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Could nucleocapsid be a next-generation COVID-19 vaccine candidate - author's reply

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Title Page

Title: Could nucleocapsid be a next-generation COVID-19 vaccine candidate - author's reply

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This is in response to a letter to the editor by Saldivar-Espinoza *et al*., commenting on a short perspective that we wrote in the September 2022 issue of the International Journal of Infectious Disease (IJID) entitled, "Nucleocapsid as a next-generation COVID-19 vaccine candidate" (**Oronsky et al., 2022**) . The main point of this perspective was that nucleocapsid, being highly conserved among coronaviruses, less mutable than spike, and strongly immunogenic especially for T cells (**Lineburg et al., 2021**), which persist for much longer than waning antibodies, is a primary target for a broader and more cross-protective vaccine.

Based on an analysis of the GISAID (Global Initiative to Share All Influenza Data) repository Salvidar-Espinoza *et al*. responded with thought-provoking evidence that nucleocapsid (N) is, in fact, highly mutation prone in certain regions, second only to spike (S), which, in theory, may jeopardize the efficacy, and universality, of coronavirus (CoV) vaccines that incorporate N as an antigen. However, we are skeptical about the clinical relevance of this analysis for the following reasons.

Mutations are common in RNA viruses like SARS-CoV-2 (**Duffy, 2018**). Their rapid evolution is responsible for the accumulation of genetic changes, particularly when the host is immunodeficient. However, most mutations impact viral fitness deleteriously, especially those involving indispensable proteins like N, which not only binds to and protects the RNA genome from degradation but also functions as an antagonist of interferon (IFN), one of the main mechanisms of the host innate immune defense (**Wu et al., 2021**).

That this protein shares 90% homology with the severe acute respiratory syndrome coronavirus (SARS-CoV-1) N protein implies a conserved mechanism of nucleocapsid formation for CoVs (**Bai et al., 2021**). It also suggests that any nonsynonymous mutations in N, which change the amino acid sequence, and decrease the interaction between nucleocapsid, and RNA or that interfere with nucleocapsid formation as a result will negatively impact the fitness of the virus and *vice versa*. That said, genes do not operate in isolation but in concert with an array of other genes, and the synergic and antagonistic effects of epistatic interactions may profoundly impact whether these N mutations evolve under positive or negative selection, due to their impact on basic reproduction number (R_0), immune evasion, generation time etc.

Accordingly, it is difficult, if not impossible, to attempt to predict the fate of individual mutations, and their relationship to a fitness phenotype, the better to determine which specific epitopes to include in a CoV vaccine given the myriad variables that are involved including population size, disease prevalence, within-host factors, and epistatic gene-gene interactions. For this reason, we are in favor of a self-replicating adenoviral-based vaccine that encodes the whole

nucleocapsid gene, including the regions "in the central disordered linker proximal to the N-G215C 28 mutation" identified by Saldivar-Espinoza *et al*. as the most conserved and stable.

Conflict of Interest

None

Funding Source

None

Ethical Approval statement

Not applicable

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