International Journal of Pathogen Research

4(3): 40-62, 2020; Article no.IJPR.58283
ISSN: 2582-3876

SARS-CoV-2 Recombinant Spike Protein-based Vaccine: A Promising Candidate against the Recent Imperial Coronavirus Disease (COVID-19)

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Authors' contributions

This study was collaboratively executed by all authors. Author POC conceived the idea and prepared the manuscript. Substantial contributions to the conception and plan of the study was made by authors CBO and POC. Author OMO collected the relevant information required for drafting the manuscript. All authors read and made final approval of the manuscript.

Article Information

DOI: 10.9734/IJPR/2020/v4i330115
Editor(s):
(1) Dr. Khadiga Ahmed Ismail Eltris, Professor, Ain Shams University, Egypt.
Reviewers:
(1) Chinmoy Mishra, Odisha University of Agriculture and Technology, India.
(2) Jose Gnana Babu, RGUHS, India.
Complete Peer review History: http://www.sdiarticle4.com/review-history/58283

Review Article

Received 05 June 2020
Accepted 21 June 2020
Published 29 June 2020

ABSTRACT

In time past, to date combating against diseases and fatal disorders (of known or idiopathic cause) is a major effort among the human race. The emergence of several, novel and pathogenic viral infections have posed a great threat to humanity and could wipe us out of existence if there are no counter measures. Among the increasing number of pathogenic viruses in this past decade, the advent of the recent imperial SARS-CoV-2 coronavirus type cannot be underestimated as it is not just a malady endemic to a nation, but have also triggered an emergency of public health across the globe. SARS-CoV-2 a memorial of the initial Severe Acute Respiratory Syndrome (SARS) reported in China in (2003) is the etiological agent of the mysterious COVID-19 reported to originate from Wuhan, Hubei province, China in 2019. Though the virus exhibit mild pathogenicity compared to other previously emerged human coronaviruses (HCoV-OC43, HCoV-HKU1, MERS-CoV, and SARS-CoV), however, the high transmissibility and infectivity among human is alarming. In spite the evidences from the increasing number of substantiated global cases and deaths resulting from the epidemic outbreak to date, curative measures to curtail and treat this disease are

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still lacking. Just like SARS-CoV, it has been revealed that SARS-CoV-2 also uses similar receptor for infectivity and shares similar disease pathogenesis. This knowledge presents a therapeutic target against COVID-19. The presence of cross-reactive epitopes in the spike protein subunit of SARS-CoV and SARS-CoV-2 present the use of neutralization antibodies from convalescent SARS challenged patients against COVID 19. However, limited cross-neutralization due to lower sequence conservation in the Spike protein subunit could render this approach ineffective. Realizing the urgent need for developing potent therapeutics against the imminent risk of COVID-19 on humanity, this review article, suggests the use of SARS-CoV-2 recombinant Spike protein-based vaccine as an immunotherapeutic target to combat COVID-19 based on garnered knowledge from researches on consanguineal coronaviruses (SARS-CoV, and MERS-CoV), and current trends in vaccine development against this infection.

Keywords: SARS-CoV-2; spike protein; receptor; Neutralizing antibody (nAb); COVID-19; coronavirus; pathogenicity; vaccine.

1. INTRODUCTION

Since the dawn of time, humanity have suffered several health challenges resulting from both known and unknown aetiology, and our race have faced an imminent risk of eradication within this recent decade with the emergence of many novel diseases and disorders. Nevertheless, human race is even more endangered with the emergence of the recent coronavirus outbreak in late 2019. This viral disease popularly known as COVID-19 is caused by the newly discovered (SARS-CoV-2) coronavirus, and has been considered an epidemic by the world health organisation (WHO) due to the magnitude of its transmission among humans. The outbreak of this viral malady, does not just pose a threat on public health only but have also incurred a global economic imbalance, while manifesting an insurgence of overwhelming fear among every individual within the planet. The increasing rise in the number of substantiated global cases between the time of epidemic hit of COVID-19 to date, have caused a surge in mortality rate worldwide. Following the reports on the viral epidemic scale till May 2020, it was discovered that the outbreak has escalated to over 109 countries. The report on morbidity rate, by May 18, 2020, shows that the epidemic has so far resulted in 311,847 deaths with over 4.6 million substantiated cases globally [1], surpassing the verified combined cases and deaths of precedented coronaviruses distemper (SARS, and MERS) that arose in early 2003, and 2012 respectively [2]. Due to, the unending tenure of COVID-19, SARS-CoV-2 coronavirus remains an all-time human viral pathogen, and might be regarded as a detonated time bomb designed by nature to exterminate mankind. Considering the menaces imposed on human life force by this virus, the urgent requirement for both prophylactic and therapeutic measures to prevent SARS-CoV-2 infection, have acquired considerable interest. Currently, lockdown and curfews in affected countries have so far proved to be the safest option to prevent the continuous spread of SARS-CoV-2 infection.

To effectively develop a potent therapeutic target against SARS-CoV-2 infection, a better understanding of the following would be of pertinence; (the viral similarity with previously reported human coronaviruses within the same genetic line, the genome complexity of the virus, the entire proteomes of the virus, evolutionary reservoirs of the virus and mechanism of interspecies transmission, the pathogenicity, and antigenicity of the virus). More recently, extensive routine studies carried out on SARS-CoV-2 coronavirus, reveals that it is also a descending line of the β-coronavirus genera. The virus shows more similarities with pre-existing severe acute respiratory syndrome coronavirus (SARS-CoV) [3,4], identified in Guangdong Province, China, in late 2002 [5], compared to other human coronaviruses. Regarding the high genetic similarities between these two coronaviruses, SARS-CoV-2 can be referred to as an immediate sister to SARS-CoV. Similar to previously emerged human coronavirus (SARS-CoV and MERS-CoV), SARS-CoV-2 is also an enveloped, positive sensed single stranded RNA virus belonging to the family Coronaviridae [6-8]. Like the sister coronavirus (SARS-CoV), the genome encodes information for viral replicase proteins, structural glycoproteins including the spike (S), membrane (M), envelope (E), nucleocapsid (N), approximately 16 non-structural proteins, and relatively five-eight accessory proteins [9].

For coronavirus entry into host cells, it usually begins with an initiation phase in which a special protein on the host cellular surface called

receptor are recognised by certain protein motifs within the receptor binding domain (RBD) of the virus structural molecules, resulting in a kind of protein-protein interaction. In spite the aforementioned proteins encoded by the viral RNA, the infection is generally initiated by the attachment of the spike (S) protein to a specific receptor on the host cell, which stimulate a structural conformational change in the S protein [10]. Receptor recognition by S-glycoprotein, accompanied by RBD interaction with host cellular receptor, plays a main role in coronavirus infectivity, and pathogenicity. Consequently, it has become a target to develop potent antiviral drugs, and vaccines against COVID-19 and is now a milestone for a good deal of extensive research study for viral vaccine development. However, in spite the increasing number of studies in receptor recognition by SARS-CoV-2, there is still no licensed vaccine and target therapeutics till date to prevent from this viral malady.

More recently, among the available potent therapeutic options against SARS-CoV-2 infection, neutralizing antibodies (nAb) have been reported to be one of the most promising treatment line against the infection [11,12]. Neutralizing antibodies (nAb) functions by targeting receptor interaction site within the spike protein subunit, thereby impairing protein-receptor docking [13-18]. In spite the knowledge presented on the use of nAbs as therapeutic target against COVID-19, relatively little is still known about this approach. in a study, based on the knowledge from researches on SARS-CoV, nAbs targeting the virus was reported to exhibit significant *in-vivo* antivirus activities by decreasing virus titres in lung tissues of animal models [19-23]. Since SARS-CoV-2 and SARS-CoV have over 70% sequence homology and share the same human receptor, this might provide useful information on whether neutralizing antibodies (nAb) could be a therapeutic target against COVID-19. Also, there might be a likelihood of sharing cross-reactive epitopes [24].

Consequently, the possibilities for common epitopes between SARS-CoV, and SARS-CoV-2, have triggered the use of neutralizing antibodies from convalescent SARS patients against SARS-CoV-2 coronavirus in some recent research study. In a recent study, [25] reported that human antibody (CR3022) that neutralizes SARS-CoV [26], could also bind to the RBD of SARS-CoV-2. Despite the evidences from this study and similar studies, a main limitation of this approach, results from limited cross-neutralization due to lower sequence conservation of residues in the cross-reactive epitopes between the spike RBDs of both coronaviruses [24,27]. Nevertheless, there might be possibility of neutralizing antibodies based on spike-RBD of SARS-CoV-2 coronavirus to act more efficiently as potent curative target regardless of cross-reactive epitopes. Realizing, and anticipating the urgency for potent curative measures against COVID-19, this review study attempt to discuss the promising potentialities of SARS-CoV-2 recombinant spike protein-based vaccine as candidate line of defence, and the possibility for development against COVID-19, based on recent research advances on SARS-CoV-2 and the knowledge from researches on SARS-CoV and MERS-CoV.

2. GENETIC INSIGHTS INTO CORONAVIRUS: GENOME ORGANIZATION, AND REPLICATION

Coronaviruses, are group of pleomorphic, spherical, enveloped viruses of the family *Coronaviridae* (order *Nidovirales*) [4,28]. They infect a wide variety of livestock, and companion animals causing several health distemper that range from mild respiratory abnormality to severe health complications. The genera coronavirus gained a prominence as human pathogen with the outbreak of severe acute respiratory syndrome (SARS) that was epicentred in china, in late 2002. Currently, the evidences from genomic organization of coronaviruses, indicates the presence of a positive-sensed single-stranded RNA genome, encapsulated within the membrane envelope of the viral structure [29]. Enclosed within the viral membrane are transmembrane (M) glycoprotein, spike (S) glycoprotein and the envelope (E) protein, surrounding a disordered or flexible, presumably helical, nucleocapsid [30,31]. So far, four coronavirus genera entitled (α, β, γ, and δ) have been identified, nonetheless, only two among the aforementioned are reported as human coronaviruses (HCoVs). A good number of extensive research has revealed HCoVs in the α coronavirus (HCoV-229E and NL63) and β coronavirus (MERS-CoV, SARS-CoV, HCoV-OC43 and HCoV-HKU1) genera [4]. Reportedly, evidences based on molecular characterization of the newly identified coronavirus (SARS-CoV-2) popularly known for the recent viral malady (COVID-19), in late December 2019, reveals that it is also member of the β-CoVs.

More extensive researches, to explore and better understand the genome of coronaviruses indicates that the viral genome ranges approximately between (26-32 Kb) [4], and consists of six major open-reading frames (ORFs), and a number of other accessory genes [7]. Similar to a mature eukaryotic mRNA, the genome contains a 5'capped structure along with a 3' polyadenyl tail, that enables its translations into replicase polyproteins. Unlike any other RNA viruses, they express the largest and most complex polyproteins [32]. The replicase protein is encoded by approximately two-thirds of the viral genome, and is localized within the first open reading frame, consisting of two large overlapping open reading frame (ORF1a/b) (Fig. 1). In contrast, the other one-third of the RNA genome, is occupied by both the structural and accessory proteins [33]. Translation of the replicase originates from ORF1a and is continued in the ORF1b following a (–1) ribosomal frameshift signal [4]. Following translation, the replicase gene is expressed as two distinct polyproteins (pp1a and pp1ab), that are subsequently processed into approximately sixteen non-structural proteins by the viral encoded proteases [primarily by chymotrypsin-like protease ($3CL^{pro}$), and papain-like protease (PLPs)] [34]. The resulting individual non-structural proteins (nsp1-nsp16) encoded by these polyproteins, thereafter assembles to become the viral replicase-transcriptase complex (RTC) that subsequently produces the anti-sense genome, nascent viral genome, and the sub-genomic RNA in the host cell cytoplasm [33]. These replication complexes (RTC) are formed when nsps rearranges membranes originating from the rough endoplasmic reticulum (RER) into double-membrane vesicles (DMVs) which in turn serves as site for viral replication and discontinuous transcription [34,35]. As opposed to the first ORF, the other ORFs on the remaining third of the viral genome encode four main structural proteins of pertinence in the virion structure namely: spike (S), envelope (E), nucleocapsid (N) and membrane (M) proteins.

More recently, a minority of the coronaviruses have been reported to encode several uncharacterized accessory proteins. Unlike all structural and non-structural proteins encoded by the viral genome, these proteins do not participate in viral replication, nonetheless, they play an important role in the viral invading mechanism. They do these by distinguishing coronavirus infections from each other, with variableness across the family, thus, enabling the viruses to adapt to current and novel hosts [36].

Reportedly, hemagglutinin esterase (HE) has been found in a subgroup of coronaviruses, predominantly among the β-CoVs. Although this protein is not essential for replication [4], it however contains activity that might promote viral entry and pathogenesis by binding to sialic acid receptors on host cell surface.

Receptor recognition, accompanied by specific binding of structural glycoprotein with host cell surface, is a peculiar attribute by most viruses to access host cell cytoplasm, and inject genetic materials. For coronaviruses, entry into host cell, is generally mediated by interaction between the receptor binding domain (RBD) within the spike (S) glycoprotein and distinct receptor, accompanied by virus uptake into endocytic vacuoles. To date, different receptors for several coronaviruses have been identified. The α-coronaviruses uses aminopeptidase N (APN) for cellular attachment to host cell surface [13]. Insights on receptor recognition, also indicate that human coronavirus-NL63 (HCoV-NL63), and SARS-CoV share angiotensin-converting enzyme 2 (ACE2) as their receptor [37,38], with ACE2 currently reported as the receptor for the newly discovered SARS-coronavirus (SARS-CoV-2) [39]. More also, murine hepatitis virus gain access to host cell via carcinoembryonic antigen-related adhesion molecule (CEACAM1a) [40], and dipeptidyl peptidase 4 (DPP4) for MERS-CoV that emerged in 2012 [41].

Following attachment of RBD within the spike (S) glycoprotein of coronaviruses with it receptor, excisable RNA genome from the viral particle, ingresses into host cells by Proteolytic cleavage of the Spike glycoprotein, commonly accompanied by the fusion of the viral and endosomal membranes [42]. Once the RNA genome is in the host cytoplasm subsequent to viral entry, it becomes an mRNA for the first open reading frame (ORF1), resulting in translation, and processing of viral replication proteins [32], including the RNA-dependent RNA polymerase (RdRP) [34]. As earlier said, the resulting proteins assemble on DMV, thus, becoming sites for viral RNA replication, and in addition the replication complexes. The viral RNA is used as a template by RdRP, and the replication complexes to generate the sub-genomic mRNA from a minus strand intermediate [43,44]. Subsequently, the sub-genomic mRNAs are transcribed by neighboring downstream ORFs,

followed by translation into structural and non-structural viral proteins. Following complete synthesis of the structural glycoproteins encoded by the sub-genomic mRNA, the (S, E, and M) proteins are imported into the endoplasmic reticulum (ER), and are shuttled along the secretory pathway into the Endoplasmic Reticulum-Golgi Intermediate Compartment (ERGIC) [45,46] (Fig. 2). Consequently, viral RNA genomes, encapsulated by the N protein also enters into the ERGIC containing the viral structural proteins [47], resulting in the export of mature virions from infected cell via exocytosis.

3. HUMAN CORONAVIRUSES (HCOVS): ORIGIN AND INTERSPECIES TRANSMISSION

Prior to the initial SARS outbreak epicentred in china in late 2002, studies on the origin and interspecies transmission of human coronavirus (HCoVs) were relatively little, since many of the coronavirus infection were more common among animals [48] than human. The emergence of SARS-CoVs and other recent HCoVs, have prompted an extensive study in order to validate the intermediate species before human-human transmission. Nonetheless, before the severe acute respiratory syndrome (SARS) epidemic caused by the memorial SARS coronavirus, there were only reports of two HCoVs namely (HCoV-229E and HCoV-OC43). To date, a total of seven HCoVs have been identified. Amidst these distinct viruses, only two HCoVs: (HCoV-229E and HCoV-NL63) belongs to the α-coronavirus group. In contrast, the other five HCoVs are in the β-coronavirus genera including the recent SARS-coronavirus version (SARS-CoV-2). In spite the increasing rise in the number of HCoVs, the biological vector for infection, is still a mystery. An understanding of the origin and transmission pathways of these viruses would enable a rationale for the development of therapeutic target and vaccines to curtail their infection.

Similar to other highly pathogenic viruses, it was evident that a zoonotic origin of the virus leading to interspecies spill over to humans was possible [49-54]. For years, HCoVs continues to cross the species barrier infrequently. Recent advances in evolutionary surveillance and genetic studies indicates a zoonotic potential, resulting from the adaptability of the viral spike glycoprotein to recognise and bind receptors of other species [54]. To effectively discuss the zoonotic origins of HCoVs, it is of great pertinence to emphasize the definitions and characteristics of evolutionary, natural, reservoir, intermediate and amplifying hosts of HCoVs [55]. Animals are presented as the evolutionary host of a HCoVs if they harbour a progenitor sharing high sequence homology [55]. Similarly, the reservoir host sustains the viruses prior to infection. Since the infection is spontaneous, both hosts can be regarded as the natural host, and may convey the viruses to other receptive host that possibly allow temporary replication of the virus prior to human infection. In order to identify the natural reservoir, so as to understand the pattern of transmission of the infection among interspecies, a wide variety of animal have been closely observed. While most of this animals have been surveyed, bats continue to be among the most exuberant source for most viral diseases [56]. Reportedly, bats have been the natural reservoir of a broad consortium of coronaviruses including the HCoVs [57]. Relevant studies to explore these reservoirs have indicated a bat origin for the α-HCoVs [58]. Surveillances of HCoV-229E reveals that the virus originated from bats, and camels acting as the intermediate hosts [58-62]. Molecular evolutionary analysis has revealed Bovine CoV (BCoV) as the closest genetic counterpart to HCoV-OC43 [62]. The outbreak of SARS-CoV led to surveillances of numerous exotic mammals in order to identify the biological source of the infection. Evidences from close observation of these animals indicates that masked palm civets and a raccoon dog within a live-animal market in Guangdong, China could be the host for transmission due to the presence of the viral RNA within the mammals [63]. To support the progress of this finding, epidemiological surveillances indicated a strong link between SARS-CoV and exposure to masked palm civets [64]. However, the disbelief as to whether these small mammals were the true hosts of the virus, arose since masked palm civets from the wild or from farms with no wet-market exposure were mostly negative for the virus [65]. The evidences of two novel SL-CoVs, named RsSHC014 and Rs3367 found in horseshoe bats, and are able to bind ACE2 to enter cells as SARS-CoV [66], therefore, reveals that SARS-CoV was either transmitted to humans from Chinese horseshoe bats directly or via masked palm civets or raccoon dogs in the live-animal markets of China [67]. Additionally, rodents were reported as the reservoir of two HCoVs: HCoV-OC43 and HCoV-HKU1 [68,69]. The presence of neutralizing antibodies against MERS-CoV in dromedary camels led to speculations that these animal

could be the true hosts of MERS-CoV. However, initial identification from an evolutionary studies authenticated bats as the evolutionary host of MERS-CoV [70–72], raising the possibility that dromedary camels could have been the intermediate host before infection in human. The emergence of the newly-identified SARS-coronavirus, is alarming. The increasing rise in mortality and morbidity, since the outbreak have acquired concerning interest. Consequently, researchers thought if the biological origin prior to interspecies transmission of the virus could be demystified, that it might provide reasonable explanation to better understand the viral nature, and a strong premium for preventive and curative options. Though bats have remained an evolutionary origin of most coronaviruses including the HCoVs based on earlier propositions, however, there was doubt as to whether bats could have been the natural reservoirs of SARS-CoV-2 since they were not available for sale in Huanan seafood market in Wuhan were the virus was first identified. To decode the viral origin, information from a study of CoV isolated from nine inpatients, revealed that SARS-CoV-2 was related to two bat-derived SARS-like coronaviruses (bat-SL-CoVZC45 and bat-SL-CoVZXC21) with an identity of 88%, compared to SARS- CoV (about 79%) and MERS-CoV (about 50%) [73]. Further studies to authenticate this finding, indicates an overall sequence similarity of 96.2% to a bat SARS-related Coronavirus (SARSr-CoV; RaTG13) [7]. This however, presents a evidence that HCoVs are still in the population of bats, thus, supporting the initial suggestions that bats are the host reservoir of HCoVs.

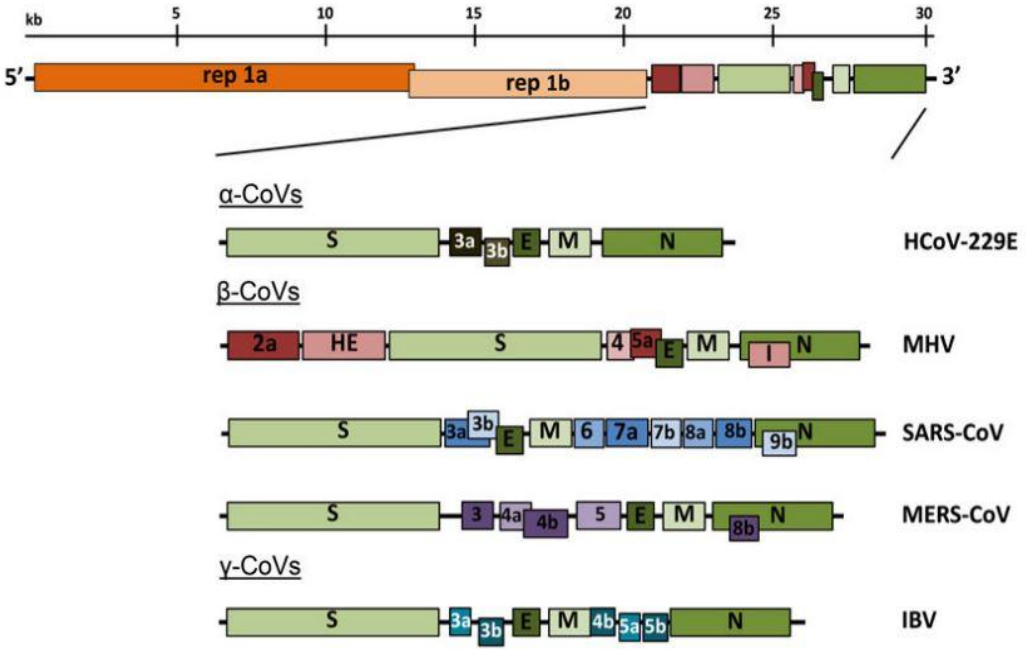

Fig. 1. A schematic illustration of the genomic organization of representative α, β, and γ-CoVs; the order of the genes is in the 5'-3' direction. The 5'- terminal depicts two-thirds of the viral genome consisting of two large overlapping open reading frame(ORF1a/b) encoding non-structural proteins (nsps) that forms the viral replicase transcriptase complex for viral replication and transcription. The other ORFs on the remaining one-third in the 3'-terminal encodes the structural glycoproteins and a number of accessory proteins as illustrated by the expanded region below. The alpha-numerals embedded in the expansion indicate the non-structural proteins respectively

Legend: α-CoVs-Alpha Coronaviruses; β-CoVs-Beta Coronaviruses; γ-CoVs-Gamma Coronaviruses; HCoV-229E-Human coronavirus 229E; SARS-CoV-Severe acute respiratory syndrome coronavirus; MERS-CoV-Middle east respiratory syndrome coronavirus; MHV-Mouse hepatitis virus; IBV-infectious bronchitis virus; HE-Hemagglutinin esterase; S-Spike; E-Envelope; M-Membrane; N-Nucleocapsid

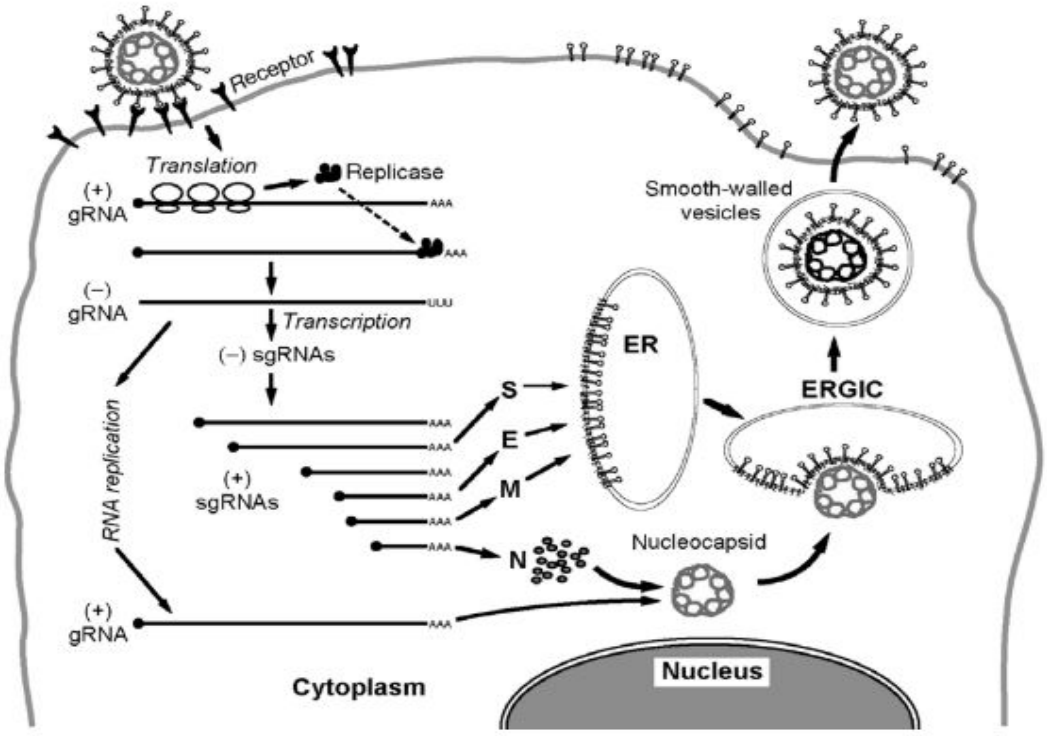

Fig. 2. A schematic representation of the life cycle of Coronaviruses (CoVs) in host cells. For CoVs entry into host cell cytoplasm, it begins by recognition of their respectivecellular receptor by the viral Spike-glycoprotein, generally accompanied by docking between the receptor-binding domain (RBD) in the S1 subunit of the viruses with host cellular receptor. The S2 subunit mediates viral fusion with the cell membrane through the endosomal pathway. Excisable RNA genome from the viral particle, ingresses into host cells and is translated into viral replicase polyproteins pp1a and 1ab, which are then cleaved and processed into small products by viral encoded proteases. RNA-dependent RNA polymerase (RdRP) generate series of sub-genomic mRNA from a minus strand intermediate [43,44]**, which are subsequently transcribed by neighboring downstream ORFs, followed by translation into structural and non-structural viral proteins. Subsequently, these proteins and the RNA genome assembles into progeny virons in the Endoplasmic Reticulum-Golgi intermediate Compartment (ERGIC) and are exported out of host cells in vesicles by exocytosis**

Legend: gRNA-Genomic ribonucleic acid; sgRNA-Sub-genomic ribonucleic acid; ER-Endoplasmic reticulum; *ERGIC- Endoplasmic Reticulum-Golgi intermediate Compartment; S-Spike; E-Envelope; M-Membrane; N-Nucleocapsid*

4. SARS-CORONAVIRUSES: MECHANISM OF PATHOGENICITY

The viral spike glycoprotein is a main determinant of host tropism, nonetheless, it presents a great premium for developing therapeutic targets and vaccines. The primary step for infection by SARS-CoVs is driven by the interaction of the S-protein with host cellular receptor (Fig. 3). More recent study has suggested a common receptor by SARS-CoV-2 and SARS-CoV for ingression into host cell cytoplasm. Both SARS-CoV-2 and SARS-CoV uses angiotensin converting enzyme 2 (ACE2), expressed on epithelial cells in the respiratory tracts and several human organs [25,73]. In a review study, it was suggested that SARS-CoV-2 might pass through the mucous membranes, especially (nasal and larynx mucosa), and then enter the lungs through the respiratory tract [74]. For both viruses to gain access into host cytoplasm, the spike (S) glycoprotein facilitates the binding of the two coronaviruses to human ACE2 [75,76], and is subsequently cleaved into two distinct polypeptide subunits (S1 and S2) by the host cellular proteases. A good number of

study indicate that the entry-activating proteases for SARS-CoVs include cell surface protease TMPRSS2 and lysosomal proteases cathepsins [77,78]. The S1 subunit of SARS-CoVs contains the receptor binding domain (RBD) that binds to the receptor [34]. Consequently, the binding leads to a conformational change in the highly conserved regions of the spike (S) glycoprotein, and the S2 subunit mediates fusion of the virion with host cellular membranes [34].

Following viral entry, its antigen is processed and packaged by antigen presenting cells (APC). Subsequently, antigenic peptides from the viral particles are presented by major histocompatibility complex (MHC), and are recognized by virus-specific cytotoxic T-cells [79]. In most SARS-CoVs infection mostly SARS-CoV-2, primary symptoms are usually fever and cough [80]. However, severe organ damages may manifest in infected patients due to the predominance of ACE2 receptors on a vast cellular surfaces providing more option for attachment between the viral spike proteins and host cellular receptor. More recent clinical manifestation has indicated severe organ damages including acute cardiac and kidney injuries, arrhythmias, gut, and liver function abnormalities suggesting myocardial, renal, enteric and hepatic damage in SARS-CoV-2 infected patients [1]. Similarly, evidences from severely ill SARS-CoV patients have suggested systemic manifestations with damages to the heart, gastrointestinal, liver, kidney, and other tissues [81,82]. Following antigen presentation, the host cellular and humoral immunity are subsequently triggered by virus-specific B and T lymphocytes [79]. To elicit an immune response against a viral infection, it often begins through the activation of pattern recognition receptors (PRRs) expressed by the innate immune cells [83]. These PRRs apprehend and bind evolutionarily conserved molecular structures called pathogen associated molecular patterns (PAMPs) that are produced during viral invasion [79]. Consequently, the recognition initiate a signaling cascade leading to expression of type 1 interferon (IFN) and other inflammatory cytokines (e.g tumor necrosis factor and interleukins) and chemokines (e.g CCL2 and CXCL8) [83]. The expression of IFN-1 (INF-α/β) is the main requirement for innate protection against viral infection, and the molecules exhibit strong antiviral potential that are of pertinence at the early stages of infection [84,85], by suppressing viral replication. However, many coronaviruses have developed multiple immune evasion mechanisms to abscond the early induction of IFN-α/β [83]. To escape or inhibit the IFN-1 pathway, both SARS-CoV and MERS-CoV stimulate the production of double-membrane vesicles (DMVs) that lack PRRs, and then replicate in these vesicles, thereby avoiding the host recognition of their dsRNA [86]. In addition, accessory protein 4a of MERS- CoV may possibly hinder the stimulation of IFN at the stage of MDA5 activation through direct interaction with double-stranded RNA, enabling the virus to flee detection by host immune cells [87]. Similarly, SARS-CoV genome encodes nsp-16, and contains (nucleoside-2' O)-methyl transferase activity. These activities allows the modification of viral RNA by adding a 2' O-methyl group, thus, disenabling recognition by MDA-5 [88,89]. Although relatively little is still known on the immune evasion mechanism of SARS-CoV-2, however, there might be possibility that the virus shares similar mechanism with precedented HCoVs (SARS-CoV and MERS-CoV).

In spite the advantageous role of type 1 interferon, a delay in its production often result in an uncontrollable viral replication, leading to damages of the airway epithelial and lung parenchyma, and thus a deleterious inflammatory cytokine storm [83,90]. Delayed response of INF-α/β often predominates in aged SARS-CoV infected individuals, and mouse models [91,92]. Consequently, this might result in intense disease manifestations in infected patients. Furthermore, a rapid surge in viral replication and pro-inflammatory cytokine/ chemokine response induces apoptosis in lung epithelial and endothelial cells, and damage the pulmonary microvascular and alveolar epithelial cell barriers, thus, causing vascular leakage and alveolar edema, eventually leading to hypoxia in the body [90]. More recently, acute respiratory distress syndrome (ARDS) have been reported the main cause of death from SARS-CoV-2 epidemic [79], and is often a common immunopathological event for newly emerged HCoVs (SARS-CoV-2, SARS-CoV and MERS-CoV) infections [93]. The rapid release of several pro-inflammatory cytokines (IFN-a, IFN-g, IL-1b, IL-6, IL-12, IL-18, IL-33, TNF-a, TGFb, etc.) and chemokines (CCL2, CCL3, CCL5, CXCL8, CXCL9, CXCL10, etc.) by immune effector cells in SARS-CoVs infection is a main contributor to ARDS occurrences [94-97]. This results in severe pulmonary damages, followed by death due to respiratory insufficiency.

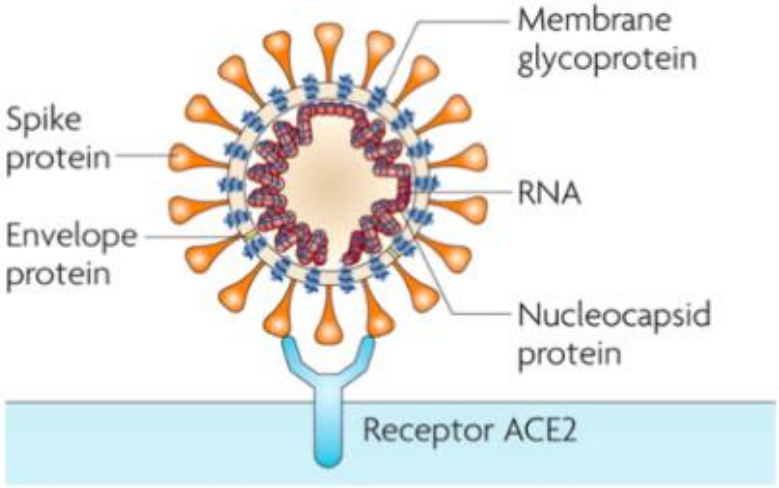

Fig. 3. A schematic representation of SARS-CoV-2: a positive-sensed single-stranded RNA genome, encapsulated within the membrane envelope of the viral structure [29]**. Enclosed within the viral membrane are transmembrane (M) glycoprotein, spike (S) glycoprotein and the envelope (E) protein, surrounding a disordered or flexible, presumably helical, nucleocapsid** [30,31]

Legend: RNA-Ribonucleic acid; ACE2-Angiotensin converting enzyme 2

5. ADVANCES FOR TARGET THERAPEUTICS, AND VACCINES AGAINST SARS-COV-2 INFECTION

Many efforts have been expended to develop potent therapeutic drugs, and promising candidate vaccines against the newly-emerged SARS-CoV-2 infection. In spite the adopted approaches and methods to protect against, and cure the respiratory malady in humans, the availability of effective drug targets and vaccines is still lacking. To date, there is still no licensed curative measure to counteract many CoV infection. Thus, the quest for both prophylactic and therapeutic targets continues indefinitely. To manage the viral infection, a good number of therapeutic options have been suggested based on various experimental facts from more recent routine studies. The use of broad-spectrum antibiotics, virally target inhibitors, combination therapy, convalescence plasma therapy, anti-inflammatory therapy remains the most promising management options against this infection, and have acquired considerable interests. Reportedly, viral proteases (3CLpro and PLpro) mediating cleavage and processing of viral peptides into functional sub-units to initiate viral replication and packaging within the host cell cytoplasm [29], presents a target against infection. In view of this context, drugs targeting these proteases in other viruses have been explored. Among the studied protease inhibitors against HCoVs, a combination of HIV drugs lopinavir and ritonavir showed promising antiviral activity against SARS-CoV in 2003-2004 when compassionately used as treatment [98]. Thus the combination has been suggested a treatment target. Though individual use of these protease inhibitors might be a treatment option for ongoing COVID-19 infection [99]. However, ritonavir contains activities that could boost the half-life of lopinavir by inhibiting cytochrome P450, resulting in clinically significant drug-drug interactions [100]. In a clinical trial, secondary outcomes from treatment with lopinavir/ritonavir indicated a clinical improvement in COVID-19 patients with hypoxemia, however, the inability of the combination to meet its primary objective [101], might be a limitation. More recently, nucleotide analogues with antiviral potentialities have been adopted to treat SARS-CoV-2 infection. Remdesivir showed an appreciable level of success against coronaviruses in animal models [102]. Though the mechanism of action of this nucleotide analog is not clearly understood, however, the possibility of interfering with viral RNA synthesis by inhibiting RNA-dependent RNA polymerase (RdRP) might exist [103]. Conceivably, the combination of remdesivir and anti-malaria drug (chloroquine) have been reported to effectively inhibit SARS-CoV-2 *in-vitro* [104]. Realising the promising potentialities

of remdesivir to protect from SARS-CoV-2 infection, have presented a milestone for more nucleotide analog trials. Currently, nucleotide analog including favipiravir, ribavirin and galidesivir have been suggested to be of clinical relevance against SARS-CoV-2 [105,106].

To mediate SARS-CoV-2 entry into host cell cytoplasm, ACE2 receptor is cleaved by serine protease (TMPRSS2), however, protease inhibitors such as camostat mesylate presents a target for another therapeutic option [107]. Intensive study on COVID-19 have revealed inhibition of a spike-mediated host cell invasion of SARS-CoV-2 by polyclonal antibodies (pAb) [108]. The quest for a broad-spectrum antiviral targets against SARS-CoV-2 infection have recently necessitated the use of drugs with both antiviral and anti-inflammatory properties. Drug targets able to inhibit clathrin-mediated endocytosis, have been reported to reduce viral infection *in-vitro* [109,110]. Baricitinib inactivates clathrin-mediated endocytosis by inhibiting AAK1 activity, thus, might possibly reduce SARS-CoV-2 infection [111]. A combination of arbidol, with antibiotics (moxifloxacin or levofloxacin, nemonoxacin, linezolid, azithromycin or amoxicillin), corticosteroids, and oxygen therapy has been used to prevent from COVID-19 [112]. The presence of cross-reactive epitopes within the spike glycoprotein of SARS-CoV-2 and consanguineal coronaviruses (SARS-CoV) presents providence for COVID-19 therapy. Consequently, monoclonal antibody (CR3022) raised against precedented SARS distemper showed cross-neutralization by binding RBD of SARS-CoV-2 [25]. Acute respiratory distress syndrome (ARDS) have been reported in recent clinical manifestations, and has been the main cause of death from SARS-CoV-2 infection [79]. This correlate with an uncontrolled inflammatory innate immune responses resulting in an exuberant release of several pro-inflammatory cytokines [94-97]. Drug molecules able to inhibit downstream signalling components might putatively block cytokine storm-related immunopathology resulting from COVID-19 [113]. Chloroquine phosphate has preliminary acquired favourable results to protect from SARS-CoV-2 associated pneumonia in china [114]. The molecule putatively acts by inhibiting the production and release of TNF and IL-6, thus, indicating its promising potentialities to suppress the cytokine storm in COVID-19 infected patients [114]. More also, a couple of clinical trials have adopted convalescent plasma containing neutralization antibodies to treat infected patients with acute respiratory distress syndrome associated with COVID-19, and a number of clinical improvement have been recorded [115]. In spite the promising potential of the highlighted drug targets, non-have been able to ascertain complete treatment against COVID-19. Hence they suffer the fate of not being licensed, providing an option to explore more therapeutics.

Currently, the development of COVID-19 vaccines is still ongoing. For novel viral infection such as COVID-19, effective vaccines are essential so as to the reduce disease severity, viral shedding, viral transmission, and also regulate its outbreaks [79]. Nonetheless, developing a new vaccine is not only cumbersome but also time consuming, since it would require several safety evaluations before availability [116]. In the lack of vaccines against SARS-CoV-2 infection, many vaccination strategies based on existing knowledge's from SARS-CoV and MERS-CoV are now being explored. More recent adopted approaches include live-attenuated virus, viral vectors, inactivated virus, subunit vaccines, recombinant DNA, and proteins vaccines [117], with a good number of them still in the preclinical stages using animal models. Though a number of study have revealed the potentialities of one or more of these vaccines as promising candidate to prevent from COVID-19, however, there are still little clinical evidences to substantiate their efficacy. Hence more vaccine options are continuously explored until a suitable target is obtained.

6. CONSIDERATIONS TO SARS-COV-2 RECOMBINANT SPIKE PROTEIN-BASED VACCINE AS PROMISING CANDIDATE AGAINST COVID-19

The availability of effective vaccines has improved the quality of public health by preventing infectious diseases [118]. In spite representing a major triumph over classical medicine, there is still no vaccine to combat new emerging pathogens. The newly identified novel coronavirus (SARS-CoV-2) is still on the loose with no licensed vaccine to prevent from its infection. Consequently, several research groups around the world have extensively explored heterogeneous approaches to develop potent vaccine to curtail COVID-19. However, a major hurdle in the development of COVID-19 vaccines, might possibly result from undesired immunopotentiation in the form eosinophilic infiltration after immunization [119]. Another

general concern is the likelihood for antibody-dependent-enhancement (ADE), resulting in increased cell infectivity. Ultimately, understanding the mechanisms of vaccine-induced immunity would rationalize an effective vaccine target against COVID-19. As earlier discussed, the spike glycoproteins of coronaviruses mediate viral entry into host cells, and are the main inducer of neutralizing antibody to stimulate a protective cellular immunity against CoVs [120]. Thus, it has preliminary acquired favourable interest for developing COVID-19 vaccines. In this context, recombinant vaccine containing viral spike glycoprotein may present a great premium to combat COVID-19.

Based on garnered knowledge from SARS and MERS vaccine development, vaccines based on recombinant spike protein have demonstrated efficacy in protecting immunized animal models from infection. Early effort to protect against SARS revealed that RBD protein induced a long-term, and a high-level SARS-CoV spike-specific antibodies concomitantly with neutralization antibodies that protected most of the vaccinated mice against SARS-CoV, after immunization against infection [121]. In a study, it was demonstrated that recombinant RBD formulated with Sigma's adjuvant system could elicit neutralizing antibodies that enhanced protective immunity in vaccinated animal models, and conceivably reduced eosinophilic infiltration [10,120,122-124]. In another study aimed at comparing the immunogenicity of recombinant receptor-binding domain (rRBD) protein in three expression systems (mammalian (293T) cells, insect (Sf9) cells, and *E. coli*), and their protection against SARS-CoV infection in mouse model, it was revealed that all three rRBDs could effectively elicit immune response by inducing highly potent neutralizing antibody to effect complete protective immunity against SARS-CoV infection in the mouse models [124]. It has been noted that vaccination by RBD-Fc induced high S-specific antibody titre with a long-term potent neutralizing activity against SARS-CoV infected mouse models. The result of the study later revealed that four of five vaccinated mice had gained immunity against subsequent SARS-CoV infection during the preclinical trial [123]. Similarly, in a study to optimise expression conditions for scale-up production of RBD vaccine candidates, two RBD protein variants (RBD193-WT, and RBD219-WT) and their respective deglycosylated forms (RBD193-N1, RBD193-N2, and RBD193-N3), (RBD219-N1, RBD219-N2, and RBD219-N3) expressed in yeast expression system were compared for antigenicity, functionality, and immunogenicity using alum as adjuvant. The result of the study showed that deglycosylated form (RBD219-N1) of rRBD protein induced the most potent RBD-specific antibody responses to prevent against SARS-CoV in immunized mice [125]. Reportedly, BALB/c mice immunized with recombinant protein encoding the N-terminal domain of MERS-CoV spike protein demonstrated an appreciable level of humoral immune response in MERS-CoV challenged mouse models [126]. In an early study to develop a vaccine for prevention against MERS-CoV infection, Du et al. [127] demonstrated the safety and efficacy of a recombinant protein containing a 212-amino acid fragment (residues 377-588) in the truncated receptor-binding domain (RBD: residues 367-606) of MERS-CoV spike protein conjugated with human IgG Fc fragment (S377-588-Fc) for the treatment of MERS-CoV challenged mice models in a preclinical trial. The evidences from their study indicated that the recombinant conjugate protein could be highly expressed in the culture supernatant of transfected 293T cells, and the purified S377-588-Fc protein could efficiently bind to dipeptidyl peptidase 4 (DPP4), the receptor of MERS-CoV, thus potently inhibiting MERS-CoV infection. Furthermore, the result from their study showed that the recombinant S377-588-Fc could prevent from infection by inducing strong MERS-CoV S-specific antibodies in vaccinated mice, which prevent the priming of MERS-CoV RBD with DPP4 receptor, thus, suggesting its potential for further development as a therapeutic modality for treating MERS-CoV infection in human.

Accordingly, in a study carried out to evaluate a recombinant receptor-binding domain (rRBD) protein vaccine in animal cohort involving nine *Rhesus macaque* model randomly assigned to three groups designated as (high-dose, low-dose and mock groups) Lan et al. [128] reported that immunization with different doses of the rRBD with or without alum adjuvant at different time points (0, 8, 25 weeks) elicited robust and sustained protective immunological responses in the monkeys, and was able to alleviate pneumonia with evidences of both reduced tissue impairment and viral clearance of airways as demonstrated by analysis of the nasal, oropharyngeal and rectal swabs prior and after the monkeys were challenged with MERS-CoV at day 14. The result from their study presented further development of an effective human vaccine against MERS-CoV infection.

The urgency for developing a safe, and cost-effective vaccine following SARS-CoV outbreak in china, in late 2002 prompted the consideration of expressing spike proteins of SARS-CoV in plant systems. The idea behind this great invention was based on the premise that ingested transgenic plant lines expressing the rRBD proteins might significantly elicit specific antibodies to protect from the infection. In view of this context, Pogrebnyak et al. [129] demonstrated the expression of N-terminal fragments of SARS-CoV spike protein in tomato and low-nicotine tobacco plants. Their study revealed an increased level of SAS-CoV-specific IgA in mice models after ingestion of the tomato fruits expressing the recombinant N-terminal fragment (rNTF) protein. In contrast, mice sera parenterally binding with tobacco-derived S1 protein revealed the presence of SARS-CoV-specific IgG as indicated by Western blot and ELISA analysis.

Though their study presented a sound hypothesis for continuous trial in human, nonetheless, little have been reported on this approach. Additionally, there have been other substantial evidences on preclinical success of recombinant protein-based vaccine against SARS-CoV and MERS-CoV. In a unique case study involving a pre-clinical development stage to prevent from SARS-CoV infection using mice models, Zhou et al. [130] have described that recombinant protein-based vaccine developed in the truncated region of S-glycoprotein, containing either the ecto-domain, or a His-tagged full-length version would elicit a strong immune response when cloned and expressed in a serum-free insect cell line (ExpresSF+®). The results of their study showed that immunization of mice with both proteins formulated without an adjuvant system at 3, 9, 27, and 50 µg per dose in phosphate saline elicited a strong immune response after two or three vaccinations with the antigen. Similarly, their study revealed that formulations of the truncated S protein with an adjuvant system, and aluminium hydroxide at 1 µg per dose, and 5 µg per dose significantly enhanced immune responses manifested by higher titres of serum ELISA and viral neutralizing antibodies. The findings however paved a way for consideration of human trial. The data obtained from several preclinical trials with distinct animal models to prevent from SARS-CoV and MERS-CoV infection by recombinant protein-based vaccine anticipated a further development to evaluate the safety in humans. In the earlier days of SARS-CoV outbreak, Cao et al. [131] launched a report to characterize the immunogenicity and antigenicity of a RBD-based vaccine in human. In their study, two panels of serum samples from recovered SARS patients were used. The first panel was a collection of 35 convalescent sera from SARS challenged patients 30 - 60 days after onset of illness, and the second from 19 SARS patients, enrolled in March 2003 for a follow-up study at the Peking Union Medical College Hospital, Beijing. To effectively study the antigenicity and immunogenicity of SARS-CoV in humans, they demonstrated a three years' follow-up in a cohort of 19 patients, enrolled during the SARS outbreak in 2003, all of which were verified by clinical laboratories to be serologically positive for SARS-CoV infection. All sequential blood samples were collected at month 3, 12, 18, 24, and 36, respectively, after the onset of clinical symptom, and the serum samples were tested at 100-fold dilutions for RBD-specific IgG antibodies by ELISA with RBD-His as a coating antigen. The result of the trial later revealed that RBD-specific antibodies could relatively maintain higher titres in the recovered SARS patients throughout the 3 years' follow-up. Unfortunately, it was discovered that one of the same sample was undetected, thus giving a positive rate of 94.74% by the end of year 2 and year 3 respectively. The findings from this study, suggested further development of recombinant spike protein-based vaccines for subsequent clinical trials.

Similar to other vaccine types currently suggested against COVID-19, vaccines based on recombinant spike proteins also have advantages and disadvantages. However, the superiority of recombinant spike protein-based vaccines is their ability to prevent or minimize host immunopotentiation [132]. Although, it was evident in some study that recombinant vaccines based on complete-length of the spike protein might induce certain antibodies that mediate enhancement of viral infection as seen in the case of SARS-CoV [133], thus, raising safety concerns for further development against CoVs [134]. Comparatively, this vaccine types still possess the highest safety profile [135], since they are developed from antigenic subunits, unlike conventional vaccines which are developed from whole pathogens by either attenuation or inactivation, and may regain their pathogenicity due to incomplete inactivation or attenuation, thereby resulting in the actual natural infection. To protect against SARS and MERS, a main hurdle faced from vaccination of

animal models by recombinant spike protein-based vaccines is the requirement for repeated booster doses during infection due to reduced level of immunogenicity [117,120]. However, a combination of the vaccine with established or newly developed adjuvants may represent a safer and faster means to scale through early clinical development, thus limiting the prejudice of lower immunogenicity [136]. Owing to the striking evidences above, the possibility to repurpose this approach to generate neutralizing antibodies specifically targeting SARS-CoV-2 to prevent its infection may exist.

Following the current situation report of SARS-CoV-2 outbreak, over 37 biopharmaceutical companies and academic sectors are in an ultimate race to develop a safe and effective vaccine by using several distinct vaccine platforms including live-attenuated, inactivated, mRNA, DNA, adenoviral vector and recombinant protein [137]. To beat these promising vaccine platforms currently raised against COVID-19, and to get commissioned for development and use in several affected countries, recombinant vaccines containing the spike glycoprotein of SARS-CoV-2 must scale through three phases of human clinical trials after the availability of sufficient pre-clinical data, so as to ensure the safety and efficacy of the vaccine. Towards the goal for developing COVID-19 vaccines, the coalition for epidemic preparedness innovation (CEPI) has provided funding to develop vaccines containing SARS-CoV-2 recombinant spike protein being run by collaborative efforts of several reputable biopharmaceutical companies and academic sectors. Currently, the Coalition for Epidemic Preparedness Innovations (CEPI) has funded Novavax with approximately $384 million for the manufacture of a virus-like nanoparticles based on recombinant expression of the Spike protein (NVX-CoV2373) [138]. The vaccine has scaled through preclinical trials demonstrating a strong antibody level that neutralizes infection in SARS-CoV-2 challenged non-human primates producing at least an order of magnitude higher antibody titres than three other vaccine counterparts (BBIBP-CorV, CoronaVac, and AZD1222) developed by Sinopharm, Sinovac, and AstraZeneca respectively [139], and is currently undergoing a phase 1 trial (NCT04368988) in Herston and Melbourne, Australia, and USA. To evaluate the safety and efficacy of this vaccine, a cohort of 130 healthy adults has been enrolled in a scheduled placebo-controlled observer blinded study, and the results is anticipated to be available in July [140], and if successful could be repurposed for clinical trials involving larger cohort in many COVID-19 affected countries. Correspondingly, Clover Biopharmaceuticals is at the verge of developing of a recombinant protein vaccine based on the trimeric spike protein of SARS-CoV-2 against COVID-19, and are in a collaborative effort with the Coalition for Epidemic Preparedness Innovation (CEPI) for fund in a total investment of $39.6 million for the COVID-19 vaccine [141]. The approach relies on their proprietary Trimer-Tag© vaccine technology platform, and the vaccine has been anticipated for use in phase 1 clinical trial in a cohort of chosen healthy adults and elderly participants from Australia after an extensive preclinical research [142], and if successful, in clinical trials could be scaled up to meet the world demand. Accordingly, recombinant protein vaccine based on oral vaccine platform by Vaxart has been indicated to prevent from SARS-CoV-2 infection. Preclinical testing with a single dose of the vaccine elicited IgG anti-SARS-CoV-2 antibodies in all animal models two weeks after immunization [143]. Consequently, the vaccine has entered a phase 1 clinical trial for safety in human. Reportedly, the Coalition for Epidemic Preparedness Innovations (CEPI) is in cohesive effort with the University of Queensland to develop a protein-based vaccine using molecular Clamp platform [144]. Furthermore, a vaccine able to confer protective immunity against SARS-CoV-2 using approaches based on SARS-CoV spike RBD is at the verge of production [123,145]. The vaccine which is spearheaded by the collaborative effort of Texas Children's centre for Vaccine Development at Baylor College of Medicine and its product development partners, has been reported to induced high-level neutralizing antibodies against both pseudotyped virus and a clinically challenged SARS-CoV mouse models when formulated with Alhydrogel® adjuvant system [145]. Following the current status of COVID-19 vaccine development till around June 02, 2020, a list containing a total of 131 vaccine candidates has been so far reported with only 10 candidates in clinical evaluation, and the rest either in their preclinical evaluation or at the verge of being repurposed for clinical trials. Among these vaccine candidates, a consortium of over 20 recombinant spike protein-based vaccines have been shortlisted one of which has been recognized by World Health Organization (WHO) in the front run have been developed and manufactured by Novavax as shown in Table 1 [146].

Table 1. Candidate recombinant protein-based vaccines raised against SARS-CoV-2 and their stages of development shortlisted from WHO draft on vaccine candidates as at June 12, 2020 [146]

Vaccine platform	Type of candidate vaccine	Developer	Development stage
Protein Subunit	Full length recombinant SARS CoV-2 glycoprotein nanoparticle vaccine adjuvanted with Matrix M	Novavax	Phase 1/2 NCT04368988
Protein Subunit	RBD protein fused with Fc of IgG + Adj.	Chulalongkorn University/GPO, Thailand	Pre-Clinical
Protein Subunit	Drosophila S2 insect cell expression system VLPs	ExpreS^{2}ion	Pre-Clinical
Protein Subunit	S protein	WRAIR/USAMRIIDS	Pre-Clinical
Protein Subunit	S protein + Adjuvant	National Institute of Infectious Disease, Japan	Pre-Clinical
Protein Subunit	VLP-recombinant protein + Adjuvant	Osaka University/ BIKEN/ NationalInstitutes of Biomedical Innovation, Japan	Pre-Clinical
Protein Subunit	Native like Trimeric subunit Spike Protein vaccine	Clover Biopharmaceuticals Inc./GSK/Dynavax	Pre-Clinical
Protein Subunit	Adjuvanted protein subunit (RBD)	Biological E Ltd	Pre-Clinical
Protein Subunit	S protein	AJ Vaccines	Pre-Clinical
Protein Subunit	Molecular clamp stabilized Spike protein	University of Queensland/GSK/Dynavax	Pre-Clinical
Protein Subunit	S protein	EpiVax/Univ. of Georgia	Pre-Clinical
Protein Subunit	S protein (baculovirus production)	Sanofi Pasteur/GSK Heat	Pre-Clinical
Protein Subunit	S1 or RBD protein	Baylor College of Medicine	Pre-Clinical
Protein Subunit	Recombinant protein, nanoparticles (based on S-protein and other epitopes)	Saint-Petersburg scientific research institute of vaccines and serums	Pre-Clinical
Protein Subunit	Oral E. coli-based protein expression system of S and N proteins	MIGAL Galilee Research Institute	Pre-Clinical
Protein Subunit	Nanoparticle vaccine	LakePharma, Inc.	Pre-Clinical
Protein Subunit	Recombinant spike protein with Advax™ adjuvant	Vaxine Pty Ltd/Medytox	Pre-Clinical
Protein Subunit	COVID-19 XWG-03 truncated S (spike) proteins	Innovax/Xiamen Univ./GSK VIDO-InterVac,	Pre-Clinical
Protein Subunit	Spike-based	University of Alberta	Pre-Clinical
Protein Subunit	Recombinant S1-Fc fusion protein	AnyGo Technology	Pre-Clinical
Protein Subunit	Recombinant protein	Yisheng Biopharma	Pre-Clinical
Protein Subunit	Recombinant S protein in IC-BEVS	Vabiotech	Pre-Clinical
Protein Subunit	Peptides derived from Spike protein	Axon Neuroscience SE	Pre-Clinical
Protein Subunit	Adjuvanted recombinant protein (RBD-Dimer)	Anhui Zhifei Longcom Biopharmaceutical/ Institute of Microbiology, Chinese Academy of Sciences	Pre-Clinical
Protein Subunit	RBD-based	Neovii/Tel Aviv University	Pre-Clinical
Protein Subunit	RBD-based	Kentucky Bioprocessing, Inc.	Pre-Clinical

Legend: Inc-Incorporation; VLPs-Virus like particles; Adj-Adjuvant; RBD-Receptor-binding domain; Fc-Fragment crystallizable; IgG-Immunoglobulin G; S2-Subunit 2; S-Spike; N-Nucleocapsid; SARS-CoV-2; Severe acute respiratory syndrome coronavirus 2

In a more recent fight against COVID-19, Sanofi Pasteur is currently leveraging with GSK to develop an adjuvanted COVID-19 vaccine. With reassuring preliminary preclinical data, the partnership is currently in support of clinical trials to determine the likeliness of one of its two vaccines to help COVID-19 challenged patients [147]. Importantly, the Danish biotech company "ExpreS^{2}ion Biotechnology ApS" in collaboration with the University of Copenhagen, is in the fore front in an ultimate race for vaccine development against COVID-19. The cohesive effort of this company and its consortium members is in a fray to develop a COVID-19 candidate recombinant protein spike-antigen based vaccine using DrosophilaS2 insect cell expression system VLPs. Reportedly, their platform which was similarly used to generate two malaria vaccines currently in phase1/ 2a clinical trials has been used to generate this COVID-19 candidate vaccine [148], and an ongoing preclinical trial with animal models have been anticipated with no report currently available.

7. CONCLUSION

SARS-CoV-2 has infrequently cross the species barrier, and is gradually reaching a peak in human population with no arsenals to prevent from its infection. The spike glycoprotein presents a target for promising candidate therapeutics and vaccines and has been a main focus in more recent suggested curatives owing to its potentials to trigger a host immune response. Conversely, the inability of many proposed treatments to successfully complete all phases of clinical trials with human has depreciated the providences for further development of many of these therapeutic options in an attempt to protect against SARS-CoV-2 infection and subsequent threat by this same virus. Although a good number of available data on preclinical and clinical trial has revealed that single use or synergistic effect of many of the proposed antivirals, vaccines, or other therapeutic targets, could prevent from the viral infection, providing a beacon of hope for continuation of human race, by reducing the stipulated timeframe for an actual cure. Nonetheless, there is still a chance to speed up the development of an actual cure that could be effected in several COVID-19 affected countries before the speculated time. In the lack of potent broad-spectrum therapeutics, and vaccines, the number of infected cases would increasingly rise uncontrollably until a more promising curative measure is developed to counteract both the present and future menaces that could emanate from SARS-CoV-2 infections. Anticipating the development of a potent vaccine against the imminent threat of SARS-CoV-2 infection on humanity, we have discussed in this review the possibility for remodelling the knowledge from recombinant protein-based vaccine developed from the spike protein of SARS-CoV, MERS-CoV, and other coronaviruses for the development of SARS-CoV-2 specific vaccine. With the striking evidences of success against COVID-19 through this vaccine platform, we believe the knowledge from this review study is a milestone that would present a premium for further development of recombinant protein-based vaccines that can be repurposed in more clinical trials, necessitating its development for use in COVID-19 affected countries upon successful evaluation of safety, dosage, and efficacy in human trials, and thus rapidly ending the quest for an actual COVID-19 cure.

8. RECOMMENDATIONS

The evidences from this study indicate the possibility for remodelling similar vaccine strategy against COVID-19, and may convincingly amount in a reasonable level of success against SARS-CoV-2. In the lack of an effective vaccine to prevent from this pandemic, recombinant spike protein-based vaccines represent promising arsenals to combat against COVID-19. Considerably, vigorous preclinical testing of this vaccine platform with various animal models, before an actual clinical trial would rule out the likelihood of undesired immunopotentiation that may result in adverse immune responses.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sdiarticle4.com/review-history/58283