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Evolving potential vaccine candidates amid COVID-19 pandemic: Pipeline to Lifeline

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Abstract

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), is globally explored to decode its genomic functionality along with elucidating immunogenic pathways to design and develop an efficient vaccine. Potential immunogenic targets have been screened and validated through preclinical evaluation using experimental models. Computational platform and molecular docking studies are also being conducted to study the immunodynamic mechanisms which involve suitable epitopes of host target cells that respond to the potential vaccine candidate eliciting an immune-mediated reaction. Although SARS-CoV-2 possesses genetic similarities with previously known human coronaviruses, the emergence of novel mutational changes in the immunodominant region of the receptor-binding domain of viral spike protein resulted in high transmissibility and fatality. On a periodical basis, the World Health Organization (WHO) publishes the update on evolving vaccine candidates and encouraging several vaccine developers including multinational companies to join the worldwide campaign against the COVID-19 pandemic. As per the latest WHO landscape draft of evolving vaccine candidates, around 180 teams with respective vaccine candidates across the world are working by utilizing multiple developmental platforms, out of which 35 candidates have entered clinical phase trial and 145 candidates are under the preclinical phase of evaluation. They are also being tested for undesired immunopotentiation without compromising their safety and efficacy. These vaccine candidates along with their advantages and various challenges have been reviewed in this article.

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INTRODUCTION

Viruses have been considered as one of the biological entities responsible for a highly contagious yet infectious form of the disease with the potential to create havoc to human living platforms. Threats of viral attacks are still being recorded as a Public Health Emergency of International Concern (PHEIC) which stimulates international alliances across the globe to prepare for the outbreak, meanwhile encouraging the discovery and development of potential vaccine candidates through solidar-

ity trial. Recently, the entire world is grappling against the viral outbreak, which is a novel form of human coronavirus (hCoV), COVID-19. Soon after the sporadic outbreak, the virus stepped into cluster transmission and community spread, thereby transmitting across 216 countries following the human chain. Based on the genome sequence of SARS-CoV-2 from several countries, the novel virus appears to be extremely contagious when compared to previous outbreaks of human coronaviruses such as SARS-CoV and the Middle East Respiratory Syndrome-Coronavirus (MERS-CoV) due to mutations in the Receptor Binding Domain (RBD) of one of the Spike protein S1. The S1 subunit is the most variable domain among all the known spike proteins of human coronaviruses (hCoVs) which is also the prominent RBD directly involved in the host-antigen immune reactions. Six RBD amino acids are found to have a critical role in binding to Angiotensin-Converting Enzyme 2 (ACE2) receptors present in the host cells and are different from all known SARS-CoV like viruses which are designated as L455, Q493, F486, N501, S494, and Y505. Out of the six residues, five differ between SARS-CoV-2 (Figure 1) and SARS-CoV, which makes SARS-CoV-2 extremely contagious to humans. The emergence of these novel variations has been attained through natural selection from the reservoir host that was found to be lethal for humans (Wan *et al.*, 2020).

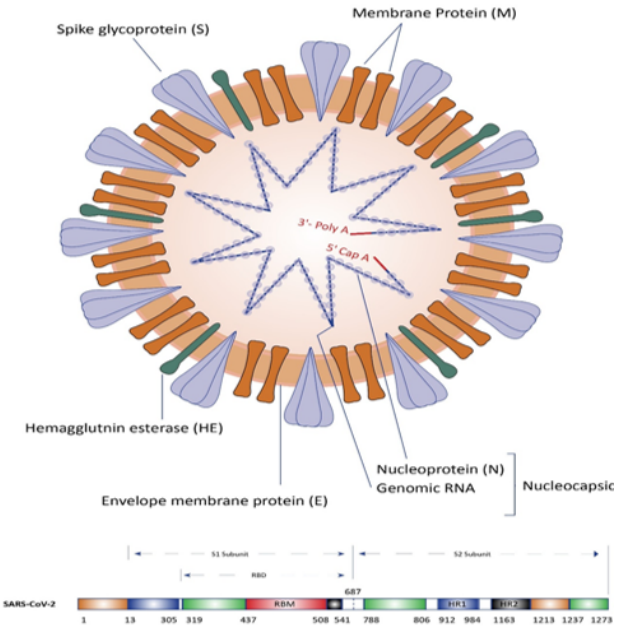

Figure 1: Schematic representation of SARS-CoV-2 structure and Spike protein fragment

A schematic representation of SARS-CoV-2 as depicted in Figure 1 indicates virion, a nucleocapsid composed of positive-sense single-stranded genomic RNA with 5' Cap and-3'-PolyA and phosphorylated nucleocapsid (N) protein within phospholipid bilayer. The viral surface proteins include spike glycoprotein trimer (S), Envelope membrane protein (E), and Membrane glycoprotein (M), which are embedded in a lipid bilayer envelope. The Hemagglutinin-esterase (HE) proteins are located along with other surface proteins in the phospholipid bilayer. The spike protein fragment, which is shown in the figure includes the S1 subunit, S2 subunit, Receptor Binding Domain (RBD), Receptor Binding Motif (RBM), Heptad Repeats (HR) 1 and 2. The site holds antigenicity and a role in eliciting an immune response.

COVID-19: The Need for Vaccines

The outbreak of COVID-19 has triggered scientific communities across the globe for the development of an effective vaccine as well as drugs to manage the disease. As per the latest WHO report updated on 11 September 2020, around 216 countries (territories) are affected with SARS-CoV-2 with total confirmed cases of 2,80,40,853 and confirmed deaths of 9,06,092. To break the chain of transmission and limit the spread towards unaffected regions, interventions in the form of effective vaccines or targeted therapies are in need to combat the COVID-19 global threat. A handful of potential vaccine candidates are in the front line after successfully being tested through different phases of the clinical trial. Yet, several vaccine candidates are still in the pipeline of development utilizing various platforms. To date, no specific and targeted therapeutic module has been approved with international agreement to treat and manage COVID-19 cases.

Multifaceted approaches have been regulated from WHO and concerned authorities to curb the spread of SARS-CoV-2 transmission by isolating positive cases and treating them along with tracing their contacts. Isolating only symptomatic cases was not found to be efficient enough in limiting the spread, since several asymptomatic or presymptomatic cases have been identified as positive with laboratory tests. Same way, high-risk groups such as front line workers, children, geriatric patients above 60 years of age with underlying comorbid conditions must be monitored periodically. Hence, an early and effective vaccine is the need of the time to manage the spread of SARS-CoV-2 and protect from community transmission for saving millions of lives.

SARS-CoV 2-Virus to Vaccine, The most awaiting developmental paradigm

Usually, the development of a vaccine is a long-going process of precisely designed scientific study,

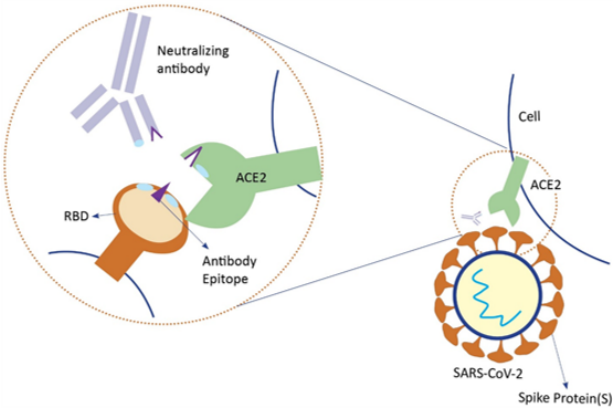

Figure 2: Schematic representation of the interaction between host cell receptor ACE2 and RBD domain of spike protein of SARS-CoV-2 with the production of Neutralizing antibodies

including several stages of preclinical and clinical trials that take years to even a decade. The WHO is updating the development and progress of several vaccines as a landscape draft on a periodical basis which helps in tracking vaccine trial status. To cope with rapidly spreading SARS-CoV-2, WHO designed "R & D Blueprint for novel coronavirus" which documented a "Large, international, multi-site, individually randomized-controlled clinical trial" to evaluate the benefits and risks of promising vaccine candidates within 3-6 months of trial. As per the latest WHO draft landscape of COVID-19 vaccine candidates update, around 180 vaccine candidates have been registered across the globe to conquer the existing SARS-CoV-2 threat, out of which 35 candidates are undergoing different stages of human phase clinical trials, and around 145 vaccine candidates are in the pipeline of preclinical evaluation.

In the modern history of infectious disease and vaccines, this is the first unprecedented condition when vaccine candidates have entered phase I clinical trials within three months of the outbreak of the virus. The international coalition developed a special strategy to compress the lengthy duration of phase II-III trials from years to months after completing preliminary study from laboratory animals and healthy humans by implementing challenged study which can bypass typical phase III trial (Eyal *et al.*, 2020). To develop the COVID-19 vaccine at a pandemic speed from the limited immunological information available, scientific communities across the world are trying to adapt existing developmental platforms to study possible host responses against various developing vaccine candidates. Several computational study designs based on existing information are being utilized to understand the possible molecular mechanism involved in immunological reactions. Immuno-informatics and molecular docking tools are being used extensively to study and identify appropriate epitopes that can elicit an immune response so that the same can be used for the development of potential vaccine candidates against COVID-19 (Baruah and Bose, 2020). Several strategies have been embraced in the development of the coronavirus vaccine, out of which surface-exposed spike (S) glycoprotein or S protein is gaining more attention as a potential target that can efficiently induce neutralizing antibodies. Several developmental platforms have been utilized to discover as well as manufacture scalable vaccines for global supply with pandemic speed is described below.

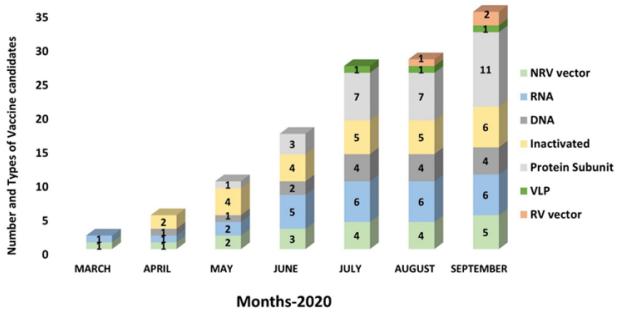

Figure 3: Evolving vaccine candidates for Clinical phase trial- Monthly trend in vaccine development as per the WHO published landscape draft

Whole Virus Vaccines

The category includes live-attenuated vaccines and inactivated vaccines prepared from the whole viral structure. Attenuated whole viruses could be a promising developmental platform for effective vaccines based on its several successful histories in delivering efficient vaccines. Furthermore, it utilizes a novel genetic technique that increases the likelihood to develop comparatively better vaccines despite a high demand to meet global needs. The possibility of working with an inactivated attenuated virus is high due to its feasibility in growing than the wild type virus. A half dozen inactivated vaccines are in the pipeline at different phases of clinical trials, out of which three vaccine candidates are running ahead with phase 3 clinical trial which includes Sinovac, Wuhan Institute of Biological products/Sinopharm, and Beijing Institute of Biological products/Sinopharm (Table 1). The other three vaccine candidates are yet in the middle phase of the clinical trial (phase I/II) which includes the Institute of Medical Biology & Chinese Academy of Medical Sciences; Research Institute for Biological Safety Problems & Rep of Kazakhstan; and Bharat Biotech. There are still 12 vaccine candi-

dates, including both inactivated and live attenuated under the preclinical phase of evaluation. Using live

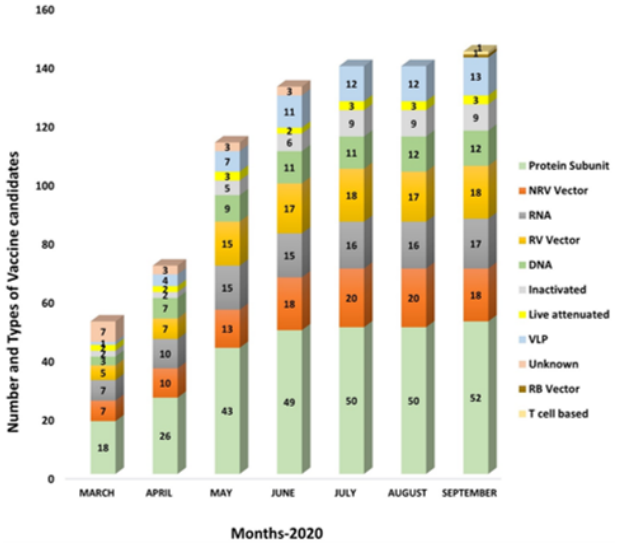

Figure 4: Evolving vaccine candidates for Preclinical evaluation-Monthly trend in vaccine development as per the WHO published landscape draft

attenuated vaccine platform, three developers are conducting preclinical phase evaluation among which Codagenix in Farmingdale, New York, is working in collaboration with Serum Institute of India Pvt. Ltd., an Indian based global vaccine manufacturer, using gene editing principle to weaken SARS-CoV-2. Codagenix has developed a "Codon Deoptimization" technology for viral attenuation which is utilized to explore the COVID-19 vaccine development strategy.

The vaccine named CodaVax-COVID was found to be safe and effective in animal models with a single dose. It is constructed to produce immunity against all SARS-CoV-2 proteins and not just the spike surface protein to elicit a vigorous immune response coupled with long-lasting cellular immunity. Another live attenuated vaccine candidate is in the process of development by Indian Immunologicals Ltd. in collaboration with Griffith University, Australia, adopting codon deoptimization technology. Recently, Bharat Biotech International Limited, an Indian based vaccine developer has developed India's first indigenous potential COVID-19 vaccine candidate COVAXIN^{TM} in collaboration with the Indian Council of Medical Research (ICMR) using an inactivated vaccine platform. Inherent immunogenicity factor has been one of the significant advantages for whole virus vaccines having the ability to potentiate toll-like receptors (TLRs) including TLR 3, TLR 7/8, and TLR 9. However, live attenuated virus vaccines must be tested extensively to ensure their safe usage (Chen *et al.*, 2020). This is especially an issue with coronavirus vaccines that renders increased infectivity following whole virus vaccine immunization.

Viral-vector Vaccines

Viruses can be genetically engineered to deliver antigens that can elicit desired immune response inside the host, so-called viral vectors which can be used for the production of vaccines. Viral vector vaccines are presented with advantages over traditional vaccines due to their efficiency in enhancing a wide range of immunogenicity without the application of an adjuvant system which finally prompts a strong cytotoxic T lymphocyte (CTL) response to destroy the virus-infected cells. These vaccines function by supplying the genes to the target cells, highly effective in gene transduction, and efficiently elicit long-term immune response due to high antigenicity, and hence vectored vaccines are more of prophylactic use. The most promising method involves "prime-boost" strategies with other types of vaccines, including DNA vaccines or recombinant antigens. As per the latest WHO published draft, 44 vaccine candidates utilized a viral vector platform in developing both non-replicating and replicating viral vector vaccines, out of which seven candidates are under different phases of the clinical trial.

Four non-replicating viral-vector vaccine candidates are leading ahead with phase III clinical trials, and vaccine developers include the University of Oxford/AstraZeneca, CanSino Biological Inc./Beijing Institute of Biotechnology, Gamaleya Research Institute, and Janssen Pharmaceutical Companies. Around 37 viral vector vaccine candidates are still under preclinical examination that includes 18 non-replicating and 19 replicating vaccine candidates. The University of Oxford in collaboration with AstraZeneca, a UK based multinational biopharmaceutical company, utilized a non-replicating viral vector named AZD1222 derived from the virus (ChAdOx1) which is a deteriorated version of a common cold virus (adenovirus) with the spike coding region cloned into the E1 locus that causes infections in chimpanzees (Astra Zeneca, 2020). It contains genetic materials of SARS-CoV-2 spike protein that is genetically modified to make it non-pathogenic towards humans. Due to non-replicating nature, it does not divide inside vaccinated individuals. Yet, it showed a strong immune response from a single dose in an experimental animal which are capable of inducing the human immune system similarly to spike proteins of wild type SARS-CoV-2. CanSino Biological Inc., in collaboration with the Beijing Institute of Biotechnol-

ogy, has developed a recombinant Adenovirus type 5 vector.

In the pipeline of potential vaccine development, another promising candidate from Gamaleya Research Institute of Epidemiology and Microbiology, Health Ministry of the Russian Federation, Moscow is under phase III trial. The candidate named Gam-COVID-Vac is under randomized, double-blinded, placebo-controlled multicentric trial (Clinical Trials, 2020) Janssen Vaccines and Prevention BV belongs to one of the leading vaccine developers working with non-replicating viral vector vaccine named Ad26.COV2.S reached phase III trial.

Council of Scientific and Industrial Research (CSIR) funded Indian based Bharat Biotech company in collaboration with Thomas Jefferson University, Philadelphia is conducting extensive preclinical trials in experimental mice and is ready for a human phase trial.

The vaccine candidate was making use of established deactivated rabies vaccine as a mode of delivery for coronavirus proteins which is known to produce a strong immune response to all groups of the population including children and pregnant women (Biotech and Jefferson, 2020). Another invention of Bharat Biotech in partnership with the University of Wisconsin-Madison (UW-Madison), is CoroFlu, which is based on FluGen's flu vaccine candidate M2SR, which will be incorporated with SARS-CoV-2 gene sequence to develop immunity against COVID-19.

Subunit Vaccines

These are also called acellular vaccines since they do not contain the whole viral structure. Instead, these vaccines contain only the immunogenic part of the whole virus that might be polysaccharides or surface proteins which are recognized as foreign materials by the host and able to elicit a specific immune response. Although viral subunit vaccines are safe and convenient, yet it shows relatively low efficacy due to the presence of unfavourable epitopes and developers are finding more hopes on protein subunit vaccines since many new studies have been proposed recently, that utilizes the protein subunit as a promising vaccine platform amid COVID-19. Around 63 protein subunit vaccine candidates are in the pipeline, among which 11 candidates are under different stages of phase trials, and remaining more than 50 candidates are still under the preclinical stage of evaluation. Most of them are focusing on the surface-exposed virus's spike protein or S protein for inducing neutralizing antibodies. The S1 subunit serves as the structural scaffold for interaction with host cell receptor ACE2 to induce neutralizing antibodies (Figure 2). Hence, RBD could be a prime candidate for subunit vaccine design, and the S2 subunit of S protein mediates fusion between the virion and host cell membranes leading to the release of viral RNA in the cytosol to initiate replication mechanism. During infection with SARS-CoV, the S protein is responsible for inducing protective immunity by stimulating neutralizing-antibodies and T-cell responses. Therefore, full length or a suitable part of S protein could emerge as the most promising vaccine candidate. It has also been reported that neither the absence nor the presence of other structural proteins having the potential to affect S protein immunogenicity or its binding to the ACE2 receptor which is one of the crucial steps for the virus to access the host cell and integrate with the cellular machinery process.

Figure 2 is a schematic representation of the interaction between host receptor angiotensin-converting enzyme 2 (AEC2) with receptor-binding domain (RBD) epitome of the spike protein of SARS-CoV-2 resulting in the production of neutralizing antibodies (NAbs) that blocks binding and fusion of SARS-CoV-2 with host cells and prevents the entry of viral nucleocapsid inside the host cell for subsequent replication, thereby mitigating the adverse consequences due to immune-mediated antigen-antibody reaction. The key residues between RBD and NAbs can be identified, which could provide an important implication for the specific vaccines against SARS-CoV-2.

Novavax Inc, a USA-based biotechnology company, developed a protein subunit vaccine that is leading ahead with the late stage of phase II trial. The vaccine candidate NVX-CoV2373 is a full length recombinant SARS-CoV-2 glycoprotein nanoparticle adjuvanted with patented saponin based Matrix-M revealed high immunogenicity in animal models measuring spike protein-specific antibodies (Novavax, 2020). This technology enhances antigen presentation in local lymph nodes and helps in boosting immune response by producing a high titre of micro neutralizing antibodies. NanoFlu, which is a quadrivalent influenza nanoparticle vaccine, which also incorporates Novavax's proprietary saponin-based Matrix-M adjuvant is in its phase II clinical trial in older adults (Inc, 2020). Novavax in coalition with Indian-based vaccine developer Cadila Pharmaceuticals has been working together with virus-like particles (VLP) platform which has been previously used for papillomavirus vaccine.

Moreover, the Mers corona vaccine is in herd phase III of Respiratory Syncytial Virus (RSV) maternal

Table 1: Vaccine candidates currently under Phase III Clinical trial

Vaccine platform	Type of candidate vaccine	Route of Administration	Developers/Manufacturer
Non-replicating Viral Vector	Adenovirus Type 5 Vector	IM	CanSino Biological Inc./Beijing Institute of Biotechnology
Non-replicating Viral Vector	ChAdOx1-S	IM	University of Oxford/AstraZeneca
Non-replicating Viral vector	Adeno-based (rAd26-S+rAd5-S)	IM	Gamaleya Research Institute
Non-replicating Viral vector	Ad26COVS1	IM	Janssen Pharmaceutical Companies
Inactivated	Inactivated	IM	Beijing Institute of Biological Products/Sinopharm
Inactivated	Inactivated	IM	Wuhan Institute of Biological Products/Sinopharm
Inactivated	Inactivated + alum	IM	Sinovac
RNA	LNP-encapsulated mRNA	IM	Moderna/NIAID
RNA	3 LNP-mRNAs	IM	BioNTech/Fosun Pharma/Pfizer

immunization in India. Furthermore, one of the preclinical studies from Dynavax Technologies corporation working in collaboration with the University of Queensland, Australia and CEPI (Coalition for Epidemic Preparedness), is trying to develop COVID-19 vaccines by providing its proprietary toll-like receptors 9 (TLR9) agonist adjuvant, i.e. CpG 1018, which is already contained in HEPLISAV-B® [Hepatitis B Vaccine (Recombinant), Adjuvanted], an adult hepatitis B vaccine, initiating the rapid development of a COVID-19 vaccine (Emeryville, 2020; Anges Inc, 2020). Clover Biopharmaceuticals AUS Pty Ltd, a Chinese biotechnology company, is also working in collaboration with GSK and Dynavax for its protein subunit-based coronavirus vaccine candidate SCB-2019 and is currently under phase I clinical trial (Clover Biopharmaceuticals AUS Pty Ltd, 2020). GSK's pandemic adjuvant system AS03 combined with S-Trimer modification over SCB-2019 is developed as a promising candidate that utilized patented Trimer-Tag® technology which has been shown to react with antibodies produced by multiple previously infected COVID-19 patients. S-Trimer developed by Clover resembles native trimeric viral spike protein when analyzed using a rapid mammalian cell culture-based expression system. Another collaborative study headed by Anhui Zhifei Longcom Biologic Pharmacy Co., Ltd in partnership with the Institute of Microbiology, Chinese Academy of Sciences is presently conducting a Phase II trial of its vaccine candidate which is an adjuvanted recombinant S protein (RBD-Dimer) using recombinant DNA technology (baculovirus production). GlaxoSmithKline's (GSK's) adjuvant technology further assists intending to manufacture scalable amount of vaccines amid COVID-19 health crisis (Sanofiacts, 2020). There are altogether seven protein subunit vaccine candidates which are under phase I clinical trial from several institutes and/or industries across the globe. In contrast, only 2 to 3 candidates have completed phase I trial.

Nucleic acid Vaccines

These are novel strategies utilized in vaccine development and are composed of purified closed circular plasmid DNA or non-replicating viral vector containing genes that result in the *in-vivo* expression of the encoded protein eliciting both cell-mediated and humoral immune responses. DNA and messenger RNA vaccines are two major types of nucleic acid vaccines that directly get integrated with cellular machinery responsible for protein synthesis such as the viral protein that can elicit an immune response in the form of neutralizing antibodies. Around 40 nucleic acid vaccine candidates are in the pipeline of development, which includes both DNA and RNA vaccines out of which ten candidates are at different stages of clinical phase trials (Figure 3), and around 30 candidates are under preclinical evaluation. RNA vaccine candidates are presently lead-

ing ahead in the front line of development, two of which are under phase III trial. In contrast, one of the RNA vaccine candidates is under phase II trial of development. None of the DNA vaccine candidates has entered clinical phase II trial, but almost 12 of them are in the pipeline of preclinical evaluation (Figure 4).

Evolving vaccine candidates amid COVID-19 for clinical phase trial-Figure 3 depicts the monthly developmental updates of potential vaccine candidates at different phases of clinical trials utilizing different platforms. The trending graph indicates progression in the development of the number of vaccine candidates monthly as per the WHO landscape draft. From March 2020, till September 2020, there is a progressive trend in the number of vaccine candidates being included in the clinical phase trial, meantime there is also an increasing trend in the utility of the different possible developmental platforms. In March 2020, only two vaccine candidates entered clinical phase trial utilizing two different developmental platforms which included NRV vector and RNA whereas, in the subsequent months, vaccine developers have taken an interest in utilizing other platforms that included DNA, Inactivated, Protein subunit, VLP, and Replicating viral vector. Recently, vaccine candidates utilizing the protein sub-unit have gained more attention from vaccine developers followed by RNA yet vaccine candidates utilizing the NRV-vector platform, and RNA platform is ahead in the pipeline which started at the earliest and expecting to complete soon shortly.

RNA vaccine is a novel strategy included in the field of vaccine development which works by introducing a synthetic mRNA sequence with a specific length calibrated through the matrix, that is made to code for the disease-specific antigen such as specific viral protein. Once the mRNA strand enters the host cells, it integrates with the host cellular machinery system. It produces antigen which is then displayed on the cell surface inducing acquired immunity to protect against the wild type. RNA vaccines are safe non-infectious, efficient in eliciting reliable immune response with comparatively faster and cheaper scalable production to meet global demand. The explanation for faster approval of the mRNA vaccine is that hCoVs is not being utilized in the developmental work and hence proves to be safely used. (Calina *et al.*, 2020). The RNA molecule trains the cell to synthesize disease-specific antigens which subsequently trains the body to fight against the actual viral antigens. Apart from several benefits, RNA vaccines do possess particular technical challenges, yet the majority of nucleic acid vaccines are based on the mRNA developmental platform. ModernaTX Inc., a US-based manufacturer, is working in collaboration with Biomedical Advanced Research and Development Authority and the National Institute of Allergy and Infectious Disease (NIAID) for developing mRNA based vaccine candidate (National Institute of Allergy and Infectious Diseases, 2020).

The delivery system, which is a novel lipid nanoparticle (LNP), encapsulates mRNA vaccine candidate that encodes for a prefusion stabilized form of SARS-CoV-2 spike (S) protein. LNPs usually consists of four components which include (i) an ionizable cationic lipid, promoting self-assembly into virus sized particles allowing the endosomal release of mRNA into the cytosol, (ii) lipid-linked polyethene glycol which increases the half-life of formulation, (iii) cholesterol as a stabilizing agent and (iv) naturally occurring phospholipids to support lipid bilayer. Recent studies demonstrated that LNPs are effective mRNA delivery tools for in vivo studies. BioNTech SE, a German biotechnology company, in collaboration with Pfizer, a US-based multinational company, has developed mRNA-based vaccine candidate 3LNP-mRNAs (Phase II BNT162b1 and Phase III BNT162b2). CureVac, which is also a German-based mRNA company, is developing an mRNA vaccine candidate (CVnCoV) that has entered the phase IIa trial (Curevac, 2020). Imperial College of London, UK, has also developed self-amplifying RNA vaccine candidate LNP-nCoVsaRNA, which encodes for the S glycoprotein of SARS-CoV-2, which is the antigenic viral molecule involved in immune-mediated responses. Indian based Genova Pharmaceuticals has developed an mRNA vaccine that utilizes a patented carrier molecule called Lipid iron oxide (LION) and an adjuvant called GLA-SE.

Evolving vaccine candidates amid COVID-19 for preclinical evaluation- Figure 4 depicts the monthly developmental updates of potential vaccine candidates undergoing preclinical evaluation utilizing various developmental platforms. The graph indicates the monthly trend in the discovery and development of new vaccine candidates approved for preclinical evaluation utilizing several vaccine platforms against the ongoing COVID-19 pandemic. As per the graph, a huge number of vaccine candidates utilizing the protein subunit platform are in the pipeline of development worldwide as per the latest WHO landscape draft published. Initially, at the early phase of vaccine development, protein subunit vaccine candidates were the most preferred platform for preclinical evaluation yet could not enter the clinical phase trial and lag behind NRV vector, RNA, DNA, and Inactivated platforms. In May 2020, one of the protein vaccine candidates first entered phase trial, and now in four months, the

platform is most preferred with the highest number of developing vaccine candidates. Apart from protein subunit vaccine candidates, other platforms gaining interest by vaccine developers include NRV-vector, Replicating viral (RV) vector, and RNA vaccine platforms. Several vaccine candidates using these three innovative platforms are moving ahead to enter clinical phase trials after successful preclinical studies. Virus-like particles (VLPs) platform for the development of COVID-19 vaccine candidates has recently gained more attention for vaccine development. VLPs are non-infectious multi protein structures that are engineered to self-assemble from viral structural proteins and could be a promising vaccine candidate with enhanced immunogenicity. The remaining platforms included DNA, Inactivated, and Live attenuated vaccine platforms out of which DNA vaccine platform has been preferred by developers as per the graph indicated. Two more innovative platforms which are Replicating Bacterial vector (RB vector) and T cell-based platforms were choices in developing potential vaccine candidates which include one candidate from each. Overall, there is a progression in the number of vaccine candidates being developed utilizing various platforms and extensive trials across the globe to fight against the COVID-19 pandemic, and the race is still on.

DNA vaccine, otherwise called genetic vaccine, is made up of a plasmid DNA that encodes for the antigen of interest under the control of a mammalian promoter, i.e. Cytomegalovirus (CMV) intron A that can be produced inside the bacterial cells. Inside the host cell, plasmid DNA is presented by antigen-presenting cells (APCs) such as dendritic cells resulting in both humoral and cellular immune response (Coban *et al.*, 2008). Although DNA vaccines suffer lower immunogenicity in higher primates and humans, several studies have been conducted to improve its immunogenicity by modifying the microenvironment of the vaccinated site for boosting its ability to enhance immune-mediated responses. Several DNA vaccine candidates are in the pipeline of development, but until date, none of them has entered phase III clinical trial. Only four DNA vaccine candidates are under the initial stages of clinical trials which are being manufactured by Inovio Pharmaceuticals/International Vaccine Institute, Osaka University/AnGes/Takara Bio, Cadila Healthcare Limited, and Genexine Consortium. Inovio Pharmaceuticals Inc, a US-based leading biotech company in collaboration with International Vaccine Institute, Seoul, Korea, and Coalition for Epidemic Preparedness Innovations (CEPI), are developing a DNA plasmid vaccine candidate with electroporation named INO-4800. INO-4800 contains plasmid Pgx9501, which encodes for the full-length spike glycoprotein of SARS-CoV-2 (International Vaccine Institute, 2020).

It is a dose defining trial in which intradermal administration of INO-4800 vaccine is followed by electroporation (EP) using CELLECTRA® 2000 device in healthy adult volunteers. AnGes Inc, a Japan-based nucleic acid drugs company has developed a DNA plasmid vaccine candidate (AG0301-COVID19) in collaboration with Osaka University, Japan Agency for Medical Research and Development which is conducting phase I/II trial (Anges Inc, 2020). Cadila Healthcare Limited, an Indian-based pharmaceutical company, is working by targeting major viral membrane protein using DNA plasmid which upon introduction into the host cells, elicited strong immune response mediated by both cellular and humoral aspects of the immune system. A consortium is established by Genexine, including Binex, International vaccine institute, GenNbio, KAIST, and POSTECH, to develop DNA vaccine candidate GX-19, which is a Genexine's formulation. GX-19 DNA vaccine which was developed in March 2020, works through the genomic pathway in which DNA inserts in the form of the vaccine, commands the body to produce antigen which further induces immune response inside the body in the form of neutralizing antibodies (Han-soo, 2020).

Immunomic Therapeutics Inc. (ITI), a leading US-based Biotechnology Company, in collaboration with leaders from EpiVax Inc. and PharmaJet, is working together to develop a nucleic acid vaccine candidate. The plasmid DNA vaccine is still under the preclinical stage of evaluation. The vaccine candidate further influences their investigational UNITE™ (Universal Intracellular Targeted Expression) platform. EpiVax's in silico T cell epitope prediction tool and PharmaJet's well-established Tropis® Needle-free Injection System specifically targets the delivery to the intradermal layer**.** Immunomic (ITI) in collaboration with EpiVax and PharmaJet aimed to develop and manufacture scalable vaccine candidate who could be prophylactically and therapeutically suitable while maintaining safety and immunogenicity (Colo, 2020). With DNA vaccines, developers are also analyzing different entry mechanisms that include needle injection with electroporation, needle-free system, intradermal and intramuscular route of administration.

Herd Immunity — key concept to epidemic control

Herd immunity is a form of immunity that describes the indirect protection developed within a group

of populations in which a certain group of people develops natural or acquired immunity to infection which is a key concept for epidemic control. Herd immunity states that only a certain percentage of the population needs to be immune either through overcoming natural wild type infection or induced through vaccination against the infectious agent like a virus, to break the chain of epidemic or pandemic outbreak (Fontanet and Cauchemez, 2020). Overall herd immunity indicates a level or status of population immunity in the given community at which the spread of contagious diseases like COVID-19 declines and stops even after all preventive measures have been relaxed.

However, if the immunity level is still below the herd immunity level and if all preventive measures are being relaxed, then there exists a high possibility of the start of the second wave of infection in the same community (Britton *et al.*, 2020). Through vaccination, the spread of infection can be limited, allowing herd immunity to develop so that the chain of infection from unvaccinated to vaccinated people can be controlled and managed more efficiently. The active immunization to immunologically responding population results in the development of immune memory in terms of neutralizing antibodies. These will be more target specific to fight against subsequent attacks and in persons with impaired immune systems. Herd immunity is also concerned with limiting the spread of infection during the pre-developmental period of vaccine utilizing the body's natural immune system such as in the current pandemic scenario of COVID-19.

With COVID-19 positive cases beyond 30 million across the world, the role of herd immunity in limiting the pandemic chain of transmission seems to be persuasive. Yet, some countries, including India, have recently confirmed the onset of re-infection which was analyzed using genome sequencing to demonstrate that the second wave of infection was because of the emergence of genetically distinct SARS-CoV-2 virus. However, it might help the vaccine industries for policy-making decisions in terms of scaling up the production of an efficient vaccine to meet the global demand.

COVID-19 Vaccine developmental Challenges and possible risks

The development and production of safe, efficient, and scalable vaccines to combat the COVID-19 pandemic are yet to face several challenges and multiple risks despite million dollars investments from global economic alliances. Regulatory issues in deploying vaccines among several countries across the world could be one of the significant challenges in this race against COVID-19. Hastening vaccine production during the current pandemic to meet the global need as an emergency supply might face several issues in terms of vaccine efficacy, adverse medical events if exists, priorities in supply, and many more (Diamond and Pierson, 2020). Vaccine efficacy of 70% in the case of COVID-19 is sufficient enough to control the pandemic. Still, if the efficacy does not exceed beyond 60%, the virus spread continues with the regular pattern, and the world will have to live with this for a long time.

Despite vaccines being developed through extensive trials utilizing several developmental platforms, the risk of triggering antibody-dependent disease enhancement (ADE) with subsequent upregulation of proinflammatory cytokines could be one of the possible adverse events that might be encountered for which clinical management strategies need to be established as per the guidelines. Antibody-mediated immunopathology is one of the serious concerns that might lead to excessive immune activation resulting in the release of cytokines and chemokines that potentially could enhance disease status (Iwasaki and Yang, 2020). Hence, vaccine candidates must not be equipped with undesired immunopotentiation but at the same time, adequately safe for front line healthcare workers, adults above 60 years with or without underlying comorbidities like diabetes or hypertension. Developed and approved vaccines further need optimized transport conditions apart from scalable production and a well-coordinated international network to deploy across the globe.

Suppose the vaccine is built from an inactivated form of SARS-CoV-2. In that case, churning out for billions of doses could be easy since the industrial technology possibly involved in this process is well established from at least the 1950s (Khamsi, 2020). Nucleic acid vaccine platforms may involve a more straightforward process and likely to make them easier to scale up, but no vaccine with this approach has yet been approved against pandemics. One of the significant challenges is to find appropriate animals that would be infected by the virus in the same way as humans are affected.

In contrast to the regular mice model which might show resistance to the virus, transgenic mice such as Tmprss2 knockout, Stat 1 knockout, human-ACE2 transgenic mice are preferred for experimentation in which HLA (Human Leukocyte Antigen) antigens are expressed. These mice might prove to be useful in understanding the pharmacokinetics of developing vaccine candidates against COVID-19. Developed vaccine candidates will be further subjected

to scalable production for emergency usage, and an emergency stockpiling facility will be arranged to ensure that the vaccine will be made available 24/7 to the high priority groups in case of emergency.

CONCLUSION

Several vaccine developmental studies are in the pipeline undergoing extensive human phase trials after the successful preclinical examination. Nine vaccine candidates are ahead with phase III clinical trial utilizing vaccine platforms such as non-replicating viral vector, inactivated, and RNA followed by protein subunit vaccines which are under phase II clinical trial. Besides ongoing clinical trials of multiple vaccine candidates worldwide, multiple approaches for computational study designs and dynamic molecular platforms for understanding the possible immunological reactions that might be involved in vaccine-mediated adverse events need to be carried out.

Meanwhile, the World Health Organization is periodically releasing landscape draft of COVID-19 vaccine candidates that helps in tracking the status of vaccine development globally which also helps scientific communities to design innovative study for the development of new vaccine candidates. With the declaration of global emergency, international alliances across the globe actively joined a worldwide campaign in raising funds for the development of vaccines and deployment across the globe on a priority basis.

World Health Organization is working in collaboration with scientists, investors, and health industries through the ACT (Access to COVID-19 Tools) accelerator to speed up the pandemic response by facilitating equitable access and distribution of effective vaccines to all countries and on a priority basis to health care workers, aged above 60 years belonging to high-risk groups.

On the other side, during the COVID-19 pandemic, several established antiviral drugs are subjected to phase trials to validate its efficacy in treating COVID-19 cases, while waiting for vaccines to be released from pipeline to lifeline. Continuous awareness programs and alerts through social media platforms are also being implemented. Overall, the entire world has joined a campaign to fight against the COVID-19 health crisis favouring the release of pipeline candidates as lifeline medicinal vaccines.

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Conflict of Interest

The authors would like to mention that they do not have any conflict of interest.

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