authors read and approved the final manuscript for intellectual content before submission. INA is responsible for the overall content as guarantor.

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