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# Next-Generation Vaccines Against COVID-19 Variants: Beyond the Spike Protein

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**Abstract**

Vaccines are among the most effective medical countermeasures against infectious diseases. The current Coronavirus disease 2019 (COVID-19) pandemic, caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has spurred the scientific strategies to fight against the disease. Since 2020, a great number of vaccines based on different platforms have been in development in response to the pandemic, among which mRNA, adenoviral vector, and subunit vaccines have been clinically approved for use in humans. These first-generation COVID-19 vaccines largely target the viral spike (S) protein and aim for eliciting potent neutralizing antibodies. With the emergence of SARS-CoV-2 variants, especially the highly transmissible Omicron strains, the S-based vaccine strategies have been faced constant challenges due to strong immune escape by the variants. The coronavirus nucleocapsid (N) is one of the viral proteins that induces strong T-cell immunity and is more conserved across different SARS-CoV-2 variants. Inclusion of N in the development of COVID-19 vaccines has been reported. Here, we briefly reviewed and discussed COVID-19 disease, current S-based vaccine strategies, and focused on the immunobiology of N protein in SARS-CoV-2 host immunity, as well as the next-generation vaccine strategies involving N protein, to combat current and emerging SARS-CoV-2 variants.

**Keywords**: COVID-19, SARS-CoV-2 variants, vaccine, mRNA, nucleocapsid

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## Introduction

In early 2020, the causative agent of Coronavirus disease 2019 (COVID-19) was identified as SARS-CoV-2 (Severe Acute Respiratory Syndrome Coronavirus 2) (*1, 2*). Although the precise origin of the virus is not known, it shows high similarity with some bat coronaviruses. By the time the World Health Organization (WHO) made alarming signals to the world, SRAS-CoV-2 has crossed the borders and become pandemic (*3*). As of December 2022, SARS-CoV-2 has caused 650 million infections with 6.6 million deaths globally (*4*). Compared to other beta-coronaviruses (Middle East Respiratory Syndrome Coronavirus [MERS-CoV], SARS-CoV), SARS-CoV-2 is more replicative with high communal attack rate and induces high fatality rates in hospitalized patients (*5*). Nevertheless, the infection and recovery patterns barely depend on the demographics (*6*) and the mechanisms behind are not fully understood thus far. COVID-19 symptoms are similar with the influenza (flu) symptoms, including fever, frontal headache, retro-orbital or temporal headache, gastrointestinal symptoms, ground-glass opacity, and pleural effusion (*7*), other than loss of taste or smell and presence of neurological symptoms. Laboratory findings showed that lymphopenia, hypersensitive C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), creatine kinase, transaminases (aspartate aminotransferase and alanine aminotransferase), thrombocytopenia, and others, are more common in COVID-19 patients (*8*), and thus many of them used as biomarkers (*8*). Similarly, lung radiographic findings identified ground-grass opacities, interlobular septal thickening, and a peripheral distribution in COVID-19 patients. Consecutively, severe COVID-19 patients who suffer from respiratory distress are transferred to the intensive care unit for mechanical ventilation. Of note, COVID-19 infection severity is associated with comorbidities, such as cardiovascular diseases, diabetes and obesity, and leading to high mortality (*9, 10*).

## COVID-19 Pathophysiology

We have discussed pathophysiology intensively elsewhere (*11-13*). The exhaustive research of host-virus interactions has revealed that SARS-CoV-2 enters the host by different routes, including nasal, oral, and others, and bind to the unique functional receptor, angiotensin-converting enzyme 2 (ACE2) (*14*). The ACE2 is largely expressed on alveolar epithelial cells, enterocytes, endothelial cells, and oral mucosa. Spike (S) protein of SARS-CoV-2 uses ACE2 to enter the cells and SARS-CoV-2 RNA utilizes host machinery for replication and propagation.

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However, the implications of other entry supportive receptors (neuropilin 1, B0AT1 [neutral amino acid transporter]) should not be ruled out (*15*). Due to the quick replication of the virus, it damages the infected organ, particularly the lungs and other organs. During the acute phase, immune cells – particularly dendritic cells and macrophages — secret signaling molecules, which recruit other immune cells to the infected environment (*11*). This process is not transient and leads to the hyperactivation of immune cells, secreting copious amounts of cytokines and chemokines that damages the infected organ, termed as "cytokine storm" (*16*). Further, the involvement of diversified pathways have been proposed, including cyclooxygenase-2 (COX-2) and prostaglandin E2 (PGE2) mediated pathway (*17, 18*), NETosis (programmed cell death mediated by stimulation extracellular neutrophils traps [NETs]) (*19*), and others (*20*). In addition, S protein-ACE2 interaction results in down-regulation of ACE2 on lung epithelial cells, and this is thought to be one of the primary causes of lung injury (*21*). ACE2 can simultaneously upregulate ACE1 via the negative feedback mechanisms (*22*), leading to excessive angiotensin-II. Excessive angiotensin II, in turn, binds to the AGTR1A (angiotensin II receptor type 1) receptors, leading to excessive vascular pulmonary activity consistent with lung pathology that is observed in pulmonary destruction (*22, 23*). Irrespective of the signaling pathways, COVID-19 disease progresses by lymphopenia, cytokine storms, accumulation of macrophages and neutrophils in lungs, immune dysregulation, acute respiratory distress syndrome (ARDS), and others.

**Emergence of SARS-CoV-2 variants**

The human immune system is tightly regulated during the infection and provides long-lasting antigen/pathogen-specific memory over time (*24-26*). To overcome the host immune system, continuously evolving viruses may either hijack the antiviral pathways or produces diversified mutations through genetic diversity. These mutations lead to significant changes in the viral proteins structure, including antigenic drift, folding/masking the critical antigenic residues (exposed to the host earlier), entirely producing new protein sequences, and others (*27, 28*) . SARS-CoV-2 also adopted the same approach and came in the form of new variants around the globe, such as Alpha (B.1.1.7), Beta (B.1.351), Gamma (P.1), Delta (B.1.617.2), Epsilon (B.1.427 and B.1.429), Eta (B.1.525), Iota (B.1.526), Kappa (B.1.617.1), Mu (B.1.621, B.1.621.1), Zeta (P.2), Theta (P.3), and Omicron (B.1.1.529, BA.1, BA.1.1, BA.2, BA.3, BA.4 and BA.5 lineages) (**Figure 1**) (*29-32*). Due to the changes in the viral S protein, the virus

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changes its receptor binding pattern in the host, enhancing the infection potential, escaping from conventional detection methods, producing diverse disease outcomes, and becoming less susceptible to the immune response raised against earlier SARS-CoV-2 strains or vaccines (*33*). The emergence of these variants has posed constant challenges to host immunity induced by first-generation vaccines or prior infection.

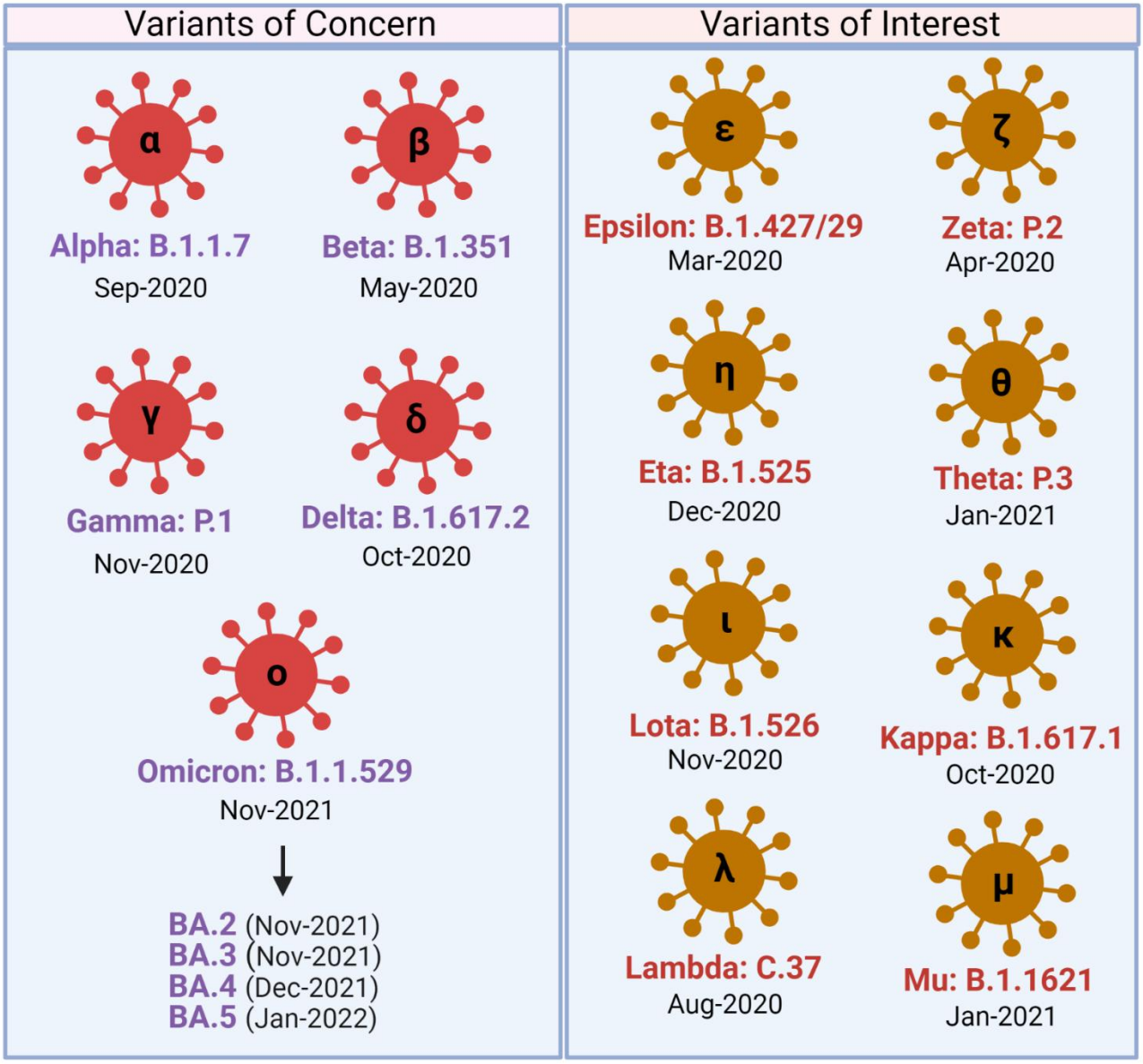


**Figure 1: Emerging SARS-CoV-2 variants.** Emergence of SARS-CoV-2 variants of concern and variants of interest is shown (https://www.who.int/activities/tracking-SARS-CoV-2-variants).

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**First-Generation COVID-19 Vaccines**

Despite unusual economic and livelihood loss globally, COVID-19 pandemic has driven a remarkable scientific and technological progress in the past a few years. Complete blueprint of the existing SARS-CoV-2 genome has been quickly revealed (*34*), and structural elucidation has been performed by mapping the host receptor interactions (*35*). Unequivocally huge success has been attained for vaccine development against SARS-CoV-2. More than 90 vaccine candidates are being developed worldwide using different strategies, including live inactivated vaccines, viral vector-based vaccines, recombinant subunit vaccines, nucleic acid (DNA or RNA) vaccines, and others (*36, 37*). Among them, two mRNA-based vaccines (Pfizer-BioNTech and Moderna) (**Figure 2**) and an adenovirus 26-based vaccine (Johnson & Johnson) were approved by the United States Food and Drug Administration (FDA) for clinical use under emergency authorization. Since then, the two mRNA vaccines have received full approval. Subsequently, additional vaccines have been approved for COVID-19, including Novavax's adjuvanted vaccine (SARS-CoV-2 S protein with Matrix-M adjuvant). Millions of doses of these vaccines have been distributed worldwide and continuously been used for immunizing both infected and non-infected populations (*38*). These first-generation COVID-19 vaccines have been extensively reviewed elsewhere (*37, 39-41*).

Despite these incredible achievements, new problems have been emerged that challenge the long-term control of the pandemic, which include emerging viral variants (**Figure 1**) with increased transmissibility and immune escape and waning immunity over time in vaccinated individuals (*32, 42*).

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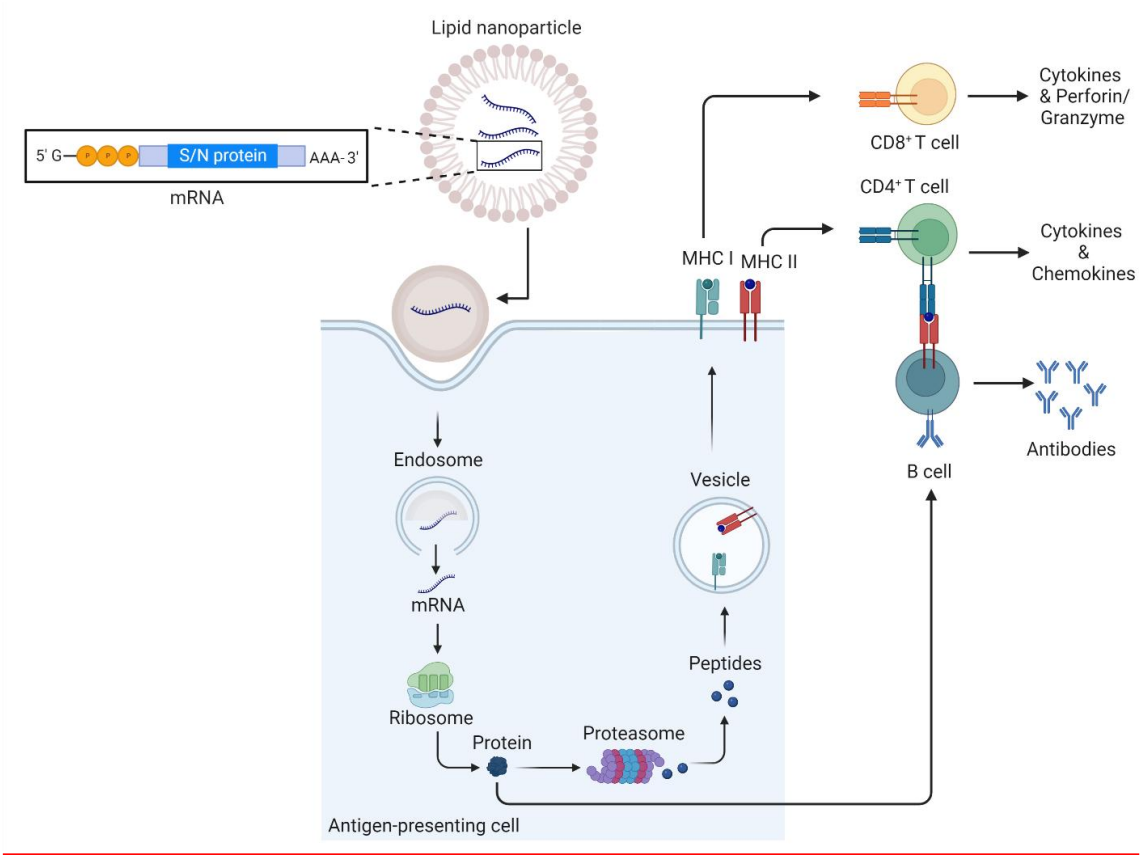


**Figure 2: Mode of action for mRNA vaccines.** mRNA encapsulated by lipid nanoparticles (LNPs) are useful for targeted delivery. Once mRNA enters the cell via endocytosis, the ionizable lipids in LNPs aid in its release into the cytosol. The host ribosomes convert mRNA into the corresponding protein, followed by protein degradation into peptides by proteasome. The presentation of peptides by the major histocompatibility complex to corresponding T lymphocytes stimulates both cellular and humoral immune responses.

Majority of the first-generation COVID-19 vaccines, either approved for clinical use or under clinical development, principally target the viral S protein, its subunits (S1 and S2), or its receptor binding domain (RBD) (**Figure 3A**), aiming for eliciting protective neutralizing antibody response. Emergence of variants with an effect on S protein structural change, especially the Delta and Omicron strains, with increased transmissibility and immune escape is predominant (*43-50*). For examples, multiple S variants were shown to have reduced sensitivity to neutralizing antibodies induced by the first-generation S-targeting vaccines (*45-47*). Clinical research also indicates that S-targeting COVID19 vaccines showed much reduced efficacy against Delta (*51*) and Omicron (*52*) strains. Thus, novel vaccine strategies that are targeting

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more conserved region of the virus to induce broader protection against COVID-19 variants are needed.

**SARS-CoV-2 nucleocapsid (N) protein biology**

N is an important structural protein of SARS-CoV-2 and is abundantly expressed in infected cells (*53*). There are hundreds of copies of N in the viral core, which encloses the genomic RNA. Like other viral structural proteins, N protein plays a vital role in CoVs life cycle, including mRNA transcription, cytoskeleton organization, RNA replication, packaging, immune regulation, and others (*54*). N protein regulates viral transcription and assembly upon enduring liquid-liquid phase separation (*55*). Initial studies elucidating N protein structure by NMR (Nuclear magnetic resonance) showed that its N-terminal domain (NTD) has an overall right-handed fold structure composed of a β-sheet core with an extended central loop (*56*). The protein is composed of two alpha helices and five beta sheets, containing a total of 419 amino acids. It is functionally divided into two domains: NTD and C-terminal domain (CTD) (**Figure 3**). NTD, which contains RNA-binding domain, acts as a linker between RNA and matrix to form virions. N binds to the positive single stranded RNA to ribonucleoprotein core that appears as "beads-on-a-string" model. CTD assists in RNA binding and contains a dimerization sequence, which is helpful in the RNA synthesis via interaction with the replication-transcription complexes (*57, 58*). The CTD dimerization sequence also facilitates viral genome incorporation into the virion. The intrinsically disordered region (IDR), between the domains, forms the physical link between the positive viral RNA genome and matrix protein (*55*). Interestingly, the presence of IDR favors the proteolysis of N protein, which is a key strategy for viral proliferation (*59*). The proteolytic products, particularly $N_{1–209}$, interact with immunophilin (i.e., Cyclophilin A) in the host cells and promote SARS-CoV-2 replication cycle (*59*) .

Due to its abundance of expression after SARS-CoV-2 infection, N protein has been used as a diagnostic marker for several antigenic tests, including IgG binding assay (*60*). Conventionally, the coronavirus N protein has also been shown to modulate the host intracellular machinery and plays regulatory roles during the viral life cycle. Therefore, the feature domains of N-protein have been the targets for antiviral development via inhibiting or blocking the RNA-binding

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activity or oligomerization capabilities. More detailed information can be found in the comprehensive review published elsewhere (*61, 62*).

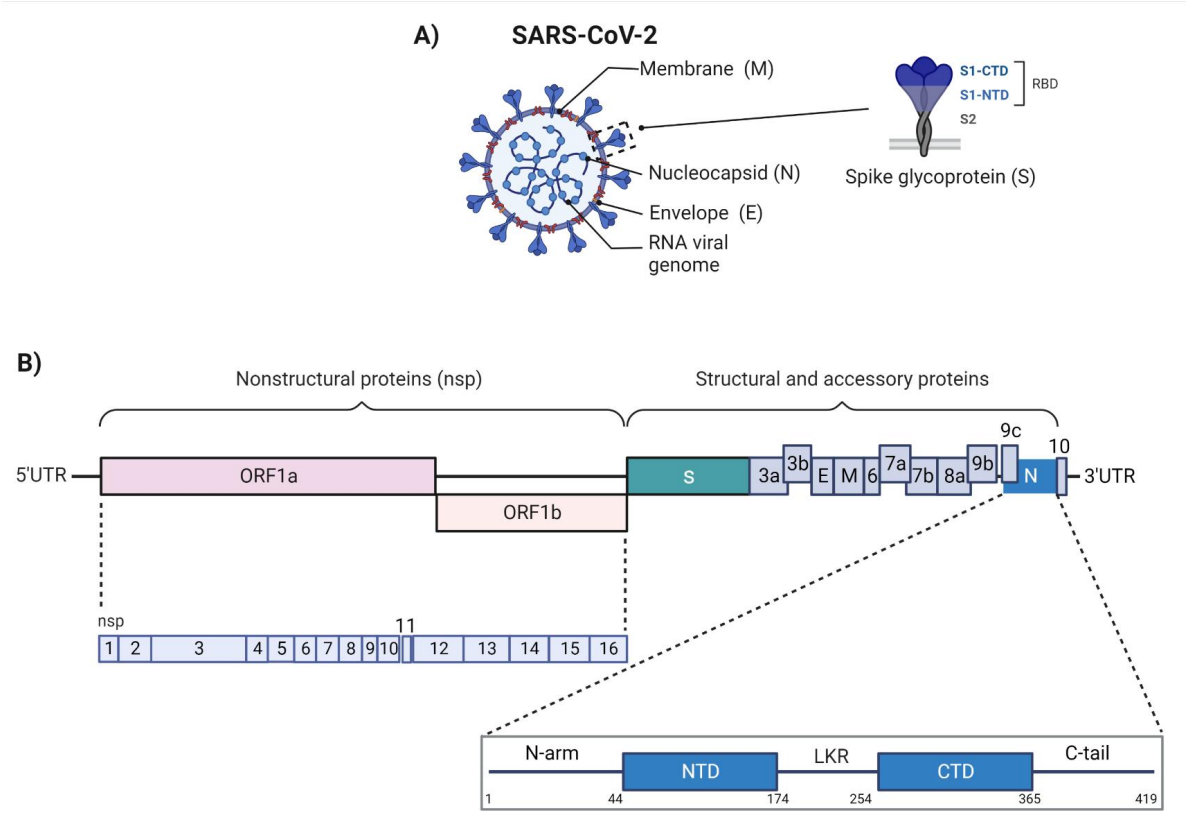


**Figure 3: Structure of SARS-CoV-2 and its nucleocapsid protein. A)** SARS-CoV-2 and its four structural proteins: S, Envelope, Membrane, and N. Physical interaction of N with the viral RNA genome. **B)** Structural organization of N protein. N-terminal domain (NTD) and C-terminal domain (CTD) are connected by an intrinsically disordered region (IDR) known as central linker region (LKR). In addition, NTD and CTD flanked by N-arm and C-tail IDRs, respectively.

**Host immunity to SARS-CoV-2 N protein**

Most of the COVID-19 patients presented a specific immune response against the full length and fragments of the N protein and, to lesser extent, against a fragment containing amino acids 300-685 of the S protein (*63*). In addition to S-specific antibodies, 82% of Convalescent patients had

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N-specific IgG after 12 months of SARS-CoV-2 infection (*64*). In terms of T cell responses, data from convalescent patients showed that the S, M, and N proteins each accounted for 27%, 21%, and 11% of the total $CD4^+$ responses, respectively (*65*). Higher frequencies of multi-cytokine production (poly-functional) by M- and N-specific $CD8^+$ T cells associated with mild COVID-19 cases (*66*). Moreover, N-specific T cell and B cell (in terms of antibody secretion) responses have been reported in different cohorts of COVID-19 infected (*65, 67, 68*). N induces robust both $CD4^+$ and $CD8^+$ T cell responses. A study identified that $CD4^+$ and $CD8^+$ T cells recognized multiple regions of the N protein in all individuals recovered from COVID-19 (*66*). Another study comparing the T cells response to SARS-CoV-1 and SARS-CoV-2 N protein showed that N-specific memory T cells from the SARS-CoV-1 infected individuals are long-lasting and cross-reactive with the SARS-CoV-2 N protein. The N-specific memory T cells remained detectable in SARS-CoV-1 infected individuals 17 years after the SARS outbreak in 2003; these T cells showed strong cross-reactivity to the N protein of SARS-CoV-2 (*66*).

Similarly, $CD8^+$ T cells specific for an immunodominant SARS-CoV-2 N epitope cross-react with selective seasonal coronaviruses (*69*). Screening of SARS-CoV-2 peptide pools revealed that the N protein induced an immunodominant response in HLA-B7+ COVID-19-recovered individuals that was also detectable in SARS-CoV-2 unexposed donors (*70*) . A single N-encoded epitope that was highly conserved across circulating coronaviruses drove this immunodominant response. A notable observation was that $CD8^+$ T cell responses against the N protein epitope ($N_{105\text{-}113}$) restricted by B*07:02 demonstrated strong antiviral activity and correlated with protection against severe disease (*70*). Indeed, the most immunodominant $CD8^+$ T cell response known so far is the $N_{105}$ peptide presented by HLA-B*07:02, which arises from a high frequency of T cells within the naive T cell repertoire that are able to recognize $N_{105}$, rather than from previously human Coronaviruses-primed cells (*70*). SARS-CoV-2 infection induces strong $CD4^+$ and $CD8^+$ T cell responses and the responses tend to be stronger in severe recovered patients compared to milder recovered patients (*10, 71*). Importantly, in the mild recovered patients, high magnitude of N-specific $CD8^+$ T cell response was observed (*71*). More detailed information on cellular immunity induced by SARS-CoV-2 infection or vaccination was reviewed elsewhere (*72*).

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**Nucleocapsid-based SARS-CoV-2 vaccines**

Besides the S protein, the N protein is well-recognized as a dominant target of antibody and T cell responses in SARS-CoV-2-infected individuals and therefore suggested as a potential immunogen to augment vaccine-mediated protective immunity (*73*). Despite use as a diagnostic marker, presence of N-specific IgG in the first week of SARS-CoV-2 infection is linked to the fast symptom resolution (*74*). Although current S-targeting vaccines confer strong protection against ancestral SARS-CoV-2, the emerging variants manifest increased immune evasion of vaccine- and/or infection-elicited S-specific neutralization (*75*). Therefore, vaccines expressing N protein has potential value in the vaccine development. Indeed, efforts were made a decade ago to investigate the N as a potential vaccine immunogen against Coronaviruses. As a result of the COVID-19 pandemic, more light has been shed on these investigations (*61, 76-79*). Several N-based vaccine immunogens have been developed using different technologies.

Adenovirus type-5 (Ad5) vector expressing N of the ancestral SARS-CoV-2 was one of the vaccine candidates studied (*80, 81*). Results showed that the vaccine was immunogenic and elicited both antibody and T-cell ($CD4^+$ and $CD8^+$) response in mice (*80*). Ad5-N alone induced a very modest protection against SARS-CoV-2 in a K18-hACE2 transgenic mouse model (expressing human angiotensin-converting enzyme 2 [hACE2] receptor); however, combining Ad5-N with Ad5 expressing S (Ad5-S) provided some synergistic effect and induced stronger protection in the mouse brain (but not in the lung) than Ad5-S alone (*80*). Another independent study testing Ad5-N vaccine showed vaccine-induced protective immunity in Syrian hamsters and K18-hACE2 mice, as evidenced by decreased animal weight loss and viral loads (*82*).

N protein-based subunit vaccines have also been developed and tested. SARS-CoV-2 N protein either alone or in combination with well-known adjuvants (e.g., Freund's adjuvant, alum, QS-21, and others) was shown to be immunogenic in animal models (*83, 84*). A synthetic peptide vaccine comprised of N epitopes (HLA class I bound cytotoxic T lymphocyte peptide) along with adjuvants (Toll-like receptor [TLR] 4 agonist (MPLA) and TLR9 agonist [CpG oligonucleotide]) has shown moderate protection against SRAS-CoV-2 in rhesus macaques (*85*).

Our group has generated a nucleoside-modified, lipid-nanoparticle (LNP)-formulated mRNA vaccine that encodes the full-length N protein of SARS-CoV-2 (Wuhan-Hu-1 strain) (mRNA-N) (*86*). Immunogenicity analysis in mice showed that mRNA-N was highly immunogenic and induced strong N-specific binding antibody and T cell (both $CD4^+$ and $CD8^+$)

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responses. As expected, no neutralizing antibody response was elicited by mRNA-N (*86*). Consistent with N-expressing vaccines using other platforms (*80, 81*), mRNA-N vaccine induced a modest protection against mouse adaptive SARS-CoV-2 and the Delta strain in mice and hamster, respectively (*86*).

**Multivalent SARS-CoV-2 vaccines expressing multiple viral proteins**

In the hope of eliciting both neutralizing antibodies and broadly protective T cell response, several multivalent SARS-CoV-2 vaccines expressing both S and N proteins have been developed, mainly using viral vectors. A synthetic Modified vaccinia Ankara (MVA) vaccine expressing SARS-CoV-2 S and N protein (COH04S1) was shown to be immunogenic (*87*) and provided protection from challenge with ancestral SARS-CoV-2 in non-human primates (NHPs) (*88*). Efficacy of this vaccine against major SARS-CoV-2 variants remains to be determined. The vaccine was evaluated in a phase-I clinical trial, and the data showed that the vaccine was well tolerated and elicited S- and N-specific immune response in healthy participants (*89*). Similarly, another independent study investigated the effect of a recombinant MVA vaccine expressing S and N (MVA/SdFCS-N) in NHPs against SARS-CoV-2 Delta variant (*90*). Among the different routes of administration, MVA/SdFCS-N via intramuscular route induced better protection against Delta variant to other routes (*90*).

Another study focused on the development of trivalent COVID-19 vaccines using adenoviral vectors of human and chimpanzee origin that expressed the S1, N, and RNA-dependent RNA polymerase of SARS-CoV-2 (*91*). The study also compared intramuscular and intranasal immunization strategies. Of note, a single intranasal mucosal immunization led to strong, systemic neutralizing antibodies. In addition, intranasal vaccination increased tissue resident memory $CD8^+$ T cells in the respiratory mucosa as well as locally trained macrophages. The intranasal strategy provided protection against the parent strain of SARS-CoV-2, and also other variants of concern (B.1.1.7 and B.1.351) (*91*).

Using the human Ad5 vector, ImmunityBio developed an approach that targets both SARS-CoV-2 S and N proteins. The S and Enhanced T-cell Stimulation Domain (N-ETSD) of the N protein have been utilized in the vaccine. Their study showed that S and ETSD together were immunogenic in mice. Subcutaneous priming and intranasal or subcutaneous boosting with this dual antigen vaccine induced Th1-biased T-cell and humoral responses at greater levels than

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Ad5-S alone (*92*). In a phase I trial, it was shown that a single dose ($1x10^{11}$ viral particle) of vaccination in healthy adults elicited strong T cell response (*93*). This platform reassures the prospect of needleless immunizations in general. However, viral challenge studies are needed to evaluate the vaccine efficacy in animal models.

Vesicular stomatitis virus (VSV) is a commonly used vector for mucosal delivery. Intranasal administration of a VSV-based vaccine expressing S and N proteins elicited mucosal immune response and protective effect in a hamster viral challenge model. Of interest, the same vaccine administered intramuscularly is only marginally protective (*94*).

In addition to viral vectors, multivalent vaccines based on DNA or proteins have also been reported. Mice immunized with a DNA vaccine encoding S/RBD and N developed broad neutralizing antibodies and N-specific T cell responses (*95*). The DNA vaccine protected the mice against lethal SARS-CoV-2 infection (*95*). In a similar vein, a DNA vaccine (GX-19N$^{TM}$) encoding RBD and N was tested in a phase I clinical trial, demonstrating evidence of safety and immunogenicity (*96*), despite efficacy of this vaccine in animal models was not clear. Hong and colleagues immunized macaques with multivalent protein subunit vaccine comprised of RBD fused with tetanus toxoid epitope P2 (RBD-P2) and N protein, and showed that addition of N induced slightly faster SARS-CoV-2 clearance than that induced by RBD-P2 alone in NHPs (*97*).

While the above multivalent vaccine approaches showed various levels of protection against SARS-CoV-2 or its variants in different animal models, critically, there is a lack of a direct comparison with the current clinically approved first-generation COVID-19 vaccine, especially the mRNA-S vaccines, in terms of protection against SARS-CoV-2 VOCs. Their efficacy against the current predominant Omicron variants also remains to be determined in the context of comparison with the clinically approved S-targeting vaccines.

As discussed earlier, the mRNA vaccine expressing the full-length N protein (mRNA-N) generated in our group showed modest protection against mouse adaptive SARS-CoV-2 and Delta strain. To compare with first-generation S-targeting vaccines, we also generated an mRNA-LNP vaccinee expressing the prefusion-stabilized SARS-CoV-2 S protein (mRNA-S) and evaluated either alone or in combination with mRNA-N (mRNA-S+N) for immunogenicity and protective efficacy against multiple SARS-CoV-2 VOCs in rodent models (*86*). Our data showed that mRNA S+N vaccination induces markedly stronger protection against multiple SARS-CoV-2 strains, including mouse-adapted strain, Delta, and Omicron, in both lower and

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upper respiratory tracts. The difference between mRNA-S alone and mRNA-S+N was most profound for protection against Omicron: while mRNA-S alone had much reduced efficacy against Omicron due to strong immune escape, mRNA-S+N vaccination led to substantial protection against Omicron (four of five animals had no detectable viral RNA and titers in the lung) (*86*), indicating induction of cross-protective immunity against VOCs by mRNA-S+N. In vivo $CD8^+$ T cell depletion supports that $CD8^+$ T cells play a critical role in protection against VOCs by mRNA-S+N vaccine (*86*). Collectively, our study suggests that the bivalent mRNA-S+N is a potential pan-COVID-19 vaccine for emerging SARS-CoV-2 variants.

**Summary and Future Directions**

As new variants of SARS-CoV-2 continue to arise, it is urgent that pan-COVID-19 vaccines be developed that induce broad protection against emerging strains. Current strategies to develop vaccines against COVID-19 VOCs, include utilization of VOC-specific S immunogens. While VOC-targeted S booster has been clinically approved in the two mRNA vaccinees, this strategy is likely challenging, since in the face of constantly mutating spike proteins, design and selection of VOC-specific sequences are considered less ideal. Given more conserved sequence of N protein across different variants and the cross-reactive, long-lasting feature of N-specific T cell immunity (*98, 99*), our study and those from other groups have clearly demonstrated benefit and utility of including N immunogen in the next-generation COVID-19 vaccines for VOCs. Our study showed that despite use of ancestral sequences, the inclusion of mRNA-N along with mRNA-S for vaccination elicited robust protection against both Delta and Omicron strains, which was not achieved by mRNA-S alone. Given the proven safety profile of mRNA-LNP platform in large human populations, the bivalent mRNA-S+N vaccine should be advanced to clinical testing.

A few important questions remain to be explored. First, durability of vaccine-elicited protective immunity is critical (*100, 101*). Whether or not the dual mRNA-S+N vaccine induces more durable protective immunity than mRNA-S alone is unclear and currently under investigation. Second, since a large human population has been vaccinated with first-generation vaccines or been naturally infected with the virus, further testing inclusion of N immunogen as a booster strategy will likely reveal additional insights into utility of this bivalent vaccine approach against

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SARS-CoV-2 VOCs. Third, further validating the safety and efficacy of the vaccine approach in larger animal models will be helpful as well before moving to clinical testing. Lastly, since COVID-19 VOCs can infect individuals through vaccine breakthrough and antibody escape, screening for broadly neutralizing or pan-neutralizing antibodies against COVID-19 VOCs is also important for the development of a pan-COVID-19 vaccine, as well as for the prevention and treatment of VOCs infections in future.

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**Conflict of interest**

No conflict of interest is declared by the authors.

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