The Olympiad of SARS-CoV-2 vaccinology: Fundamentals to Complement Technical Frontiers

Robert L Gottlieb, MD PhD[1,2,4,*] and Cedric W. Spak MD MPH[1,3,4]

[1] Baylor University Medical Center Dallas

[2] Baylor Scott and White Research Institute

[3] Texas Centers for Infectious Disease Associates

[4] Department of Internal Medicine, Texas A&M Health Science Center, Dallas

* Corresponding author:
Robert L Gottlieb, MD PhD
Baylor Center for Advanced Heart and Lung Disease
Baylor University Medical Center Dallas
3410 Worth St, Suite 250, Dallas TX 75246
+1-214-820-6856 (phone)
+1-214-820-1474 (fax)
E-mail: robert.gottlieb@bswhealth.org

In sports, a goal is a goal. . . whether or not it was a *golasso*, it counts the same as long as crosses the goal line, while observing the rules of sportsmanship. Another truth from sports is that is impossible to win a game if it remains tied at 0-0. Such is our common shared objective in the fight to eradicate or control SARS-CoV-2, and the varied Covid-19 disease states wrought by this no-longer-quite-as-novel 2019 beta-coronavirus. As the novelty has worn off, we just need this virus eradicated or controlled – the more shots-on-goal, the better. Although we are fortunate that two mRNA vaccines have received FDA emergency use authorization (1, 2, 3, 4) the global community will benefit from diverse vaccine strategies to maximize vaccine production, simplify vaccine delivery, accelerate future vaccine initiatives for other SARS-family coronaviridae, and to offer excess antigenic coverage to mitigate viral escape mutations in spike protein.

A theoretical Achilles heel shared by the four dominant commercial vaccines furthest along in large-scale phase 3 trials including both mRNA vaccines with active FDA EUAs [Pfizer/BioNTech BNT162b2, Moderna mRNA-1273] and both major replication-incompetent adenoviral vectors approaching FDA EUA submission [Janssen Ad26.COV2.S, Oxford/AstraZeneca ChAdOx1/AZD1222]) is that they focus on the SARS-CoV-2 spike as the sole antigenic target, rendering them at least theoretically vulnerable to viral escape. The risk of focusing solely on spike protein is highlighted by the observation that selected spike mutations including E484K, E484Q, F490S, and S494P result in 2-orders of magnitude less neutralization from the therapeutic neutralizing monoclonal antibody bamlanivimab, albeit with only minimal effects on the affinity of etesevimab (5). The potential clinical impact of spike mutations in escape from the mixed polyclonal humoral and cellular response to vaccination is currently unknown, but pursuit of alternative vaccination strategies that includes nucleocapsid epitopes, including those offered by killed, inactivated vaccines such as those described by Che et al. in this issue (6) and by other inactivated vaccines such as CoronaVac (Sinovac Life Sciences) (7), remain warranted. Entering 2021, traditional vaccinology remains salient and

relevant although thus far, inactivated SARS-CoV-2 vaccines are limited to early safety and surrogate immunological endpoints in phase 2 trials such as that described (6, 7), and will need to await phase 3 trials to ultimately demonstrate whether this translates into sufficient clinical efficacy of this vaccine strategy.

A crucible in this quest to find one or more vaccines is the need to ensure health equity, including the ability to widely distribute it across all continents for this global pandemic, at scale and at an affordable cost. While the 2019-2021 SARS-CoV-2 pandemic has showcased the proof-of-concept arrival of glitzy vaccines at the technologic frontiers, such as the so-called 'mRNA vaccines' (liposomal-encapsulated, modified oligoribonucleotide vaccines) including those FDA authorized vaccines developed by Pfizer/BioNTech (1. 3) and Moderna Therapeutics (2, 4), the requisite deep cold-chain storage of sub-zero temperatures functionally limits the practical distribution of modified mRNA vaccines to high-economic power countries or urban centers in less affluent countries. As data emerge that this newest technology will be effective against SARS-CoV-2, the fundamental limitation of this complex sub-zero distribution chain poses an acknowledged operational barrier to fulfilling the true sense of the humanistic interconnection evoked by the rings of the modern Olympiad, and underscores the pressing need for an alternative, practical, temperature-stable workhorse vaccine to ensure that inhabitants of each continent receive adequate protection.

Historically, polio eradication campaigns illustrate the utility and challenges of a distribution chain for traditional vaccinology approaches of the live-attenuated (Sabin-approach) and inactivated vaccine (Salk-approach), each with their relative merits and gaps. But unlike the global campaign to eradicate poliomyelitis, the development of a non-pathogenic, live-attenuated, passaged SARS-CoV-2 strain as a traditional alternative vaccinology approach is, at least for now, thwarted by our

nascent and as-yet primitive understanding of how this disease transitions from being a viral disease to a post-viral (or virally-uncoupled) entity, with its pleiotropic and protean manifestations of Covid-19. The quest for a non-pathogenic, passaged strain being an *a priori* non-starter, in that light, Che and co-authors of the current study (6) provide an informed update on their phase 2a development of a traditional "killed" or inactivated virus vaccine against SARS-CoV-2 (analogous to the Salk approach), with multiple available epitopes including antigenic stimulation to both nucleocapsid as well as spike proteins.

Fundamentally, this begins with basic tissue culture to amplify and harvest live SARS-CoV-2 in a Bio-Safety 3 equivalent laboratory – a task that requires technicians with fortitude and courage. This traditional vaccine approach harvests potentially infectious plaque-forming units that are rendered inert via traditional formaldehyde and propiolactone-inactivation, purified, and delivered as an alum-adjuvanted SARS-CoV-2 vaccine. This particular inactivated vaccine was harvested from a strain obtained from a single definitive SARS-CoV-2 case from western China, but offers the advantage that if the circulating strains change sufficiently, it still provides a broad range of viral nucleocapsid (N) and spike (S) epitopes from the whole, inactivated virions, as well as a pathway to recapitulate the vaccine in the unlikely event the world were to require seasonal vaccine.

The authors utilized a boosted protocol using one of two alternative schedules, delivering the second dose at either 14 or 28 days after the initial inoculation, and studied a 2:2:1 randomization of medium-dose (100 EU), high-dose (150 EU), versus placebo. Beyond collecting safety endpoints, they collected functional data with anti-S and anti-N geometric mean titers, and more critically, assessed neutralizing ability in a functional Vero-cell assay. The medium and high-dose regimen demonstrated neutralizing responses in 89 % and 96 % of subjects, respectively, in the 14-day boosted regimen, with the paired high-dose inocula performing comparably to the 28-day boosted protocol that

demonstrated neutralizing antibody titers in 95 % of subjects in both the medium and high-dose arms. The favorable data for the 14-day boosted regimen using high-dose inoculation would allow more rapid coverage and induction of immunity, should clinical efficacy be demonstrated in a phase 3 trial.

Ultimately, this study provides context and necessary pre-requisite data en route to a planned phase 3 trial to assess for clinical efficacy, informed by their promising initial surrogates. It is critical to note that this important contribution is looking only at humoral biomarkers and cell-culture assay of immunity without measures of cellular immunity nor clinical endpoints.

Our single-stranded RNA global opponent is a nano-sized member of the beta-coronaviridae, and is a shared nemesis to all who possess or one or more lungs. The authors have attested that they followed the tenets of the Declaration of Helsinki, followed good clinical practice, with appropriate data safety monitoring committee oversight, with voluntary participation of the subjects. As the studied cohort was aged 18-59, it remains unknown whether the same boosted protocol would remain optimal in other age groups, or in other demographic groups, but these are readily tested in a phase III trial which is clearly justified by their phase IIa data. The world awaits these next steps.

Information from the neutralizing monoclonal antibodies directed against varied epitopes of the receptor-binding domain (RBD) of the spike protein including bamlanivilab and etesevimab (Lilly) and casarivimab and imdevimab (Regeneron) as well as clinical understanding is helping us ensure that humoral therapy and vaccines do not seem to be impacted by clinically-meaningful antibody-dependent enhancement or cytotoxicity, and confirmed by the interim phase 3 data from first-generation SARS-CoV-2 mRNA vaccines from Pfizer (1, 3) and Moderna (2, 4) encoding a homogenous spike sequence. It is likely that we will ultimately have multiple therapies and multiple vaccines. Our tools to assess innate and cellular immunity remain imperfect. We are fortunate that emerging data from the Pfizer, Moderna, and Astra Zeneca vaccines inform us that the SARS-CoV-2

S (spike) protein is indeed an appropriate and viable target. Nevertheless, the traditional inactivated vaccine approach hold promise as a worthy, complementary addition.

SARS-CoV-2 is a consequential and devastating virus, yet it is unlikely to be a particularly "fit," having limited genomic size and a constrained sequence space to sample for escape mutations. If spike mutations happen to decrease efficacy of spike-specific vaccine strategies, the world may require comparative efficacy data from multiple workarounds. Chimeric derivatives of mRNA or vectored vaccines encoding various different spike variant cassettes may suffice, but inactivated vaccines offer a back-up of alternative epitopes just in case. Like saline for cholera, or like a goal in sports, vaccinology does not need to be fancy… it just needs to work. The world eagerly awaits the next steps – objective data from phase 3 trials of this, and similar, inactivated SARS-CoV-2 vaccines.

NOTES

Funding Source: None

Relevant Conflicts of Interest: None related to SARS-CoV-2 vaccine. However, RLG has received gift-in-kind from Gilead to support NCT03383419 (TROJAN-C) as well as a nominal safety consulting fee.

REFERENCES

1. Pfizer-BioNTech COVID-19 Vaccine. https://www.fda.gov/emergency-preparedness-and-response/coronavirus-disease-2019-covid-19/pfizer-biontech-covid-19-vaccine. Retrieved 23 Jan 2021.
2. Moderna COVID-19 Vaccine. https://www.fda.gov/emergency-preparedness-and-response/coronavirus-disease-2019-covid-19/moderna-covid-19-vaccine. Retrieved 23 Jan 2021.
3. Polack FP, Thomas SJ, Kitchin N, et al. Safety and Efficacy of the BNT162b2 mRNA Covid-19 Vaccine. N Engl J Med. 2020;383(27):2603-2615. doi:10.1056/NEJMoa2034577
4. Baden LR, El Sahly HM, Essink B, et al. Efficacy and Safety of the mRNA-1273 SARS-CoV-2 Vaccine [published online ahead of print, 2020 Dec 30]. N Engl J Med. 2020;NEJMoa2035389. doi:10.1056/NEJMoa2035389
5. Gottlieb RL, Nirula A, Chen P, et al. Effect of Bamlanivimab as Monotherapy or in Combination With Etesevimab on Viral Load in Patients With Mild to Moderate COVID-19: A Randomized Clinical Trial. JAMA. Published online January 21, 2021. doi:10.1001/jama.2021.0202
6. Che Y, Liu X, Pu Y, et al. Randomized, double-blinded and placebo-controlled phase II trial of an inactivated SARS-CoV-2 vaccine in healthy adults [published online ahead of print, 2020 Nov 9]. Clin Infect Dis. 2020;ciaa1703. doi:10.1093/cid/ciaa1703

7. Zhang Y, Zeng G, Pan H, et al. Safety, tolerability, and immunogenicity of an inactivated SARS-CoV-2 vaccine in healthy adults aged 18-59 years: a randomised, double-blind, placebo-controlled, phase 1/2 clinical trial [published online ahead of print, 2020 Nov 17]. Lancet Infect Dis. 2020;S1473-3099(20)30843-4. doi:10.1016/S1473-3099(20)30843-4