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Perspective

Nucleocapsid as a next-generation COVID-19 vaccine candidate

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ABSTRACT

Multiple new variants of the SARS-CoV-2 virus have emerged globally, due to viral mutation. The majority of COVID-19 vaccines contain SARS-CoV-2 spike protein, which is susceptible to mutation. It is known that protection against COVID-19 after two doses of mRNA vaccine continuously wanes over time. If viral variants contain mutated spike protein, current vaccines may not provide robust protection. This perspective suggests the inclusion of SARS-CoV-2 nucleocapsid protein in future COVID-19 vaccines and boosters, as nucleocapsid is much less vulnerable to mutation and may provide stronger immunity to novel viral variants.

The SARS-CoV-2 coronavirus exemplifies "evolution in action" as demonstrated by the continuous emergence of new genetic variants, including Alpha (B.1.1.7), Beta (B.1.351), Gamma (P.1), Delta (B.1.617.2), and Omicron (B.1.1.529), with the latter seeming to outcompete the others. These new variants are defined by multiple spike (S) protein mutations that mediate increased transmissibility, replication efficiency, and immune evasion. The ongoing high frequency of mutations in various regions of the spike sequence essentially renders it a "moving target" and supports a rationale to replace or coexpress spike with the nucleocapsid (N) gene in the second generation of vaccine candidates. In contrast to spike, which is external, the internal N gene is more conserved and stable. The presence of fewer mutations over time is consistent with its importance to the viral life cycle, including RNA packaging, replication, and transcription (Dutta et al., 2020). The sequence conservation of the nonsurface N protein potentially makes it an ideal vaccine target for cytotoxic CD8+ T cells, which are positively associated with effective viral clearance and less severe disease (Moss, 2022). Indeed, robust T-cell responses to nucleocapsid have been characterized (Nguyen et al., 2021; Le Bert et al., 2020).

In a worst-case scenario, the evolvability and heterogeneity of the SARS-CoV-2 virus in response to the selection pressure imposed by use of spike-centric vaccines and monoclonal antibodies will promote the rise of variants that are fully resistant to current vaccines, including Pfizer-BioNTek, Moderna, Janssen (Johnson & Johnson), AstraZeneca/Oxford, CanSino, Sputnik V, Novavax, and others that narrowly target the S protein (Ahn et al., 2022). Nevertheless, messenger RNA (mRNA) boosters have been highly effective against symptomatic Delta infection, although less so against symptomatic Omicron infection (Ferdinands et al., 2022). However, with both variants, mRNA boosters still manage to enhance cross-neutralizing antibodies and substantially protect against COVID-19–related hospitalization and death. (Andrews et al., 2022).

Repeated administration of the same mRNA spike vaccine could be properly termed a "homologous" prime-boost strategy, as the first dose primes the immune response, and subsequent doses amplify it. Homologous prime-boost is effective for the augmentation of humoral responses, but studies have shown that the antibody response tends to wane over time (Bates et al., 2022), and well before T-cell responses wane (Negi et al., 2021; Ramshaw and Ramsay, 2000).

In contrast, a "heterologous" prime-boost (a "mix-and-match" approach), in which for example a spike-based vaccine is sequentially administered with a nucleocapsid-based vaccine (or *vice versa*), may boost cell-mediated immunity. This approach has been well-documented against other pathogens (Masopust et al., 2006). However, several vaccine variables would need to be compared and optimized before the implementation of a heterologous prime-

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boost. These include the number, scheme, and schedule of injections; safety profile; and type and order of vaccines.

In addition to mRNA, purified inactivated viruses such as CoronaVac and BBIBP-CorV, manufactured by the Chinese companies Sinovac and Sinopharm (China National Pharmaceutical Group Corporation), respectively, are options because they incorporate not only the S protein but other viral proteins, including the matrix (M), envelope (E), and nucleocapsid (N) (Dinc et al., 2022). Studies have demonstrated that in populations that were primed with inactivated viruses and in which the seropositivity rate was low (28%), booster vaccinations significantly increased immunogenicity; heterologous prime-boost was more effective than homologous prime-boost (Cheng et al., 2022). The drawbacks of inactivated vaccines are the low levels of induced immunity (unless vaccine is administered with an adjuvant) and the large dose required for each immunization (Sharma et al., 2020).

Despite the use of mRNA and inactivated vaccines, the emergence of multimutational immune escape variants has already been described in immunocompromised patients with prolonged SARS-CoV-2 replication. These patients serve as potential "breeding grounds" or "Petri dishes" for viral evolution, propagation, and subsequent spillover into the general population (Hensley et al., 2021; Avanzato et al., 2020). Immunocompromised patients include those with HIV-1, leukemia, lymphoma, and systemic autoimmune and inflammatory rheumatic diseases who are treated with immunosuppressive or immunomodulatory therapies. For example, Truong et al. (2021) published a case series of patients with B-cell acute lymphoblastic leukemia in whom multiple escape variants were detected over the course of persistent COVID-19 infection. Nussenblatt et al. (2022) documented an immunocompromised patient with a SARS-CoV-2 infection that persisted for almost one year (355 days), during which time virus accumulated a unique in-frame deletion in spike and a complete deletion of ORF7b and ORF8 .

These and several other case reports lend credence to the adage that "no one is safe until everyone is safe", and this is especially the case with immunocompromised patients who appear to serve as reservoirs for new mutations that are potentially transmissible to the healthy population.

Instead of a "whack-a-mole" model, which focuses vaccination efforts on a single spike variant at a time, and in which successful suppression leads to the subsequent emergence of another spike variant, a broader-protection vaccine strategy is necessary. Such a strategy may be based, for example, on the widely conserved N protein, which is essential for RNA synthesis that effectively induces T-cell responses (Silva et al., 2022; Thura et al., 2021).

CRediT authorship contribution statement

Bryan Oronsky: Conceptualization, Writing – original draft. **Christopher Larson:** Conceptualization, Writing – original draft. **Scott Caroen:** Writing – review & editing. **Farah Hedjran:** Writing – review & editing. **Ana Sanchez:** Writing – review & editing. **Elena Prokopenko:** Writing – review & editing. **Tony Reid:** Conceptualization, Writing – original draft.

Conflicts of Interest

The authors have no conflicts to declare.

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