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Unveiling the SARS-CoV-2 Spike Protein: A Comparative Analysis of Vaccine Development Approaches and Glycosylation Implications

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ABSTRACT

In December 2019, a mysterious pneumonia-causing sickness frightened the world. SARS-CoV-2 caused the acute respiratory illness. Since March 11, 2020, 220,563,227 COVID-19 cases and 4,565,483 deaths have been reported worldwide as of October 2021. SARS-CoV-2, like all coronavirus, appears to have crowns due to its S proteins and enters host cells using highly glycosylated spike (S) proteins. S1 and S2 are SARS-CoV-2 spike protein subunits. S2 controls transmembrane fusion, while S1 controls receptor binding. Antibody-mediated neutralization targets SARS-CoV-2 spike (S) proteins, which are essential for viral entry and fusion. This paper summarized how S protein was used in newly created and distributed SARS-CoV-2 vaccines and the implications for future advancements given the emergence of more lethal SARS-CoV-2 variants in this paper. It also discussed the role of S protein glycosylation in the viral entry and binding mechanism of SARS-CoV-2 and the implications for developing adaptive immunity and vaccines. The review was carried out through a deductive search strategy with keywords: COVID-19 vaccines, nCoV-2019 vaccines, coronavirus, COVID-19 vaccine development, S protein, and protein glycosylation using Google Scholar. The emergence of more transmissible and potentially more lethal SARS-CoV-2 variants, such as the Delta variant, highlights the need for continued research on vaccine development. Future research should focus on understanding the mechanism of the spike protein and how vaccines can effectively target the mutated regions. Continued monitoring and adaptation of vaccination strategies are essential to control the ongoing COVID-19 pandemic.

INTRODUCTION

Coronaviruses are single-stranded RNA viruses classified into three genera (alpha, beta, and gamma coronaviruses), which correspond to groups 1, 2, and 3 of the coronavirinae subfamily, coronaviridae family, and nidovirales order or superfamily (Weiss & Leibowitz, 2011). Coronaviruses infect a wide range of animals, both domestic and wild, and humans (Pedersen & Ho, 2020). Six strains, namely, HCoV-229E, HCoV-OC43, HCoV-NL63, HKU1, SARS-CoV, and MERS-CoV, are known to cause respiratory diseases (Hasöksüz *et al.*, 2020). SARS-CoV and MERS-CoV variants infect the lower respiratory tract and are therefore more harmful (McIntosh & Peiris, 2009); the others, on the other hand, infect the upper respiratory tract but only in moderate cases (Fehr *et al.*, 2015).

In December 2019, the globe was alarmed by reports of an emergent illness of unknown origin, characterized by pneumonia, and epidemiologically related to a seafood market in the Chinese city of Wuhan (Zhu *et al.*, 2019). A novel coronavirus (SARS-CoV-2) has been identified as the source of the acute respiratory infection by employing a "pneumonia of unknown etiology" surveillance system built in the aftermath of the 2003 SARS outbreak to allow early detection of new infections (Li *et al.*, 2020a; Zhu *et al.*, 2019). COVID-19 was declared a "public health emergency of international concern" (Li *et al.*, 2020b) by the World Health Organization (WHO) on the 30th of January 2020, and then a pandemic on the 11th of March 2020. (Olson *et al.*, 2020). On September 7, 2021, it was reported that there were 220,563,227 confirmed COVID-19 cases and 4,565,483 confirmed deaths globally (WHO, 2021).

SARS-CoV-2 is a member of the betacoronavirus genus (Perlman & Netland, 2009; Schoeman & Fielding, 2019; Vlasova *et al.*, 2007), which is one of the genera of the Orthocoronavirinae subfamily (Hasöksüz *et al.*, 2020). It is quite similar to the SARS-CoV virus, which caused a worldwide epidemic in 2003. SARS-CoV-2 share the identical tropism and mode of entry as SARS-CoV– about 80 percent (Zhou *et al.*, 2020)– because they use the same cellular receptor, angiotensin-converting enzyme 2 (ACE2) (Yan et a., 2020). Like all coronaviruses, SARS-CoV-2 appears to have crowns because of the S proteins that protrude on its surface (Zhao *et al.*, 2021). SARS-CoV-2 uses these highly glycosylated spike (S) proteins (Wrapp *et al.*, 2020).

The purpose of this research is to describe how the SARS-CoV-2 S protein was employed in the development of vaccines given its critical function in receptor binding and membrane fusion in the SARS-CoV-2 virus and discuss the glycosylation of its proteins. Specifically, this paper compared each approach on which S protein is given focus in vaccine development. By doing so, this paper can yield implications for future studies.

LITERATURE REVIEW

Structure

The S protein is a class I fusion protein that helps the virus connect to the angiotensin-converting enzyme 2

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(ACE2) receptor on the host cell surface, causing the virus and cell membrane to fuse (Pardi *et al*., 2015; Hoffman *et al*., 2020; Rauch *et al*., 2018). The overall length of S protein in SARS-CoV-2 is 1,273 amino acids, including a signal peptide at the N-terminus (Zhao *et al*., 2021). Two subunits, namely, S1 and S2, composed the S protein of SARS-CoV-2. These subunits are cleaved from a furin site in the virus' S protein, containing multiple essential amino acids. The S1 subunit is apical V-shaped and harbors one ACE2-recognition motif per monomer, the receptor-binding domain (RBD) (Sterberg & Naujokat, 2020).

SARS-CoV-2 uses a spike (S) protein that is highly glycosylated to gain entry to host cells, as previously stated (Wrapp *et al*., 2020). The S protein is glycosylated through the secretory pathway and is glycosylated by the host cellular glycosylation mechanism (Zhao *et al*., 2021). With this process comes two benefits for the virus itself. To begin with, the mannose residues within these glycans are crucial components in interacting with cell surface attachment proteins (Li *et al*., 2017; Tortorici *et al*., 2019; Robson, 2020) before attaching to ACE2. Second, it helps protect the virus from host antibodies by hiding the underlying polypeptide epitopes (Doores, 2015; Bagdonaite & Wandall, 2018).

Function

As mentioned, the S protein in SARS-CoV-2 is responsible for receptor identification and membrane fusion (Gallagher & Buchmeier, 2001), which are carried out by functional domains near the S1 and S2 termini (Hasöksüz *et al*., 2020). The S2 subunit is in charge of transmembrane fusion, whereas the S1 subunit controls receptor binding (Chen & Guo, 2020; Hasoksuz *et al*., 2002). The trimeric S protein is cleaved into S1 and S2 subunits after viral infection and S1 subunits are released during the transition to the postfusion conformation (Song *et al*., 2018; Simmons *et al*., 2013; Belouzard *et al*., 2009; Simmons *et al*., 2004).

Because it contains the receptor-binding domain (RBD), which directly connects with ACE2, a peptidase domain, the S1 subunit can bind to receptors (Li *et al*., 2005). To engage a host cell receptor, S1's receptor-binding domain (RBD) undergoes hinge-like conformational movements, briefly hiding or exposing the receptor binding determinants (Wrapp *et al*., 2020). When S1 connects to the ACE2 receptor on the host, another cleavage site on S2 is revealed and cleaved by host proteases, a step that is essential for viral infection (Belouzard *et al*., 2009; Millet & Whittaker, 2015; Simmons *et al*., 2005). The polybasic cleavage site of S may contribute to SARS-CoV-2's high virulence, as furin and furin-like proteases, which are necessary for S's proteolytic activation, are widely expressed in humans, allowing SARS-CoV-2 to infect a broader range of tissues.

Furthermore, multiple investigations have suggested that the S protein of SARS-CoV-2 may employ ACE2 to infect the host (Zhou *et al*., 2020; Hoffman *et al*., 2020; Kuba *et al*., 2005; Li *et al*., 2003). ACE2 is important because it aids in the maturation of angiotensin, a hormone that regulates blood pressure and vasoconstriction (Yan *et al*., 2020). Cardiovascular illnesses are very likely when ACE2 expression in the lungs, heart, kidneys, and intestines is reduced (Crackower *et al*., 2002; Zisman *et al*., 2002; Raizada *et al*., 2007). The S protein is a target for antibody-mediated neutralization because of its essential function. Furthermore, studying the prefusion S structure can help with vaccine development.

MATERIALS AND METHODS

The review was carried out through a deductive search strategy with keywords: COVID-19 vaccines, nCoV-2019 vaccines, coronavirus, COVID-19 vaccine development, S protein, and protein glycosylation using Google Scholar. Articles were included if they were published in English, appeared in peer-reviewed publications, and were related to the issue of the SARS-CoV-2 virus and vaccine development. Statistics from the World Health organization were also included. The flowchart outlining the search technique is presented in the figure below.

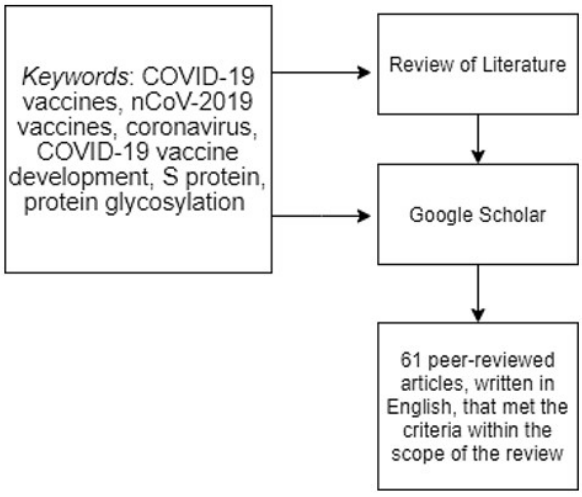

Figure 1: Search strategy employed

RESULTS AND DISCUSSION

Glycosylation of SARS-CoV-2 S Protein

Viral protein glycosylation is a successful virus strategy for modifying its proteins using the host-cell machinery. As a result, glycans play critical roles in viral infection and immune response (Zhao *et al*., 2021). Glycans on viral surface proteins play a role in viral entry, fusion, epitope shielding, viral protein folding, stability, and protection. For instance, by shielding surface antigens with glycan envelopes, glycosylation on the surface proteins of viruses can prevent antibodies from binding, which plays a significant role in viral infection (Shajahan *et al*., 2021).

As mentioned, the S protein of SARS-CoV-2 is highly glycosylated. SARS-CoV-2 has 22 N-glycosylation sites and several O-glycosylation sites on each protomer of the transmembrane homotrimeric protein (Watanabe *et al*., 2020). SARS-CoV-2 infection is influenced by the glycosylation process of both the virus and the target cells on several levels, according to recent reports (Reis

et al., 2020). Given this, it's worth examining how this process works and how it affects SARS-CoV-2 because it could lead to vaccine development breakthroughs.

For replication and protein glycosylation, SARS-CoV-2 uses the machinery of the host cell. As a result, the viral surface glycans are composed of host glycans that the immune system recognizes as self, suppressing the anti-carbohydrate immune response (Crispin *et al.*, 2015). Moreover, T cell activation and cytokine production are affected by differential hemagglutinin N-glycosylation, which poses a challenge for vaccine development (Hütter *et al.*, 2013). Furthermore, as shown in the case of influenza viruses, mutations in protein sequences can alter glycosylation by generating new or removing existing glycosylation sites (Altman *et al.*, 2019; Zost *et al.*, 2017). This could lead to the emergence of new virus strains (Altman *et al.*, 2019). This paper summarizes how protein glycosylation plays a role in SARS-CoV-2 virus replication, binding and entry, immune response, therapy, and vaccine development in the table below.

Table 1: Biologic and pathologic effects of glycosylation of SARS-CoV-2 S protein and other insights from literature

Aspect	Role of glycosylation in biology and pathogenesis of SARS-CoV-2 S protein and additional insights	Reference/s
Viral entry	Glycosaminoglycan heparan sulfate of the cellular glycocalyx is required for SARS-CoV-2 infection of these target cells. Heparan sulfate was found to interact with the receptor-binding domain of the SARS-CoV-2 spike glycoprotein, which is located next to ACE-2, causing the spike structure to open up and allow ACE-2 to bind to it.	Clausen *et al.* (2020)
	The conformation of the S protein's receptor-binding domain has been shown to be modulated by glycans at N165 and N234 of the protein.	Casalino *et al.* (2020)
	A sialic acid-binding pocket was discovered at the N-terminus of the S protein from SARS-CoV-2, suggesting that it may play a role in viral binding.	Verma (2020)
	Human lectins galectin-3, 7 and 8, Siglec-10, macrophage galactose lectin (MGL), and dendritic cell-specific intercellular adhesion molecule-3-grabbing non-integrin (DC-SIGN) bind to two N-glycans of the RBD of the S protein at N331 and N343, which may provide insight into SARS-CoV-2 tropism and binding.	Lenza *et al.* (2020)
Adaptive immunity	When compared to other blood groups, the proportion of seropositives in group O individuals was significantly lower, implying that group O individuals have a lower risk of infection.	Gallian *et al.* (2020)
	Sulfate glycans on the SARS-CoV-2 S protein could act as selectin ligands and play a role in immune regulation.	Lowe (2002)
	Variations in virus and host glycosylation are likely to influence tissue tropism and individual infection susceptibility; differences in virus infectivity and patient susceptibility may be caused by SARS-CoV-2 and ACE 2 mutations that alter N-glycosylation sites.	Reis *et al.* (2021)
Vaccine development	SARS-CoV-2 glycosylation can affect each of the current vaccine strategies in different ways (i.e., vector-based vaccine, mRNA-based vaccines).	Reis *et al.* (2021)
	Suitable glycosylation of the recombinant protein will at least partly determine how immunogenic polypeptide epitopes of vaccine glycoproteins fold.	Reis *et al.* (2020)
	The presence of glycans on protein antigens influences cellular uptake, proteolytic processing, MHC presentation, and subsequent T-cell priming, providing insight into how proper folding of recombinant vaccine glycoprotein can affect the development of an adaptive immune response	Wolfert & Boons (2013)
	The presence of non-human glycans on recombinant therapeutic glycoproteins may induce antibodies directed against non-human glycan epitopes.	Zhou & Qiu (2019)

Vaccines Based on the Sars-Cov-2 S Protein

Because the S protein is involved in receptor binding and membrane fusion, vaccinations may induce antibodies that prevent virus binding and fusion or neutralize virus infection. S protein is the major antigenic component of SARS-CoV. It is essential to generate host immunological responses, neutralizing antibodies, and protective immunity against virus infection among all structural proteins (Du *et al.,* 2009). This paper reviewed and compared each approach on which S protein is given focus in vaccine development. Some of these vaccines are already being used and distributed worldwide. Table 1 summarizes the findings.

Table 2: Summary of key findings

Vaccine Developer	Nature of the vaccine	S protein utilization approach	Reference/s
BioNTech/ Pfizer	mRNA-based	prefusion stabilized, membrane-anchored SARSCoV-2 full-length spike protein is encoded	Polack *et al.*, 2020; Walsh *et al.*, 2020
Moderna	mRNA-based	encodes the SARS-CoV-2 stabilized prefusion spike glycoprotein trimer	Baden *et al.*, 2021
University of Oxford/ Astra-Zeneca	Viral vector-based	formulated with simian adenovirus vector encoding the full-length S protein with a tPA leader sequence that is recombinant and replication-deficient	Folegatti *et al.*, 2020
Gamaleya Research Institute	Viral vector-based	made up of full-length S protein-expressing recombinant and replication-deficient human adenovirus 26 (dose 1) and human adenovirus 5 (dose 2)	Logunov *et al.*, 2020
Janssen	Viral vector-based	formulation was characterized by a recombinant, replication-deficient human adenovirus 26 producing full-length S protein with two amino acid alterations in the S1/S2 junction that eliminate the furin cleavage site and two proline substitutions in the hinge region that keep the protein in the pre-fusion conformation	Bos *et al.*, 2020
Novavax	Protein subunit	uses a recombinant nanoparticle of full-length S protein with protease resistance mutations at the S1/S2 cleavage sites and two proline substitutions to stabilize protein in a pre-fusion conformation, together with a saponin-based adjuvant (Matrix- M1)	Keech *et al.*, 2020
Sinovac Biotech	Whole cell inactivated virus	the attenuated or inactivated whole SARS-CoV-2 virus is administered to individuals to elicit the immune responses, which target the SARS-CoV-2 S protein.	Li *et al.*, 2021

mRNA-based

Three mRNA-based vaccines, BioNTech/Pfizer (BNT162b2 mRNA), Moderna (mRNA-1273), and CureVac (CVnCoV), have been reported (Sadarangani *et al.*, 2021; Kowalzik *et al.*, 2021). This type of vaccine is said to be different from the traditional kind of vaccine. In an mRNA vaccine, a transcript encoding one or more immunogens is delivered into the host cell's cytoplasm, which is translated into immunogenic proteins (Kowalzik *et al.*, 2021). mRNA vaccine manufacture does not entail infectious elements or the risk of stable integration into the host cell genome; instead, the vaccine RNA strand is destroyed (Sadarangani *et al.*, 2021). Because of this, this type of vaccine is safer.

In the case of BioNTech/Pfizer, a prefusion stabilized, membrane-anchored SARSCoV-2 full-length spike protein is encoded (Polack *et al.*, 2020; Walsh *et al.*, 2020). Two proline mutations modify this encoding S protein to lock protein in the pre-fusion conformation (Pardi *et al.*, 2015; Kariko *et al.*, 2005; Wrapp *et al.*, 2020). mRNA-based vaccines, particularly the BioNTech/Pfizer vaccine, elicit high SARS-CoV-2 neutralizing antibody titers and robust antigen-specific Th1-type CD4+ and CD8+ T-cell responses, similar to DNA-based vaccines developed for SARS-CoV, which induce neutralizing antibody and T-cell responses and protective immunity (Wang *et al.*, 2005; Huang *et al.*, 2007; Liu et (Sadarangani *et al.*, 2021). The S1-binding antibody is also present after the first of two doses (Walsh *et al.*, 2020).

Like BioNTech/Pfizer's the Moderna vaccine encodes the SARS-CoV-2 stabilized prefusion spike glycoprotein trimer, which is necessary for host cell attachment and viral entry (Baden *et al.*, 2021). S-binding antibody was also detected 14 days after the first dose, with levels increasing marginally by 28 days and significantly after the second dose (Jackson *et al.*, 2020). After the second treatment, CD4+ T cells secreting TH1 cytokines (TNF>IL-2>IFNγ) increased significantly (Jackson *et al.*, 2020).

Viral vector-based

Incorporating immunogenic full-length or truncated viral surface proteins into viral expression has been a common strategy for vaccine development against infectious agents, primarily viruses (Lundstrom, 2020). Various viral vector expression techniques have been used to target viral surface proteins. Adenoviruses (Ads), alphaviruses, flaviviruses, measles viruses (MVs), rhabdoviruses, retroviruses (RVs), lentiviruses (LVs), and poxviruses are all examples of expression vectors (SM Wold & Toth, 2013; Lundstrom, 2019). Four viral vector-based vaccines, namely, University of Oxford/Astra-Zeneca (ChAdOx1 nCoV-19), Gamaleya Research Institute (Gam-COVID-Vac), Janssen (Ad26.COV2.S), and CanSino Biologics (Ad5- nCoV), have been reported (Sadarangani *et al.*, 2021; Knoll & Wonodi, 2021).

The University of Oxford/Astra-Zeneca vaccine is formulated with a simian adenovirus vector encoding the full-length S protein with a tPA leader sequence that is recombinant and replication-deficient (Folegatti *et al.*, 2020). When mice and rhesus macaques were immunized with the chimpanzee Ad vector ChAdOx1 nCoV-19, which was designed to produce the SARS-CoV-2 S protein, it induced robust humoral and cellular immune responses and prevented pneumonia in macaques (van Doremalen *et al.*, 2020; Folegatti *et al.*, 2020). In humans, S-binding antibody was detected 14 days after the first dosage, and levels increased by 28 days; there was a significant rise after the second dose, with a peak at 14 days. Also, T cell responses peaked 14 days following the first dosage but were slightly greater 28 days later (Folegatti *et al.*, 2020).

Meanwhile, the Gamaleya Research Institute vaccine is made up of full-length S protein-expressing recombinant and replication-deficient human adenovirus 26 (dose 1) and human adenovirus 5 (dose 2) (Logunov *et al.*, 2020). S-binding antibody was found in 85–89% of people 14 days after the initial dose (Logunov *et al.*, 2020). Fourteen days after the second dose, S antibody levels are boosted (Logunov *et al.*, 2021). Based on proliferation assays and antigen-specific IFN production, CD4+ and CD8+ T cell responses were seen 14 days after the initial dosage (Lugonov *et al.*, 2021).

For the Janssen vaccine, the formulation was characterized by a recombinant, replication-deficient human adenovirus 26 producing full-length S protein with two amino acid alterations in the S1/S2 junction that eliminate the furin cleavage site and two proline substitutions in the hinge region that keep the protein in the pre-fusion conformation (Bos *et al.*, 2020). After stabilizing substitutions were inserted, the ratio of neutralizing vs. non-neutralizing antibody binding increased, indicating that the S protein was in a prefusion conformation, as observed in its experimental stages. S-binding and neutralizing antibodies were detectable in 99 percent of people 28 days after immunization, and antibody levels were maintained for at least 84 days (Janssen Biotech, 2021; Sadoff *et al.*, 2021).

Protein Subunit

To elicit an immune response, protein subunit-based vaccinations combine a protein (or part of a protein) from the targeted virus with an immune-boosting chemical termed an adjuvant (Wadman, 2020). Adjuvants are frequently required for this type of vaccine to increase the immune response and improve vaccine efficacy (Brito *et al.*, 2013). When a protein-based vaccination is taken up and processed into several epitopes by cells, it provides a more focused response to a particular antigen (Li *et al.*, 2021). The antigen in most of these vaccinations is either the S protein or its receptor-binding domain (RBD).

The Novavax vaccine, one of the vaccines that utilize protein subunit, uses a recombinant nanoparticle of full-length S protein with protease resistance mutations at the S1/S2 cleavage sites and two proline substitutions to stabilize protein in a pre-fusion conformation, together with a saponin-based adjuvant (Matrix- M1) (Keech *et al.*, 2020). Antibody to S-binding was identified 21 days after the first treatment, and significantly increased after the second dose. Meanwhile, based on IFN, IL-2, and TNF production in response to S protein stimulation, CD4+ T cell responses were detectable seven days after the second dosage, with a substantial bias towards a TH1 cell phenotype (Keech et a., 2020).

Whole Cell Inactivated Virus

Sinovac Biotech (CoronaVac), Sinoparm (BBIBP- CorV; WIBP- CorV), and Bharat Biotech are three SARS-CoV-2 vaccines that use the whole-cell inactivated virus method (BBV152) (Sadarangani *et al.*, 2021). Inactivated vaccines, also known as killed vaccines, are made by growing the virus in a culture medium and then treating it with chemicals, heat, or radiation to inactivate it (Li *et al.*, 2021). The majority of these vaccines contain aluminum hydroxide (Flanagan *et al.*, 2020; WHO, 2021). The Sinovac Biotech vaccine, for example, is made from SARS-CoV-2 produced in Vero cells, inactivated using β-propiolactone, and adsorbed onto aluminum hydroxide. In the case of vaccines developed against COVID-19, the attenuated or inactivated whole SARS-CoV-2 virus is administered to individuals to elicit immune responses (Li *et al.*, 2021). These immune responses target the SARS-CoV-2 S protein. Inactivated vaccines have a long history of use and can benefit many people, even individuals with advanced immunological senescence (Iversen & Bavari, 2021).

CONCLUSION

SARS-CoV-2 mutations have been on the rise recently. The Delta variant, for example, has been linked to a surge in cases in India and has now been found worldwide, including a significant increase in cases in the United Kingdom (Bernal *et al.*, 2021). The spike protein mutations T19R, 157-158, L452R, T478K, D614G, P681R, and D950N distinguish the delta variant (ECDPC, 2021). Several of these mutations may affect immune responses directed at the receptor-binding protein's key antigenic regions (452 and 478) and the deletion of a portion of the N-terminal domain (Li et a., 2020). P681R is located at the S1–S2 cleavage site, and it appears that strains with mutations there has greater replication, resulting in higher viral loads and transmission (Johnson *et al.*, 2020). This advent of more lethal SARS-CoV-2 variations raised questions about the efficiency of existing vaccines, which were meant to combat the initial SARS-CoV-2 strain. As a result, future vaccine development research must focus on how vaccines can effectively battle these developing strains, focusing on the mechanism of the SARS-CoV-2 S proteins, which is the site of mutations in the Delta variant.

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