



Mini review

Severe acute respiratory syndrome coronavirus 2 variants–Possibility of universal vaccine design: A review

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ABSTRACT

Both novel and conventional vaccination strategies have been implemented worldwide since the onset of coronavirus disease 2019 (COVID-19) pandemic caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Despite various medical advances in the treatment and prevention of the spread of this contagious disease, it remains a major public health threat with a high mortality rate. As several lethal SARS-CoV-2 variants continue to emerge, the development of several vaccines and medicines, each with certain advantages and disadvantages, is underway. Additionally, many modalities are at various stages of research and development or clinical trials. Here, we summarize emerging SARS-CoV-2 variants, including delta, omicron, and “stealth omicron,” as well as available oral drugs for COVID-19. We also discuss possible antigen candidates other than the receptor-binding domain protein for the development of a universal COVID-19 vaccine. The present review will serve as a helpful resource for future vaccine and drug development to combat COVID-19.

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Abbreviations: SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; RBD, receptor binding domain; COVID-19, coronavirus disease 2019; S1-NTD, S1 N-terminal domain; S1-CTD, S1 C-terminal domain; FP, fusion peptide; HR1, heptad repeat 1; HR2, heptad repeat 2; ACE2, angiotensin-converting enzyme 2; TMPRSS2, transmembrane protease serine 2; HE, hemagglutinin-esterase; mAbs, monoclonal antibodies; HIV, human immunodeficiency virus; FDA, Food and Drug Administration.

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1. Introduction

In January 2020, the World Health Organization (WHO) declared coronavirus disease 2019 (COVID-19) caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) as an “international public health emergency.” However, despite efficient transmission of information regarding COVID-19 during global quarantine, we failed to overcome the pandemic [1–6]. Given the lack of vaccines and treatments at the early stages of the pandemic, clinical testing stages and time taken for new vaccine development were significantly short. As a result, many people doubt the safety of the available vaccines. If infectious viruses, such as SARS-CoV-2, continue to emerge, pandemics will occur repeatedly, and humans will suffer enormous economic and political damage. At the start of the pandemic, when no specific vaccines or treatments were available, pre-existing cocktail treatments were administered to manage COVID-19. Although these treatments reduced mortality, increased recovery, and alleviated symptom severity, most approaches failed clinical trials. In this context, many scientists worldwide are involved in active research even today to find a preventive as well as curative vaccine/medicine for COVID-19. Currently, the urgent need for countermeasures against the pandemic has prompted rapid development of COVID-19 vaccines with diverse formulations. High efficacy and safety of the messenger RNA (mRNA) vaccines BNT162b2 (BioNTech-Pfizer) and mRNA-1273 (Moderna/NIAID) against COVID-19 have been demonstrated in clinical trials [1–3]. COVID-19 vaccines currently on the market produced by UK’ AstraZeneca, China’s CanSino Biologics, Russia’s Gamaleya Institute, and Belgium’s Janssen (Johnson & Johnson) also use this adenoviral-delivery strategy, although interest in this technology long predates the pandemic. However, in some cases, the level of antibodies decreases after infection or vaccination due to the continuous emergence of SARS-CoV-2 variants, and in a few cases, even previously immunized people are re-infected. In addition, variants including alpha, beta, delta, omicron, and the most recent “stealth omicron” have emerged in succession [4] (Fig. 1). Representative mutations include alpha mutations (B.1.1.7, September 2020), with seven mutated S proteins; beta mutations (B.1.351, October 2020), with nine mutated spikes; and omicron mutations (B.1.1529, November 2021), with 30 mutated S proteins [5,6]. The recent Omicron variant substantially increased the number of confirmed cases in 2022. With many mutated S proteins, the ability of this variant to overcome immune responses, such as infection-blocking antibodies and T-cell responses, is high [5]. These trends underscore the need for a periodic immune-enhancing vaccine against COVID-19 [7–9]. Consequently, concerns regarding the increased infectiousness of the virus and controversies on the effectiveness of vaccines are growing [10,11]. In this context, interest in the development of oral antivirals, in addition to vaccines, is increasing. One of the most anticipated oral antivirals on the market to treat COVID-19 is Paxlovid, produced by Pfizer. It is an oral medication that can reduce the severity of COVID-19 when administered promptly. In addition, several novel oral antivirals, including nirmatrelvir/ritonavir (Paxlovid), molnupiravir, and flvoxamine, are available [12]. Recently, a universal influenza vaccine was developed, which may take the

threat of both seasonal and pandemic influenza “off the table” [13,14]. As defined by the National Institute of Allergy and Infectious Diseases (NIAID) in 2018, a “universal” vaccine implies at least 75% effectiveness in all age groups for a minimum of 1 year against all strains of influenza A [15]. Such knowledge and tools of successful universal vaccine research for influenza may be used in the development of a novel universal COVID-19 vaccine. Here, we discuss possible antigen candidates other than the receptor-binding domain (RBD) protein for universal COVID-19 vaccine development. The present review will serve as a critical reference for future vaccine and drug development against COVID-19.

2. Structure and function of the coronavirus spike protein

SARS-CoV-2 is an enveloped, positive-sense, single-stranded RNA virus. The 5′-two-third of the genome encodes polyproteins (*pp1a* and *pp1ab*), which are cleaved by 3C-like and papain-like proteases into 16 nonstructural proteins, including an RNA-dependent RNA polymerase [16]. The 3′-end of the genome encodes four essential structural proteins, namely spike (S), envelope (E), matrix/membrane (M), and nucleocapsid (N), along with a set of accessory proteins (Fig. 2A). Most vaccine candidates against SARS-CoV-2 aim to block infection by inducing neutralizing antibodies against the S protein, thereby preventing uptake through the human angiotensin-converting enzyme 2 (ACE2) receptor [17–19]. S protein is a structural glycoprotein expressed on the SARS-CoV-2 surface, and it is a critical determinant of viral host tissue tropism (Fig. 2B). It mediates virus entry into the target cells upon ACE2 receptor binding [16,20] and is thus an important therapeutic target [21,22]. Moreover, spike antigenicity has been highlighted through the observation of spike-specific CD4⁺ and CD8⁺ T cells in blood samples of recovered COVID-19 patients [23]. Consequently, the S protein of SARS-CoV-2 is a major target of prophylactic vaccination strategies [17–19]. Specifically, the S protein is cleaved into two subunits, namely S1 and S2, by extracellular proteases, including furin, host surface-associated transmembrane protease serine 2 (TMPRSS2), and endocytic cathepsin L [19,24–26] (Fig. 2B). Two cleavage sites at the S1 and S2 boundaries (S1/S2) and one in the S2 domain (S2′) play essential roles in virus entry into the host target cells [19,24–26]. S1 binds ACE2 through its RBD, and S2 is further cleaved and activated by TMPRSS2 and/or cathepsin L [19,24]. The S protein exists in two structurally distinct conformations: pre-fusion and post-fusion states (Fig. 3). In its pre-fusion state, it is a “closed” trimer, with RBDs buried in the inner S1 head trimer at the interface between each protomer [27]. Each spike protomer harbors three segments: a large ectodomain (ED), a transmembrane anchor (TM), and a short intracellular tail (IC) [28]. ED comprises three critical elements: S1, RBD, and S2 (Fig. 2B). S1 consists of two subdomains, including an N-terminal domain (S1-NTD) and a C-terminal (S1-CTD) domain. RBD is located in S1-CTD and buried in the inner S1 head trimer. S2 constitutes a fusion peptide (FP), two heptad repeats (HR1 and HR2), a TM domain, and a cytoplasmic fusion (CT) domain, which are responsible for virus fusion and entry [28]. FP is a short part with 15–20 conserved amino acids in the viral family, mainly composed of hydrophobic residues, such as glycine or alanine. When the S

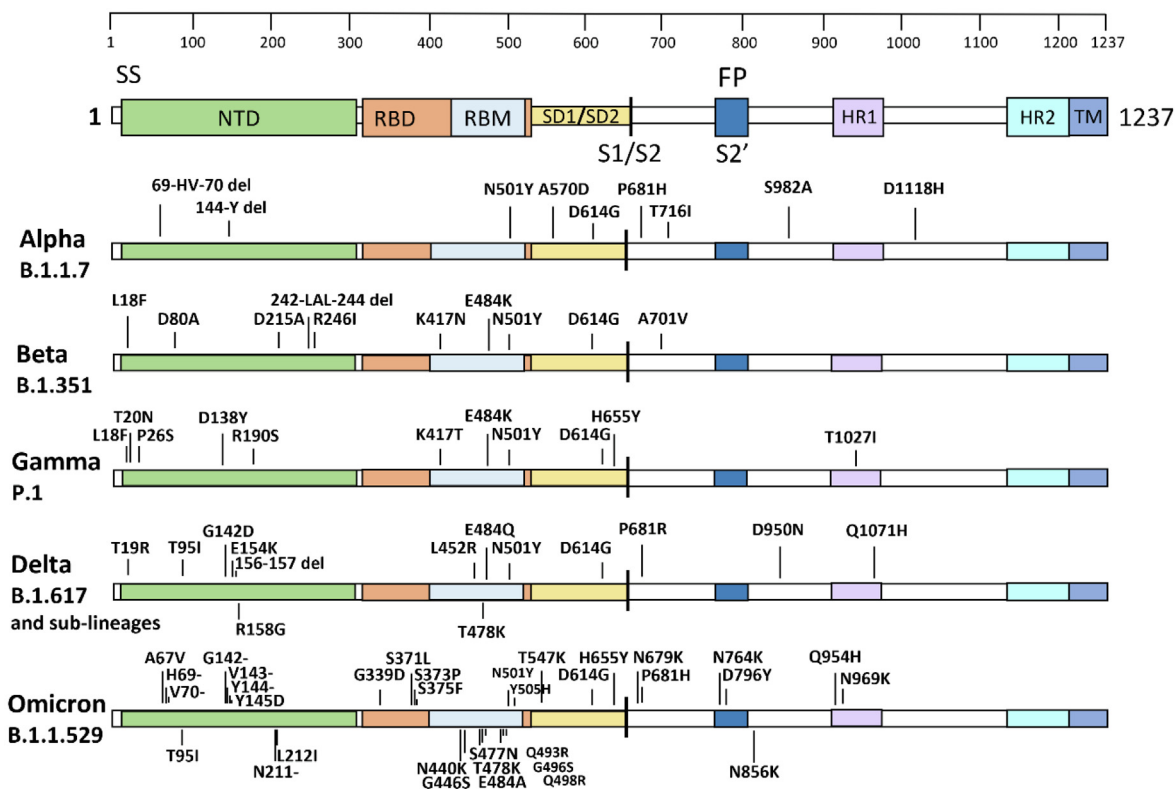


Fig. 1. Mutated amino acid sequences parts of SARS-CoV-2 variants [16,17,18]. Structural mutations of S glycoprotein in SARS-CoV-2 variants: B.1.1.7., B.1.351, P.1, B.1.617 and its sub-lineages, and B.1.1.529. Schematic representation of S glycoprotein (PDB:7DK3) and its mutations present in variants. Abbreviations: NTD, N-terminal domain (13–305); RBD, receptor-binding domain (319–541); RBM, receptor-binding motif (437–508); SD1, subdomain 1; SD2, subdomain 2; FP, fusion peptide (788–806); HR1, heptad repeat 1 (912–984); HR2, heptad repeat 2 (1163–1213); TM, transmembrane domain (1213–1237).

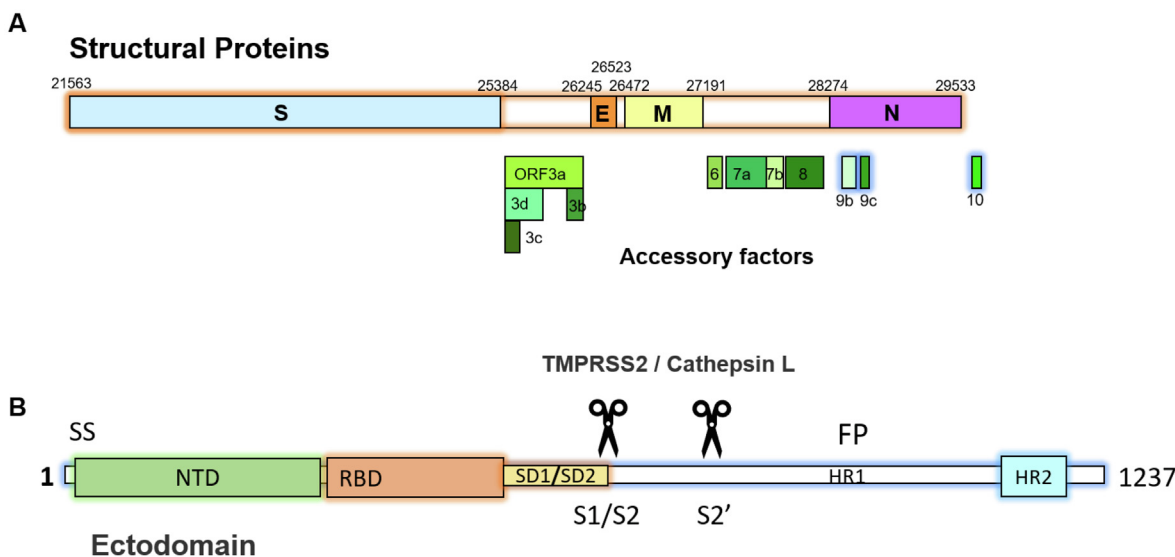


Fig. 2. Domain arrangement of SARS-CoV-2. (A) Domain arrangement of structural and accessory proteins of SARS-CoV-2. S, spike; E, envelope; M, matrix/Membrane; N, nucleocapsid. (B) Domain arrangement of S protein ectodomain (ED). S1: S1-NTD, S1 N-terminal domain; S1-CTD, S1 C-terminal domain; RBD, receptor binding domain; S2: FP, fusion peptide; HR1, heptad repeat 1; HR2, heptad repeat 2. Scissor Symbols: Protease (TMPRSS2/or Cathepsin L) cleavage site.

protein assumes its pre-hairpin conformation, it is immobilized on the target membrane, and FP is important for mediating membrane fusion through disrupting and ligating the lipid bilayer of the host cellular membrane [29]. While the S protein is an important target protein for specific drug development, RBD is part of a highly mutation-prone region and is not an ideal target site for the development of broad-spectrum antivirals [30]. In contrast, the HR

region of S2 plays an essential role in SARS-CoV-2 infection and is preserved across SARS-CoV-2 mutants [31]. Peptides derived from the HR2 region of fusion protein of enveloped viruses competitively bind viral HR1, thereby effectively inhibiting viral infection [32,33]. Hemagglutinin-esterase (HE) is a family of viral glycoproteins that mediate reversible adhesion to O-acetylated sialic acids [34]. However, HE as a candidate therapeutic agent target-

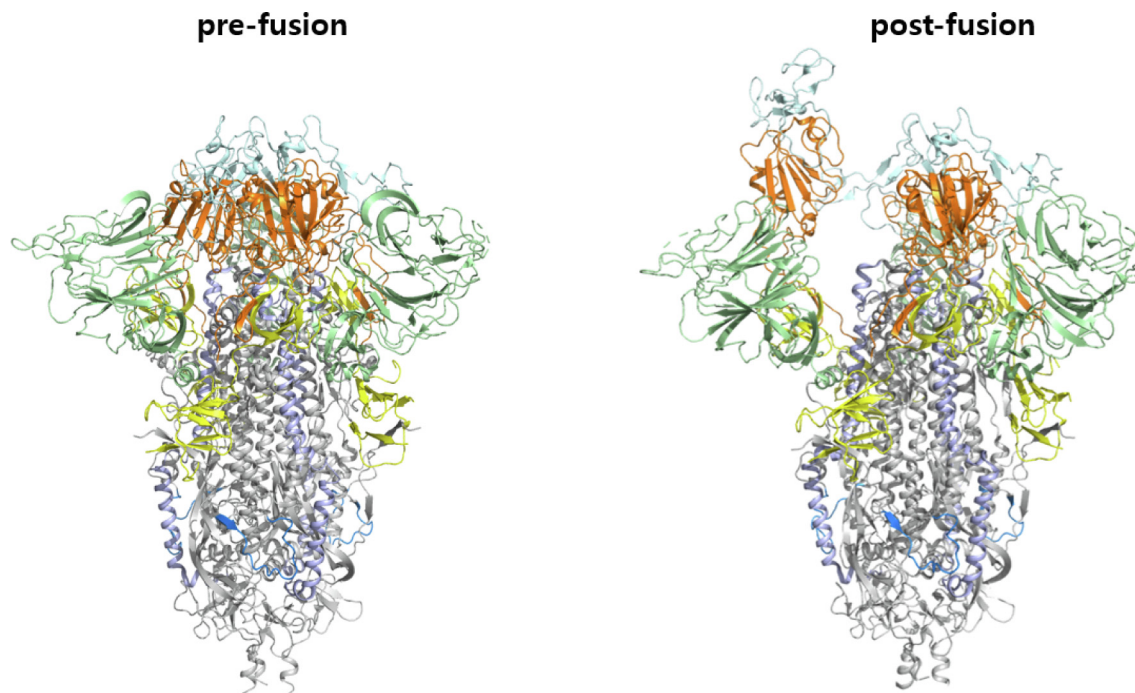


Fig. 3. Overall structure of two conformations of S protein. Graphical representation of the two conformations of S protein. The domains are represented by the same color as described in Fig. 1. NTD, RBD, and SD1/SD2 of the S1 subunit are indicated in pale green, orange, and yellow, respectively. RBM in RBD is indicated in pale cyan. FP and HR1 of the S2 subunit are indicated in marine and light blue, respectively. (PDB: 7ddd, pre-fusion; PDB: 7ddn, post-fusion). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

ing SARS-CoV-2 is not appropriate because the SARS-CoV-2 genome lacks HE. Therefore, HR1 may be important for the study of fusion inhibitors to treat COVID-19.

3. SARS-CoV-2 variants

Several SARS-CoV-2 variants, including alpha, beta, delta, omicron, and the recent “stealth omicron” have emerged in succession [4]. Representative mutations include alpha mutations (B.1.1.7, September 2020), corresponding to seven S protein mutations; beta mutations (B.1.351, October 2020), corresponding to nine spike mutations, and omicron mutations (B.1.1529, November 2021), corresponding to 30 S protein mutations [5,6] (Figs. 1, 4). Recently, the omicron variant has caused many confirmed cases till date. Due to many mutations, the ability of the variant to avoid immune responses, such as infection-blocking antibodies and T-cell responses, is high [5]. According to initial data, the delta variant was almost exclusively dominant until the end of November 2021, and the WHO designated the newly classified variant of concern as Omicron in only 2 days [35]. The parent omicron variant, which was designated BA.1 or B.1.1.529, was, until recently, the most transmissible known variant of the virus. More recently, one of its sub-lineages, designated BA.2, spread rapidly. BA.2 carries approximately 20 mutations that set it apart from the parent BA.1; however, it lacks the mutation that distinguishes omicrons from other variants in genetic PCR tests. Therefore, BA.2 has been named “stealth omicron.” This variant carries over 50 mutations in its genome, of which 30 are responsible for binding to the human ACE2 receptor for promoting infection and encoding the S protein of SARS-CoV-2 (Figs. 4, 5). As of February 2022, omicrons were the dominant variants worldwide. Omicron variants are also divided into BA.1, BA.2, BA.3, BA.4, BA.5 and descendant lines. This also includes BA.1/BA.2 circulating recombinant forms such as XE

[35]. Among these, BA.1 was once the most prevalent strain. However, BA.2 is gradually replacing BA.1 in some countries such as Denmark, Nepal, and the Philippines. BA.3 has a very limited transmissibility and shows very few cases of infection, at most a few hundred cases [36]. Therefore, we considered it prudent to summarize the variants for SARS-CoV-2 in detail.

Several coronaviruses, including SARS-CoV-2 and SARS-CoV-1, belong to the β -coronavirus group, one of the four groups (α -, β -, γ -, and δ -) of the Orthocoronavirinae subfamily of the Coronaviridae family [37]. Many members of the β -coronavirus lineage B (termed sarbecoviruses), including SARS-CoV; SARS-CoV-2 variants; and civet-, bat-, and pangolin-derived sarbecoviruses can exploit human ACE2 to enter host cells [38,39]. SARS-CoV-2 variants and pangolin-derived sarbecoviruses show a higher affinity to human ACE2 than SARS-CoV and civet- or bat-derived sarbecoviruses, which may be explained by the affinity-enhancing mutations present in the former [38,40]. Analysis of conserved protein sequences around the receptor-binding sites of 25 known sarbecovirus members relying on human ACE2 for cellular entry revealed that 11 of the 21 residues of the virus that interact with the receptor are highly conserved [41]. The two closely related viruses SARS-CoV-2 and SARS-CoV-1 share 75% similarity at the amino acid level and both rely upon human ACE2 release for viral influx [42]. There are 56 individual amino acid changes between the RBDs of SARS-CoV-2 and SARS-CoV-1 [43]. For instance, SARS-CoV-2 carries a D614G mutation located at the C-terminus of the S1 domain, near the furin cleavage site [44] (Figs. 1, 2). However, there is currently no scientific consensus on positive selection for the G614 variant, which is associated with higher infectivity and transmissibility in humans [45]. Importantly, in a hamster model, antibodies against SARS-CoV-2 D614 potentially neutralized infection with its G614 counterpart [46], suggesting that the D614G mutation would not reduce the protective ability of vaccines in clinical trials. Typically, however, as opposed to single-

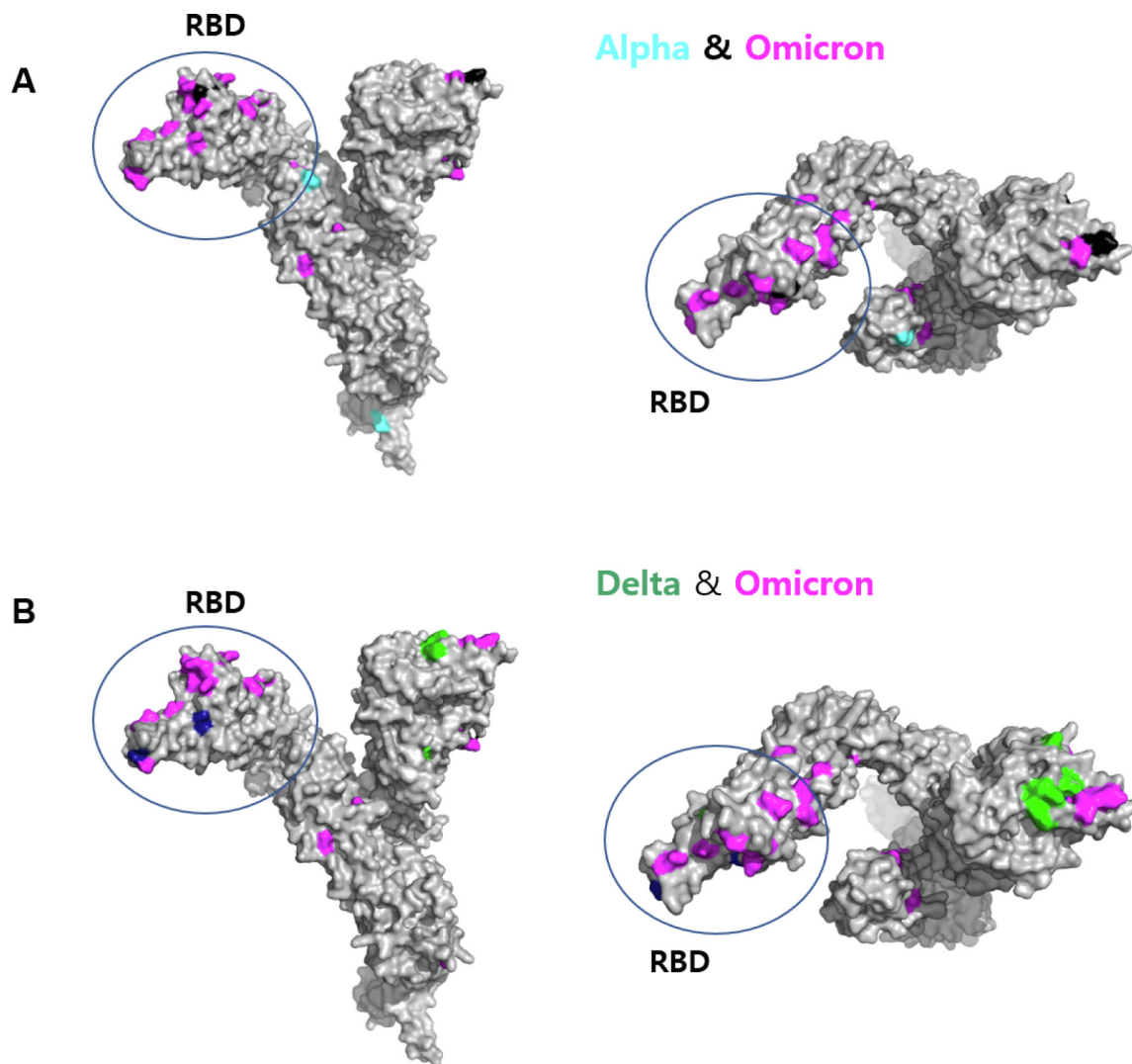


Fig. 4. Representative mutations of SARS-CoV-2 variants. (A) Comparison of alpha and omicron variants: Mutation sites of alpha and omicron variants are indicated in cyan and magenta, respectively, and common mutation sites are indicated in black. (B) Comparison of delta and omicron variants: Mutation sites of delta and omicron variants are indicated in green and magenta, respectively, and common mutation sites are indicated in deep blue. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.) (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

amino acid changes, most combinations of several mutations destroy many key epitopes, which may lead to the loss of serum activity (Figs. 3, 4, 5). Normally, the neutralization effect of spike-specific monoclonal antibodies (mAbs) on the mutant SARS-CoV-2 spike is greatly reduced. In contrast, polyclonal antibodies remain active against most mutant spike-like types, albeit being less effective against a few mutants [46]. These viruses use the same spike region of ACE2 RBD, enabling SARS-CoV-2 sera to isolate cross-neutralizing mAbs [47]. Vaccines and treatments targeting the virus must be continuously updated owing to the occurrence of several virus mutations designed to adapt to the environment [46,48–51]. Therefore, the effectiveness of currently available vaccines targeting SARS-CoV-2-induced antibodies against several variants must be examined [46].

Changes in individual amino acids make it difficult for mAbs to effectively neutralize viral mutations. mAbs are less likely to produce an effective neutralizing effect on viral mutations, because the existing antibody neutralization function decreases every time an amino acid mutation occurs in the binding part of the antibody. Interestingly, regarding new vaccine development, some antibodies, such as S2-targeting mAbs, act as common antibodies against

both SARS-CoV-2 and SARS-CoV-1 [52]. If the common epitope of S2 is an antibody RBD target that produces fewer mutations and is likely to exist in all SARS-CoV-2 mutants, a universal vaccine for multiple variants can be developed by targeting the new epitope of SARS-CoV-2.

4. Insights into a universal vaccine

4.1. S2 region of SARS-CoV-2 in public clonotype

Generally, viruses use various mechanisms, such as molecular mimicry and 2'-O methylation to induce immune escape process, which acts against the host immune system [53,54]. Since the emergence of SARS-CoV-2, its RBD has evolved through several mutations from alpha to omicron. Most current research on mAbs is focused on the S protein of SARS-CoV-2. Many studies have identified antibodies against the S1 and S2 regions, most of which are neutralizing antibodies targeting the RBD of S1 regions to repress ACE2 receptor binding [55–59]. However, vaccines containing S1 are less effective against several variants because many amino acids in S1-RBD are mutated. For instance, the Pfizer-

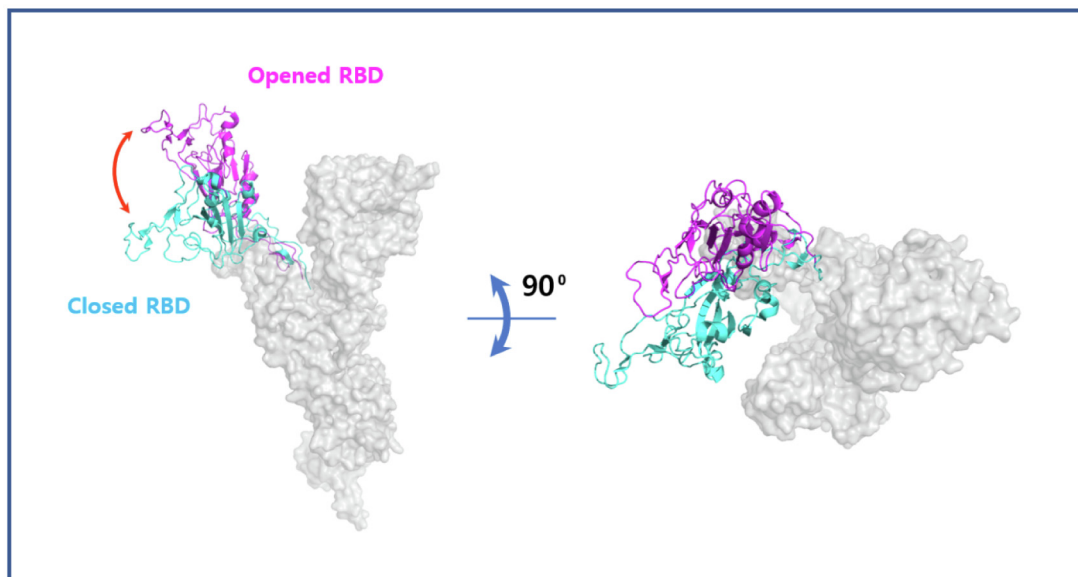


Fig. 5. Comparison of the pre-fusion and post-fusion conformations of S protein. Superimposed structures of pre- and post-fusion conformations. The closed RBD of the pre-fusion conformation is represented as a cyan cartoon model and the open RBD of the post-fusion conformations is represented as a magenta cartoon model. The remaining parts are represented as gray surface models. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.) (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

BioNTech (or BNT162b2) vaccine showed 95% efficacy against wild-type SARS-CoV-2, 87% against B.1.1.7 (alpha) variants, and 72.1% against B.1.351 (beta) variants [60]. Therefore, universal vaccine development has gained much attention to ensure efficacy against persistent viral mutations (Fig. 4).

Interestingly, 37 common antibodies (public clonotypes) were recently detected in people with COVID-19 or vaccinated individuals, of which 27 were shared among individuals who had recovered from infection and those who had been vaccinated [41]. Clonotypes provide important information regarding vaccine responses in a large human population. Shared public clonotypes form a significant proportion of human B-cell responses induced by the S trimer and produce neutralizing antibodies against RBD in the S2 of SARS-CoV-2 [41,61]. Among these, two single antibodies recognize the conserved regions of S proteins that are not mutated in SARS-CoV-2 and SARS-CoV-1 [62]. Among the public clonotypes, antibodies targeting S2 include immunoglobulin heavy variable (IGHV)1-58 [63], IGHV3-53, IGHV3-66, IGHV3-53 [64], IGHV3-30, and IGHV3-30-3 [56]. Majority of the public clonotypes are IgGs, while some are subsets of IgA. Of the 37 public clonotypes identified, 16 can bind RBD, and 11 of these 16 are neutralizing spikes. Moreover, the remaining 21 clonotypes bind S2 and antigens other than RBD (Table 1). In addition to SARS-CoV-2, three bat-related β -coronaviruses (SARS-CoV, MERS-CoV, and SARS-CoV-2) have

caused pandemics over the past 20 years, and some additional bat-related SARS-like coronavirus strains (including β -coronaviruses), such as WIV-1-CoV, hold the potential to cause zoonotic pandemics [65,66]. Thus, much efforts are being devoted to develop a universal vaccine against not only SARS-CoV-2 variants but also all β -coronaviruses and sarbecoviruses. In this context, we suggest S2 region as a potential target for developing novel vaccination strategies in the future.

Many genetically similar antibodies, including multiple public clonotypes, have been detected between Pfizer-BioNTech-vaccinated individuals and natural SARS-CoV-2-infected convalescent donors. These antibodies mainly encode VH genes, such as IGHV3-53, IGHV3-66, IGHV1-58, IGHV3-30, and IGHV3-30-3. Of the 37 public clonotypes identified, 16 can bind RBD, and 11 of these 16 are neutralizing spikes. All neutralizing public clonotypes recognize RBD. Meanwhile, the remaining 21 of the 37 public clonotypes can bind antigenic sites (including S2 domains) other than RBD. Overall, these results suggest that many public clonotypes have been detected between vaccinated and infected individuals.

4.2. Hemagglutinin (HA) region of the influenza virus

The influenza virus has been in vogue worldwide since 1918. Currently available seasonal influenza vaccines provide

Table 1
Convergent antibody response (public clonotype) to SARS-CoV-2 [67].

37 Public clonotypes	16 Combined with RBD	11 Neutralize spikes
		5
	21 Bind to S2 and antigens other than RBD	

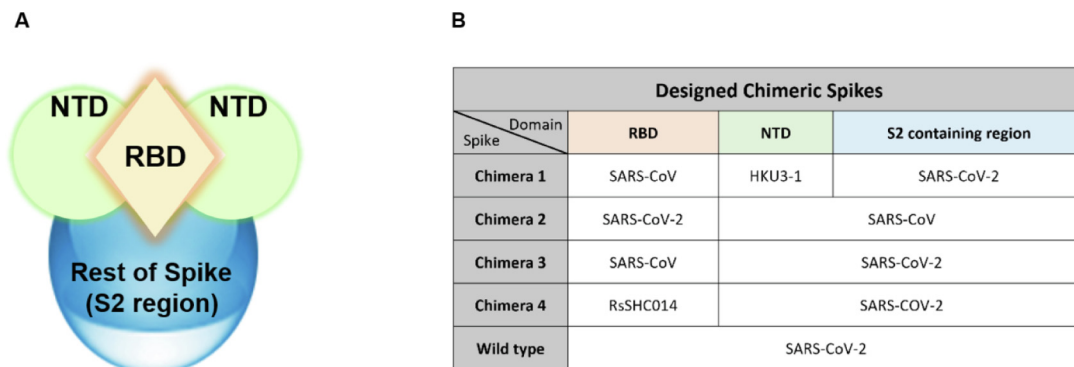


Fig. 6. Sarbecovirus chimeric spike designs. (A) Simple structure model of coronavirus S glycoprotein. Blue area indicates the whole S2 region. RBD, receptor binding domain; NTD, N-terminal domain. (B) Schema of designed chimeric spike proteins. Each part of chimera 1 comprises three different strains and the others (chimeras 2–4) comprise of two different strains. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.) (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

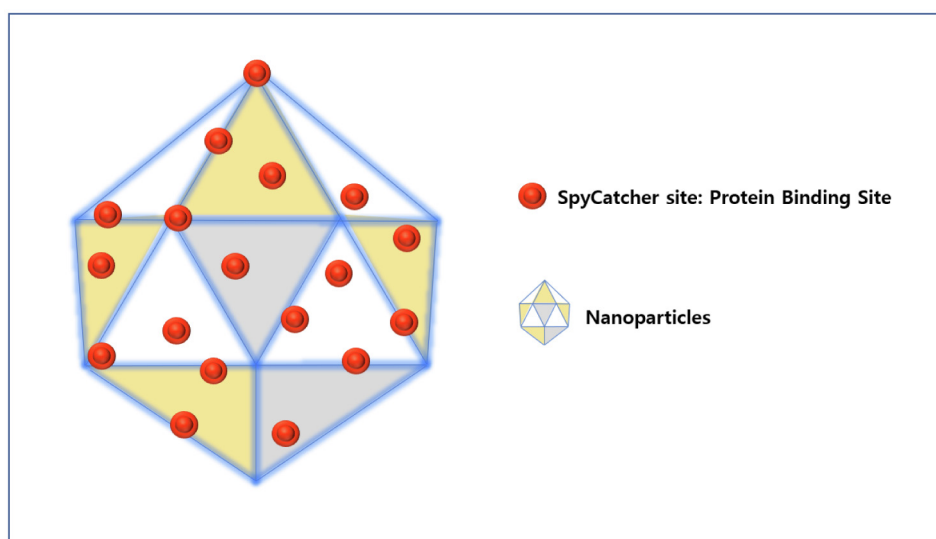


Fig. 7. Mosaic nanoparticle vaccine platform. Each red point represents a SpyTagged coronavirus RBD, and the hexagon represents SpyCatcher-VLP (SpyCatcher003-mi3). SpyTag003 is attached to the SpyCatcher003-mi3 structure to form a mosaic particle. Aspartic acid on SpyTag and lysine on SpyCatcher form an isopeptide bond with high specificity. There are approximately 60 possible sites for SpyTag attachment, which are briefly described. Three in SpyTag003 and SpyCatcher003-mi3 indicates the “third generation” of the technology, concentrated on rate-forming bonds [79,80]. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.) (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

narrow-spectrum and short-term yet effective protection against these viruses. The key target in the development of a universal vaccine is the conserved region. Generally, the human immune system recognizes HA, neuraminidase (NA), nucleoprotein (NP), and matrix proteins 1 (M1) and 2 (M2). Among these, conserved regions, such as the stem region of HA and M2, are targeted for universal vaccine development. In the case of influenza virus, attempts have been made to remove the HA1 subunit of HA using chemical or genetic methods for the development of a universal vaccine [68,69]. However, chemical removal of HA1 causes several problems, including protein damage during chemical treatment and loss of part of the HA1 stem site, which impedes the action of antibodies on HA2 [68]. In addition, genetic modification to remove HA head was unsuccessful because it did not produce a reliable full-stem protein. Although headless HA produced by insect cells has been successfully used against H1N1, H5N1, and H6N1 strains [69], the structural instability of the stem region remains unresolved [70,71]. Thus, headless HA may be an important candidate for the development of universal vaccines.

4.3. Current status of research into universal vaccines against other viruses

Chimeric vaccines use chimeric antigens, which are a “mix” of various regions of antigens. Although chimeric vaccines against many viruses, such as dengue fever [72], yellow fever [73], and influenza [74] viruses, have been studied extensively, none has passed all essential clinical procedures. Sarbecovirus, including SARS-CoV-2, SARS-CoV, and many other viruses with zoonotic potential, harbor S proteins, which comprise NTD, RBD, and S2. Since antibodies can be derived from all regions of S protein in addition to RBD [75], this region has been exploited to induce a wide range of antibodies through chimeric vaccines and positive results have been reported [71]. In the case of coronaviruses, S protein is the target of “mixed antigens.” Chimeric S proteins are known to elicit a broad-range cellular immune response, offering an opportunity to develop a universal vaccine. For instance, four types of chimeric S proteins have been designed (Fig. 6). In a previous study on primed and boosted aged mice, all chimeric S

proteins showed high cross-activity against SARS-CoV, SARS-CoV-2, HKU-1, and RsSHC014 strains. HKU-1 is a coronavirus discovered in Hong Kong in 2004 [76]. RsSHC014 is a bat-derived SARS-like coronavirus discovered in China in 2013 [77]. Parvathaneni et al. [78] conducted a similar study.

Mosaic vaccines are similar to chimeric vaccines in that they induce a wide range of immune responses against multiple strains. In a previous study, whole S proteins from selected strains were conjugated in a particle using the SpyTag/SpyCatcher technique, similar to a mosaic (Fig. 7) [79]. Mosaic vaccines have been actively studied, particularly in the context of human immunodeficiency virus (HIV) [80]. In 2009, the effectiveness of RV144, a vaccine for HIV type 1, was demonstrated, which prompted additional studies on mosaic vaccines. RV144 follows the principle of heterologous prime-boost by combining ALVAC-HIV and AIDSVAX B/E vaccines [80], and it could successfully reduce HIV infection by up to 31.2% [81]. In a previous nanoparticle-based study [82], four mosaic vaccines for COVID-19 were developed by combining different RBDs. Of these, two were derived by combining four different RBDs from β -coronavirus strains; one was derived by combining eight different RBDs; and the remaining comprised SARS-CoV-2 RBD alone, acting as the wildtype SARS-CoV-2 antigen [83]. Each preliminary vaccine was administered to mice, and their immune response was analyzed. Compared with the control homotypic SARS-CoV-2 vaccine, mosaic vaccines induced a broader immune response in mice [82].

FP is a region of the S protein that aids the evasion of viruses into the host cell. FP bears highly conserved amino acid residues within a virus family [83]. This feature renders FP a suitable target for the development of universal vaccines. Trials of FP vaccines against porcine epidemic diarrhea virus (PEDV)—which belongs to *Pedacovirus*, a subgenus of β -coronaviruses—and SARS-CoV-2 have been conducted [84]. Despite the use of an innovative vaccine platform, the results highlighted the feasibility of a universal FP vaccine.

Finally, T-cell vaccines, which are peptide vaccines, can be a solution to manage SARS-CoV-2 variants. These vaccines can be effective in patients with B-cell deficiency (immune deficiency) who lack the ability to produce antibodies. Nathan et al. [85] used a structure-based network and HLA class 1 stability peptide analysis to successfully identify highly conserved regions of CD8⁺ T-cell epitopes. Other vaccines are considered to provide broad immunity against variants and other sarbecoviruses.

5. Antiviral chemicals for SARS-CoV-2

Interest in the development of oral antiviral drugs is growing given the need for periodic immune-boosting vaccines against SARS-CoV-2, concerns regarding increase in the infectivity of the virus, and doubts on the effectiveness of the vaccine. One of the most anticipated oral antivirals on the market to treat SARS-CoV-2 is Paxlovid (Pfizer), which is an oral medication that can reduce the severity of COVID infection, when administered promptly. In addition, several novel oral antivirals or candidates, including nirmatrelvir/ritonavir (Paxlovid), molnupiravir, and flvoxamine, were available as of February 2022 [13]. Nirmatrelvir is an orally bioavailable protease inhibitor that acts against M^{PRO}—a viral protease playing an essential role in viral replication by cleaving two viral polyproteins [86]. Nirmatrelvir exhibits antiviral activity against all coronaviruses known to infect humans [87]. Nirmatrelvir is packaged with ritonavir (as Paxlovid)—a strong cytochrome P450 (CYP) 3A4 inhibitor and a pharmacokinetic boosting agent of HIV protease inhibitors. Ritonavir co-administration is essential to increase nirmatrelvir concentration in the target therapeutic range. Molnupiravir is a small-molecule

Table 2
Mutated amino acid sequences of COVID-19 variants [32,92–94].

Peptide	Sequence
EK1	SLDQINVTFLDLEYEMKLEEAIAIKLEESYIDLKEL
EK1C4	SIDQINVTFLDLEYEMKLEEAIAIKLEESYIDLKELGSGSG-PEG4 (cholesterol)
IPB02	ISGINASVVNIQKEIDRLNEVAKNLSLIDLQELK (cholesterol)

ribonucleoside prodrug of N-hydroxycytidine, which acts against SARS-CoV-2 and other RNA viruses and serves as a potent barrier to virus resistance development [97]. Flvoxamine is a selective serotonin reuptake inhibitor approved by the Food and Drug Administration (FDA) for the treatment of obsessive-compulsive disorder and other conditions, including depression. However, flvoxamine is not FDA-approved for the treatment of any infectious disease. Trials to confirm the anti-inflammatory effects of flvoxamine observed in non-clinical studies in humans and its clinical relevance in the COVID-19 setting are ongoing. However, the COVID-19 Treatment Guidelines Panel has insufficient evidence to support or reject the use of flvoxamine as COVID-19 treatment. Here, we summarize the target groups of related oral chemicals or candidates for COVID-19.

5.1. Fusion inhibitors

In SARS-CoV, the S2 subunit is an essential component of the fusion process during the entry of the virus into the host cell. The HR1 and HR2 domains interact to form helical bundles in the S2 subunit. This helical bundle binds the cell membrane and allows fusion [88]. Based on their high similarity, the sequences of HR2 domains of SARS-CoV-2 and SARS-CoV-1 are conserved. The SARS-CoV-2 HR2 protein (residues 1168–1203) inhibits virus entry into host cells or fusion. Specifically, the IC₅₀ of SARS-CoV-2 HR2 protein against SARS-CoV-2 pseudovirus was 0.98 mM [33,89]. OC43-HR2P is a modified peptide derived from the HR2 domain of HCoV-OC43, a type of β -coronavirus 1 that infects humans and cattle [90]. Compared with SARS-CoV, MERS-CoV, and SARS-CoV-2, HCoV-OC43 manifests milder symptoms [91]. EK1 is a fusion inhibitor targeting the HR1 domain of HCoV S protein in pan-coronaviruses (Table 2). EK1 is more soluble in phosphate-buffered saline and water than the existing OC43-HR2P [32]. A peptide-based inhibitor, such as EK1, can inhibit the fusion of SARS-CoV-2 with the host. Based on crystal structure, the HR2 and HR1 domains of SARS-CoV-2 interact to form a helical bundle, suggesting that amino acid mutations in HR1 can improve its interaction with HR2 [92]. EK1C4, a lipopeptide originating from EK1, suppressed the fusion of SARS-CoV-2 with host cells. EK1C4 is formed by adding polyethylene glycol, cholesterol, and a fusion linker to the amino acid sequence of EK1 (Table 2). EK1C4 inhibited the entry of SARS-CoV-2 pseudovirus into the host cells, with an IC₅₀ of 15.8 nM, which was approximately 149 times higher than that of EK1 [93]. IPB02, a lipopeptide fusion inhibitor based on a different sequence, inhibited the fusion to SARS-CoV-2 pseudovirus to prevent infection [94] (Table 2).

EK1 is a peptide drug, while EK1C4 and IPB02 are lipopeptide drugs. Lipidation is useful because it can enhance *in vivo* activity. EK1C4 covalently binds cholesterol at the C-terminus of EK1. The spacer based on polyethylene glycol and GSG is a glycine/serine sequence. Since the efficacy of the drug could be increased by extending the GSG linker to GSGSG for a longer period, increasing the length of the linker in lipopeptide is the key. IPB02 is an HR2-derived fusion inhibitor, with cholesterol covalently bound to its terminal end.

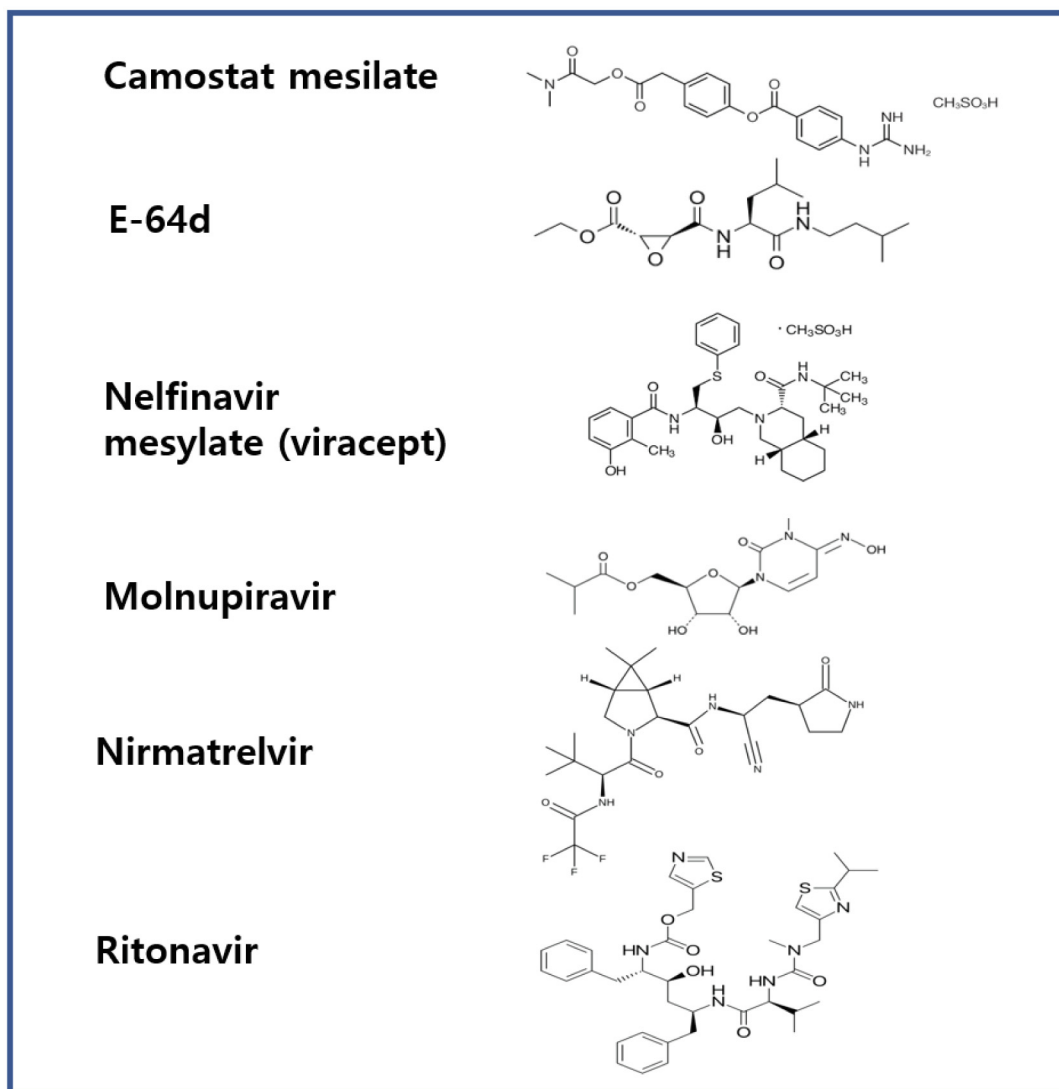


Fig. 8. Structural formulas of antiviral chemicals for COVID-19 treatment. Chemical structures of the antiviral drugs summarized in the present review are shown. Mesylate is the abbreviation for methanesulfonate. Camostat mesilate and E-64d are protease inhibitors targeting cleavage sites. Camostat mesilate inhibits TMPRSS2—a serine protease involved in SARS-CoV-2 fusion. E-64d inhibits SARS-CoV-2 infection by inhibiting cathepsin L. Viracept, used in HIV treatment, is a form of mesylate attached to nelfinavir, and it inhibits the spike protein-mediated fusion of SARS-CoV-2. Molnupiravir was originally used to treat influenza, but its effectiveness in COVID-19 treatment has also been demonstrated. Nirmatrelvir is an oral protease inhibitor. The combination of nirmatrelvir and ritonavir (Paxlovid) inhibits SARS-CoV-2.

5.2. Protease inhibitors targeting cleavage sites

Entry into the host cell requires cleavage between the S2 subunit and S1 and S2 of SARS-CoV-2. Degradation by TMPRSS2 and cathepsin L and B plays an essential role in SARS-CoV-2 entry into the host cell. TMPRSS2 is a serine protease that promotes virus entry and activation by cleaving S proteins. Membrane fusion cannot proceed without proteolysis; thus, TMPRSS2 is important for SARS-CoV2 fusion with the host cells. Camostat mesilate is a potential serine protease inhibitor that targets TMPRSS2 (Fig. 8). A study on the cellular entry mechanism of SARS-CoV-2 demonstrated that camostat mesilate inhibited virus entry into host cells [93]. E-64d, a cathepsin L inhibitor, blocked SARS-CoV-2 infection. Future trials involving patients with COVID-19 may help identify the efficacy of E-64d therapy [95] (Fig. 8). In addition to peptide fusion inhibitors, nelfinavir mesylate (Viracept), an anti-HIV protease inhibitor, inhibits both S- and S-mediated cell–cell fusion of SARS-CoV-2. Viracept was the first reported small-molecule fusion inhibitor, other than peptide fusion inhibitors (Fig. 8). In addition,

nelfinavir inhibits the function of TMPRSS2, which is involved in S protein cleavage [96]. This finding has allowed for the clinical application of anti-SARS-CoV-2 therapeutics, particularly at the early stages of infection. Among phospholipids, furin with phosphatidylcholine (PC) hydrolyzes peptide substrates at basic residues [97]. The S protein of SARS-CoV-2 has furin cleavage sites at the S1/S2 boundary, and they can enhance replication efficiency of the virus upon entry into the host cell [98]. Cleavage sites similar to the furin cleavage sites of SARS-CoV-2 can affect the viral life cycle and pathogenicity. Accordingly, inhibitors targeting multiple cleavage sites, including furin inhibitors, can be used as drug therapies for COVID-19.

5.3. Future prospects of antiviral medicine for COVID-19 treatment

As an important component for interaction with the host, the S protein of SARS-CoV-2 is a significant target for vaccine or neutralizing antibody development. As such, if the mechanism of interaction with the host is elucidated, the enzyme involved is inhibited,

and the ion channel is blocked, a potent antiviral effect can be produced. Of note, EK1C4 targeting the highly conserved HR1 domain of the S2 subunit was expected to exhibit therapeutic potential against SARS-CoV-2 (Table 2). In addition, a combination of different antiviral agents has been proposed as a solution to combat the disease. Similarly, a recent study has demonstrated the sensitivity of the omicron variant of SARS-CoV-2 to the combination of molnupiravir and nirmatrelvir [99] (Fig. 7). In particular, nirmatrelvir is an antiviral medicine developed by Pfizer and an oral protease inhibitor. In December 2021, the potent inhibitory effect of Paxlovid, prepared by combining nirmatrelvir and ritonavir, against SARS-CoV-2 was proven, and it was approved for emergency use by the FDA [100]. Molnupiravir is an oral antiviral drug developed for the treatment of influenza; it inhibits infection by inducing errors in viral RNA replication [101]. Molnupiravir is currently approved in the UK for the treatment of COVID-19 [102] (Fig. 7). Based on their chemical functions, both molnupiravir and nirmatrelvir exhibit antiviral properties, and the combination of these two produces a synergistic effect, which may be a potential solution to manage emerging SARS-CoV-2 variants. Novel effective antiviral drugs can be developed through the combination of existing drugs for the treatment of COVID-19. Finally, if issues related to the accessibility and cost effectiveness of oral antiviral agents are resolved, they may prove revolutionary in the management of emerging variants of SARS-CoV-2.

6. Summary and outlook

For the prevention and treatment of COVID-19, rapid development of neutralizing antibodies against SARS-CoV-2 in convalescent patients represents an important scientific challenge at present. Several vaccines have been developed and distributed at an unprecedented rate to combat the COVID-19 pandemic. However, the persistent emergence of new SARS-CoV-2 variants has impeded treatment using neutralizing antibodies and reduced the effectiveness of vaccines. In this context, development and implementation of novel vaccine designs and technologies are anticipated. Periodic screening for the efficacy of neutralizing antibodies and vaccines against new emerging pathogenic variants is warranted. In this review, we have summarized oral medicines and emerging SARS-CoV-2 variants, such as delta and omicron. In addition, we have briefly discussed the possible antigen candidates (mainly the S2 region) other than RBD for the development of a universal COVID-19 vaccine. This review will be helpful for future vaccine and drug development against COVID-19.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Author contributions

K.Y.H. and S.K. wrote manuscripts; S.K. performed mutants analysis and prepared the figures; E.Y. and D.K. analyzed the COVID-19 variants and vaccine data. H.J., Y.K. and Y.J. analyzed the COVID-19 oral drugs.

References

- [1] Baden LR, El Sahly HM, Essink B, Kotloff K, Frey S, Novak R, et al. Efficacy and safety of the mRNA-1273 SARS-CoV-2 vaccine. *N Engl J Med* 2021;384:403–16. <https://doi.org/10.1056/NEJMoa2035389>.
- [2] Polack FP, Thomas SJ, Kitchin N, Absalon J, Gurtman A, Lockhart S, et al. Safety and efficacy of the BNT162b2 mRNA Covid-19 Vaccine. *N Engl J Med* 2020;383:2603–15. <https://doi.org/10.1056/NEJMoa2034577>.
- [3] Walsh EE, Frenck RW, Falsey AR, Kitchin N, Absalon J, Gurtman A, et al. Safety and immunogenicity of two RNA-based Covid-19 vaccine candidates. *N Engl J Med* 2020;383:2439–50. <https://doi.org/10.1056/NEJMoa2027906>.
- [4] Chavda VP, Apostolopoulos V. Omicron variant (B.1.1.529) of SARS-CoV-2: threat for the elderly? *Maturitas* 2022;158:78–81. <https://doi.org/10.1016/j.maturitas.2022.01.011>.
- [5] El-Shabasy RM, Nayel MA, Taher MM, Abdelmonem R, Shouair KR, Kenawy ER. Three waves changes, new variant strains, and vaccination effect against COVID-19 pandemic. *Int J Biol Macromol* 2022;204:161–8. <https://doi.org/10.1016/j.ijbiomac.2022.01.118>.
- [6] Konings F, Perkins MD, Kuhn JH, Pallen MJ, Alm EJ, Archer BN, et al. SARS-CoV-2 variants of interest and concern naming scheme conducive for global discourse. *Nat Microbiol* 2021;6:821–3. <https://doi.org/10.1038/s41564-021-00932-w>.
- [7] Falsey AR, Frenck Jr RW, Walsh EE, Kitchin N, Absalon J, Gurtman A, et al. SARS-CoV-2 neutralization with BNT162b2 vaccine Dose 3. *N Engl J Med* 2021;385:1627–9. <https://doi.org/10.1056/NEJM2113468>.
- [8] Levin AT, Hanage WP, Owusu-Boaitey N, Cochran KB, Walsh SP, Meyerowitz-Katz G. Assessing the age specificity of infection fatality rates for COVID-19: systematic review, meta-analysis, and public policy implications. *Eur J Epidemiol* 2020;35:1123–38. <https://doi.org/10.1007/s10654-020-00698-1>.
- [9] Keehner J, Horton LE, Binkin NJ, Laurent LC, SEARCH Alliance, Pride D, et al. Resurgence of SARS-CoV-2 infection in a highly vaccinated health system workforce. *N Engl J Med* 2021;385:1330–2. <https://doi.org/10.1056/NEJM2112981>.
- [10] Ingraham NE, Ingbar DH. The omicron variant of SARS-CoV-2: understanding the known and living with unknowns. *Clin Transl Med* 2021;11:e685.
- [11] UK Health Security Agency. Accessed 2022. 02.13, SARS-CoV-2 variants of concern and variants under investigation in England, <https://www.gov.uk/government/publications/investigation-of-novel-sars-cov-2-variant-variant-of-concern-20201201>; updated 2021.
- [12] Wen W, Chen C, Tang J, Wang C, Zhou M, Cheng Y, et al. Efficacy and safety of three new oral antiviral treatment (molnupiravir, fluvoxamine and Paxlovid) for COVID-19: a meta-analysis. *Ann Med* 2022;54:516–23. <https://doi.org/10.1080/07853890.2022.2034936>.
- [13] National Institute of Allergy and Infectious Diseases. Universal influenza vaccine research [accessed 2022.02.13], <https://www.niaid.nih.gov/diseases-conditions/universal-influenza-vaccine-research>; updated 2019.
- [14] Nachbagauer R, Feser J, Naficy A, Bernstein DI, Guptill J, Walter EB, et al. A chimeric hemagglutinin-based universal influenza virus vaccine approach induces broad and long-lasting immunity in a randomized, placebo-controlled phase I trial. *Nat Med* 2021;27:106–14. <https://doi.org/10.1038/s41591-020-1118-7>.
- [15] Malik JA, Ahmed S, Mir A, Shinde M, Bender O, Alshammari F, et al. The SARS-CoV-2 mutations versus vaccine effectiveness: new opportunities to new challenges. *J Infect Public Health* 2022;15:228–40. <https://doi.org/10.1016/j.jiph.2021.12.014>.
- [16] Liu C, Zhou Q, Li Y, Garner LV, Watkins SP, Carter LJ, et al. Research and Development on therapeutic agents and vaccines for COVID-19 and related human coronavirus diseases. *ACS Cent Sci* 2020;6:315–31. <https://doi.org/10.1021/acscentsci.0c00272>.
- [17] Fehr AR, Perlman S. Coronaviruses: an overview of their replication and pathogenesis. *Methods Mol Biol* 2015;1282:1–23. https://doi.org/10.1007/978-1-4939-2438-7_1.
- [18] Zhou P, Yang XL, Wang XG, Hu B, Zhang L, Zhang W, et al. A pneumonia outbreak associated with a new coronavirus of probable bat origin. *Nature* 2020;579:270–3. <https://doi.org/10.1038/s41586-020-2012-7>.
- [19] Hoffmann M, Kleine-Weber H, Schroeder S, Krüger N, Herrler T, Erichsen S, et al. SARS-CoV-2 cell entry depends on ACE2 and TMPRSS2 and is blocked by a clinically proven protease inhibitor. *Cell* 2020;181:271–280.e8. <https://doi.org/10.1016/j.cell.2020.02.052>.
- [20] Guo YR, Cao QD, Hong ZS, Tan YY, Chen SD, Jin HJ, et al. The origin, transmission and clinical therapies on coronavirus disease 2019 (COVID-19) outbreak – an update on the status. *Mil Med Res* 2020;7:11. <https://doi.org/10.1186/s40779-020-00240-0>.
- [21] Yin Y, Wunderink RG. MERS, SARS and other coronaviruses as causes of pneumonia. *Respirology* 2018;23:130–7. <https://doi.org/10.1111/resp.13196>.

- [22] Cui J, Li F, Shi ZL. Origin and evolution of pathogenic coronaviruses. *Nat Rev Microbiol* 2019;17:181–92. <https://doi.org/10.1038/s41579-018-0118-9>.
- [23] Zhu N, Zhang D, Wang W, Li X, Yang B, Song J et al. A novel coronavirus from patients with pneumonia in China. *N Engl J Med* 2019;382:727–33. <https://doi.org/10.1056/NEJMoa2001017>.
- [24] Walls AC, Park YJ, Tortorici MA, Wall A, McGuire AT, Veesler D. Structure, function, and antigenicity of the SARS-CoV-2 spike glycoprotein. *Cell* 2020;181:281–292.e6. <https://doi.org/10.1016/j.cell.2020.02.058>.
- [25] Hoffmann M, Kleine-Weber H, Pöhlmann S. A multibasic cleavage site in the Spike protein of SARS-CoV-2 is essential for infection of human lung cells. *Mol Cell* 2020;78:779–784.e5. <https://doi.org/10.1016/j.molcel.2020.04.022>.
- [26] Xia S, Lan Q, Su S, Wang X, Xu W, Liu Z, et al. The role of furin cleavage site in SARS-CoV-2 spike protein-mediated membrane fusion in the presence or absence of trypsin. *Signal Transduct Target Ther* 2020;5:92. <https://doi.org/10.1038/s41392-020-0184-0>.
- [27] Li F, Li W, Farzan M, Harrison SC. Structure of SARS coronavirus spike receptor-binding domain complexed with receptor. *Science* 2005;309:1864–8. <https://doi.org/10.1126/science.1116480>.
- [28] Plante JA, Liu Y, Liu J, Xia H, Johnson BA, Lokugamage KG, et al. Spike mutation D614G alters SARS-CoV-2 fitness. *Nature* 2021;592:116–21. <https://doi.org/10.1038/s41586-020-2895-3>.
- [29] Huang Y, Yang C, Xu XF, Xu W, Liu SW. Structural and functional properties of SARS-CoV-2 spike protein: potential antiviral drug development for COVID-19. *Acta Pharmacol Sin* 2020;41:1141–9. <https://doi.org/10.1038/s41401-020-0485-4>.
- [30] Ma X, Zou F, Yu F, Li R, Yuan Y, Zhang Y, et al. Nanoparticle vaccines based on the receptor binding domain (RBD) and heptad repeat (HR) of SARS-CoV-2 elicit robust protective immune responses. *Immunity* 2020;53:1315–1330.e9. <https://doi.org/10.1016/j.immuni.2020.11.015>.
- [31] Xia S, Yan L, Xu W, Agrawal AS, Algaissi A, Tseng CK, et al. A pan-coronavirus fusion inhibitor targeting the HR1 domain of human coronavirus spike. *Sci Adv* 2019;5:eaa4580. <https://doi.org/10.1126/sciadv.aav4580>.
- [32] Xia S, Zhu Y, Liu M, Lan Q, Xu W, Wu Y, et al. Fusion mechanism of 2019-nCoV and fusion inhibitors targeting HR1 domain in spike protein. *Cell Mol Immunol* 2020;17:765–7. <https://doi.org/10.1038/s41423-020-0374-2>.
- [33] Visser EA, Moons SJ, Timmermans SBPE, de Jong H, Boltje TJ, Büll C. Sialic acid O-acetylation: from biosynthesis to roles in health and disease. *J Biol Chem* 2021;297:.. <https://doi.org/10.1016/j.jbc.2021.100906>.
- [34] Zeng Q, Langereis MA, van Vliet AL, Huizinga EG, de Groot RJ. Structure of coronavirus hemagglutinin-esterase offers insight into corona and influenza virus evolution. *Proc Natl Acad Sci USA* 2008;105:9065–9. <https://doi.org/10.1073/pnas.0800502105>.
- [35] World Health Organization. Update on omicron [accessed 2022.06.07]. Tracking SARS-CoV-2 variants. <https://www.who.int/activities/tracking-SARS-CoV-2-variants>; updated 2022.
- [36] Fan Y, Li X, Zhang L, Wan W, Zhang L, Zhou F. SARS-CoV-2 Omicron variant: recent progress and future perspectives. *Signal Transduction Targeted Ther* 2022;7(1):141. <https://doi.org/10.1038/s41392-022-00997-x>.
- [37] Li F. Receptor recognition mechanisms of coronaviruses: a decade of structural studies. *J Virol* 2015;89:1954–64. <https://doi.org/10.1128/JVI.02615-14>.
- [38] Liu C, Ginn HM, Dejnirattisai W, Supasa P, Wang B, Tuekprakhon A, et al. Reduced neutralization of SARS-CoV-2 B.1.617 by vaccine and convalescent serum. *Cell* 2021;184:4220–4236.e13. <https://doi.org/10.1016/j.cell.2021.06.020>.
- [39] Wrobel AG, Benton DJ, Xu P, Calder LJ, Borg A, Roustan C, et al. Structure and binding properties of Pangolin-CoV spike glycoprotein inform the evolution of SARS-CoV-2. *Nat Commun* 2021;12:837. <https://doi.org/10.1038/s41467-021-21006-9>.
- [40] Starr TN, Greaney AJ, Hilton SK, Ellis D, Crawford KHD, Dingsen AS, et al. Deep mutational scanning of SARS-CoV-2 Receptor binding domain reveals constraints on folding and ACE2 binding. *Cell* 2020;182:1295–1310.e20. <https://doi.org/10.1016/j.cell.2020.08.012>.
- [41] Chen EC, Gilchuk P, Zost SJ, Suryadevara N, Winkler ES, Cabel CR, et al. Convergent antibody responses to the SARS-CoV-2 spike protein in convalescent and vaccinated individuals. *Cell Rep* 2021;36:.. <https://doi.org/10.1016/j.celrep.2021.109604> 109604.
- [42] Gralinski LE, Menachery VD. Return of the coronavirus: 2019-nCoV. *Viruses* 2020;12:135. <https://doi.org/10.3390/v12020135>.
- [43] Ortega JT, Serrano ML, Pujol FH, Rangel HR. Role of changes in SARS-CoV-2 spike protein in the interaction with the human ACE2 receptor: an in silico analysis. *Excli J* 2020;19:410–7. <https://doi.org/10.17199/excli2020-1167>.
- [44] Korber B, Fischer WM, Gnanakaran S, Yoon H, Theiler J, Abfalterer W, et al. Tracking changes in SARS-CoV-2 Spike: evidence that D614G increases the infectivity of the COVID-19 virus. *Cell* 2020;182:812–827.e19. <https://doi.org/10.1016/j.cell.2020.06.043>.
- [45] Callaway E. The coronavirus is mutating - does it matter? *Nature* 2020;585:174–7. <https://doi.org/10.1038/d41586-020-02544-6>.
- [46] Rees-Spear C, Muir L, Griffith SA, Heaney J, Aldon Y, Snitselaar JL. The effect of spike mutations on SARS-CoV-2 neutralization. *Cell Rep* 2021;34:.. <https://doi.org/10.1016/j.celrep.2021.108890> 108890.
- [47] Brouwer PJM, Daniels TG, van der Straten K, Snitselaar JL, Aldon Y, Bangaru S, et al. Potent neutralizing antibodies from COVID-19 patients define multiple targets of vulnerability. *Science* 2020;369:643–50. <https://doi.org/10.1126/science.abc5902>.
- [48] Meng B, Kemp SA, Papa G, Datt R, Ferreira IATM, Marelli S, et al. Recurrent emergence of SARS-CoV-2 spike deletion H69/V70 and its role in the Alpha variant B.1.1.7. *Cell Rep* 2021;35(109292). <https://doi.org/10.1016/j.celrep.2021.109292>.
- [49] Oude Munnink BBO, Worp N, Nieuwenhuijse DF, Sikkema RS, Haagmans B, Fouchier RAM, et al. The next phase of SARS-CoV-2 surveillance: real-time molecular epidemiology. *Nat Med* 2021;27:1518–24. <https://doi.org/10.1038/s41591-021-01472-w>.
- [50] Tegally H, Wilkinson E, Giovanetti M, Iranzadeh A, Fonseca V, Giandhari J et al (2020) Emergence and rapid spread of a new severe acute respiratory syndrome-related coronavirus 2 (SARS-CoV-2) lineage with multiple spike mutations in South Africa. medRxiv. Preprint at <https://doi.org/10.1101/2020.12.21.20248640>.
- [51] Welkers MRA, Han AX, Reusken CBEM, Eggink D. Possible host-adaptation of SARS-CoV-2 due to improved ACE2 receptor binding in mink. *Virus Evol* 2021;7:veaa094. <https://doi.org/10.1093/ve/veaa094>.
- [52] Zheng Z, Monteil VM, Maurer-Stroh S, Yew CW, Leong C, Mohd-Ismail NK, et al. Monoclonal antibodies for the S2 subunit of spike of SARS-CoV-1 cross-react with the newly emerged SARS-CoV-2. *Euro Surveill* 2020;25. 10.2807/1560-7917.ES.2020.25.28.2000291, 32700671.
- [53] Albarnaz JD, Ren H, Torres AA, Shmeleva EV, Melo CA, Bannister AJ, et al. Molecular mimicry of NF-κB by vaccinia virus protein enables selective inhibition of antiviral responses. *Nat Microbiol* 2022;7:154–68. <https://doi.org/10.1038/s41564-021-01004-9>.
- [54] Daffis S, Szretter KJ, Schriewer J, Li J, Youn S, Errett J, et al. 2'-O methylation of the viral mRNA cap evades host restriction by IFIT family members. *Nature* 2010;468:452–6. <https://doi.org/10.1038/nature09489>.
- [55] Liu L, Wang P, Nair MS, Yu J, Rapp M, Wang Q, et al. Potent neutralizing antibodies against multiple epitopes on SARS-CoV-2 spike. *Nature* 2020;584:450–6. <https://doi.org/10.1038/s41586-020-2571-7>.
- [56] Robbiani DF, Gaebler C, Muecksch F, Lorenzi JCC, Wang Z, Cho A, et al. Convergent antibody responses to SARS-CoV-2 in convalescent individuals. *Nature* 2020;584:437–42. <https://doi.org/10.1038/s41586-020-2456-9>.
- [57] Seydoux E, Homad LJ, Maccamy AJ, Parks KR, Hurlburt NK, Jennewein MF et al (2020) Characterization of neutralizing antibodies from a SARS-CoV-2 infected individual. bioRxiv. <https://doi.org/10.1101/2020.05.12.091298>.
- [58] Wec AZ, Wrapp D, Herbert AS, Maurer DP, Haslwanter D, Sakhkhar M, et al. Broad neutralization of SARS-related viruses by human monoclonal antibodies. *Science* 2020;369:731–6. <https://doi.org/10.1126/science.abc7424>.
- [59] Zost SJ, Gilchuk P, Chen RE, Case JB, Reidy JX, Trivette A, et al. Rapid isolation and profiling of a diverse panel of human monoclonal antibodies targeting the SARS-CoV-2 spike protein. *Nat Med* 2020;26:1422–7. <https://doi.org/10.1038/s41591-020-0998-x>.
- [60] Han X, Ye Q. The variants of SARS-CoV-2 and the challenges of vaccines. *J Med Virol* 2022;94:1366–72. <https://doi.org/10.1002/jmv.27513>.
- [61] Soto C, Bombardi RG, Kozhevnikov M, Sinkovits RS, Chen EC, Branchizio A, et al. High frequency of shared clonotypes in human T cell receptor repertoires. *Cell Rep* 2020;32:.. <https://doi.org/10.1016/j.celrep.2020.107882> 107882.
- [62] Hisham Y, Ashhab Y, Hwang SH, Kim DE. Identification of highly conserved SARS-CoV-2 antigenic epitopes with wide coverage using reverse vaccinology approach. *Viruses* 2021;13:787. <https://doi.org/10.3390/v13050787>.
- [63] Dong X, Zost S, Greaney A, Starr TN, Dingsen AS, Chen EC et al. (2021) Genetic and structural basis for recognition of SARS-CoV-2 spike protein by a two-antibody cocktail. bioRxiv. <https://doi.org/10.1101/2021.01.27.428529>.
- [64] Yuan M, Liu H, Wu NC, Lee CD, Zhu X, Zhao F, et al. Structural basis of a shared antibody response to SARS-CoV-2. *Science* 2020;369:1119–23. <https://doi.org/10.1126/science.abc2321>.
- [65] Menachery VD, Yount BL, Debbink K, Agnihothram S, Gralinski LE, Plante JA, et al. A SARS-like cluster of circulating bat coronaviruses shows potential for human emergence. *Nat Med* 2015;21:1508–13. <https://doi.org/10.1038/nm.3985>.
- [66] Martinez DR, Schäfer A, Leist SR, De la Cruz G, West A, Atochina-Vasserman EN, et al. Chimeric spike mRNA vaccines protect against Sarbecovirus challenge in mice. *Science* 2021;373:991–8. <https://doi.org/10.1126/science.abi4506>.
- [67] Kirkpatrick E, Qiu X, Wilson PC, Bahl J, Krammer F. The influenza virus hemagglutinin head evolves faster than the stalk domain. *Sci Rep* 2018;8:10432. <https://doi.org/10.1038/s41598-018-28706-1>.
- [68] Graves PN, Schulman JL, Young JF, Palese P. Preparation of influenza virus subviral particles lacking the HA1 subunit of hemagglutinin: unmasking of cross-reactive HA2 determinants. *Virology* 1983;126:106–16. [https://doi.org/10.1016/0042-6822\(83\)90465-8](https://doi.org/10.1016/0042-6822(83)90465-8).
- [69] Wohlbold TJ, Nachbagauer R, Margine I, Tan GS, Hirsh A, Krammer F. Vaccination with soluble headless hemagglutinin protects mice from challenge with divergent influenza viruses. *Vaccine* 2015;33:3314–21. <https://doi.org/10.1016/j.vaccine.2015.05.038>.
- [70] Impagliazzo A, Milder F, Kuipers H, Wagner MV, Zhu X, Hoffman RM, et al. A stable trimeric influenza hemagglutinin stem as a broadly protective immunogen. *Science* 2015;349:1301–6. <https://doi.org/10.1126/science.abc7263>.
- [71] Yassin HM, Boyington JC, McTamney PM, Wei CJ, Kanekiyo M, Kong WP, et al. Hemagglutinin-stem nanoparticles generate heterosubtypic influenza protection. *Nat Med* 2015;21:1065–70. <https://doi.org/10.1038/nm.3927>.
- [72] Murrell S, Wu SC, Butler M. Review of dengue virus and the development of a vaccine. *Biotechnol Adv* 2011;29:239–47. <https://doi.org/10.1016/j.biotechadv.2010.11.008>.

- [73] Halstead SB, Russell PK. Protective and immunological behavior of chimeric yellow fever dengue vaccine. *Vaccine* 2016;34:1643–7. <https://doi.org/10.1016/j.vaccine.2016.02.004>.
- [74] Krammer F, Pica N, Hai R, Margine I, Palese P. Chimeric hemagglutinin influenza virus vaccine constructs elicit broadly protective stalk-specific antibodies. *J Virol* 2013;87:6542–50. <https://doi.org/10.1128/JVI.00641-13>.
- [75] Voss WN, Hou YJ, Johnson NV, Delidakis G, Kim JE, Javanmardi K, et al. Prevalent, protective, and convergent IgG recognition of SARS-CoV-2 non-RBD spike epitopes. *Science* 2021;372:1108–12. <https://doi.org/10.1126/science.abg5268>.
- [76] Woo PCY, Lau SKP, Chu CM, Chan KH, Tsoi HW, Huang Y, et al. Characterization and complete genome sequence of a novel coronavirus, coronavirus HKU1, from patients with pneumonia. *J Virol* 2005;79:884–95. <https://doi.org/10.1128/JVI.79.2.884-895.2005>.
- [77] Ge XY, Li JL, Yang XL, Chmura AA, Zhu G, Epstein JH, et al. Isolation and characterization of a bat SARS-like coronavirus that uses the ACE2 receptor. *Nature* 2013;503:535–8. <https://doi.org/10.1038/nature12711>.
- [78] Parvathaneni K, Torres-Rodriguez K, Meng W, Hwang WT, Frey N, Naji A, et al. SARS-CoV-2 spike-specific T-cell responses in patients with B-cell depletion who received chimeric antigen receptor T-cell treatments. *JAMA Oncol* 2022;8:164–7. <https://doi.org/10.1001/jamaoncol.2021.6030>.
- [79] Keeble AH, Howarth M. Power to the protein: enhancing and combining activities using the Spy toolbox. *Chem Sci* 2020;11:7281–91. <https://doi.org/10.1039/D0SC01878C>.
- [80] Reks-Ngarm S, Pitisuttithum P, Nitayaphan S, Kaewkungwal J, Chiu J, Paris R, et al. Vaccination with ALVAC and AIDSVAX to prevent HIV-1 infection in Thailand. *N Engl J Med* 2009;361:2209–20. <https://doi.org/10.1056/NEJMoa0908492>.
- [81] Haynes BF, Gilbert PB, McClrath MJ, Zolla-Pazner S, Tomaras GD, Alam SM, et al. Immune-correlates analysis of an HIV-1 vaccine efficacy trial. *N Engl J Med* 2012;366:1275–86. <https://doi.org/10.1056/NEJMoa1113425>.
- [82] Cohen AA, Gnanapragasam PNP, Lee YE, Hoffman PR, Ou S, Kakutani LM, et al. Mosaic nanoparticles elicit cross-reactive immune responses to zoonotic coronaviruses in mice. *Science* 2021;371:735–41. <https://doi.org/10.1126/science.abf6840>.
- [83] Martin I, Ruyschaert JM. Common properties of fusion peptides from diverse systems. *Biosci Rep* 2000;20:483–500. <https://doi.org/10.1023/a:1010454803579>.
- [84] Maeda DLNF, Tian D, Yu H, Dar N, Rajasekaran V, Meng S, et al. Killed whole-genome reduced-bacteria surface-expressed coronavirus fusion peptide vaccines protect against disease in a porcine model. *Proc Natl Acad Sci USA* 2021;118. <https://doi.org/10.1073/pnas.2025622118>.
- [85] Nathan A, Rossin EJ, Kaseke C, Park RJ, Khatri A, Koundakjian D, et al. Structure-guided T cell vaccine design for SARS-CoV-2 variants and sarbecoviruses. *Cell* 2021;184:4401–4413.e10. <https://doi.org/10.1016/j.cell.2021.06.029>.
- [86] Pillaiyar T, Manickam M, Namasivayam V, Hayashi Y, Jung SH. An overview of severe acute respiratory syndrome-coronavirus (SARS-CoV) 3CL protease inhibitors: peptidomimetics and small molecule chemotherapy. *J Med Chem* 2016;59:6595–628. <https://doi.org/10.1021/acs.jmedchem.5b01461>.
- [87] Owen DR, Allerton CMN, Anderson AS, Aschenbrenner L, Avery M, Berritt S, et al. An oral SARS-CoV-2 Mpro inhibitor clinical candidate for the treatment of COVID-19. *Science* 2021;374:1586–93. <https://doi.org/10.1126/science.aba4784>.
- [88] Düzgünes N, Fernandez-Fuentes N, Konopka K. Inhibition of viral membrane fusion by peptides and approaches to peptide design. *Pathogens* 2021;10:1599. <https://doi.org/10.3390/pathogens10121599>.
- [89] Xiang R, Yu Z, Wang Y, Wang L, Huo S, Li Y, et al. Recent advances in developing small-molecule inhibitors against SARS-CoV-2. *Acta Pharm Sin B* 2021;2211–3835. <https://doi.org/10.1016/j.apsb.2021.06.016>.
- [90] Lim YX, Ng YL, Tam JP, Liu DX. Human coronaviruses: a review of virus–host interactions. *Diseases* 2016;4. <https://doi.org/10.3390/diseases4030026>.
- [91] King A. An uncommon cold (2020) *New Sci* 246:32–5. ISSN 0262-4079. [https://doi.org/10.1016/S0262-4079\(20\)30862-9](https://doi.org/10.1016/S0262-4079(20)30862-9).
- [92] Kathiravan MK, Radhakrishnan S, Namasivayam V, Palaniappan S. An overview of spike surface glycoprotein in severe acute respiratory syndrome–coronavirus. *Front Mol Biosci* 2021;8. <https://doi.org/10.3389/fmolb.2021.637550>.
- [93] Hoffmann M, Hofmann-Winkler H, Smith JC, Krüger N, Arora P, Sørensen LK, et al. Camostat mesylate inhibits SARS-CoV-2 activation by TMPRSS2-related proteases and its metabolite GBPA exerts antiviral activity. *EBiomedicine* 2021;65. <https://doi.org/10.1016/j.ebiom.2021.103255>.
- [94] Zhu Y, Yu D, Hu Y, Wu T, Chong H, He Y. SARS-CoV-2-derived fusion inhibitor lipopeptides exhibit highly potent and broad-spectrum activity against divergent human coronaviruses. *Signal Transduct Target Ther* 2021;6:294. <https://doi.org/10.1038/s41392-021-00698-x>.
- [95] Zhao MM, Yang WL, Yang FY, Zhang L, Huang WJ, Hou W, et al. Cathepsin L plays a key role in SARS-CoV-2 infection in humans and humanized mice and is a promising target for new drug development. *Signal Transduct Target Ther* 2021;6:134. <https://doi.org/10.1038/s41392-021-00558-8>.
- [96] Musarrat F, Chouljenko V, Dahal A, Nabi R, Chouljenko T, Jois SD, et al. The anti-HIV drug nelfinavir mesylate (Viracept) is a potent inhibitor of cell fusion caused by the SARSCoV-2 spike (S) glycoprotein warranting further evaluation as an antiviral against COVID-19 infections. *J Med Virol* 2020;92:2087–95. <https://doi.org/10.1002/jmv.25985>.
- [97] Drescher S, van Hoogevest P. The phospholipid Research Center: current research in phospholipids and their use in drug delivery. *Pharmaceutics* 2020;12:1235. <https://doi.org/10.3390/pharmaceutics12121235>.
- [98] Tang T, Jaimes JA, Bidon MK, Straus MR, Daniel S, Whittaker GR. Proteolytic activation of SARS-CoV-2 spike at the S1/S2 boundary: potential role of proteases beyond Furin. *ACS Infect Dis* 2021;7:264–72. <https://doi.org/10.1021/acsinfectdis.0c00701>.
- [99] Li P, Wang Y, Lavrijsen M, Lamers MM, de Vries AC, Rottier RJ, et al. SARS-CoV-2 Omicron variant is highly sensitive to molnupiravir, nirmatrelvir, and the combination. *Cell Res* 2022;32:322–4. <https://doi.org/10.1038/s41422-022-00618-w>.
- [100] Robbins R, Zimmer C. Clears Pfizer's Covid pill for high-risk Patients 12 and older. *The New York Times*. 12. F.D.A.; 2021.
- [101] Toots M, Yoon JJ, Hart M, Natchus MG, Painter GR, Plemper RK. Quantitative efficacy paradigms of the influenza clinical drug candidate EIDD-2801 in the ferret model. *Transl Res* 2020;218:16–28. <https://doi.org/10.1016/j.trsl.2019.12.002>.
- [102] Medicines and Health Care Products Regulatory Agency. First oral antiviral for COVID-19, Lagevrio (molnupiravir), approved by MHRA, <https://www.gov.uk/government/news/first-oral-antiviral-for-covid-19-lagevrio-molnupiravir-approved-by-mhra>; 2021. Government of The United Kingdom.