Virus-Like Particles-Based COVID-19 Vaccines

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Virus-like particles (VLPs) are a versatile, safe, and highly immunogenic vaccine platform. The use of a very flexible vaccine platform in COVID-19 vaccine development is an important feature that cannot be ignored. Incorporating the spike protein and its variations into VLP vaccines is a desirable strategy as the morphology and size of VLPs allows for better presentation of several different antigens.

virus-like particles   vaccines   COVID-19   SARS-CoV-2

1. Introduction

The SARS-CoV-2 (Severe Acute Respiratory Syndrome Coronavirus 2) is the causative agent of COVID-19 (Coronavirus Disease 2019) [1][2][3] and is responsible for the recent pandemic, which has already reported 248,467,363 cases and 5,027,183 deaths worldwide as of 5 November 2021 [4]. As new cases continue to increase worldwide, it is urgent to develop inexpensive and versatile vaccines to handle emerging variants that can affect pre-existing natural immunity and the efficacy of already approved vaccines [5]. According to the World Health Organization, 129 COVID-19 vaccines are under clinical trials, and eight are approved for emergency or definitive use worldwide, including inactivated, mRNA, and viral vector vaccines (**Table 1**) [6][7]. Although we already have these available vaccines, there is still a need for improved versions of COVID-19 vaccines. Hence, adapting vaccines to variants of concern (VOCs) along with decreasing vaccine costs will be the goals for the next step towards eradication. A technology that has the potential to address some of these issues is the virus-like particles (VLPs) vaccine platform, as it is adaptable, resembles viral structures, is highly immunogenic, and can be less expensive than other platforms.

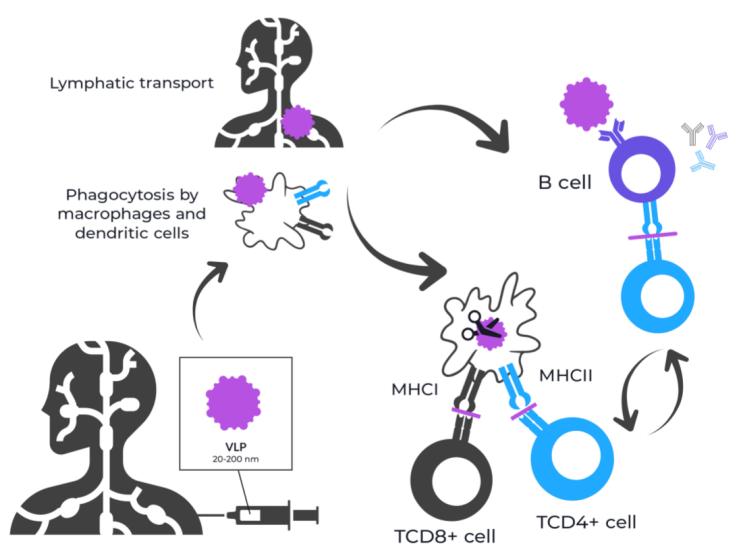**Table 1.** Summary of WHO COVID-19 approved vaccines.

Name	Platform	Adjuvant	Dosage	Efficacy *	References
Coronavac (Sinovac)	Inactivated	Alum	2 doses	83.5% (95% CI, 65.4–92.1)	[8][9][10][11]
BBIBP-CorV (Sinopharm)	Inactivated	Alum	2 doses	72.8% (95% CI, 58.1–82.4)	[12][13]
BBV152-Covaxin (Bharat Biotech)	Inactivated	Alum	2 doses	77.8% (95% CI, 65.2–86.4)	[14][15]
AZD1222–Vaxzevria	Viral	No	2	74.0% (95% CI, 65.3–	[16][17][18]

Name	Platform	Adjuvant	Dosage	Efficacy *	References
(Oxford/AstraZeneca)	vector		doses	80.5)	
Covishield (Oxford/AstraZeneca formulation)	Viral vector	No	2 doses	74.0% (95% CI, 65.3–80.5)	[16][17][18]
Ad26.COV2.S (Johnson &Johnson-Janssen)	Viral vector	No	1 dose	66.9% (95% CI, 59.0–73.4)	[19][20][21] [22]
mRNA-1273 (Moderna)	mRNA	No	2 doses	94.1% (95% CI, 89.3–96.8)	[23][24]
BNT162b-Comirnaty (Pfizer/BioNTech)	mRNA	No [27]	2 doses	95% (95% CI, 90.3–97.6)	[25][26]

r nonviral tured and processed by antigen-presenting cells (APCs) and presented by both MHC-I and MHC-II to T helper and cytotoxic T lymphocytes (**Figure 1**A). The structural repetitiveness and particle size of VLPs enhance recognition and direct

* Against symptomatic COVID-19.

activation of B cells. [28][29][30][31]. Taken altogether, these properties lead to robust humoral and cellular immune responses, which are exciting for vaccination against infectious diseases [32][33][34][35][36][37][38].

VLPs are classified according to their structural composition (nonenveloped-neVLPs or enveloped-eVLPs) and to the native virus components (homologous or heterologous) [39] (**Figure 1**B). Homologous VLPs comprise particles that self-assemble using proteins derived from the native virus only [40]. On the other hand, heterologous VLPs contain proteins from different sources to increase immunogenicity [41]. Moreover, available bioinformatics tools can help to optimize the rational design of new and pre-existing VLPs to achieve the best immunogenic performance [32][42][43][44][45][46].

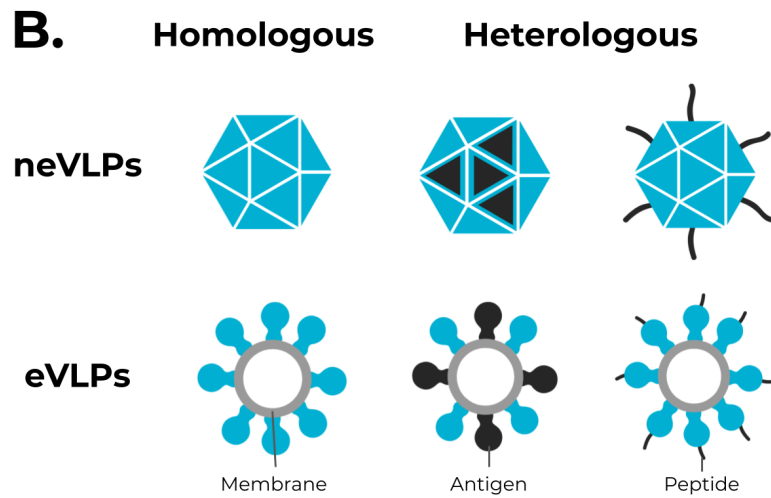**A.** **Adaptive immune response generated by VLP immunization**

Figure 1. The adaptive immune

response generated by VLPs immunization and VLPs classification. (A) After immunization, VLPs are phagocytized by dendritic cells or macrophages. Then, they are carried out to lymphatic vessels, where the antigenic regions will be processed and presented by class II MHC molecules (CD4+ T cells) and, through cross-presentation, by class I (CD8+ T cells). Immunological pathway activation by immunization with VLPs will activate robust cellular (cytokines) and humoral (B cell-antibodies) immune responses. (B) VLPs are classified as nonenveloped (neVLPs) or enveloped VLPs (eVLPs) based on the absence or presence of a lipidic membrane, respectively. These particles can also be classified as homologous or heterologous VLPs according to their composition. Homologous VLPs are assembled using proteins from the native pathogen only (blue), and heterologous VLPs can be assembled using proteins or peptides from different sources (black and blue).

2. SARS-CoV-2, VOCs, and Structural Vaccinology

The SARS-CoV-2 positive-sense single-stranded RNA genome (29 kb in length) encodes four structural and 16 non-structural proteins [3]. The structural proteins are the membrane (M), envelope (E), spike (S), and nucleocapsid (N) proteins, as seen in other coronaviruses (**Figure 2**A).
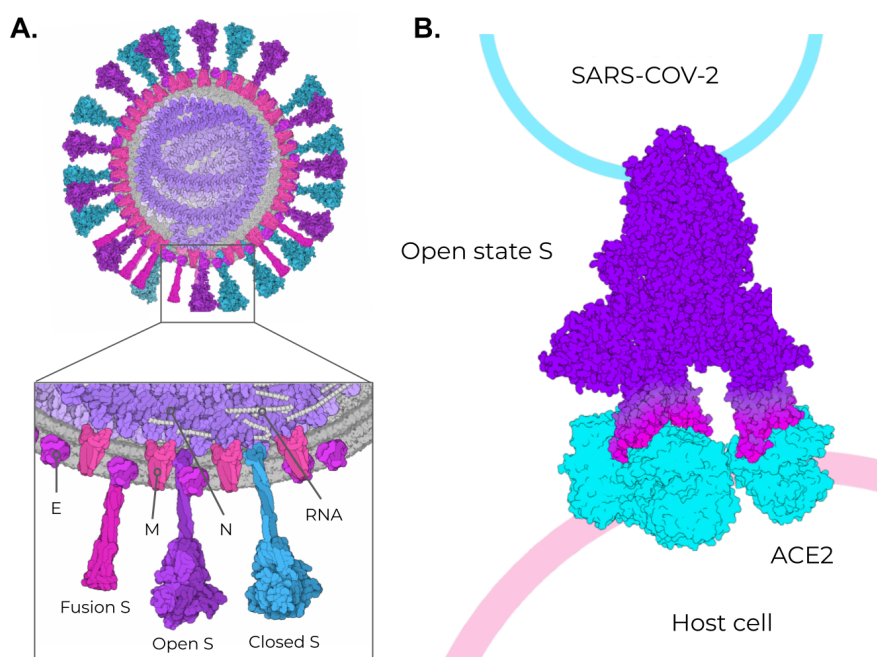
Figure 2. SARS-CoV-2 structural proteins and the different states of the Spike protein. (A) Schematic representation of the SARS-CoV-2 viral particle. The structure of the SARS-CoV-2 viral particle is composed of four structural proteins: Membrane (M), Envelope (E), Nucleocapsid (N), and Spike (S). The S protein is found in two different states on viral particles: open state (minor population) and closed state (major population). In addition, during the membrane fusion process (host cell entry), the S protein can be found in the fusion state (fusion S). (B) Schematic representation of the binding of open-state S (PDB ID 7498) to the ACE2 receptor present in the host cell. The illustrations were made in free software (CellPaint 2.0 [47] and 3D Protein Imager [48]). The binding figure was made using the crystal structure of ACE2 bound to Spike available at the Protein Data Bank (PDB ID 7A98).

Among these structural proteins, the main target for vaccine development is the SARS-CoV-2 S protein, which gives the characteristic crown-shaped structure of coronaviruses [49][50]. S is a highly glycosylated [51] homotrimer transmembrane protein (UNIPROT ID P0DTC2) composed of 1273 amino acids per chain. Known human sarbecoviruses (SARS-CoV-2 and SARS-CoV-1) and the alphacoronavirus NL63 invade the host cell through an interaction between the S protein and its receptor, the angiotensin-converting enzyme 2 (ACE2) [51][52][53][54][55] (**Figure 2**B). In general, the S protein consists of two major regions in addition to the signal peptide (SP) (1–12): the S1 subunit (13–685), and the S2 subunit (686–1273), which contains the transmembrane region (TM) (1214–1234) followed by the cytoplasmic domain (CD) (1235–1273) (**Figure 3**A). The S protein and the ACE2 receptor binding are mediated by the receptor-binding motif (RBM; 437–508), located in the receptor-binding domain (RBD; 319–541) [56] (**Figure 3**A, purple and cyan, respectively). The fusion machinery in S2 is composed of two fusion peptides (816–837 and 835–855) and two heptad regions (920–970 and 1163–1202). The first site of cleavage targeted by host proteases, such as furin and TMPRSS2, is located in the S1/S2 interface (685–686) [57][58][59]

(**Figure 3**A, red)**.** Removing the S1/S2 site promotes conformational changes that open the second cleavage site at S2 (815–816). The subsequent cleavage of the S2 site promotes the projection of needle-shaped fusion peptides into the host membrane [60][61], leading to cell fusion in 60–120 s in feline coronavirus [62]. The S protein presents a closed and open conformation [45][63] (**Figure 3**B, upper and bottom panel, respectively). With one or more RBDs projected outward, the open state constitutes the major conformation population of viable virions [63]. The increased exposure and steric freedom enable stronger interactions with the ACE2 receptor [45][64]. Therefore, mutations that stabilize this open conformation lead to positive selection, making the virus more transmissible [65][66][67].
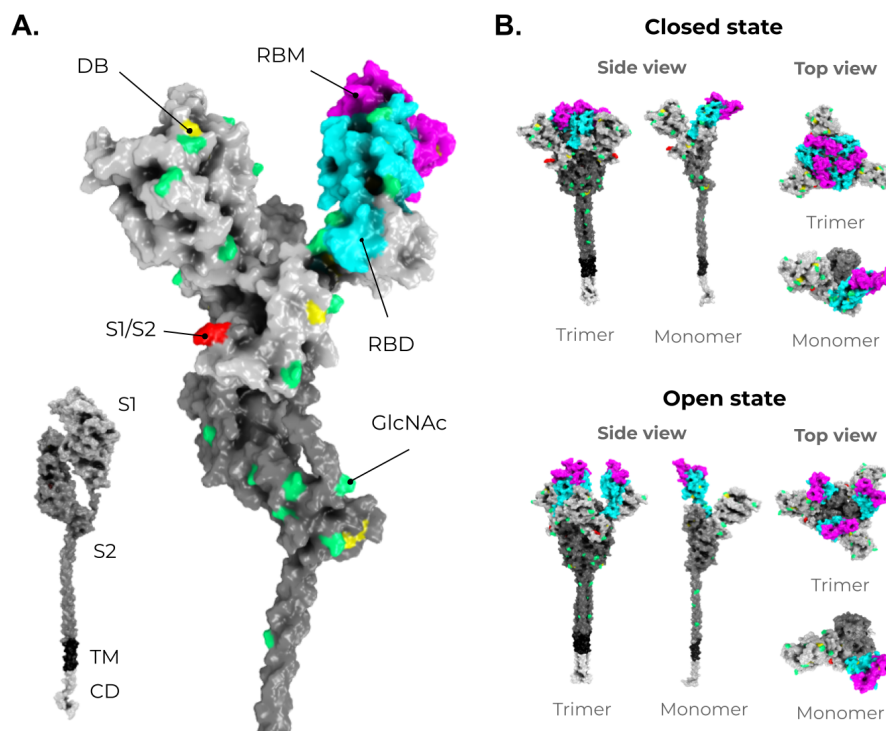

Figure 3. Structure and domain organization of the SARS-CoV-2 Spike (S) protein. (A) The S structure comprises a cytoplasmic domain (CD, white), a transmembrane domain (TM, black), and an ectodomain, which is divided into two subunits, S1 (gray) and S2 (dark gray). The magnification shows the several disulfide bridges (DB, yellow) and the glycosylation sites (GlcNAc, green) through the S protein ectodomain. It is highlighted in red, the S1/S2 interface. The receptor-binding domain (RBD, in cyan) and the receptor-binding motif (RBM, magenta) are also shown in S1. (B) As mentioned in Figure 2, the S protein shows two conformers on viable viruses (closed and open state). The upper panel shows the S protein in the closed state (trimeric and monomeric state). The bottom panel shows the S protein in the open state (trimeric and monomeric state). Illustrations were made in PyMol [68] using the wild-type structures available from Zhang et al. [69][70].

A better understanding of the effects of these conformational changes allows us to closely monitor the emerging VOCs. The VOCs that have attracted the most attention so far are the Alpha, Beta, Gamma, and Delta variants [66], which were initially identified in the UK [67], South Africa [71], Brazil [72], and India [73], respectively (**Figure 4**).
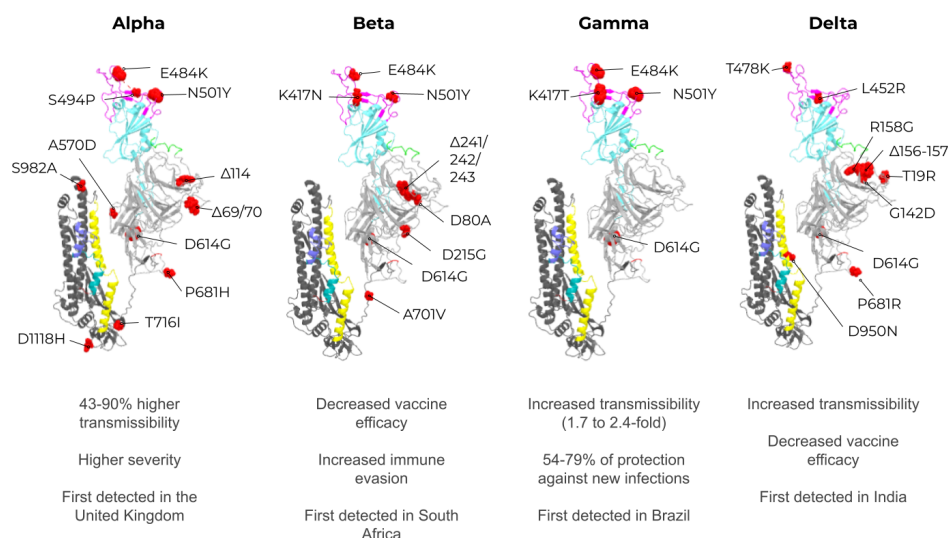
Figure 4. Mapping mutations of SARS-CoV-2 variants of concern (VOCs) and phenotypes. Red: mutations; Cyan: receptor-binding domain (RBD); Magenta: receptor binding motif (RBM); Light gray: S1; Dark gray: S2; Yellow: Heptad repeat 1; Green cyan: fusion peptide 1; Slate: Fusion peptide 2; Green: Signal peptide. Illustrations were made in PyMol [68] using resources from Zhang et al. [69][70].

3. Enveloped VLPs against SARS-CoV-2

Some studies have shown that the minimal requirement for the assembly of SARS-CoV-2 VLPs and other coronaviruses is the combination of M and either the E or N proteins. However, most particles include the N protein and the highly immunogenic S protein for better assembly and expression (**Figure 5**A) [74][75]. To date, Vero E6 cells presented the highest expression of S-containing VLPs when compared to HEK293 cells [75]. All of these initial approaches for SARS-CoV-2 VLP production show a promising use of this platform in vaccine development. However, industrial viability and large-scale production were not considered, and these are crucial features for further development and are still very challenging in the eVLP production field [58].

Although homologous VLPs are an attractive strategy for producing these particles, the combination of antigenic SARS-CoV-2 proteins with other highly expressed heterologous proteins (that could be used as alternative VLPs scaffolds) are an exciting strategy to address issues of industrial production. The NDVLP-S2P (**Figure 5**B) is a heterologous chimeric eVLP vaccine candidate against SARS-CoV-2 that uses the structure of a well-characterized enveloped virus, the Newcastle disease virus (NDV) [41], and is being developed by the National Institute of Allergy and Infectious Diseases (NIAID). The transmembrane domain of the NDV fusion protein was fused to SARS-CoV-2 S2P, allowing the correct display of S2P on the VLP surface [41][45][76]. The NDV-S2P VLPs were more immunogenic than the trimeric S protein alone, showing that the presentation of antigens on the surface of the VLPs is advantageous [41]. Another heterologous SARS-CoV-2 eVLPs vaccine candidate is the CoVLP from Medicago/GSK (**Figure 5**C) [37][77]. This vaccine is based on VLPs that display a mutated S2P protein, which

comprises a plant signal peptide, GSAS substitutions in the S1/S2 site, and TM/CD regions of the Influenza H5 A/Indonesia/5/2005. The CoVLP vaccine is formulated with AS03 [78] and given in a two-dose regimen. After the second dose, immunized volunteers showed higher serum SARS-CoV-2 nAb titers than in convalescent plasma. This vaccine candidate is already in phase 3 clinical trials (NCT04636697). VBI-2902a [76] is an MLV-based eVLPs vaccine candidate containing the S protein in the prefusion state fused with the VSV-G transmembrane cytoplasmic domain (VSV-GTC) (**Figure 5**D). This vaccine is being developed by VBI Vaccines and is in ongoing clinical trials 1/2 (NCT04773665).
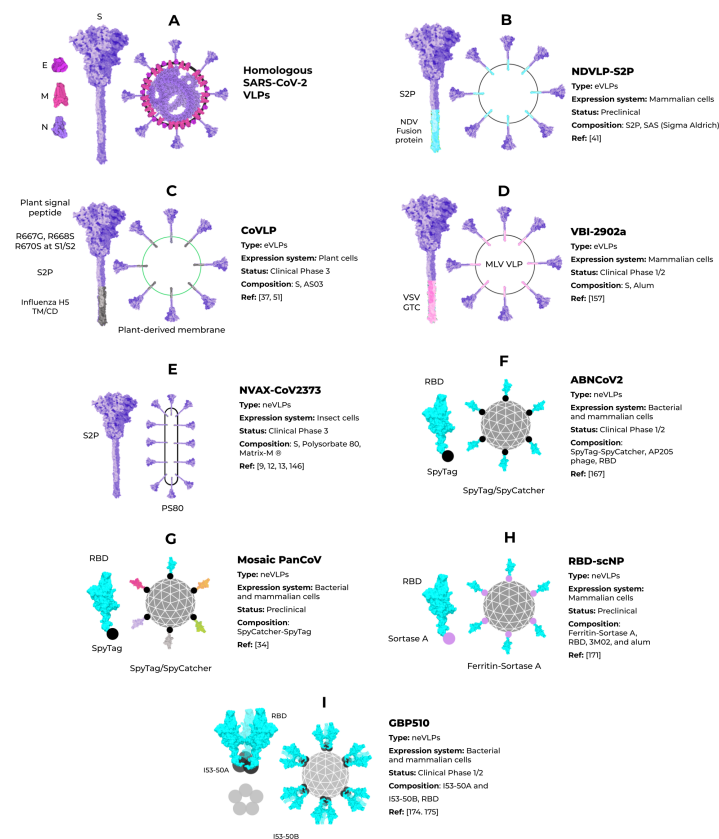

Figure 5. Enveloped and nonenveloped VLPs against SARS-CoV-2.

4. Non-Enveloped VLPs against SARS-CoV-2

Unlike eVLPs, neVLPs do not contain any lipid membranes. They can be produced in simpler expression systems, such as those using bacteria (i.e., *Escherichia coli*) and yeast (i.e., *Saccharomyces cerevisiae* and *Pichia Pastoris*) cells. The Hepatitis B virus vaccine, Engerix-B® [79], and the Human papillomavirus vaccine, Gardasil® and Gardasil 9® [33], are neVLPs-based vaccines approved by the FDA. They are produced in *Saccharomyces cerevisiae*, an efficient expression system that is industrially scalable and cheaper than mammalian and insect cell systems. Despite these clear advantages, bacteria and yeast cells lack complex post-translational modifications (PTM) needed to produce some proteins, such as the highly glycosylated SARS-CoV-2 S protein [80][81]. Thus, the choice of the best expression system could be a determinant for protein/VLP production, even considering neVLPs.

Cervarix® is another HPV neVLP-based vaccine [82] that is highly immunogenic [83] and effective [84][85] against HPV types 16 and 18, which are the main serotypes that cause cervical cancer [86]. The Cervarix® vaccine is produced using insect cells infected with recombinant baculovirus [40][87][88].

An up-and-coming SARS-CoV-2 neVLP-based vaccine candidate is the Novavax NVAX-CoV2373 (**Figure 5**E) that consists of S2P protein (1–1273) locked in the prefusion state and expressed using a baculovirus/insect cell system [32]. Later, the recombinant S protein is incorporated into polysorbate 80 detergent. This vaccine is formulated in combination with Matrix-M® adjuvant [32]. The NVAX-CoV2373 vaccine was shown to be immunogenic and safe [35][36] and conferred 89.7% protection against symptomatic cases [89]. Using a different approach, the ABNCoV2 (**Figure 5**F) is a SARS-CoV-2 neVLPs-based vaccine candidate from the Radbound University and AdaptVac. A SpyTag-SpyCatcher was used to display the S RBD in a bacteriophage scaffold system [90]. The ABNCoV2 is another highly immunogenic candidate and induced high titers of nAbs in mice [90]. This vaccine candidate is currently in phase 1/2 clinical trials (NCT04839146).

Although these different approaches show great potential for developing a SARS-CoV-2 VLPs-based vaccine, some improvements could be made to design broad-spectrum vaccines against other coronaviruses of interest. A pancoronavirus vaccine is an exciting strategy against zoonotic coronaviruses that represent a threat to humans and may soon be responsible for the next pandemic [91][92].

It is already known that SARS-CoV-2 vaccines do not generate significant broadly neutralizing antibodies against other family members, supporting the need for a functional pancoronavirus vaccine [93]. Cohen and colleagues designed different mosaic nanoparticle vaccines based on the S RBD from several human and animal (bat and pangolin) sarbecoviruses, including the SARS-CoV-2 RBD (**Figure 5**G) [34]. Each vaccine-induced strikingly higher cross-neutralizing antibodies than the SARS-CoV-2 S protein alone. Following the same idea, a Sortase A tagged ferritin-based VLPs conjugated to the SARS-CoV-2 S RBD (**Figure 5**H) was tested with alum adjuvants [94]. The protective immunity induced by this vaccine was robust against SARS-CoV-2 Alpha and Beta variants, as well as other human and bat sarbecoronaviruses. Interestingly, the cross-neutralizing antibody titers were more significant than the mRNA vaccine encoding the SARS-CoV-2 S protein. Despite this, the data suggest that mRNA and other S-based vaccines may afford some protection against other sarbecoronaviruses. Lastly, the candidate GBP510 (**Figure 5**I) is based on two rationally designed proteins, I53-50A and I53-50B [95][96], which self-assemble into trimers and pentamers, respectively. Combining these proteins forms a versatile 120 subunit scaffold, 28 nm wide, for the multivalent display of antigens linked to I53-50A subunits, such as SARS-CoV-2 RBD [87]. These particles were highly immunogenic in mice and resulted in 10-fold higher antibody titers at a 5-fold lower dose than the S2P protein alone [87]. In further preclinical trials with rhesus macaques, GBP510 was tested with different adjuvants, including alum, AS03, CpG, Essai O/W, and AS37. Although highly immunogenic against the original strain and the Alpha variant, a 4.5- to 16-fold reduction in neutralization was observed against the Beta variant [88]. GBP510 is already in phase I/II clinical trials (NCT04742738 and NCT04750343) sponsored by SK Bioscience Co. LTD and the Coalition for Epidemic Preparedness Innovations (CEPI).

5. Conclusions

As the cases of COVID-19 continue to grow and variants of SARS-CoV-2 emerge, the need for easily adaptable vaccine platforms remains one of the major goals in the pursuit of eradicating the disease. VLPs are an attractive platform for developing vaccines against infectious diseases such as COVID-19, due to their impressive versatility and immunological applications. The main advantage of using this vaccine platform is that VLPs can closely resemble the native virus in their size, shape, and antigen display. Additionally, VLPs can be adapted to contain more than one antigen of interest, a very important feature to produce efficient and broad vaccines.

Several VLP-based vaccines have been tested in human clinical trials since the approval of the Hecolin®, Cervarix®, Gardasil® and Gardasil 9® vaccines. These vaccines consolidated this platform and opened doors for its use to produce vaccines against several diseases, including COVID-19. As seen with other available vaccines, rational and adaptable vaccine development directly leads to a reduction of hospitalizations, deaths, and spread. The recent advances in VLP production and structural vaccinology provide this platform with all the needed features to produce novel, potent, and broad-spectrum coronavirus vaccines that could help in the fight against current and future pandemics.

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