Book Chapter

SARS-CoV-2 Variants Associated Challenges in the Ongoing Vaccination of COVID-19

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Abstract

The coronavirus disease of 2019 (COVID-19) is caused by the Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2), which belongs to the *Coronaviridae* family and is a positive-stranded RNA virus. This virus originated in Wuhan city of China and became cause of multiwave pandemic that killed 5,631,457 people worldwide with 364,191,494 confirmed cases of COVID-19 until January 28th, 2022. As per WHO, A total of 9,854,237,363 vaccination doses has been given as of January 27, 2022. The havoc intensified with emergence of SARS-CoV-2 variants (Alpha, Beta, Gamma, Delta, Lambda, Eta, Epsilon, Mu, and Omicron) due to mutation appears during replication in human host. The most promising approach for combating viruses and their emerging variants lies in prophylactic vaccines. Several vaccine candidates are being developed using various platforms, including nucleic acids, live-attenuated-virus, inactivated virus, viral vectors, and protein-based subunit vaccines. In this unprecedented time, ten vaccines against SARS-CoV-2 have been phased out following WHO approval, while 194 are in preclinical stage and 140 are under clinical development process. Many of them are directed to elicit neutralizing antibodies against viral spike protein (S), to inhibit viral entry through ACE-2 receptor of host cells. Inactivated vaccines, in contrary, provide a wide range of viral antigens for immune activation. Being intracellular pathogen, the cytotoxic CD8+ T cell (CTL) response remain crucial for all viruses including SARS-CoV-2 needs to be explored in detail. In this review, we have tried to describe and compare approved

vaccines against SARS-CoV-2. We have discussed immune responses induced by various candidate vaccine, benefits, potential limitations, effectiveness against variants, future challenges, vaccine safety issues and their possible resolutions. Most of the current vaccines developed against SARS-CoV-2 are showing either promising or compromised efficacy against new variants. Multiple antigen-based-vaccines (multivariant-vaccines) should be developed on different platforms to tackle future variants. Alternatively, the recombinant-BCG, containing SARS-CoV-2 multiple antigens, as live-attenuated-vaccine should be explored for long-term-protection. Irrespective of their efficacy, all vaccines are efficient in providing protection from disease severity. It also expected that other variants may emerge to cause additional pandemic waves. We must insist on the vaccine compliance for all age groups and work on vaccine hesitancy globally to achieve herd immunity and to curb this pandemic.

Keywords

SARS CoV-2; COVID-19; Immune Response; Vaccine; Multivariant Vaccines

Introduction

Coronaviruses are named after crown-like spikes present on their outer surfaces. Out of seven known human coronavirus (HCoVs), four viruses, HCoV-229E (alphacoronavirus genus), HCoV-NL63 (alphacoronavirus genus), HCoV-OC43 (betacoronavirus genus) and HCoV-HKU1 (betacoronavirus genus) causes mild upper respiratory tract disease in adults. However, SARS-CoV and Middle East respiratory syndrome coronavirus (MERS-CoV) caused pandemic in 2002 -2003 and 2012, respectively [1,2]. The SARS-CoV-2, a seventh member of the HCoV family, is an etiological agent of the COVID-19 pandemic as announced by WHO on March 11, 2020 [3]. Surprisingly, all pandemic-causing HCoVs, including SARS-CoV-2 belong to betacoronavirus genus and are zoonotic in nature [4,5].

Considering SARS-CoV-2 evolutionary linkages, its genomic structures coincide with other HCoVs in the range of 65.04 % to

82.45 %, with the SARS-CoV showing the highest similarity [6,7]. The SARS-CoV-2 is extremely infectious and transmissible due to its high propensity for attaching to angiotensin-converting enzyme-2 (ACE-2) receptors of the host cells, resulting in its quick spread from epicentre in Wuhan, China, to more than 200 countries worldwide. It can be transmitted directly by contacting an infected person's respiratory droplets or indirectly by coming into contact with anything used or touched by an infected person [8]. COVID-19 symptoms include anything from a simple respiratory infection to severe pneumonia, acute respiratory distress syndrome (ARDS), hypoxia, multiorgan failure, and death. Additionally, lymphopenia was also reported in a meta-analysis study of COVID-19 patients and was linked with a higher mortality, ARDS, severe clinical symptoms, and ICU admission. It is recorded that patients who died from COVID-19 had significantly lower lymphocyte counts than survivors [9]. Because of the similitudes with SARS-CoV the international committee on taxonomy of viruses named the novel virus as SARS-CoV-2 [6,10]. SARS-CoV-2 is a spherical or pleomorphic wrapped particle containing single-stranded positive-sense RNA associated with a nucleoprotein inside a capsid [11]. The envelope provides platform for club-framed glycoprotein projections. Some coronaviruses additionally contain a hemagglutinin-esterase protein (HE). The RNA genome of size around 30 kb encodes 16 nonstructural proteins (Nsp1 to Nsp16) and 4 structural proteins named spike (S), envelope (E), membrane (M) and nucleocapsid (N) (Figure 1A and 1B). Open reading frame (ORF)1ab (265-21555 bp) encodes polyproteins PP1ab (codes for Nsp1 to Nsp16) or shorter protein (PP1a; codes for Nsp1 to Nsp11) depends on a-1 ribosomal frameshift event. The polyproteins are cleaved either by papain-like proteinase protein (PLpro) or main protease (Mpro) to yield 16 nonstructural proteins [12]. Out of 16 nonstructural proteins, the PLpro (Nsp3), 3C-like proteinase (Nsp5, Mpro), RNA-dependent RNA polymerase (Nsp12, RdRp), helicase (Nsp13), endoRNAse (Nsp15), 2'-O-Ribose-Methyltransferase (Nsp16) and other nonstructural proteins show similarity with other coronaviruses. However, the remaining 3’ genome codes for S (~180 kDa glycoprotein), E (9-12 kDa protein), M (23-35 kDa

protein) and RNA binding basic N protein (~45.6 kDa). Apart from structural proteins, the 3'-end also encodes nine accessory proteins, Orf3a, Orf3b, Orf6, Orf7a, Orf7b, Orf8, Orf9b, Orf9c and Orf10, these proteins are involved in viral replication, assembly and egress, they also have potential to serve as antigens [13]. The S protein trimerizes to provide the viral crown structure, making it a highly exposed protein of the virus. Thus, the trimeric S antigen is a major protective antigen that elicits highly potent neutralizing antibodies [14]. Spike protein is 1273 amino acid glycoprotein mainly composed of 2 major subunits S1 (aa 14-685) and S2 (aa 686-1273). The N terminal of S1 subunit there is a signal peptide (SP; aa 1-13), the S1 subunit contain N terminal domain (NTD, aa 14-305) and receptor binding domain (RBD; aa 319-541), the RBD contains RBM (aa 437-508) and a C terminal domain (CTD) (Figure 1). The RBD of S1 subunit binds to ACE2 receptor of host cell. However, the S2 subunit responsible for fusion and entry, have fusion peptide (FP; aa 788-806), heptapeptide repeat sequence-1 (HR-1; aa 912-984), HR-2 (aa 1163-1213), transmembrane domain (TM; aa 1213-1237) and cytoplasmic tail (CP; aa 1237-1273) domains (Figure 1B). Based on the important role of spike protein in viral transmission, most of the vaccines including adenovirus, mRNA and DNA based candidates are generating neutralizing antibodies against spike protein to block the viral entry (Figure 1C) [15]. On the other hand, the inactivated virus provides all the antigens, hence, the immune response developed into vaccinated individuals are not only against spike but against numerous viral antigens as well. Other than S protein, the N, M, non-structural proteins (nsps), and accessory proteins, may also have antigenic potential to serve as candidate vaccine [16]. Infact, promising antigenic determinants (epitopes) in M, N, and S proteins, 4, 8, and 13 epitopes, respectively, have been identified using an extensive immunoinformatics analysis of SARS-CoV-2 proteins.. Among thses, M protein (165–181 and 306–322), N protein (314–330), S protein (817–833, 891–907, 897–913, and 1182–1209) were found to be non-allergenic, non-toxic, and have a low chance of producing autoimmune reactions in 87 percent of the world's population [17]. These antigens could be used for developing effective candidate vaccines against SARS-CoV-2.

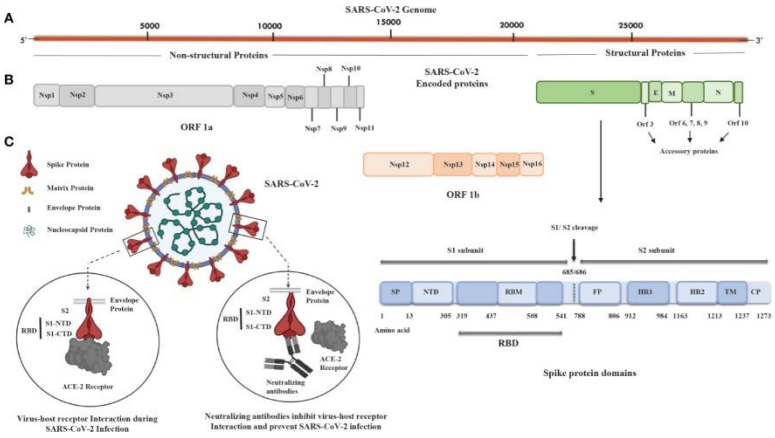

Figure 1: SARS-CoV-2 genome, encoded proteins, and basic mechanism of virus fusion and entry: **A.** Illustration of the SARS-CoV-2 genome that is around 30 kb in size and has a 5' cap and 3' poly A tail. **B**. SARS-CoV-2 Proteins; Non-structural proteins (Nsp), ORF1a and ORF1b (Nsp1-Nsp16) and structural proteins like Spike (S), Envelope (E), Matrix (M) and Nucleocapsid (N), spike protein having 1273 aa (~180 kDa) contain Signal peptide (SP), N-terminal domain (NTD), Receptor binding motif (RBM) in receptor binding domain (RBD), Fusion peptide (FP), Heptad repeat (HR)-1, HR-2, Transmembrane domain (TM) and Cytoplasmic tail (CP) domains. There are accessory proteins Orf3, Orf6,7,8,9 and Orf10 located in between S, E, M and N proteins. **C**. The spike protein subunit 1 (S1; aa 14-685) of SARS-CoV-2 consist of RBD domain divided into two parts; S1 N-terminal domain (S1-NTD) and S1 C-terminal domain (S1-CTD) and spike protein subunit 2 (S2; aa 686-1273) contain TM and CP domains. S1-CTD interacts with Angiotensin-converting enzyme-2 (ACE-2) receptor of host cells and facilitates fusion and entry of virus. The neutralizing antibody against S1-CTD blocks the entry of the SARS CoV-2 into host cells. ORF1a; Open reading frame 1a, ORF1b; Open reading frame 1b, aa; Amino acid.

In response to the outbreak, rapid diagnostics, speedy therapy and vaccine research and development (R&D) is critical to curb the pandemic and preventing new viral outbreaks [18-20]. Many attempt has been carried out on various platform to develop vaccine against SARS-CoV-2 [21]. Around 284 different candidate vaccines against COVID-19 are in development and are in the race to be introduced in the market after their successful phase-III trials with prophylactic and safety data [22]. Due to the great impact on the lives of people and economy by COVID-19, various vaccines got emergency approval after or before phase-III trials (**Table 1, 2**). Currently, various platforms e.g, nucleic acid (RNA/DNA), attenuated live, protein subunit,

viral vector, whole virus inactivated and VLP based vaccines are being used to develop safe and prophylactic vaccine against SARS-CoV-2 (Figure 2A-2G). The vaccines by Pfizer, Moderna, Oxford/AsrtaZeneca (Vaxzevria/Covishield), Bharat Biotech (Covaxin), Gamaleya (sputnik V), Sinopharm and Sinovac are in the market (Table 2) [23-28]. However, the detailed phase-III clinical trial-based vaccine efficacy and safety data remain elusive for some approved vaccines and many candidates that are now under clinical trials (Figure 3 and Table 2).

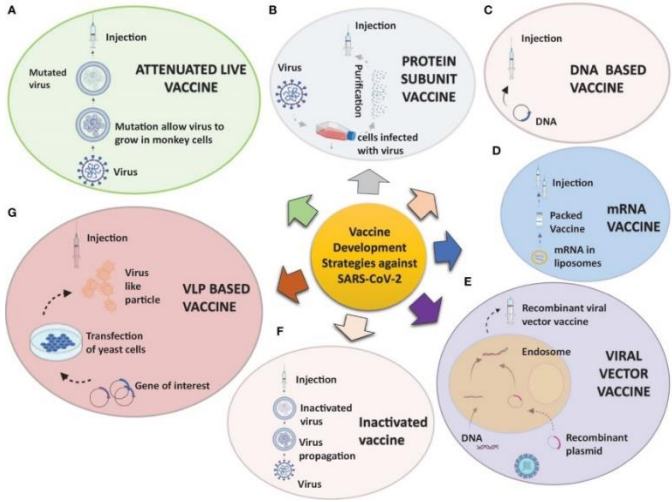

Figure 2: Strategies being utilized to develop vaccine candidates against SARS-CoV-2: A concise overview of various aspects of the SARS-CoV-2 vaccine development process varying from traditional to novel platforms. A. **Attenuated live pathogen vaccine**: A debilitated (infection incompetent) form of live pathogen obtained by lengthy cell culture passaging in non-human cell lines or animals are administered. **B. Protein subunit vaccines:** Are prepared either from antigen purification of pathogens replicated in cell cultures or from recombinant expressed antigens. **C and D. Nucleic acid vaccines**: mRNA (C) or DNA (D) codifying for an immunogenic protein of the pathogen express and present the antigen in antigen presenting cells. The mRNA is mixed with nanoparticles or other stabilizing agents and DNA is inserted in a vector. **E. Viral vector vaccines**: Recombinant viral vectors are produced by genetic manipulation of measles or adenoviral platform to express antigen of interest. **F. Inactivated pathogen vaccines:** It contains whole pathogen that has been subjected to heat or chemical treatment for inactivation. **G. Virus-like particles (VLP) vaccines**: These are virus like particles (20-200 nm) assembled and released by many baculovirus or mammalian expression system e.g. recombinant yeast cells, vaccinia virus expression system or even tobacco plants transfected with tobacco mosaic virus platform.

Table 1: Platform of COVID-19 Vaccine: Platforms being used in development of vaccine against SARS CoV-2, these candidate vaccines are either in pre-clinical or in various clinical phase trial stages and has not yet been licensed or launched by firms [22].

Platform	Type of candidate vaccine	Developer	Clinical phase trial
DNA	INO-4800+electroporation	Inovio Pharmaceuticals + International Vaccine Institute	NCT04642638 Phase II/III
	AG0301-COVID19	AnGes + Takara Bio + Osaka University	NCT04655625 Phase II/III
	nCov vaccine	Zydus Cadila	CTRI/2020/07/026352 Phase III
RNA	ARCT-021	Arcturus Therapeutics	NCT04668339 Phase II
	SARS-CoV-2 mRNA vaccine (ARCoV)	Academy of Military Science (AMS), Walvax Biotechnology and Suzhou Abogen Biosciences	NCT04847102 Phase III
	mRNA-1273.351. A lipid nanoparticle (LNP)-encapsulated mRNA-based vaccine that encodes for a full-length, prefusion stabilized S protein of the SARS-CoV-2 B.1.351 variant.	Moderna + National Institute of Allergy and Infectious Diseases (NIAID)	EUCTR2021-000930-32 Phase II
	CVnCoV Vaccine	CureVac AG	NCT04674189 Phase III
Protein Subunit	Recombinant SARS-CoV-2 vaccine (CHO Cell)	Anhui Zhifei Longcom Biopharmaceutical + Institute of Microbiology, Chinese Academy of Sciences	ChiCTR2000040153(Phase III
	VAT00002: SARS-CoV-2 S protein with adjuvant	Sanofi Pasteur + GSK	PACTR202011523101903 Phase III

	FINLAY-FR-2 anti-SARS-CoV-2 Vaccine (RBD chemically conjugated to tetanus toxoid plus adjuvant)	Instituto Finlay de Vacunas	RPCEC00000354 Phase III
	CIGB-66 (RBD+aluminium hydroxide)	Center for Genetic Engineering and Biotechnology (CIGB)	RPCEC00000359 Phase III
Non-Replicating Viral Vector	GRAd-COV2 (Replication defective Simian Adenovirus (GRAd) encoding S)	ReiThera + Leukocare + Univercells	NCT04791423 Phase II/III
Replicating Viral Vector	DelNS1-2019-nCoV-RBD-OPT1 (Intranasal flu-based-RBD)	University of Hong Kong, Xiamen University and Beijing Wantai Biological Pharmacy	Phase II ChiCTR2000039715
Inactivated	SARS-CoV-2 vaccine (vero cells)	Institute of Medical Biology + Chinese Academy of Medical Sciences	NCT04659239 Phase III
	QazCovid-in® - COVID-19 inactivated vaccine	Research Institute for Biological Safety Problems, Rep of Kazakhstan	NCT04691908 Phase III
	Inactivated SARS-CoV-2 vaccine (Vero cell)	Beijing Minhai Biotechnology Co	NCT04852705 Phase III
	VLA2001	Valneva, National Institute for Health Research, United Kingdom	NCT04864561 Phase III
	COVID-19 inactivated vaccine	Shifa Pharmed Industrial Co	IRCT20201202049567N3 Phase II/III
VLP	Coronavirus-Like Particle COVID-19 (CoVLP)	Medicago Inc.	NCT04636697 Phase II/III

Table 2: List of marketed vaccines against SARS-CoV-2: Vaccines developed by several companies and institution on multiple platforms have been introduced in most of the countries. The vaccine efficacy is influenced by a variety of factors, including the vaccine type, proper packaging, the number of dosages and booster required after a certain time interval, as well as the route of administration. Vaccines with the potential to boost ongoing pandemic response have been introduced in many countries, either on an approved or emergency basis [29-31].

Developer	Vaccine Name	Vaccine Type	SARS-CoV-2 Antigen	Doses and Route	Storage	Efficacy	Status	References
Pfizer/BioNtech	Comirnaty, tozinameran, BNT162b1 BNT162b2	mRNA encapsulated in lipid nanoparticle (LNP)	SARS-CoV-2 RBD and S protein full length in prefusion conformation for BNT162b1 and BNT162b2 respectively	Twice (3 weeks apart), I.M.	-70 °C to -80 °C (6 month)	95%	Emergency use in U.S, E.U. etc, Approved in several countries	[32]
Moderna/ NIAID	mRNA-1273	mRNA encapsulated in lipid nanoparticle (LNP)	Full length S protein with prefusion conformation	Twice (4 weeks apart), I.M.	4 °C (30 days), –20 °C (6 months)	94.5%	Approved in Switzerland. Emergency use in U.S, U.K, E.U, others	[24]
Oxford University/ AstraZeneca	AZD1222/ ChAdOx1 nCoV-19 (Covishield in India)	Attenuated adenoviral vector (non-replicating) from chimpanzee ChAd	full-length codon-optimized S protein	Twice (4 weeks apart), I.M.	2-8 °C	70%	Emergency use in U.K, E.U, India and other countries	[24]
Bharat Biotech/ICMR	Covaxin, BBV152 A, B, C	Whole virion Inactivated SARS-CoV-2 vaccine + adjuvant	Whole virus	Twice (2 weeks apart), I.M.	2-8 °C	81%	Emergency use in India	[33]
Gamaleya	Sputnik V, Gam-Covid-Vac	two non-replicating viral vectors, adenovirus type 5 (rAd5) and adenovirus type 26 (rAd26)	SARS-CoV-2 full-length glycoprotein S	Twice (3 weeks apart), I.M.	-20 °C	92%	Early use in Russia. Emergency use in other countries including India	[34]
Beijing Institute of Biological Products/Sinopharm	BBIBP-CorV	Inactivated SARS-CoV-2 vaccine (Vero cell)	Whole virus	Twice (3 weeks apart), I.M.	2-8 °C	79%	Approved in China, U.A.E, Bahrain. Emergency use in Egypt, other countries	[35]
Sinovac	CoronaVac, PiCoVacc	Inactivated virus	Whole virus	Twice (2 weeks apart), I.M.	2-8 °C	50–91%	Approved in China. Emergency use in Brazil, Singapore, Malaysia, and Philippines	[36]
CanSino/ BIB	Convidecia, Ad5-nCoV	Adenovirus based viral vector (Ad5)- Non-Replicating	SARS-CoV-2 full length S protein	Single, I.M.	2-8 °C	79%	Emergency use in China and Mexico	[35]
Johnson & Johnson	Ad26.COV2. S	Adenovirus based viral vector (Ad26)- Non-Replicating	SARS-CoV-2 full length S protein	Single, I.M.	-20 °C (2 years) 2 to 8 °C (3 months)	76.7–85.4%	Applied for emergency use authorization in US	[37]
Novavax	NVX-CoV2373	S Protein adjuvanted with recombinant	Trimeric SARS-CoV-2 full length S protein	Twice (3 weeks apart), I.M.	2-8 °C (3 months)	89.3% (P-3, UK),	Early use in UK and Australia	[38]

		novavax protein			-20 °C (2 years)	90% (South Africa Trial)		
Vector Institute	EpiVacCorona	Chemically synthesized peptide antigens of SARS-CoV-2 proteins	Peptide Subunit Vaccine	Twice (3 weeks apart), I.M.	2-8 °C (2 years)	100%	Early use in Russia	[39]
WIBP/Sinopharm	WIBP-CorV	Inactivated virus propagated in Vero cells	Whole virus	Twice (3 weeks apart), I.M.	2-8 °C	72.5%	Bahrain, Jordan, Egypt, UAE	[40]

Abbreviations: ICMR, Indian Council of Medical Research; WIBP, Wuhan Institute of Biological Products; BIB, Beijing Institute of Biotechnology; NIAID, National Institute of Allergy and Infectious Diseases; U.S, United State, EU, European Union; U.A.E, United Arab Emirates; U.K, United Kingdom; I.M, Intramuscular, P-3 (Phase-III trial)

To instigate immune response, the protein antigens are achieved either by direct injecting protein subunit vaccines or by expression of genetic material delivered through viral or non-viral vectors into muscle cells (Figure 4A-4B). Apart from antigens, the vaccine preparations also contain low toxic preservatives like 2-phenoxyethanol to prevent vaccine contamination. The stabilizers e.g, Sugars (lactose, sucrose), amino acids (glycine), gelatin or proteins are used to prevent chemical reactions to achieve vaccine stability. Surfactants prevent clumping to keep the vaccine components homogeneous and diluents (mostly distilled water) are used to achieve desired antigen concentration. The aluminium salts like aluminium phosphate, aluminium hydroxide or potassium aluminium sulphate could be used as an adjuvant to enhance immune response. Vaccines may also contain, traces of compounds used during vaccine manufacturing (https://www.who.int/news-room/feature-stories/detail/how-are-vaccines-developed).

Antigens in circulatory system recognized by antibodies on B cells leading to isotype switching and antibody secreting plasma cell formation in germinal centers of secondary lymphoid organs. Some of the antibodies are capable to neutralize virus (neutralizing antibodies), crucial for clinical outcome (Figure 4C-4D). Eventually, memory B cells are generated to onslaught future infections (Figure 4E). The T cells on the other hand recognize antigens presented on (major histocompatibility complex) MHC-I or MHC-II of antigen presenting cells (APCs) to activate $CD8^+$T or $CD4^+$T cells, respectively (Figure 4F). The activated helper T (effector $CD4^+$T) cells secrete Th1 or Th2 cytokines for cell mediated or humoral immune response against pathogen (Figure 4G-4H). However, the activated $CD8^+$T cells develop into cytotoxic T cells responsible for killing of infected cells (Figure 4J-4K). Both CD4 and CD8 T cells develop central memory response (Figure 4I and 4L). Most immunizations require booster doses to strengthen the immunological response induced by vaccines. (Figure 5). A recent study suggests that S, M, and N proteins strongly and nsp3, nsp4, and ORF8 were partially able to activate $CD4^+$ T-cells. On the other hand, the SARS-CoV-2 M and S proteins were strongly recognized by

$CD8^+$ T-cell, and other antigens, such as nsp6, ORF3a, and the N protein also showed significant reactivity [41].

The Wuhan strain of SARS-CoV-2 has undergone various mutations, resulting in B.1.1.7/alpha (UK), B.1.351/beta (South Africa), P.1/gamma (Brazil), B.1.617/delta (India), B.1.429 and B.1.427/epsilon (USA/California), B.1.525/eta (UK, Nigeria), P3/theta (Philippines), B.1.526/lota (USA/New York), B.1.617.1/kappa (India) and C.37/lambda (Peru) lineage (https://www.gisaid.org/hcov19-variants/). The complexity in the immune response elicited during COVID-19 infection and the probability of genomic changes render a huge challenge for the scientific fraternity regarding vaccines in the market and those under development. Cutting edge strategy needs to be adapted to achieve prophylactic vaccine against this dreadful virus. To achieve the aim of limiting the pandemic, we would address the various vaccine types, their components, immune response against various strain, hurdles, and future challenges.

Strategies Utilized in COVID-19 Vaccine Development

There are various strategies being utilized worldwide for vaccine development. An example of some of the forms of SARS-CoV-2 vaccine candidate and vaccine concepts were presented in the figure 2. Apart from emergency authorized vaccines for COVID-19, there are 100 candidate vaccines in the clinical evaluation phase, while 184 vaccines are in the preclinical evaluation stage [22]. These vaccines are being developed in various countries around the globe including United States of America, Germany, Austria, United Kingdom, China, Australia, France, India, and Hong Kong [42]. Development of efficacious and safe vaccines is urgently needed to curb the current pandemic. Vaccine developers must guarantee that people of all age group including with comorbidities such as asthma and diabetes could be the recipient of COVID-19 vaccine, as these categories of patients are particularly at high risk. It is therefore important to determine the level of safety provided by the vaccines, and patients may require more than one dose of the vaccine to preserve continued immunity to the virus. The production of a vaccine against SARS-CoV2 is a challenging task due to many

problems faced during the design phase. A variety of methods including next-generation and traditional approaches are being used to develop vaccine, each one with different advantages and disadvantages (Table 2). The basic mechanism and details of immunological responses induced by different vaccines are shown in detailed in figure 4. Also, the predicted antibody response to first, second and probable booster dose of COVID-19 vaccination were represented in the figure 5. Live attenuated coronavirus vaccines provide the best protection, but their clearance would be hampered by biosafety concerns. In contrast, inactivated coronavirus vaccines have performed well in primate models and up to pre-clinical levels [43]. Since the immune systems of elderly people vary from those of healthy middle-aged adults they sometimes do not respond equally well to immunization. Overall, there is no such thing as "a one-size-fits-all". In general, we hope that adjuvants can aid ongoing vaccination campaigns across the world. Adjuvants are important for eliciting a stronger, long lasting, and broader immune response, especially in people with compromised immune systems. Figure 3A, B, and C shows the number of coronavirus vaccines in the pre-clinical and clinical stages based on the various platforms accessible and in a phase-by-phase approach. Figure 3D summarize the coronavirus vaccine under time it takes from development, preclinical testing, needing it to pass through phases 1, 2, and 3 trials. Few vaccines have been dropped because of their inability to induce a robust immune response, owing to negative side effects.

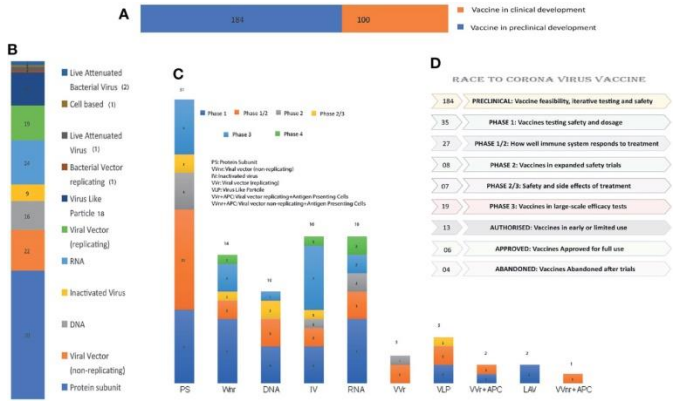

Figure 3: Summary and number distribution of candidate vaccines in preclinical and clinical trials: A. Clinical stages of candidate vaccines: Number of vaccines under clinical (depicted by orange color; 100) and preclinical development (depicted by blue color; 184). B. Candidate vaccines of different platforms under clinical trial from bottom to top: Protein Subunit, VVnr, DNA, Inactivated Virus, RNA, VVr, VLP, BVr, LAV, Cell based and LABv with their respective candidate vaccine numbers. C. Candidate vaccines of various platforms their numbers under clinical phase-1, phase-2 or phase-III: Phase vise distribution along with the numbers in the platform used is marked in the bar graph. D. Summary of various vaccines clinical trial status is presented in the graph: Vaccine production from inception to commercialization is a prolonged process that requires multiple clinical trials, some of which have failed due to adverse effects.

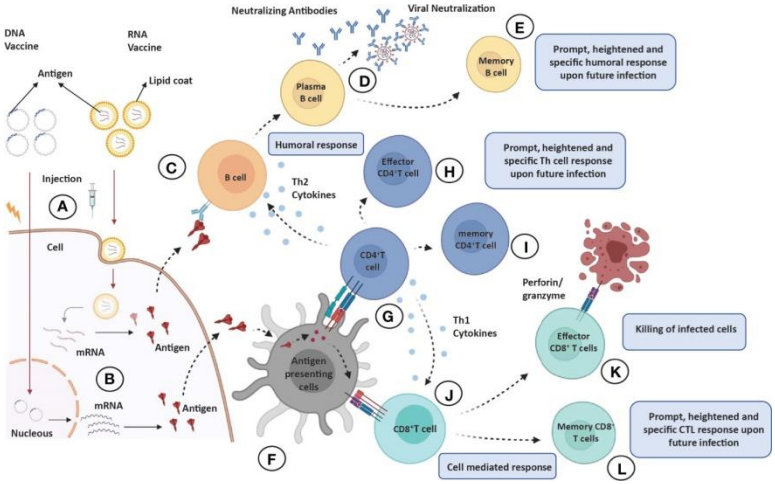

Figure 4: Overview of immune response elicited by various vaccine candidates. When administered into skin or muscle cells (A), the nucleic acid expresses and code for an immunogenic protein which mimics viral infection (B). (C-E) Humoral responses: Antigen produced by skin/muscle cells are released in blood to activate antibody response; antigen recognition by Naïve B cells (C) leading to clonal selection and Plasma cell (antibody secreting B cells) formation (D) and eventually production of long-lasting memory B cells (E). F. Antigen processing and presentation: The antigen produced skin/muscle cells are captured by antigen-presenting cells (APCs; Dendritic cells or Macrophages) for processing and presented by MHC-II or MHC-I molecules on their surface. (G-I) $CD4^+$T helper cells effector and memory response: The presented MHC-II molecule and antigen complex on APCs recognized by TCR and CD4 molecules on $CD4^+$ Th cells (G) leading to production of effector CD4+ Th cells (H) which produces sufficient level of cytokines (Th2 cells produce TH2 cytokines IL-4 or IL-10 for humoral response and Th1 cells produce IL-12, IFN-Y for CTL response) and eventually long-lasting memory CD4+ cells are generated (I). (J-L) $CD8^+$T helper cells effector and memory response: The presented antigen and MHC-II molecule complex on APCs recognized by TCR and CD8 molecules on $CD8^+$ T cells (J) leading to production of effector $CD8^+$T cells also known as Cytotoxic T lymphocytes (CTL) which is responsible for killing the infected or self-altered cells (K) and finally long-lasting memory $CD8^+$T cells are generated (L). All memory cells provide long lasting, heightened and antigen specific responses upon future infections.

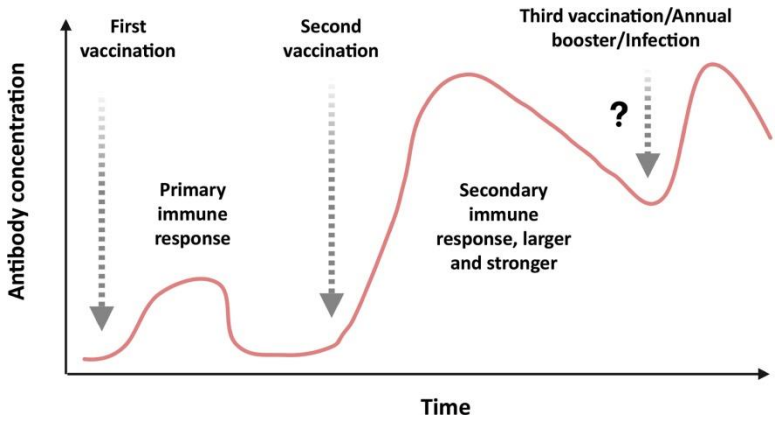

Figure 5: Antibody response to first, second, probable third/annual booster dose of COVID-19 vaccination or infection: Initially, just after first immunization the vaccine does not elicit sufficient neutralizing antibody to prevent the infection of SARS-CoV-2, upon administration of second booster dose of vaccine the vaccine elicits stronger neutralizing antibody response with high titre that could prevent the infection efficiently. In some cases, third booster or annual boosters are required to revive the immune response against pathogen.

Inactivated Vaccine

Various vaccines for viral and bacterial diseases including Pertussis, Rabies, Hepatitis A, Influenza, Polio, and Japanese Encephalitis are based on inactivated form of the vaccines. Usually, they do not provide protection as strong as live vaccines and require booster shots. Sixteen inactivated vaccines of SARS-CoV-2 are in advance clinical trial. The Bharat Biotech Covaxin (BBV152) is an inactivated COVID-19 vaccine being used in India. These vaccines require a BSL-3 facility for large-scale virus propagation, rendering this platform the most time-consuming and difficult. However, the benefit of using this platform is multiple antigen-based vaccine which remains helpful in any viral mutation on single protein. These inactivated vaccine leads to strong neutralizing antibody response and gives more potent $CD4^+$ T_h1 cell response as compared to $CD8^+$ T cells.

Live Attenuated Vaccine

Live vaccines are made from attenuated or weakened forms of the pathogen to stimulate strong and long-lasting neutralizing antibody response along with both $CD4^+$ T helper and $CD8^+$ T cell mediated immunity by mimicking natural infection. However, extensive safety studies are required and could not be given to immunocompromised individuals. Currently, live attenuated vaccines are being used for rotavirus, chickenpox, yellow fever and measles, mumps, rubella (MMR). There are several benefits of a live-attenuated vaccine, including mounting an immune response to multiple virus antigens and the capacity to scale for mass production. The COVI-VAC, currently, in clinical phase-I trial is a single dose intranasal, live attenuated vaccine generated using Codagenix's proprietary deoptimization technology in collaboration with Serum Institute of India.

Viral Vector

Viral vector vaccines make use of modified version of pathogenic virus as a vector to provide immunity. Scientists all over the world are attempting to produce a COVID-19 vaccine dependent on replicating viral vector, such as damaged measles, whilst the others are focusing on non-replicating viral vectors, such as adenovirus.

Replicating

Three candidates from replicating viral vector platform and 2 based on replicating viral vector along with artificial antigen presenting cell (APC) has reached in clinical phase. When cells are infected with the replicating viral vector vaccine, it not only produces vaccine antigen but also produces more viral particles that infect another cell. DelNS1-2019-nCoV-RBD-OPT1 is an intranasal spray vaccine based on H1N1 Influenza A virus. The dose is given twice 28 days apart. It is currently evaluated in phase II clinical trial. A vaccine candidate developed by Israel Institute for Biomedical Research make use of recombinant vesicular stomatitis virus (rVSV), where VSV-G protein is replaced with the SARS-CoV-2 S protein, creating a

recombinant replicating virus. The candidate has reached Phase I/II clinical trial for safety and efficacy evaluation.

Non-Replicating

Non-Replicating viral vaccines not able to produce new viral particles and are only involved in production of vaccine antigen. Fifteen non replicating viral vector and 1 with artificial antigen presenting cell is being evaluated in human clinical trial. CanSino Biologics and the Beijing Biotechnology Institute (BIB) developed the novel coronavirus vaccine (Adenovirus Type 5 Vector) candidate (Ad5-nCoV) which has now been authorized and made accessible as a marketed vaccine. The vaccine candidate is based on the adenovirus-based viral vector vaccine technology framework of CanSinoBIO, which was previously used to successfully produce the globally groundbreaking Ebola virus infection vaccine [44]. The neutralizing antibody response mediated by such vaccine platforms is determined by pre-existing antivector immunity with enhanced $CD4^+$ Th1 cell response and potent $CD8^+$ T cells response.

DNA based Vaccine

DNA based vaccine also known as third generation vaccine contains DNA encoding specific proteins or antigen of pathogen. Ten DNA vaccine candidates are in human clinical trials while 16 are in pre-clinical trials. The proteins encoded by the DNA are translated into host cells which are identified as a foreign material by host immune system thus inducing an immune response. INOVIA pharmaceuticals, a biotechnology company is currently focused on developing DNA vaccine, which is in Phase 2/3 clinical testing in the U.S. for COVID-19. Using INOVIO's patented DNA medicine platform, INO-4800 was developed quickly after the genetic sequence of the coronavirus causing COVID-19 was released. DNA-based vaccine that can be administered by a nasal spray is being produced by researchers at the University of Waterloo, Ontario, Canada. The vaccine will operate using engineered bacteriophage, a process that will enable the vaccine to activate the immune response in the lower respiratory tract of the nasal cavity and target tissues [45]. DNA-

based vaccines provide sufficient neutralizing antibodies response along with CD4+ Th1 cell response. However, CD8+ T-cell response is less robust [46].

RNA based Formulation

New technology based on RNA where mRNA encoding pathogen's proteins or antigens are used to elicit an immune response. Sixteen vaccine candidates based on mRNA formulation are being tested in clinical phase. The mRNA-1273 is a novel lipid nanoparticle (LNP)-encapsulated mRNA-based vaccine that encodes for a full-length, prefusion stabilized spike (S) protein of SARS-CoV-2. The candidates for the vaccine include nucleoside modified mRNA (modRNA), mRNA-containing uridine (uRNA), and self-amplified mRNA (saRNA). A lipid nanoparticle (LNP) formulation is combined with any mRNA format. These vaccine candidates for COVID-19 either contain the larger spike sequence or the smaller optimized receptor binding domain (RBD) of the spike protein. The vaccines based on mRNA give adequate neutralizing antibody response while the T_h1 and T_h2 cell response depends on the adjuvant used.

Protein Subunit Vaccine

Protein subunit vaccine uses protein fragments of pathogen to elicit immune response instead of introducing whole pathogen. Currently, it is being used for hepatitis B, human papillioma virus (HPV), whooping cough, pneumococcal disease, meningococcal disease and shingles. These vaccines provide strong immune response against a particular antigen of pathogen and is safe for all recipients including individuals with weakened immune system. Thirty-one such candidates are assessed in human clinical trial before being available to general population. While some researchers want to inject coronavirus proteins directly into the bloodstream, it's also conceivable to employ protein fragments or protein shells that look like the coronavirus's outer coat. Scientists and researchers are developing vaccines for viral protein subunits, the majority of which focus on the virus's spike protein, or a central portion

known as the receptor binding domain (RBD). ExpreS2ion, a biotech company uses its clinically validated Drosophila S2 insect cell expression system, ExpreS2, to produce SARS-CoV-2 viral antigens in the clinically validated cell lines. The goal is to generate and test vaccine antigens in mice to demonstrate immunogenicity and efficacy in vitro. The vaccine is in pre-clinical trials now. Protein subunit vaccines are well recognized for eliciting a strong neutralizing antibody response, however the Th1 and Th2 cell response is influenced by the adjuvant employed.

Virus Like Particle Vaccine

Virus-like particles (VLPs) are protein-based vaccines that stimulate high immune responses due to VLPs repetitive structures. These molecules mimic viruses but does not contain any viral genetic material and are not infectious. They are a very effective way of creating vaccines against diseases such as human papillomavirus (HPV), hepatitis B, malaria, and many more. There are 5 VLP based vaccine candidates currently being evaluated in human clinical trial against SARS-CoV-2. RBD SARS-CoV-2 HBsAg VLP vaccine, is a subunit vaccine which uses RBD in SARS-CoV-2 S protein conjugated with the hepatitis B surface antigen to stimulate the immune system to produce anti-RBD antibody. The Central Committee on Research Involving Human Subjects (CCMO) in the Netherlands has approved ABNCoV2 capsid virus-like particle (cVLP) based COVID-19 vaccine developed by AdaptVac, a PREVENT-nCoV consortium member. The candidate vaccine is in the Phase I/II study.

Approved COVID-19 Vaccine

Several COVID-19 vaccines have shown greater than 90 percent efficacy in preventing COVID-19 infections. A handful of vaccinations are presently authorized by both the national regulatory body and the World Health Organization. Some vaccine are in the process of being approved by WHO [47].

Pfizer-BioNTech (BNT162b1 and BNT162b2)

Very first time the mRNA has been used as vaccine platform initially established by Weissman group to develop zika virus vaccine [48]. Pfizer-BioNTech developed BNT162b1 and BNT162b2 vaccines against SARS-CoV-2. BNT162b1 is SARS-CoV-2 spike protein RBD encoding modified messenger RNA (mRNA) incorporating 1-methyl-pseudourine which dampens innate immune sensing to induce mRNA translation. The RBD antigen is modified by addition of a T4 fibritin derived "foldon" trimerization domain to increase its immunogenicity by multivalent display [49]. The other candidate, BNT162b2, encodes the full-length SARS-CoV-2 spike protein with two proline changes that lock it in the prefusion conformation and make it more closely mimic the intact virus to elicit prominent virus-neutralizing antibodies. Apart from mRNA (30 μg), the Pfizer-BioNTech vaccine includes lipids and cholesterol, potassium chloride, monobasic potassium phosphate, sodium chloride, dibasic sodium phosphate dihydrate and sucrose. Storage is -70 to -80 degrees Celsius, later, as an alternative, -25ºC to -15ºC was advised for two weeks [32]. The European Medicines Agency (EMA) as a National Regulatory Authority (NRA) approved it and later it was approved by WHO after evaluating the data on clinical trials. (www.pfizer.com).

Moderna (mRNA1273)

The Moderna COVID-19 (mRNA1273) Vaccine is a sterile, preservative-free frozen suspension for intramuscular injection comprised of following ingredients: mRNA, lipids (SM-102, 1,2-dimyristoyl-rac-glycero tromethamine, tromethamine hydrochloride, acetic acid, sodium acetate, and sucrose), PEG2000-DMG, cholesterol, and 1,2-distearoyl-snglycero-3-phosphocholine [DSPC] [50] (www.modernatx.com). Moderna vaccine currently is licensed by EMA as well as WHO based on experimental evidence. It is a SARS-CoV-2 glycoprotein S-2P, with a transmembrane anchor and an intact S1-S2 cleavage site, antigen encoding mRNA-based vaccine. The S2-P is stabilized in its prefusion conformation by 986 and 987 position proline substitutions at the top of the central helix in S2 subunit. The

lipid nanoparticle capsule composed of four lipids was formulated in a fixed ratio of mRNA and lipid. The dose is given intramuscularly in two doses 4 weeks apart. It can be stored in refrigerators for 30 days and at -20 ºC for 6 months. Primary efficacy analysis of the Phase-III COVE study of mRNA-1273 involved 30,000 participants including 196 cases of COVID-19, of which 30 cases were severe. Vaccine efficacy against COVID-19 was 94.1% while vaccine efficacy against severe COVID-19 was 100%. mRNA-1273 continues to be generally well tolerated with no serious safety concerns identified to date [51].

Covaxin

Bharat Biotech's Covaxin gained certification from the Drugs Controller General of India (DCGI) as a National Regulatory Authority (NRA), and it is currently under consideration of WHO for possible approval. This vaccine is developed by Bharat Biotech, India using a coronavirus sample isolated from an asymptomatic patient (Strain: NIV-2020-770), by National Institute of Virology, India [33]. It falls under inactivated whole virion vaccine category that uses adjuvant Alhydroxiquim-II to boost immune response for long-lasting immunity. It contains 6µg of whole-virion inactivated SARS-CoV-2 antigen and 250µg aluminium hydroxide gel, 15µg TLR 7/8 agonist (imidazoquinolinone), 2.5mg TM 2-phenoxyethanol, and phosphate buffer saline up to 0.5 ml (https://www.bharatbiotech.com). The Phase-I and Phase-II clinical trials were conducted on 800 subjects and the result demonstrated that vaccine is safe and induced a robust immune response. The Phase-III study enrolled 25,800 participants between 18-98 years of age, including 10% over the age of 60, with analysis conducted 14 days post 2nd dose. According to phase-III analysis of COVAXIN on 127 symptomatic cases, the vaccine showed a point estimate of 81% vaccine efficacy against mild and moderate COVID-19 disease. The efficacy against severe COVID-19 disease was 100%, with an impact on reduction in hospitalizations. The efficacy against asymptomatic COVID-19 infection was 70%, suggesting decreased transmission in COVAXIN recipients [52].

Oxford-AstraZeneca (AZD1222)

The chimpanzees-adenovirus vectored vaccine containing gene for expressing spike protein ChAdOx1-nCoV-19 vaccine (AZD1222) is developed by Oxford-AstraZeneca and manufactured by the Serum Institute of India. The vaccine is administered in two separate doses of 0.5 ml each given between four to twelve weeks apart and can be stored at temperature 2 °C to 8 °C. In India, this vaccine is named as Covishield that includes L-histidine, L-Histidine hydrochloride monohydrate, Polysorbate 80, Ethanol, Sucrose, Sodium chloride, Disodium edetate dihydride (EDTA) and water for infection. Initially the booster dose was given 4 weeks post immunization, however, due to shortage of vaccine and published date sets allowed the booster dose could be administered 6 to 8 weeks apart. Further studies are required to adapt the best immunization policy for this vaccine. (www.astrazeneca.com). Analysis data from four ongoing blinded, randomized, controlled trials done across the UK, Brazil, and South Africa showed an overall efficacy of 70.4%. 11,636 participants (7548 in the UK, 4088 in Brazil) were included in the interim primary efficacy analysis. Participants aged 18 years and older were randomly assigned (1:1) to ChAdOx1 nCoV-19 vaccine or control [53].

Sputnik V by Gamaleya (Gam-Covid-Vac)

Also known as Gam-Covid-Vac, developed by the Gamaleya Research Institute, part of Russia's Ministry of Health. It is an adenoviral based vaccine (dual viral vector) that uses weakened virus which in turn deliver small parts of pathogen ultimately stimulating an immune response. Gene encoding S-protein of SARS-CoV-2 carried via rAd26 vector (1st dose) that provides humoral and cellular immunity and rAd5 vector (2nd dose) induces formation of memory cells. The active components are a modified replication-defective adenovirus of a different serotype modified to include the protein S-expressing gene of SARS-CoV-2. The ingredients include Tris-(hydroxymethyl)-aminomethane, Sodium chloride, Sucrose, Magnesium chloride hexahydrate, Disodium EDTA dihydrate, Polysorbate 80, Ethanol, and Water. The interim report of the phase-III data

including results for more than 20 000 participants, aged 18 years and older, 75% of whom were assigned to receive the vaccine, and the follow-up for adverse events and infection. No serious adverse events considered related to the vaccine were recorded and vaccine efficacy, based on the numbers of confirmed COVID-19 cases from 21 days after the first dose of vaccine, was reported as92% [54] (https://sputnikvaccine.com).

Sinovac (CoronaVac)

CoronaVac vaccine developed by Sinovac using inactive SARS-CoV-2 virus (CZ02 strain) along with Aluminium hydroxide, disodium hydrogen phosphate dodecahydrate, disodium hydrogen phosphate monohydrate, sodium chloride as adjuvant and stabilizing agents. Two doses of vaccine about 14 to 28 days apart are administered. One main advantage associated with vaccine developed by Sinovac is that it can be stored at 2-8 ^{o}C. China has approved Sinovac vaccine for emergency use since July 2020 through the national medical product and administration (NMPA) approval and later it got approval by WHO. Other countries like Indonesia, Turkey, Brazil, Chile have also authorized emergency use of this vaccine (http://www.sinovac.com). The phase-III trials conducted in Brazil and Turkey evaluated the efficacy of the vaccine candidate in healthcare workers who provide treatment to COVID-19 patients. Both trial studies were randomized, double-blind, and placebo-controlled. There were 12,396 health workers over 18 years old enrolled. A total of 253 positive cases were collected during the observation period. After 14 days following vaccination with 2 doses of vaccine following a 0, 14-day schedule, the efficacy rate against diseases caused by COVID-19 was 50.65% for all cases, 83.70% for cases requiring medical treatment, and 100.00% for hospitalized, severe, and fatal cases [55].

Sinopharm (BBIBP-CorV)

China has approved Sinopharm multivariant vaccine (Sinopharm's BBIBP-CorV). Which is an WHO approved inactivated virus vaccine. Of the three coronavirus variants

obtained from patients in China, the variant which multiplied the most quickly in monkey kidney cells was chosen and was inactivated using chemical beta-propiolactone. It was then treated with aluminium based adjuvant to increase its immunogenicity. Unlike the moderna and Pfizer vaccine, this vaccine is easy to transport and can be stored at 2-8 ºC [35]. Being multivariant, the immune response induced by BBIBP-CorV vaccine could not be impacted by mutation in virus. (http://www.sinopharm.com). According to China National Biotec Group Company (CNBG) study, more than 40,000 people in the United Arab Emirates and Bahrain aged 18 and above without a known history of COVID-19 participated in the trials. The vaccine showed efficacy rate of 79% against symptomatic COVID-19 cases, with rare serious adverse effects reported [56].

CanSino (Convidecia)

CanSino vaccine from China is approved by the national medical product and administration (NMPA), China, and is under the process to get approval from WHO. The vaccine developed by CanSino, Ad5-nCoV is a non-replicating human adenoviral (Ad5) based vaccine which expresses full length spike glycoprotein of coronavirus. It was originally tested in mice and ferrets and was the first vaccine to enter human trials in March 2020. The phase III trial NCT04540419 with 500 participants determined a single intramuscular shot of 5×10^{10} virus particles proved to be well tolerable and immunogenic [26]. Mexico became the first country to give emergency use approval to this vaccine developed by CanSino Biologics and People's Liberation Army scientists (http://www.cansinotech.com). CanSino Biologics conducted its Phase III trials in Argentina, Chile, Mexico, Pakistan, Russia, and Saudi Arabia with 40,000 participants. the company announced the interim analysis data of phase III clinical trial of Convidecia shows that the vaccine candidate has overall efficacy of 65.28% at preventing all symptomatic COVID-19 disease 28 days after single-dose vaccination and efficacy of 90.07% at preventing severe disease 28 days after single-dose vaccination (https://www.precisionvaccinations.com/vaccines/convidicea-vaccine).

Janssen (Janssen COVID-19 Vaccine)

Janssen got licensed from European Medicine agencies (EMA) and approved by WHO. Vaccine developed by the Janssen and Prevention BV subsidiary of Johnson and Johnson, the Ad26.CoV.S is based on human adenovirus Ad26 and expresses the full length spike protein [27]. Vaccine contains genetically modified organisms (GMOs) and ethanol derived from corn or vegetables (https://www.janssenmd.com). The vaccine can remain stable for two years at -20°C, and a maximum of three months at temperatures of 2 to 8°C. The phase-III trial was conducted across eight different countries. U.S. Food and Drug Administration (FDA) has issued Emergency Use Authorization (EUA) to this vaccine in individuals 18 years of age and older. In an international, randomized, double-blind, placebo-controlled, phase-III trial, Johnson & Johnson randomly assigned adult participants in a 1:1 ratio to receive a single dose of Ad26.COV2.S (5×10^{10} viral particles) or placebo. In the per-protocol at-risk population, 468 centrally confirmed cases of symptomatic Covid-19 with an onset at least 14 days after administration were observed, of which 464 were moderate to severe–critical (116 cases in the vaccine group vs. 348 in the placebo group), which indicated vaccine efficacy of 66.9% [57].

Novavax (NVX-CoV2373)

Novavax has been licensed by the European Medicines Agency (EMA) and is now being reviewed by the World Health Organization (WHO). The vaccine developed by Novavax is protein subunit-based vaccine developed by using SARS-CoV-2 spike protein subunit in its glycosylated form. The vaccine can be stored stably at 2-8 ^{o}C and requires 2 doses three weeks apart. To increase the immune response, it is mixed with Novavax' patented saponin-based Matrix-M adjuvant. (https://www.novavax.com). In the 15,000-subject U.K. phase-III clinical trial (NCT04583995), Novavax saw 56 cases of COVID-19 in the placebo arm and six cases in the NVX-CoV2373 group at the interim analysis, resulting in an overall efficacy of 89.3%. All the cases in the vaccine cohort were mild or moderate. Twenty-seven percent of subjects were aged over

65 years. The company said that 32 of the COVID-19 cases were infected with the B.1.1.7 variant. A post hoc analysis put the efficacy against B.1.1.7 at 85.6%, compared to an efficacy of 95.6% versus older variants. The Novavax clinical trials represent the first major controlled clinical tests of how a COVID-19 vaccine performs against the B.1.1.7 and B.1.351 variants. Overall, the results suggest NVX-CoV2373 may be as effective as any prophylactic studied to date against older variants [58].

Vector Institute (EpiVac Corona)

EpiVac corona got approval from Russian National Regulatory Authority (NRA) and currently in the process to get approval by WHO. The EpiVac corona vaccine uses chemically synthesized peptide antigens of SARS-CoV-2 along with aluminium hydroxide as adjuvant. The vaccine is administered intramuscularly twice 21-28 days apart [39]. The Phase I and II studies tested the safety, side-effects and immunogenicity of the potential vaccine in 100 people aged 18-60, according to the state trials register. The immunological effectiveness of the EpiVacCorona vaccine was found out to be 100% [59].

WIBP/Sinopharm (WIBP-CorV)

Another vaccine by Sinopharm used a whole inactivated virus. The double-blind, randomized, phase-III trial designed by the Wuhan Institute of Biological Products Co, Ltd. and the Beijing Institute of Biological Products Co, Ltd. on 3469 participants including adults of 18 years and older age without prior known history of SARS-CoV, SARS-CoV-2, or Middle East respiratory syndrome infection (via on-site inquiry) were enrolled showed an efficacy rate of 72.8% against symptomatic COVID-19 cases [56].

Challenges in Vaccine Design

Various precedented and unprecedented hurdles encountered by designing and manufacturing agencies to develop an effective and prophylactic vaccine against novel viruses. Moreover, for a

new vaccine candidate it takes 10-15 years to be introduced for public use post rigorous and cumbersome clinical trials. Furthermore, most companies are unwilling to engage in vaccine production because vaccine profit margins are incompatible with those for drug development. Here, we have discussed various challenges in SARS-CoV-2 vaccine development and proposed possible solutions for respective problems (Figure 6).

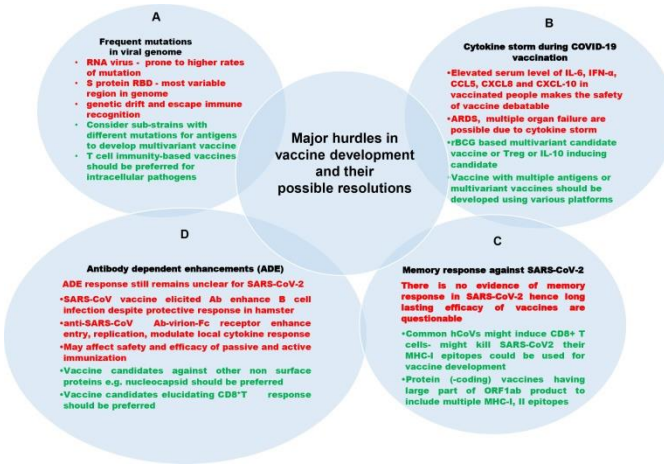

Figure 6: Challenges in developing vaccines against SARS-CoV-2 (Black color text) and their possible resolutions (Green color text). A. Mutation in viral genome: The RNA viruses including SARS-CoV-2 are more prone to mutation in their genome leading to viral immune evasion. B. Cytokine storm during COVID-19 vaccination: Occasionally the vaccination may lead to cytokine storm and other complications raised by it. C. Lack of memory response: Any vaccine provides long lasting efficacy only because of memory responses of adaptive immunity. No vaccine against SARS-CoV-2 is documented to have established memory response post vaccination. The neutralizing antibody fades away few months post vaccination. D. Antibody-dependent enhancements: There is possibility of antibody mediated increase in infection called antibody dependent enhancement.

Mutation

The novel coronavirus, like other RNA viruses, evolves by multiplying genetic material with higher rates of mutation [60,61] resulting in greater genetic variation over time. Additionally, its capacity to shift genetic material from human to human and mutate in the human body allows it to be more

transmissible [62]. The rise of these variants may impact vaccine development and therapies as they spread throughout the population all over the world (Table 3) within a short span of time. Out of three types of possible mutations; base deletions, insertions, and substitutions, SARS-CoV-2 have shown deletion and substitution mutations. Missense mutations account for 34.3 percent, nonsense mutations for 6.7 percent, and silent mutations for 0.8 percent of SARS-CoV-2 genome [63]. Researchers found that highly variable region of the spike protein in circulating viruses, accounting for the top 10% of entropy (rate of mutation). The change in the amino acid in the antigenic part may hampers the neutralizing antibody functionality and could affect the degree of protection by vaccines. Importantly, to prevent the derailing of promising vaccines or antibody-based prophylactics or treatments, it is critical to understand how and whether SARS-CoV-2 may evolve to evade antibody-dependent immunity. Intriguingly, the most variable region in the coronavirus genome is the RBD of the spike protein [64]. Greaney et al, have performed mutational scanning to detect the possible mutations in RBD of spike protein that could affect the neutralizing antibody functionality [65]. It is therefore imperative to consider various clan, variants and sub-strains with different mutations to reduce the possibilities that will impact vaccine potential. The N439K mutation in RBD, is second most common mutation in RBD and sixth most common mutation in spike, it increases binding affinity of spike with ACE-II by two-fold because of salt bridge formed at the binding interface with a positively charged amino acid. This has been observed in over 30 countries. The variant was first identified in March 2020 in Scotland, and as of January 2021, a second lineage B.1.258 has independently emerged in other European countries (Table 3). The N439K mutation did not change the clinical spectrum of disease, however, *in vivo* studies, the viral loads were increased in compared to wild type viruses with N439 residue. Also, D614G is one of the most prevalent non-synonymous mutation of spike protein that dominate the global pandemic (Table 3). The D614G mutation, like the N439K mutation, has been shown to enhance viral infectivity in vitro and enhance viral fusion with the ACE2 receptor, but there has been no evidence of a connection between this mutation with disease severity [66-68].

Table 3: Major SARS-CoV-2 variants: Scientists are looking into how effective a vaccination might be in protecting persons infected with SARS-CoV-2 variants. The table displays the most common variants, countries first detected in countries, newly identified mutations in spike protein, and the risks they bring to public health [69].

Type	First detected in country	Variation in spike	Risk and impact on vaccine efficacy	References
VOC 202012/01 GRY (B.1.1.7)	U.K.	N501Y 144Y del 69/70 deletion P681H, D614G	Increased risk of death compared with other variants. ~50% increased transmission, Minimal impact on neutralization by convalescent and post-vaccination sera	[70-81]
VOC GH/501Y.V2 (B.1.351)	South Africa	Shares some mutations with B.1.1.7 K417N, E484K N501Y, D614G	No evidence to suggest that this variant has any impact on disease severity or impact on neutralizing antibodies.	[73,82-84]
VOC GR/501Y.V3 (P.1)	Brazil, Japan	17 unique mutations, K417T, E484K, and N501Y	The emergence of this variant raises concerns of a potential increase in transmissibility or propensity for SARS-CoV-2 re-infection of individuals.	[72,85,86]
VUI G/484K.V3 (B.1.525)	UK, Nigeria	E484K Q677H F888L	E484K mutation associated with potential immune escape.	[87]
VOC G/452R.V3 (B.1.617+)	India	L452R, E484Q, D614G	Slightly reduced neutralization by post-vaccination sera	[88,89]
VUI GH/452R.V1 (B.1.429)	USA	S13I, W152C, L452R, D614G	~20% increased transmissibility, Reduced neutralization by convalescent and post-vaccination sera	[90]

Global Variants of SARS-CoV-2

Various mutations, leading to rapidly spreading SARS-CoV-2 variants in the United Kingdom (B.1.1.7), South Africa (B.1.351), India (B.1.617), Brazil (P1), USA (B1.429) and UK/Nigeria (B.1.525) strains, propagate even more rapidly which could contribute to more cases of COVID-19 (Table 3). It is obvious that rise in the number of cases would put pressure on health care infrastructure, and may lead to more fatalities. Current research indicates that, antibodies produced by vaccines administered to indigenous people respond to these variants, however, more evidence and research is required to confirm this. In various studies the mutations or their combinations showed diminished neutralizing activity of serum from mRNA vaccinated people [91-93].

In addition, there is always a possibility that these mutations may hamper the therapeutic antibody functions and therefore impact the efficacy of the vaccines. Furthermore, a human infected with one strain is more likely to become immune to another variant or strain, and if it failed to do so, then it clearly necessitates the development of new vaccination strategies to produce preventive vaccines that can combat the risk of many of these variants.

Antibody dependent Enhancements (ADE)

Virus-specific antibodies control infections by neutralizing the viruses. However, in antibody-dependent enhancement (ADE) the antibodies may be helpful to the virus by facilitating its entry into the cells through Fc receptors. Some vaccines against Dengue and Zika viruses have been reported with ADE effects [94]. Previously, it has been shown that neutralizing antibodies against RBD of the MERS-CoV and SARS-CoV, facilitated the viruses entry into Fc receptor-expressing human cells *in vitro* [95]. The incidence of SARS-CoV-2 ADE has not yet proven [95-97]. An alternative target to overcome ADE may be the non-surface proteins like nucleocapsid (N) protein. Since N protein is not on virus surface, its antibodies cannot promote virus entry. Despite defensive responses in the hamster model, antibodies elicited by the SARS-CoV vaccine strengthened infection of B

cell lines [98]. On the other hand, anti-nucleocapsid antibodies, neither neutralized infection nor induced ADE. Therefore, the interaction with Fc receptors of virion-complexed anti-SARS-CoV antibodies can result in both increased viral cell entry and replication and clinically impactful regulation of the local cytokine response. ADE infection has become a major concern for the prevention of diseases by vaccination. ADE response studies should be included for all candidate vaccine as major safety parameter for approval of vaccine.

Immune Evasion by Coronavirus

Studying the immune responses to SARS-CoV-2 and its approaches to immunoevasion will enhance our understanding of pathogenesis, viral clearance, and lead to the development and assessment of vaccines and immune therapeutics. The subsequent immune reactions, immunopathology, and processes of immune evasion are under evaluated. SARS-CoV-2 has developed various mechanism to avoid innate immune detection like many other viruses, including low levels of cytosine-phosphate-guanosine (CpG) in the genome, glycosylation to shield the interacting receptor to the host, RNA shielding and viral protein generation that effectively hinder a virus. These mechanisms together allow efficient infection and increased viral load. Innate immune evasion is enabled by cap-methylation of viral RNA as well as inhibition of steps in the IFN form I/III pathways are included in such evasion. SARS-CoV-2 has established the most severe cytosine-phosphate-guanosine (CpG) deficiency of all betacoronaviruses in accordance with other viruses [99], thus preventing ZAP action. The processing of capping the 5' end is another technique for defending mRNA used by the host and several viruses. Capping restricts degradation and greatly inhibits identification by cytosolic pattern recognition receptors (PPRs) for both host and virus RNA. The post translational modifications of structural and non-structural proteins alter the target epitope leading to compromised vaccine efficacy [100]. Importantly, RNA viruses including SARS-CoV-2 go through frequent mutation to have multiple variant that leads to immune evasion by viruses. This immune evasion gives hard time to develop efficient vaccine,

especially in case of variants, but, allows the development of multiple epitope-based vaccine candidates, so, in case one epitope does not respond the other could take over and eventually enhance the vaccine efficacy.

Cytokine Storm in COVID-19 Vaccination

Cytokine storm, a deadly unregulated systemic inflammatory response resulting from the release of large amounts of pro-inflammatory cytokines (IFN-α, IFN-γ, IL-1β, IL-6, IL-12, IL-18, IL-33, TNF- α etc.) and chemokines (CCL2, CCL3, CCL5, CXCL8, CXCL9, CXCL10, etc.) by immune effector cells in response to SARS-CoV-2 infection. The uncontrolled cytokine response, is one of the main mechanisms for ARDS, responsible for mortality in COVID-19 patients [101,102]. Similar ARDS like immunopathology was observed in SARS-CoV and MERS-CoV [101]. Moreover, individuals with serious MERS-CoV infection display elevated serum levels of IL-6, IFN-α, and CCL-5, CXCL-8, CXCL-10 compared to those with mild-moderate disease, comparable to those with SARS-CoV [103]. In extreme cases of SARS-CoV-2 infection, as is the case with SARS-CoV and MERS CoV infections, the cytokine storm forces the immune system to attack the body aggressively, causing ARDS and multiple organ failure, and eventually death [104]. The vaccines should be able to suppress cytokine storm or at least hamper its deleterious impact e.g. the IL-10 a Th-2 cytokine could be helpful in controlling cytokine storm [105].

Most of the candidate vaccines for SARS-CoV-2 aim to boost immune system as maximum as possible to induce strong immunization. This may lead to uncontrolled cytokine storm among some of vaccinated individuals, which may lead to ARDS, one of the most common cause of death in COVID-19 patients. Vaccination in 33 African green monkeys and 200 mice induced protective immunity against SARS-Cov-2. Approximately 7% monkeys along 5% experimental mice showed cytokine storm in their lungs upon post mRNA vaccination challenge by SRSCoV-2. It seems developing a vaccine for SARS-Cov-2 which could regulate cytokine storm are needed [106].

Memory Response against SARS-CoV-2

The efficacy of vaccine against pathogen is confirmed by the memory response at the time of the second or subsequent attacks. The reinfection in COVID-19 patients demonstrate lack of suitable memory response after prior infection probably due to viral immune evasion strategy. The candidate vaccines should be capable to elicit B and T cell memory responses. Ironically, any approved vaccine does not have a well-established memory response data and it is imperative to be deciphered for all candidate vaccines. There are some potential ways for this likely immune memory to be manipulated. For instance, it may be best to use SARS-CoV-2 genes that encode MHC-I epitopes that fit those of common coronaviruses when using RNA for immunization [107]. For the most part, when considering potential strategies for SARS-CoV-2 vaccination, consideration should be given to pre-existing MHC-I-based immunity resulting from previous common coronavirus infections. Common human coronaviruses can also induce some MHC-II-mediated immune memory by $CD4^+$ helper T cells with regard to SARS-CoV-2 recognition for shared epitope use by various coronaviruses. $CD4^+$ helper T cells can help stimulate cells involved in cytotoxic immune responses mediated by antibodies or cells. Recent studies have also demonstrated the previously SARS-CoV infected patients have shown long lasting SARS nucleoprotein (NP) specific memory T cells displayed cross reactivity to SARS-CoV-2 NP [108]. The promiscuous common T cell epitopes which could elicit both CD4 and $CD8^+$ T cells against SARS-CoV, SARS-CoV-2 and MERS should be taken into consideration for vaccine development. For all vaccines, the studies should be conducted on vaccine recipient individual samples for detection of long-term protection specifically memory T and B cells. Additionally, the virus neutralization assay should be performed to detect functional antibodies in the serum of vaccine recipient individuals.

Animal Model for Vaccine Study

Animals play a crucial role in the development of COVID-19 treatments and vaccines study. The race to generate and expand

mice for COVID-19 research is going on in the lab. The introduction of a safe and predictable COVID-19 animal model of infection is indeed a welcome development for preliminary antiviral and vaccine evaluations, and it is likely to help understand the immune evasion mechanism adopted by various strains of the virus. The hamster model is not new to corona virology, but it has the ability to offer a more stable and predictable model of infection than the murine models [109]. In the murine model, hyper-accentuated immune responses after SARS-CoV-2 vaccination have previously been published [110]. In addition to that, transgenic animals are often employed as models in biomedical research in the lab. Genetically engineered animals, mostly mice, account for almost 95% of those utilized. They are the tools for studying the disease susceptibility, progression, and treatment response. Following the discovery of ACE2 as the SARS-CoV host receptor, there has been a lot of interest in establishing human disease-like mouse models. This resulted in the creation of transgenic mice with human hACE2, K18-hACE2, expressed in the epithelia of tissues and organs such as the lungs, liver, kidney, spleen, heart, and intestine [111]. They are very susceptible to SARS-CoV infection, with high viral lung titres, considerable weight loss, and morbidity. ACE2 mouse model becomes the toolset for cardiovascular and pulmonary research and critical to advancing medicine and technology to search for drugs and a vaccine to fight the COVID-19 pandemic [112].

Storage, Transportation and Handling of Vaccines

Most of the vaccines, especially the mRNA vaccines as observed require significantly low storage temperature that is below freezing point which is difficult to maintain, particularly for longer durations and poses a huge limitation in developing countries. The vaccines developed by Pfizer, Moderna and Johnson & Johnson require storage of vaccine vials at -70ºC to -80 ºC, -20 ºC (6 months) and -20 ºC (2 years) respectively, thus making them extremely fragile and difficult to store and handle specially in developing countries. Apart from the requirement of ultra-low freezing temperatures, the vaccines must be protected from direct exposure to sunlight and ultraviolet light making the

handling all the way more difficult. The storage and transportation requirement puts extra financial burden on vaccine recipient. The vaccines having room temperature storage are always cost effective and ease point of care units. The advantages and disadvantages of the major vaccine platforms were mentioned in the table 4.

Table 4: Advantages and disadvantages of the major vaccine platforms [113].

Vaccine Type	Advantages	Disadvantages
Nucleic acid vaccines	Scalability. Fast design and development. Extremely safe. No infectious agent handling is required. Can induce humoral and cellular responses.	Currently, few nucleic acid vaccines approved including vaccine for SARS-CoV-2. DNA vaccines require a special delivery platform. mRNA vaccines exhibit instability and require storage at less than -20 °C.
Viral-vectored vaccines[π]	Can induce robust humoral and cellular responses with a single dose. Good safety profile.	Pre-existing immunity against a human viral vector can attenuate immune responses. Some candidates require storage at less than -20 °C.
Protein Subunit vaccines[£]	Safety during production. Can be safely administered to Immunosuppressed people. No infectious agent handling is required.	Small size of antigens diminishes their uptake by antigen-presenting cells (APCs). Low immunogenicity. Need several booster doses and adjuvants. Do not elicit cellular responses. Antigen integrity needs to be confirmed. Production limited by antigen production scalability.
Polysaccharide vaccines[¥]	Provides an alternative for vaccines against pathogens	Boost doses seldomly enhance the responses.

	with an abundance of polysaccharide antigens (mostly bacteria).	Only IgM isotype and IgG2 subtype are induced leading to limited antibody mediated effector functions. Poor memory responses. Works poorly on Children.
Conjugate vaccines[≠]	Enhances the poor immunologic responses produced by polysaccharide vaccines as it induces T-dependent responses.	Absence of cellular responses. Adjuvant and booster doses needed.
Virus-like particles vaccines[±]	They combine the efficacy of attenuated vaccines and the safety of subunit vaccines. Scalability of production. Their size makes them ideal for uptake by APCs.	The assembly of the particles is sometimes challenging.

Example of some vaccine used for this strategy [[#]Oral Polio, Yellow fever, Chickenpox, Mumps, Measles, Rotavirus, Rubella, Vaccinia, Bacillus Calmette-Guerin (BCG); [€]Rabies, Polio, Hep A; [£]Hep B, Hep C, Influenza, Acellular pertussis vaccine, Human Papilloma Virus (HPV); [¥]Pneumococcal polysaccharide vaccine (PPSV or PPV-23), *Neisseria meningitidis* polysaccharide vaccine (meningococcal vaccine); [≠]*Streptococcus pneumoniae* vaccine, *Neisseria meningitidis* conjugated vaccine (meningococcal vaccine), Typhoid vaccine, *Haemophilus influenzae* type b vaccine; [±]HPV, Hep B; [π]Ebola].

Possible Resolutions to Overcome Hurdles in Vaccine development against SARS CoV-2

Vaccines Focused on T cell Immunity and Memory

Most of the vaccine candidates against SARS-CoV-2 are focused on developing neutralization antibodies against viral spike protein to diminish the viral entry into host cells. However, to develop a prophylactic vaccine, against intracellular pathogen

like viruses, the cytotoxic T lymphocyte (CTL) response remains indispensable [114]. A group recently suggested that the severe infection of COVID-19 provides better memory T cells than milder infection [115]. Earlier studies have demonstrated that only 50% of the SARS survivors showed viral specific-B cell and -T cell responses [116,117-119]. However, T cell response was long lasting (6-17 years), associated with less severe disease, cleared the virus rapidly and does not involve into antibody-dependent enhancement (ADE) response. On the other hand, B cell response was short lived (3 years), associated with severe disease (MERS survivors with higher antibody titre must stay longer in ICU), does not clear the virus rapidly and found to be involved in ADE [116-123]. High serum level in SARS-CoV-2 infection is associated with longer stay of patients in ICU and need of ventilators because of severe disease condition [124,125]. Thus, they may serve as candidates for designing SARS-CoV-2 vaccines. Another eight immunodominant CD4+ T-cell epitopes have been suggested for use in a subunit vaccine, to potentially elicit effective T- and B-cell responses. They are distributed across the S protein (232–246 and 233–247), E protein (55–69, 56–70, and 57–71), and M protein (97–111, 98–112, and 99–113).109 These predictions warrant further investigation and may aid effective vaccine design against SARS-CoV-2. In addition to a strong antibody response, the coronavirus vaccines should also be capable to induce virus-specific CD8+ T-cell immunity. In addition, the focus should also be made on memory response of both humoral as well as cell mediated responses.

Development of Multivariant Vaccines

Most viruses evolve and change over time, and SARS-CoV-2 is no exception, with its composition constantly changing and raising concerns about the efficacy of existing COVID-19 vaccinations in use. With the appearance of three new strains, B.1.1.7 (UK), B.1.351 (South Africa), and P.1 (Brazil), as well as B.1.617 (India), there is a need to develop multivariant vaccine (Table 3). Likewise, annual influenza vaccinations are administered to people in many countries every year. The constant mutation in the influenza virus yearly design of new

vaccines predicted on the basis of current influenza viral strain. Both trivalent and quadrivalent vaccines targeting three and four strains of virus respectively are used as flu vaccines [126]. Gritstone Oncology Inc. in Emeryville, California with support from the Bill and Melinda Gates Foundation and National Institute of Allergy and Infectious Diseases (NIAID), is working on a multivariant vaccine that targets epitopes on the spike protein, as well as other areas of the virus for attack by T-cells. It is an mRNA vaccine using adenovirus vectors for delivery. The Gates Foundation supported preclinical development of the vaccine, while NIAID has partnered with the company on an early-stage clinical trial. Preclinical tests revealed that more regions of the SARS-CoV-2 virus elicited multiple immune responses, including high-titer neutralising antibodies and $CD8^+$ T cell responses against the spike protein, as well as a broad $CD8^+$ T cell response against epitopes from multiple viral genes beyond the spike. There are currently no approved multivariant vaccines, but efforts are underway to develop in future. We recommend that we proceed forward with the development of a SARS-CoV-2 vaccine by aiming to build a multivariant vaccine for improved protection.

BCG as a Platform for Live Attenuated Vaccine

Recently, various reports suggests that countries with non-BCG vaccine recipient population (Italy, Nederland, USA) have shown higher case fatality rates (CFR) in compare to long-standing universal BCG policy practicing countries [127,128]. In addition, elderly population [129], suggestive of the notion that BCG protects the vaccinated elderly population. Because of, known protective immunological benefits, decreased incidences, hampered disease transmission and progression, and lowered mortality are suggestive of BCG vaccination as a potential nonspecific safe tool against COVID-19, however, various other factors make BCG efficacy against COVID-19 debatable [127-132].

BCG Vaccination Renders Nonspecific and Variable Immune Response

BCG vaccination in healthy volunteers increases IFN-γ, enhances monocyte derived cytokines TNF and IL-1β release, elevates activation markers CD11b and PRRs like Toll-like receptor-4, CD-14 and scavenger receptors [127-132]BCG-vaccination in infants, surprisingly, increases in 11 cytokines and chemokines in response to different non-specific innate immunity stimuli which includes, epidermal growth factor (EGF), eotaxin, IL-6, IL-7, IL-8, IL-10, IL-12p40, monocyte chemotactic protein-3 (MCP-3), macrophage inflammatory protein-1α (MIP-1α), soluble CD-40 ligand and platelet-derived growth factor (PDGF). Moreover, in monocytes the heterologous production of Th1 (IFN-γ) and Th17 (IL-17 and IL-22) immune responses to non-mycobacterial stimulation remained strongly elevated even 1 year after BCG vaccination [135].

The SARS-CoV-2 infection renders hyper-inflammation mediated pulmonary dysfunction due to pro-inflammatory cytokines (IL-1β, IL-4, IL-6, IL-8, MCSF, CXCL-10, and TNF-α) burst in patients [136,137]. Surprisingly, these elevated cytokine levels have been reported along with lymphopenia in COVID-19 patients, suggestive of, major contribution by uncontrolled innate responses. The anti-inflammatory cytokine IL-10 could be of great help in combating COVID-19 because of anti-inflammatory nature and its capability to obstruct inflammatory cytokines production including IFN-γ and IL-2. BCG has been shown to induce, T and B independent, monocytes/macrophages and NK cells mediated non-specific trained immunity response to a secondary infection by innate immune system either to the same or different microorganisms [138,139]. SARS-CoV-2 infection in BCG immunized individuals. Moreover, it would be imperative to compare the cytokine profiles of patients from BCG immunized and non-immunized individuals.

Thus, BCG induces sustained changes in the immune system associated with a nonspecific response to infections that could be beneficial against COVID-19. However, the beneficial role of

BCG against COVID-19 remains debatable, because of the variation in testing rate, population density, median age, TB incidence, urban population, public policy and community spread check measures in different countries [132]. The BCG as adjuvant has been proved very efficient in producing non-specific immune responses, in case of COVID-19, we should perform studies to evaluate the efficacy of various adjuvants including BCG to elicit immunoprophylactic responses by candidate vaccines. The suitable adjuvant would not only instigate immune responses, but they will also facilitate memory response that could be a boon for vaccine against dreadful pathogen.

Discussion and Future Prospects

The most daunting threat in a century for mankind is the coronavirus disease 2019 (COVID-19) pandemic caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Less than a year after COVID-19 declared as a pandemic, several vaccine candidates had been authorized for emergency use in various countries. Despite the availability of vaccinations, recent pandemic waves have created concerns about i) the effectiveness of existing vaccines against new virus variants, ii) achieving global herd immunity against COVID-19, iii) safety of the vaccine in autoimmune disorder individuals iv) vaccine safety for pregnant women, toddlers and young adults, v) possibilities of cytokine storm in vaccinated people, vi) efficacy of vaccines in comorbid conditions, vii) appropriate timing of booster doses for best immune response viii) B cell memory responses ix) T cell immunity and memory response x) effectiveness of vaccines in reducing viral transmission, xi) vaccine distribution to underprivileged xii) effect of vaccination on infected people xiii) vaccine hesitancy and xiv) vaccine swapping in absence of second dose or improving immune response continues to explore significant scientific and policy challenges [140,141].

Multiple waves of infection place a huge burden on the state, doctors, and scientists in establishing a successful fight against SARS-CoV-2. Some of the countries have achieved sufficient

number of vaccinations to curb the pandemic. In contrary, some countries confirm lowered vaccination either due to unavailability of vaccine as per requirement or because of vaccine hesitancy. Because of severe impact of pandemic in terms of infection intensity and mortality, the vaccination drives in between this pandemic has reduced vaccine hesitancy among population, this has contributed to achieving herd immunity. Herd immunity generally requires protective immune response in 60-70% of population attained either through infection with the virus or by vaccination. The global scientific coordination and partnership is key to successful vaccination against COVID-19. It is particularly effective since nationalistic approaches to immunisation will continue to raise issues like vaccine development and production, pricing, allocation, and deployment, and only a global resource collaboration can fulfil these goal [53]

The vaccines used are safe, but there have been reports of safety concern with some of the vaccines, which has caused havoc and has been a source of vaccine hesitancy. Scientific evidence on vaccine safety should be established not just in the general population, but also for pregnant woman, toddlers and teens, people with comorbid diseases like diabetes and hypertension, and those who have been infected prior to vaccination. Since most of the vaccines are based on neutralizing antibodies against spike protein of SARS-CoV-2, the details are available for neutralizing antibodies, however, the T cell immunity remains untouched for most of the vaccine candidates. Furthermore, the B and T cell memory response has not been established for most of the approved vaccines. The B and T cell memory responses and T cell mediated immunity should be deciphered for all the used vaccines with immediate attention.

Globally, the spread of different variants has worsened the situation, we need vaccine with prophylactic efficacy to combat COVID-19. The multivariant vaccines might be more efficient in eliciting better and strong immune response for better protection against virus variants. We can use various platforms to achieve multivariant vaccines. Nonetheless, direct virus could be employed to develop live attenuated vaccines; however, there is

always the possibility of reversal. One of the major concerns during SARS-CoV-2 infection is cytokine storm which leads to various physiological damages including pneumonia, pulmonary embolism, blood clotting in various organs including brain, gastrointestinal tract, kidney and liver, also it results into ARDS. This is suggestive that unregulated immune response due to viral immune evasion strategies renders these damages. The cytokine storm could be controlled by inducing Treg cells and IL-10 cytokine strategically. Nevertheless, the BCG has been proved to elicit nonspecific controlled immune response, it would be an inclusive strategy to develop formulations of rBCG by incorporating multiple antigens of SARS-CoV-2. The live attenuated nature of rBCG could be helpful in generating long term protection.

Interestingly, a number of studies are currently being conducted in many parts of the world on the use of two separate vaccines for better protection against SARS-CoV-2 and its variant form. According to a German study, immunizing with AstraZeneca's ChAdOx1-nCov-19 vaccine as a first shot and BioNTech/BNT162b2 Pfizer's as a second shot after 10 weeks apart boosts the immune response. BNT162b2 induced significantly higher frequencies of spike-specific CD4 and CD8 T cells and, in particular, provided high titers of neutralizing antibodies against the B.1.1.7, B.1.351, and P.1 lineage of the virus [142,143]. There has been speculation that using covaxin as a first shot and AstraZeneca (covishield) as a booster shot will help to protect people better. However, further research has been required on the interchangeable vaccine approach.

Considering worst hit by multiple waves, it is suggestive that individuals should take the vaccines whichever is available at their end, to curbs the disease severity even if it does not provide the best protection. Importantly, prophylactic vaccines against SARS-CoV-2 have great potential to prevent future pandemics. Nonetheless, we must continue to improve the present vaccine by increasing neutralizing antibodies, and a focus on multivariant vaccines that include T cell immunity would be beneficial in combating numerous variations of this dreadful pathogen.

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