

OVS CoVPN COVID-19 Vaccine Efficacy Trial Immune Correlates Statistical Analysis Plan

Operation Warp Speed (OWS) / Coronavirus Prevention Network
(CoVPN) Biostatistics Team

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1 Prelude

The scientific community has responded to the COVID-19 pandemic with admirable global cooperation and solidarity, characterized by rapid sharing of results and data in an effort to urgently re-focus research toward the common goal of developing prevention and treatment modalities to help turn the tide of the COVID-19 pandemic. As spoken by WHO Director-General Dr. Tedros Adhanom Ghebreyesus at the 2020 Aspen Security Forum, “Our best way forward is to stick with science, solutions and solidarity and together we can overcome this pandemic.” [World Health Organization \(2020\)](#)

In this spirit, we are making this Statistical Analysis Plan (SAP) publicly available at a relatively early and intermediate stage. The SAP is a work in progress that will continue to be developed and refined over the coming weeks. Our hope is that fellow statistical scientists and scientists of other disciplines will bring new insights and offer input to maximize the scientific knowledge pertaining to immune correlates of protection that can be learned from COVID-19 vaccine efficacy trials. We invite collaboration and are eager to explore opportunities for working with others on COVID-19 immune correlates analyses.

We envisage three applications of this SAP. First, as the Coronavirus Prevention Network Statistical Center and OWS Biostatistics Team our group is responsible for statistical design and analysis of immune correlates for the Operation Warp Speed (OWS) phase 3 trials, and this SAP serves as a master protocol-type SAP for harmonized immune correlates analyses across the OWS trials. Second, researchers conducting additional clinical trials may work collaboratively with our group to co-conduct the immune correlates analysis. Third, researchers conducting clinical trials may use this SAP as a resource for immune correlates analyses conducted on their own, either by implementing this SAP or components therein, or by selecting methods and code from it to adopt for their own SAPs.

We are implementing the SAP in R. The R scripts are hosted on a Github code repository CovidCorrSAP (hosted by Dr. Youyi Fong) and will be made publicly available as soon as they are ready. The first publicly available down-

load will be focused on immunogenicity characterization and correlates of risk analysis and the second one will be focused on correlates of protection analysis. This collaborative Github repository will include reproducible reports implementing the SAP on a mock/practice COVID-19 vaccine efficacy trial data set.

Please direct communication related to this SAP to Peter B. Gilbert at the Fred Hutchinson Cancer Research Center (pgilbert@fredhutch.org).

2 Introduction

2.1 Antibody Assays and Day 57 Markers

This SAP describes the statistical analysis of antibody markers measured at a key time point post last vaccination as immune correlates of risk and as various types of immune correlates of protection against primary and secondary endpoints in OWS / CoVPN COVID-19 vaccine efficacy (VE) trials. For definiteness, we assume this time point for antibody measurements is Day 57, a typical time point for a two-dose vaccine; for a one-dose vaccine the key time point would likely be around Day 28. The antibody markers of interest are measured using one of three kinds of humoral immunogenicity assays [more detail on assay types (2) and (3) can be found in [Sholukh et al. \(2020\)](#)]:

- (1) **bAbs: Binding antibodies** to the vaccine insert SARS-CoV-2 proteins;
- (2) **Pseudovirus-nAbs: Neutralizing antibodies** against viruses **pseudotyped** with the vaccine insert SARS-CoV-2 proteins; and
- (3) **Wild Type Live virus-nAbs: Neutralizing antibodies** against **live** “vaccine insert-matched” wild type SARS-CoV-2 (or recombinant “vaccine insert-matched” SARS-CoV-2 harboring a reporter gene within the viral genome).

For example, the following assays are expected to be used:

- (1) **bAb assay**: The MSD-ECL Multiplex Assay (MSD-ECL = meso scale discovery-electrochemiluminescence assay) measures binding antibody to anti-

gens corresponding to: Spike (an engineered version of the Spike protein harboring a double proline substitution (S-2P) that stabilizes it in the closed, prefusion conformation [McCallum et al. (2020)]); the Receptor Binding Domain (RBD) of the Spike protein; and Nucleocapsid protein (N), which is not contained in any of the COVID-19 vaccines. This assay has a standard curve to interpolate arbitrary units/ml; an 8 point dilution curve on each sample with 5-fold dilutions starting at 1:20; an 8 point dilution curve on VRC control sera; and includes Positive, Negative and Intermediate controls. Binding antibody to N are not of interest as a potential immune correlate; these data are included only for immunogenicity evaluation.

(2) **Pseudovirus-nAb assay:** A firefly luciferase (ffLuc) reporter neutralization assay for measuring neutralizing antibodies against SARS-CoV-2 Spike-pseudotyped viruses; and

(3) **Wild Type Live virus-nAb assay:** An assay measuring antibody-mediated neutralization of live wild-type SARS-CoV-2 (WA isolate, passage 3, Vero-E6 cells).

Throughout this SAP we assume that all three types of assays have validated versions that are applied uniformly to samples collected in one or several late-stage SARS-CoV-2 vaccine efficacy trials. Samples from the same trial are expected to be assayed by the same lab that performs one of these immunoassays.

Based on each immunoassay applied to paired serum samples collected from participants on Day 1 (baseline, pre-vaccination) and Day 57, a set of multiple markers will be defined for immunogenicity and immune correlates analyses. For example, the bAb-related markers will be defined as \log_{10} IgG concentration (IU/ml) at Day 57 and by difference in \log_{10} concentration (Day 57 minus Day 1) representing \log_{10} fold-rise in IgG concentration, to the Spike, RBD, and N antigen targets. The lower limit of quantitation (LLOQ) of the binding antibody assay is 34 AU/ml and the upper limit of quantitation (ULOQ) is 19,136,250 AU/ml, where AU stands for arbitrary units from a standard curve that will aligned to the WHO standards before use on efficacy trials samples. Once these standards appear, units will be reported in IU/ml

(International units/ml). Values of bAb below the LLOQ are assigned the value $\text{LLOQ}/2 = 17 \text{ IU/ml}$. Values greater than the ULOQ are assigned the value ULOQ.

The pseudovirus-nAb markers are defined as Day 57 \log_{10} serum inhibitory dilution 50% titer (ID50) and 80% titer (ID80), as well as the \log_{10} fold-rise of these markers over Day 1. The ID50 and ID80 values are estimated based on a starting serum dilution of 1:20, with eight 5-fold dilutions. Thus 1:20 is the lower limit of detection (LLOD). The LLOQ for ID50 and ID80 is based on precision, with values $\text{LLOQ} = 49$ for ID50 and $\text{LLOQ} = 43$ for ID80. ID50 and ID80 values below the LLOQ are assigned the value $49/2 = 25$ and $43/2 = 22$, respectively. If there is an upper limit of quantitation (ULOQ), then values greater than the ULOQ are assigned the value of the ULOQ.

The WT live virus-nAb marker is defined as Day 57 \log_{10} serum MN50 calculated using the Spearmanâ€™s Karber method, as well as the \log_{10} fold-rise of this marker over Day 1. The LLOQ for MN50 for the Battelle assay is 117.35 and the ULOQ is 18,976.19, and the LLOD is 62.16. Values below the LLOQ are assigned the value $117.35/2 = 59$ and values greater than the ULOQ are assigned the value of the ULOQ.

For two-dose vaccines, the immunogenicity and correlates analyses may also include the same antibody markers measured at the second-dose sampling time point, which we refer to as the Day 29 time point. In this SAP we include contingency sub-sections marked '[\[With Day 29 Markers\]](#)' to describe how the SAP is augmented to include the Day 29 antibody markers in the analysis.

3 Study Cohorts and Endpoints

3.1 Study Cohort for Correlates Analyses

Finalization of the primary study cohort for correlates analysis will take place before unblinding case/non-case and treatment arm information for correlates analyses. The default is for the primary study cohort to be the same as the cohort used in the primary analysis of vaccine efficacy against the primary

endpoint in the protocol, except that availability of a Day 1 and Day 57 blood sample for antibody testing is also required. It may also be required to have results from all tests for SARS-CoV-2 infection on Day 57 samples [serology and/or nucleic acid amplification test (NAAT)].

Typically the primary analysis cohort is baseline SARS-CoV-2 negative participants in the per-protocol cohort, with the per-protocol cohort defined as those who received all planned vaccinations without any specified protocol deviations, and who were NAAT negative at the terminal vaccination visit. We refer to this cohort representing the primary population for correlates analysis as the Per-Protocol Baseline Negative Cohort. We will wait to fully understand all of the antigen and serology testing data that are available in the data set to finalize the definition of the primary analysis cohort. If a vaccine has high vaccine efficacy, it is possible that rare vaccine breakthrough cases will be individuals who were infected before the second vaccine dose, or soon after the first dose, but had unusually long time periods between SARS-CoV-2 acquisition and symptomatic infection (COVID) diagnosis. This situation could complicate the interpretation of correlates analyses. Therefore, we may conduct some correlates analyses that use a stringent criterion to include vaccine breakthrough cases in the analyses, such as requiring antigen negative tests at both Day 29 and Day 57 and all serologies negative through Day 57.

For trials with the primary analysis of vaccine efficacy in baseline negative individuals, CoR and CoP analyses are only done in baseline negative individuals, and the analysis of data from baseline positive individuals is for purposes of immunogenicity characterization, given too-few anticipated vaccine breakthrough study endpoints for CoR/CoP assessment (although if there are many baseline positive vaccine breakthrough endpoint cases that baseline positive subgroup analyses may be considered). In baseline negative individuals, antibody marker data in placebo recipients is relevant for verifying the expectation that almost all Day 57 marker responses will be negative, given the lack of SARS-CoV-2 antigen exposure.

3.1.1 [With Day 29 markers]

If Day 29 markers are included, analyses of Day 29 correlates are done in the same cohort as studied for the analyses of Day 57 markers, except the time origin in correlates analyses is set at the Day 29 visit and the set of participants included in the analysis is augmented with intercurrent cases. Intercurrent cases are defined as participants who were diagnosed with the COVID primary endpoint sometime between 7 days post Day 29 visit and 6 days post Day 57 visit (if the Day 57 visit is missed, then between 7 days post Day 29 visit and 34 days post Day 29 visit). These intercurrent cases would be missed for the Day 57 per-protocol correlates analyses for which cases are counted starting 7 days after the Day 57 visit. Note that intercurrent cases may or may not qualify for the per-protocol cohort depending on the timing of the COVID case and on protocol violations. Analyses that include both Day 29 and 57 markers use the same cohort as for analyses of Day 57 markers only.

3.2 Study Endpoints

Endpoints for per-protocol correlates analyses are included if they occur at least 7 days after the Day 57 visit, to help ensure that the endpoint did not occur prior to Day 57 antibody measurement. Thus participants with a per-protocol endpoint diagnosed earlier are excluded from the per-protocol correlates analyses.

Figure 1 defines five study endpoints that are assessed in COVID-19 vaccine efficacy trials, where all trials to our knowledge use COVID (symptomatic infection) as the primary endpoint. While the severe COVID endpoint is of paramount clinical importance, likely the number of events at the time of the first correlates analysis will be too small to assess correlates against this endpoint, such that correlates analyses will be done once more endpoints have accrued through longer-term follow-up.

In contrast, depending on the estimate of vaccine efficacy, there may be enough data to assess correlates against the endpoints non-severe COVID, SARS-CoV-2 infection (COV-INF), asymptomatic infection (ASYMP-COV-

INF) at or shortly after the time of the first correlates analysis, and viral load at COVID diagnosis. Similar statistical methods can be used for each endpoint, with some distinctions that we discuss below in “General Statistical Issues in Correlates Assessment.”

When a correlates analysis is done, all available follow-up for participants is included through to the time of the data base lock for the correlates analysis, for every CoR and CoP analysis that is conducted. This means that the time of right censoring for a given failure time endpoint will be the first event of loss to follow-up or the date of administrative censoring defined as the last date of available follow-up. For CoP analyses, which use both vaccine and placebo recipient data and leverage the randomization, follow-up is censored at the time of unblinding.

3.2.1 [With Day 29 markers]

If Day 29 markers are included, analyses that study Day 29 markers count study endpoints starting 7 days post Day 29 visit, instead of starting 7 days post Day 57 visit. Analyses that include both Day 29 and Day 57 markers (for correlates analyses this only includes the multivariable correlates of risk superlearning objective) use the same cohort and endpoints as analyses of Day 57 markers only.

4 Objectives of Immune Correlates Analyses of a Phase 3 Trial Data Set

4.1 Characterize Vaccine Immunogenicity

There are two objectives to characterize the binding and neutralizing antibody immunogenicity of the vaccine:

Stage 1 To characterize vaccine immunogenicity (bAb, nAb) at Day 1, 29, 57

Stage 2 To characterize vaccine immunogenicity/durability (bAb, nAb) over time (Day 1, 29, 57, 209, 394, 759)

4.2 Correlates of Risk and Correlates of Protection

We broadly classify the proposed analyses into two related categories: correlates of risk (CoR) and correlates of protection (CoP) analyses. CoR analyses seek to characterize correlations/associations of markers with future risk of the outcome amongst vaccinated individuals in the study cohort. CoP analyses seek to formally characterize causal relationships among vaccination, antibody markers and the study endpoint, and use data from both vaccine and placebo recipients. Table 1 summarizes these objectives and statistical frameworks that are commonly used to these ends.

The advantage of CoR analyses is that it is possible to obtain definitive answers from the phase 3 data sets, that is one can credibly characterize associations between markers and outcome. The advantage of CoP analyses is that the effects being estimated have interpretation directly in terms of how an antibody marker can be used to reliably predict vaccine efficacy (the criterion for use of a non-validated surrogate endpoint for accelerated approval). The disadvantage of CoR analyses are that a CoR may fail to be a CoP, for example due to unmeasured confounding, lack of transitivity where a vaccine effect on an antibody marker occurs in different individuals than clinical vaccine efficacy, or off-target effects ([VanderWeele \(2013\)](#)). The disadvantage of CoP analyses is that statistical inferences rely on causal assumptions that cannot be completely verified from the phase 3 data, such that compelling evidence may require multiple phase 3 trials and external evidence on mechanism of protection (e.g., from adoptive transfer or vaccine challenge trials). Our approach presents results for both CoR and CoP analyses, seeking clear exposition of how to interpret results, the assumptions undergirding the validity of the results, and diagnostics of these assumptions and assessment of robustness of findings to violation of assumptions.

We conjecture that an antibody marker could qualify as a non-validated surrogate endpoint (meeting accelerated approval criteria) based on meeting all three conditions: (1) demonstration of a strong and robust CoR with confounding control; (2) external data supporting functionality and connection to a mechanism of protection; and (3) CoP analyses supporting that the

biomarker is likely to be a CoP and not only a CoR. Mechanisms of protection as in (2) may be learned through passive antibody transfer studies and vaccine challenge studies in animals and/or humans.

Table 1: Correlates of Risk (CoRs) and Correlates of Protection (CoPs) Objectives for Day 57 Markers

Objective Type	Objective
CoRs (Risk Prediction Modeling)	To assess Day 57 markers as CoRs in vaccine recipients <ul style="list-style-type: none"> a. Relative risks of outcome across marker levels b. Absolute risk of outcome across marker levels c. Machine learning risk prediction for multivariable markers
CoP: Correlates of VE	To assess Day 57 markers as correlates of VE in vaccine recipients <ul style="list-style-type: none"> a. Principal stratification effect modification analysis b. Assesses VE across subgroups of vaccine recipients defined by Day 57 marker level in vaccine recipients
CoP: Controlled Effects on Risk and VE	To assess Day 57 markers for how assignment to vaccine and a fixed marker value would alter risk compared to assignment to placebo
CoP: Stochastic Interventional Effects on Risk and VE	To assess Day 57 markers for how stochastic shifts in their distribution would alter mean risk and VE (Hejazi et al., 2020)
CoP: Mediators of VE	To assess Day 57 markers as mediators of VE <ul style="list-style-type: none"> a. Mechanisms of protection via natural direct and indirect effects a. Estimate the proportion of VE mediated by a marker or markers

4.2.1 [With Day 29 markers]

If Day 29 markers are included, then each of the objectives for Day 57 markers is repeated for Day 29 markers. In addition, the multivariable CoR machine learning objective includes models that include both Day 29 and Day 57 markers.

4.3 Synthesis of the Phase 3 Correlates Analyses for Decisions

Establishment of an immunologic biomarker for approval/bridging applications is generally not based on pre-fabricated criteria nor a single type of correlates analysis. Therefore, the goal of the correlates analysis is to generate evidence about correlates from many perspectives, and to synthesize the evidence to support certain decisions. Consequently, we believe there is value in assessing all of the types of correlates presented in Table 1 in each trial, given that the analyses address distinct questions. Obtaining a set of results from multiple distinct approaches that provide complementary and coherent support may increase the rigor and robustness of an evidence package supporting potential use of an antibody marker as a validated surrogate (for traditional approval) or as a non-validated surrogate (for accelerated approval) (Fleming and Powers (2012)); these uses of a biomarker are summarized below. However, the assumptions needed for valid inferences are somewhat different across the methods, and some of these assumptions have testable implications; therefore examination of the assumptions may lead to favoring some methods over others, and affect the synthesis and interpretation of results, and moreover if diagnostics support that some necessary assumptions are infeasible then certain analyses will be canceled, as described below.

Section 16 summarizes the approach to use and interpretation of the set of multiple correlates of protection methods. Furthermore, depending on the number of study endpoints in the vaccine and placebo arms at the time a trial delivers primary results, some of the Day 57 marker correlates types defined in Table 1 will be evaluable at the first correlates analyses, whereas others will not be evaluable until additional evaluable vaccine breakthrough endpoints have been observed.

As detailed in Table 4, some CoR analyses are done after there are at least 25 evaluable vaccine breakthrough cases, which is considered to be a minimal number to achieve worthwhile precision. On the other hand, the most non-parametric/flexible CoR analyses require more cases, as do the CoP analyses in general, given the need to adjust for all potential confounders in order to fully identify the causal effects parameters of interest and the greater chal-

lenge in estimation (compared to CoR analysis) posed by the need to deal with missing potential outcomes.

Finally, we note that meta-analysis of multiple VE trials will provide important empirical support for potentially establishing an immunologic surrogate endpoint, which underscores the necessity of standardizing the VE trials (common study endpoints, common labs and immunoassays, common statistical methods and data analysis).

4.4 Additional Objectives Not Covered in this SAP

An additional objective that will be assessed, but will be described in a separate SAP, is to assess antibody markers over time beyond Day 57 through two years post vaccination, to assess “outcome-proximal correlates.” While we do not formally develop methods to address this aim in this SAP, here we very briefly outline approaches that may be pursued to address such aims. For example, a CoR analysis might assess longitudinal markers in vaccine recipients through estimation of the hazard ratio of outcome across levels of the current value of the marker modeled via a linear mixed effects model (e.g., [Fu and Gilbert \(2017\)](#)). A CoP analysis might assess longitudinal markers as mediators of VE (mechanisms of protection), for example by assessing the proportion of VE mediated by the longitudinal marker(s) profile (e.g., [Zheng and van der Laan \(2017\)](#)).

To potentially help addressing these aims, antibody markers should be measured at the time of COV-INF and COVID diagnosis. An open question is whether and how these measurements may be used in outcome-proximal correlates analyses, for example by assuming that the observed marker values on or near the day of diagnosis were present on the date of SARS-CoV-2 acquisition. Justification for this assumption for the COVID endpoint would derive from information that COVID tends to occur within only several days of SARS-CoV-2 acquisition, implying insufficient time for the infection to make new antibodies that would complicate the interpretation of the vaccine-elicited antibodies. It is possible that this condition could only be verified in a subset of cases, in which case validation-set missing data statistical methods may be fruitful. More validation work will be required before methods would

be used treating marker values at diagnosis as present at endpoint diagnosis and caused solely by the vaccine (i.e., not also caused by natural infection). For the COV-INF endpoint it is less feasible / possible to use the COV-INF sampling marker value to infer the marker value at acquisition, given the unknown period of weeks or months that may have elapsed between acquisition and seroconversion.

Outcome-proximal correlates analyses may be especially relevant if Day 57 antibody markers tend to be generally high in vaccine recipients and this fact leads to a failure of the correlates analyses to identify a Day 57 correlate: if antibodies wane over time then the outcome-proximal correlates analyses could be more sensitive to detect a correlate.

Additional objectives that may be addressed with the at-diagnosis samples include: (1) to characterize abnormal responses, possibly relevant for safety signals; and (2) to assess the effect of pre-existing antibodies on the active immune response to infection and disease.

5 Applications of Immune Correlates Analyses: Vaccine Approval Pathways and Standards of Evidence

Suppose that one or more phase 3 trials demonstrates beneficial vaccine efficacy against the primary clinical endpoint (e.g., symptomatic infection, i.e. COVID) meeting pre-specified success criteria, and correlates analyses of Day 57 antibody marker data are conducted based on the clinical data and antibody data from the phase 3 trial(s). These correlates analyses, combined with additional data supporting the role of antibody markers as mechanisms of protection or as surrogates of mechanisms of protection, can buttress two potential applications of an antibody marker (Table 2).

Table 2: Two Potential Vaccine Approval Pathways Based on a Day 57 Antibody Marker Endpoint

Traditional Approval	<p>If the marker is scientifically well-established to reliably predict vaccine efficacy, then subsequent efficacy trials may use the marker as the primary endpoint</p> <ul style="list-style-type: none"> a. Same vaccine for different populations b. Possibly new vaccines in the same class for the same or different populations
Accelerated Approval	<p>If the marker is judged reasonably likely to predict vaccine efficacy but not yet scientifically well established, then accelerated approval based on the marker endpoint may be possible (requires verification of beneficial clinical VE in post marketing studies)</p> <ul style="list-style-type: none"> a. Same vaccine for different populations b. Possibly new vaccines in the same class for the same or different populations

Fleming and Powers (2012) defined a *validated surrogate* as a marker that is appropriate for use as an outcome measure for traditional approval of a specific class of interventions against a specific disease, when such interventions are deemed safe and have demonstrated strong evidence that risks from off-target effects are acceptable. They also defined a *non-validated surrogate* as a marker appropriate for use as an outcome measure for accelerated approval as one established to be “reasonably likely to predict clinical benefit” for a specific disease setting and class of interventions. These definitions provide two goalposts for immune correlates analyses of COVID-19 VE trials.

Table 3 summarizes one possible set of requirements for a Day 57 antibody marker to be accepted as a *validated surrogate* for a COVID-19 disease endpoint for use in approving COVID-19 vaccines for specific populations (e.g., SARS-CoV-2 seronegative adults) using Fleming and Power’s definition. These potential requirements are conjectures provided for conceptualization purposes, and are not based on COVID-19 regulatory guidance documents.

Table 3: Potential Traditional Approval Requirements for a Day 57 Antibody Marker

Requirements (1.-6. Required)	Endpoints and Evidence Bar
1. Strong evidence for CoR and CoP in vaccine recipients in animal and/or human challenge models	COVID and VL endpoints: Highly significant and predictive <u>and</u> Severe COVID : Point estimates in the right direction <u>and</u> COV-INF, ASYMP-COV-INF: No countervailing evidence ¹
2. Strong evidence that the marker is a mechanistic CoP or tightly correlated with a mechanistic CoP (likely deriving from animal challenge studies of vaccines or passively transferred antibodies)	Study endpoints used in challenge models such as subgenomic SARS-CoV-2 RNA
3. Supportive evidence from natural history studies of CoRs of re-infection in SARS-CoV-2 infected individuals	Same endpoints as in Phase 3 trial (COVID , severe COVID , ASYMP-COV-INF, COV-INF, VL Dx)
4. Phase 3 trial strong evidence as a CoR in vaccine recipients	COVID and ≥ 1 other endpoint: Highly significant and predictive <u>and</u> Point estimates in the right direction for the other endpoints Consistent results Day 29, 57 markers <u>Require consistent results from multiple trials</u>
5. Phase 3 trial strong supportive evidence as CoP, for at least one CoP type, plus point estimates in the right direction for the other CoP types (consistency of evidence)	COVID Point estimates of association/causal parameters in the right direction for the other endpoints ² <u>Require consistent results from multiple trials</u>
6. Temporal ordering support for several of the above results, e.g., CoRs and CoPs are stronger for COVID occurrence proximal to vaccination than distal, synchronized with pattern of biomarker waning	COVID , severe COVID , ASYMP-COV-INF, COV-INF, VL Dx
7. Additional support from non-vaccine interventions, e.g., demonstration of a neutralization CoP for a monoclonal Ab	COVID , severe COVID , ASYMP-COV-INF, COV-INF, VL Dx

¹Countervailing evidence could be any observations that provide evidence against a CoP, e.g., relative to Bradford-Hill criteria (see Section 16).

²Because CoPs can differ by study endpoint Plotkin (2010) and vaccine efficacy can differ by study endpoint, this criterion will not necessarily be important.

A potential goalpost for a *non-validated surrogate* for accelerated approval can be conceptualized as the same as that for traditional approval, with modifications:

- The package of evidence for the seven sources listed in Table 3 may be less stringent quantitatively, and not requiring success on all of the first six categories.
- Source 4 (Phase 3 CoR in vaccine recipients) would need to have strong evidence (highly statistically significant and highly predictive).
- The support for an immune correlate may be more restricted to a given study endpoint.
- It may no longer be required to have replication of results across two or more Phase 3 trials.

It is hypothesized that a single validated assay will yield a validated or non-validated surrogate endpoint, e.g., based on binding antibody IgG concentration or serum ID50 or ID80 titer to viruses pseudotyped with the Spike vaccine insert protein (or live SARS-CoV-2). However, the goalposts could potentially also be met by a synthesis biomarker aggregated from measurements from multiple validated assays if this aggregation substantially improves the correlate (e.g., a co-correlate Plotkin (2010); Plotkin and Gilbert (2018)). However, the preferred approach, for parsimony and practical utility, would be to define a correlates of protection as a single biomarker derived from a single assay.

5.0.1 [With Day 29 markers]

If Day 29 markers are included, then a validated surrogate endpoint or non-validated surrogate endpoint could be defined based on either a Day 57 time point or a Day 29 time point, and possibly also requiring both time points integrated into the same biomarker.

6 Timeline/Sequencing of Correlates Analyses

The correlates analyses are initiated by the availability of (a) a data set defined at or after the primary analysis data set triggered by the accrual of a certain number of primary endpoints (typically approximately 150 in U.S. phase 3 studies); and (b) Day 1, 57 antibody marker data from correlates-eligible COVID primary endpoint cases from at least 25 baseline negative vaccine recipients. The latter requirement ensures that there are enough endpoint cases to achieve worthwhile precision for CoR analyses. The HVTN 505 trial serves as a precedent where 25 evaluable vaccine recipient cases provided enough data to reasonably characterize correlates of risk for a preventive candidate HIV vaccine (Janes et al. (2017); Fong et al. (2018); Neidich et al. (2019); Gilbert et al. (2020b)). In addition, simulation studies show that correlates analyses at 20 endpoints have notably lower precision.

Table 4 shows the minimum number of baseline negative vaccine recipient endpoints evaluable for correlates analyses that are required before conducting the various planned correlates analyses.

Table 4: Minimum Numbers of Evaluable Endpoints in Baseline Negative Vaccine Recipients to Initiate Correlates Analyses

Correlates Analysis Type	Number
CoRs (Risk Prediction Modeling)	
a. (Semi)parametric models with strongly parametrized associations: Cox, hinge/threshold logistic regression	25
b. Flexible parametric models: Generalized additive model	35
c. Nonparametric thresholds: Donovan et al. (2019)/ van der Laan et al. (2020)	35
d. Superlearner estimated optimal surrogate	35
CoP: Correlates of VE	50
CoP: Controlled VE	50
CoP: Stochastic Interventional VE	50
CoP: Mediators of VE	50

6.1 Timeline of Statistical Analysis Reports

We summarize the plans for analysis reports over the whole period of the study. When the Day 1, 57 antibody data from the random subcohort are available, the first immunogenicity report will be produced. When Day 1, 57 antibody data on COVID cases are also available, the first correlates of risk report will be produced, focusing on Stage 1 data only. When there is enough follow-up to measure antibody markers at the later time points (i.e., Day 209, Day 394, possibly Day 759), additional immunogenicity and correlates reports will be made, including those that assess outcome-proximal correlates of risk and protection based on Stage 2 data. The initial correlates reports will likely only include the symptomatic infection/COVID study endpoint; as data sets become available for the other endpoints the reports will add correlates analyses against the secondary endpoints.

7 General Statistical Issues in Immune Correlates Assessment

Throughout this section, we define the asymptomatic infection endpoint as seroconversion without prior occurrence of the COVID endpoint.

Issue 1: Timing of endpoint definition, accounting for diagnosis at presentation (i.e., date of virological confirmation of symptomatic COVID – COVID diagnosis) or during post-COVID-19 diagnosis follow-up.

- **COV-INF:** Defined at presentation (if COVID endpoint) or at first positive serotest visit, whichever occurs first
- **COVID:** Defined at presentation/virologic confirmation
- **Asymptomatic infection:** Defined at first positive serotest (without prior COVID endpoint)
- **Non-severe COVID:** Ascertained by post-COVID diagnosis follow-up, where the failure time could be defined by the time of resolution of symptoms

- **Severe COVID:** Occurs at presentation or at any time during post-COVID diagnosis follow-up

At COVID endpoint diagnosis, participants rollover onto a post-diagnosis follow-up track (Figure 2). This is irrelevant for analysis of the first three endpoints listed above, but for the non-severe COVID endpoint and the severe COVID endpoint special considerations are needed for proper correlates analyses. Survival analysis theory typically requires *predictable processes*, such that non-severe COVID and severe COVID would have failure times defined when the classification of the endpoint is known. However, alternatively, the analysis could be simplified by defining the failure time for all three endpoints COVID, severe COVID, and non-severe COVID to be the date of presentation, even though at that time one needs to look into the future to determine whether the COVID endpoint is severe or non-severe. Such an approach could be justified by thinking of the data as a competing risks data structure, where one observes the time to COVID, and each COVID endpoint has an associated binary endpoint “type”, severe or non-severe. The analyses will use this simplified approach. A justification of this simplified approach is that severe COVID is a very rare event among vaccine recipients, and it is the fact of having the event that is important, not whether it happened at or 9 days post COVID diagnosis, such that using a more refined failure time would be unlikely to carry additional meaningful information. If greater than 10% of COVID endpoint cases are missing the endpoint type, then methods accounting for missing endpoint types will be used (e.g., [Heng et al. \(2020\)](#)).

Issue 2: Is the endpoint appropriately analyzed using ordinary survival analysis or competing risks survival analysis?

For this issue, we consider use of a time-to-event method to assess vaccine efficacy. In general, a competing risk of a given endpoint of interest is an endpoint that, once it occurs, precludes the possibility of future occurrence of the other endpoint.

1. COVID is a competing risk for asymptomatic infection
2. Severe COVID is a competing risk for non-severe COVID

Therefore, the asymptomatic infection and non-severe COVID endpoints may be best analyzed by competing risks methods. For example, instead of estimating cumulative incidence $P(T \leq t|A = a)$ for a given treatment arm $A = a$, where T is the time from enrollment until the endpoint, we analyze cumulative incidence $P(T \leq t, J = 1|A = a)$, where T is the time to the first event of $J = 1$ (event of interest) and $J = 2$ (competing event), and cumulative VE(t) may be assessed using the parameter

$$\text{VE}(t) = 1 - \frac{P(T \leq t, J = 1|A = 1)}{P(T \leq t, J = 1|A = 0)}.$$

In addition, hazard-ratio-based VE may be defined as one minus the cause $J = 1$ -specific hazard ratio (Prentice et al., 1978, Gilbert et al., 2000).

1. Asymptomatic infection is not a competing risk for COVID, because participants experiencing the asymptomatic infection endpoint continue follow-up for the COVID endpoint (such that at asymptomatic infection diagnosis it is not known whether the infection is truly asymptomatic or pre-symptomatic), and it is not certain that seroconversion prevents future COVID (if future knowledge supports this conclusion, then asymptomatic infection could be treated as a competing risk).
2. Non-severe COVID is not a competing risk for severe COVID. At presentation, if the COVID event does not qualify as severe, then post-COVID diagnosis follow-up is required to determine whether the endpoint registers as non-severe or severe. One will only know the endpoint is not severe after post-COVID diagnosis follow-up is completed (symptoms resolve), such that the failure time is not known until the end of post COVID diagnosis follow-up. Therefore, non-severe COVID is not a competing risk for severe COVID, and the severe COVID endpoint can be analyzed using ordinary survival analysis ignoring the non-severe COVID endpoint.

In sum, the COV-INF, COVID, and severe COVID endpoints will be analyzed by ordinary survival analysis methods, whereas the asymptomatic infection and non-severe COVID endpoints will be analyzed using competing risks methods. Moreover, adding nomenclature precision, for the parent infection

endpoint, the daughter endpoints COVID and asymptomatic infection are semi-competing risks data (nomenclature in the survival analysis literature), and for the COVID parent endpoint, the daughter endpoints severe COVID and non-severe COVID are semi-competing risks data.

In addition, one non-clinical endpoint may be important for correlates assessment: SARS-CoV-2 viral load at COVID diagnosis (VL Dx) (e.g., measured by nasal swab), or alternatively area under the viral load curve (AUC-VL) from the COVID diagnosis date through to undetectable viral load, or to an alternative threshold indicating low viral load. Viral load endpoints are putative surrogates of disease progression and severity for the individual, and are also putative surrogates for secondary transmission; moreover the quantitative nature of viral load endpoints may afford an opportunity to increase statistical power.

Issue 3: Courseness level of the failure time variable

1. **COVID:** Event time defined in ‘continuous time’ on the day of virological confirmation.
2. **Asymptomatic infection:** Event time defined only at fixed infrequent visits (e.g., Month 6, 12, 18, 24).
3. **COV-INF:** Event time defined as ‘mixed continuous and discrete’, equal to the day of virological confirmation (if COVID) and by the first seropositive visit (if asymptomatic infection).
4. **Non-severe COVID:** Event time may be defined in continuous time, as the number of days from enrollment to COVID diagnosis plus the number of additional days until the COVID event is known to be non-severe. However, following the decision made for Issue 1, we simplify and define the event time at COVID diagnosis.
5. **Severe COVID:** Event time may be defined in continuous time, as the number of days from enrollment to COVID diagnosis plus the number of additional days until the COVID event is known to be severe (which may be zero days). However, following the decision made for Issue 1, we

simplify and define the event time at COVID diagnosis.

Issue 4: Binary endpoint vs. failure time endpoint

In general, in phase 3 trials with prospective follow-up for event occurrence where right-censoring occurs (either due to administrative censoring or loss to follow-up), it can be advantageous to conduct data analysis in a survival analysis paradigm. Many of the correlates analyses are specified as such. However, because the endpoints are rare, and the rate of loss to follow-up is anticipated to be very low, reliable and interpretable answers may be obtained based on simpler methods that use binary endpoints, and deal with loss to follow-up in a cruder way. If retention is very high, such that bias and precision may be minimally impacted by use of a binary endpoint, some of the correlates analyses may use a binary endpoint. In settings with competing risks, such analyses would treat the endpoint as multinomial and utilize methodology accordingly.

In sum, correlates methods are needed that consider time-to-event or binary endpoints, with or without accounting for a competing risk. In addition, the methods need to be able to handle continuous, discrete, and mixed continuous/discrete failure times.

8 Case-cohort Sampling Design for Measuring Antibody Markers

Figure 3 illustrates the case-cohort ([Prentice \(1986\)](#)) sampling design that is used for measuring Day 1, 57 antibody markers (and the later time points at a later point in time) in a random sample of trial participants. The random sample is stratified by the key baseline covariates: assigned treatment arm, baseline SARS-CoV-2 status (defined by serostatus and possibly also NAAT and/or RNA PCR testing), any additional important demographic factors such as the randomization strata (e.g., defined by age and/or co-morbidities), and underrepresented minority status within the U.S. Because the design uses a stratified random sample instead of the simple random sample proposed by [Prentice \(1986\)](#), the design may also be referred to as a “two-phase sampling

design” ([Breslow et al. \(2009b,a\)](#)), where “phase one” refers to variables measured in all participants and “phase two” refers to variables only measured in a subset (thus the “case-cohort sample” constitutes the phase-two data).

The case-cohort design enables obtaining marker data (Day 1, 57) for the random subcohort during early trial follow-up in real-time batches, thereby accelerating the time until final data set creation and hence data analysis and results on Day 57 marker correlates. The design allows using the same random subcohort to assess correlates for multiple endpoints, relevant for the COVID-19 VE trials with multiple endpoints ([Figure 1](#)). This makes the design operationally simpler than a case-control sampling design.

8.1 Prototype OWS Random Subcohort

[Table 5](#) summarizes the size of the prototype OWS random subcohort, by baseline factors used to stratify the random sampling. In this subcohort 6 baseline demographic strata are used; if a trial has a different number of baseline demographic strata, then the table would be modified, holding the total sample size of the subcohort approximately fixed. For U.S. strata, all OWS trials specify 50:50 balance by underrepresented minority status Yes:No. The subcohort sampling is implemented to create representative sampling across the entire period of enrollment. Non-OWS trial sampling designs would likely be similar, with different baseline sampling strata perhaps even the simplest case with no sampling strata and use of simple random sampling.

Table 5: Random Subcohort Sample Sizes by Baseline Strata for Antibody Marker Measurement

Bas. Cov. Strata ¹	Baseline SARS-CoV-2 Negative ²						Baseline SARS-CoV-2 Positive ³					
	1	2	3	4	5	6	1	2	3	4	5	6
Vaccine	150	150	150	150	150	150	50	50	50	50	50	50
Placebo	20	20	20	20	20	20	50	50	50	50	50	50

¹This schema specifies 6 baseline covariate strata for stratified sampling, for example

1 = U.S. Age 18-64 Minority; 2 = U.S. Age 18-64 non-Minority; 3 = U.S. Age ≥ 65 Minority;

4 = U.S. Age ≥ 65 non-Minority; 5 = non-U.S. Age 18-64; 6 = non-U.S. Age ≥ 65 .

²The vaccine group baseline negative strata are assigned large sample sizes because the correlates of risk analysis focuses on baseline negative vaccine recipients. The placebo group baseline negative strata are assigned small sample sizes given the expectation that almost all Day 57 bAb and nAb readouts will be negative/zero given the absence of prior exposure to SARS-CoV-2 antigens.

³Equal stratum sizes are assigned for the vaccine and placebo groups in order to compare bAb and nAb responses in previously infected persons, studying potential differences in natural+vaccine-elicited responses vs. natural-elicited responses.

If certain strata do not have enough eligible participants available for sampling, then additional sampling is done from other strata to keep the total random subcohort sample size to close to 1620. A separate OWS Antibody Marker sampling plan describes the algorithm, available upon request.

8.1.1 Additional sampling of participants missing the second dose

For trials with two doses of vaccine, the set of baseline negative strata for vaccine recipients is expanded to also include the subgroup that misses the second vaccination and minimally has available samples at Day 57, and similarly the corresponding subgroup to placebo recipients is added; these subgroups are defined regardless of baseline demographic factors. For the vaccine subgroup, a random sample of 150 (or the number available, whichever is smaller) participants is sampled, and for the placebo subgroup, a random sample of 20 (or the number available, whichever is smaller) participants is sampled. This additional sample may not be drawn until the last participant reaches 1 or 2 years of follow-up, to be able to ensure that sampled participants have many samples available.

The two objectives of the additional sampling are: (1) To compare long-term

antibody responses one dose vs. two doses; and (2) to increase power of the Stage 2 analysis of outcome-proximal correlates.

8.2 Correlates Objectives Addressed in Two Stages

Figure 4 depicts the two stages of the immune correlates analyses. Stage 1 includes antibody marker data from all COVID and COV-INF cases diagnosed through to the last date of: (1) the time that at least 25 evaluable vaccine breakthrough COVID endpoint cases are available for analysis; and (2) the time of a data-cut at or after the primary analysis used to define the data base for the first correlates analysis. Only Day 1, 57 antibody markers, and COVID and COV-INF diagnosis time point antibody markers, are measured in Stage 1. The objectives of Stage 1 correlates analyses focus on Day 57 markers, which are the objectives listed in Table 1. Stage 1 focuses on Day 57 markers because in general validated or non-validated surrogate endpoints for approved vaccines are based on the peak antibody time point, and this approach fits the priority to develop a validated or non-validated surrogate endpoint as rapidly as possible.

Stage 2 includes antibody marker data from all COVID and COV-INF cases diagnosed after the Stage 1 cases through to the end of the trial, including all available sampling time points (6–7 time points). For random subcohort participants, the antibody markers at all available time points other than Day 1, 57 are measured for Stage 2 correlates analyses (4–5 additional time points). The Stage 2 clinical endpoint data and antibody marker data enable assessment of longitudinal antibody markers as outcome-proximal correlates of instantaneous endpoint risk and as various types of outcome-proximal correlates of protection.

The Stage 1 random subcohort sampling plan is finalized prior to or shortly after study start. The Stage 2 sampling plan is not made until after the results on vaccine efficacy at the primary analysis are known. The Study Oversight Group may modify the scope of the set of samples for immunoassay measurements in Stage 2 based on analysis results. The essential distinguishing mark of Stage 1 vs. Stage 2 is assessment of Day 57 marker correlates that can be done using antibody data only from Day 1, 57 markers vs. assessment

of outcome-proximal correlates that requires antibody data longitudinally including at endpoint diagnosis dates.

8.2.1 Prioritize antibody marker measurement at COVID and COV-INF diagnosis sampling time points

Conduct of the immunologic assays on diagnosis date samples for all COVID and all COV-INF endpoint samples is of the highest priority, equal to the priority of conducting the assays on the Day 1, 57 samples.

8.2.2 [With Day 29 markers]

If Day 29 markers are included, then the case-cohort sampling design is the same, except all participants in the random subcohort (augmented with infected cases) also have the set of markers measured at Day 29. Thus ‘Day 1, 57’ becomes ‘Day 1, 29, 57’ throughout.

9 Unsupervised Feature Engineering of Antibody Markers (Stage 1: Day 1, 57)

9.1 Descriptive Tables and Graphics

9.1.1 Antibody marker data

Pseudovirus neutralizing antibody ID50 and ID80 titers, as well as fold-rise in ID50 and ID80 titers from baseline, are measured at each pre-defined time point. Indicators of 2-fold rise and 4-fold rise in ID50 titer (fold rise [post/pre] ≥ 2 and ≥ 4 , 2FR and 4FR) are measured at each pre-defined post-vaccination timepoint. Neutralization responders at each pre-defined timepoint are defined as participants who had baseline values below the LLOQ with detectable ID80 neutralization titer above the assay LLOQ, or as participants with baseline values above the LLOQ with a 4-fold increase in neutralizing antibody titer. While quantitative fold-rise is shown for both ID50 and ID80, response above LLOQ, 2FR and 4FR responder status are shown only for ID50. (However, for superlearner analysis of multivariable CoRs, 2FR and 4FR responder status variables are included for each of pseud virus-nAb ID50 and ID80, given the objectives of more comprehensive analysis in

building the estimated optimal surrogate.) For WT live virus-nAb MN50, the same types of variables are analyzed/reported as for pseudovirus-nAb ID50.

Binding antibody titers to full length SARS-CoV-2 Spike protein, to the RBD domain of the Spike protein, and to the Nucleocapsid (N) protein will be measured in all participants in the random subcohort (augmented with infected cases). N-specific binding antibody titers are not used for correlates analyses or for graphical reporting; these data are only used for tabular reporting. Neutralizing antibody titers are not measured to N protein.

The following list describes the antibody variables that are measured from random subcohort and infection case participants.

1. Individual anti-Spike antibody concentration at each pre-defined time point
2. Individual anti-Spike antibody fold-rise concentration post-vaccination relative to baseline at each pre-defined post-vaccination time point
3. Individual anti-RBD antibody concentration at each pre-defined time point
4. Individual anti-RBD antibody fold-rise post-vaccination relative to baseline at each pre-defined post-vaccination time point
5. Individual anti-N antibody concentration at each pre-defined time point
6. Individual anti-N antibody fold-rise post-vaccination relative to baseline at each pre-defined post-vaccination time point
7. 2-fold-rise and 4-fold rise (fold rise in anti-Spike antibody concentration $[\text{post/pre}] \geq 2$ and ≥ 4 , 2FR and 4FR) at each pre-defined post-vaccination time point
8. 2-fold-rise and 4-fold rise (fold rise in anti-RBD antibody concentration $[\text{post/pre}] \geq 2$ and ≥ 4 , 2FR and 4FR) at each pre-defined post-vaccination time point
9. 2-fold-rise and 4-fold rise (fold rise in anti-N antibody concentration $[\text{post/pre}] \geq 2$ and ≥ 4 , 2FR and 4FR) at each pre-defined post-vaccination

time point

10. Pseudovirus-nAb responders, at each pre-defined timepoint defined as participants who had baseline values below the LLOQ with detectable pseudovirus-nAb ID50 titers above the assay LLOQ or as participants with baseline values above the LLOQ with a 4-fold increase in pseudovirus-nAb ID50 titers
11. Wild type live-virus-nAb responders, at each pre-defined timepoint defined as participants who had baseline values below the LLOQ with detectable WT live virus-nAb MN50 titers above the assay LLOQ or as participants with baseline values above the LLOQ with a 4-fold increase in WT live virus-nAb MN50 titers

Summaries of the immunogenicity data will be reported in tables. In particular, the tables will include, for each pre-defined post-baseline time point:

1. For each binding antibody marker, the estimated percentage of participants defined as responders, and with concentrations $\geq 2 \times$ LLOQ or $\geq 4 \times$ LLOQ, will be provided with the corresponding 95% CIs using the Clopper-Pearson method
2. For the ID50 pseudo-virus neutralization antibody marker, the estimated percentage of participants defined as responders, participants with 2-fold rise (2FR), and participants with 4-fold rise (4FR) will be provided with the corresponding 95% CIs using the Clopper-Pearson method
3. For the MN50 WT live virus neutralization antibody marker, the estimated percentage of participants defined as responders, participants with 2-fold rise (2FR), and participants with 4-fold rise (4FR) will be provided with the corresponding 95% CIs using the Clopper-Pearson method
4. Geometric mean titers (GMTs) and geometric mean concentrations (GMCs) or will be summarized along with their 95% CIs using the t-distribution approximation of log-transformed concentrations/titers (for each of the 5 Spike-targeted marker types including pseudovirus-nAb ID50 and ID80 and WT live virus-nAb MN50, as well as for binding Ab to N).
5. Geometric mean titer ratios (GMTRs) or geometric mean concentration

ratios (GMCRs) are defined as geometric mean of individual titers/concentration ratios (post-vaccination/pre-vaccination for each injection)

6. GMTRs/GMCRs will be summarized with 95% CI (t-distribution approximation) for any post-baseline values compared to baseline, and post-Day 57 values compared to Day 57
7. The ratios of GMTs/GMCs will be estimated between groups with the two-sided 95% CIs calculated using t-distribution approximation of log-transformed titers/concentrations
8. The differences in the responder rates, 2FRs, 4FRs between groups will be computed along with the two-sided 95% CIs by the Wilson-Score method without continuity correction (Newcombe, 1998).

All of the above estimates will use inverse probability of antibody marker sampling weighting in order that estimates and inferences are for the population from which the whole study cohort was drawn.

Tables will be provided separately for (1) baseline negative individuals, (2) baseline positive individuals, (3) baseline negative individuals by subgroup defined as in Table 6, and (4) baseline positive individuals by the same subgroups as in (3). Each table will show data for all available time points and for each of the vaccine and placebo arms.

Table 6: Baseline Subgroups that are Analyzed (May Vary Slightly by Protocol)¹.

Age: < 65, \geq 65
Risk for Severe COVID: At risk, Not at risk
Age x Risk for Severe COVID:
< 65 At risk, < 65 Not at risk, \geq 65 At risk, \geq 65 Not at risk
Sex Assigned at Birth: Male, Female
Age x Sex Assigned at Birth:
< 65 Male, < 65 Female, \geq 65 Male, \geq 65 Female
Hispanic or Latino Ethnicity: Hispanic or Latino, Not Hispanic or Latino
Race or Ethnic Group:
White ² , Black, Asian, American Indian or Alaska Native (NatAmer)
Native Hawaiian or Other Pacific Islander (PacIsl), Multiracial,
Other, Not reported, Unknown
Underrepresented Minority Status in the U.S.:
Communities of color (Comm. of color), White ²
Age x Underrepresented Minority Status in the U.S.:
Age \geq 65 Comm. of color, Age < 65 Comm. of color, Age \geq 65 White, Age \geq 65 White

¹All analyses are done within strata defined by randomization arm and baseline positive/negative status, such that these variables are not listed here as subgroups for analysis.

²For purposes of analysis White is defined as Race=White and Ethnicity=Not Hispanic or Latino. All of the other Race subgroups are defined solely by the Race variable, with levels Black, Asian, American Indian or Alaska Native, Native Hawaiian or Other Pacific Islander, Multiracial, Other, Not reported, Unknown.

For comparing antibody levels between groups, the following groups are compared:

- Baseline negative vaccine vs. baseline negative placebo
- Baseline positive vaccine vs. baseline positive placebo
- Baseline negative vaccine vs. baseline positive vaccine
- Within baseline negative vaccine recipients, compare each of the following pairs of subgroups listed in Table 6: Age \geq 65 vs. age < 65; risk for severe COVID: at risk vs. not at risk; age \geq 65 at risk vs. age \geq 65 not at risk; age < 65 at risk vs. age < 65 not at risk; male vs. female; Hispanic or Latino ethnicity: Hispanic or Latino vs. Not Hispanic or Latino; Underrepresented minority status: Communities of color vs.

White (within the U.S.).

The entire analysis is done in the per-protocol cohort with both Day 1 and Day 57 marker data available (the two-phase sample).

In addition, if a substantial number of trial participants who missed the second dose of vaccine have Day 57 antibody marker data, then similar analyses will be done in this subgroup, again stratified by randomization arm and baseline negative/positive status.

9.1.2 [With Day 29 markers]

If Day 29 markers are included, then participants in the random subcohort and outcome cases will have marker data at Day 1, 29, 57. All immunogenicity analyses (graphical and tabular) of Day 57 markers are also conducted for Day 29 markers. In addition, tabular output will include point and 95% confidence interval estimates of GMT and GMC ratios (Day 57 / Day 29).

9.1.3 Graphical description of antibody marker data

The Day 1, 57 antibody marker data collected from the random subcohort participants will be described graphically. These data are representative of the entire study cohort. Importantly, only antibody data from the random subcohort are included (i.e., no data from cases outside the subcohort are included). This makes the analyses unsupervised (independent of case-control status), enabling interrogation and optimization of the antibody biomarkers prior to the inferential correlates analyses.

Plots are developed for the following purposes. All of the analyses are done separately within each of the four subgroups defined by treatment arm cross-classified with baseline negative/positive status. In addition, many of the descriptive analyses will also be done separately for each demographic subgroup of interest listed above. For descriptive plots of individual marker data points that pool over one or more of the baseline strata subgroups, two approaches to plotting will be used. First, plots show all observed data points. The limitation of this approach is that if the subgroup being plotted aggregates over multiple baseline sampling strata, then the plot describes the marker

distribution for a biased sample, not a representative sample. Therefore, a second set of plots will use weighted random sampling of participants with replacement, with inverse probability of sampling weights used to make the plot representative of the subgroup (placed in the Appendix). These plots will plot the same number of data points as the number of observed data points.

For each antibody marker readout, both Day 57 and baseline-subtracted Day 57 readouts are of interest. We will refer to the latter as ‘delta.’ All readouts, including delta, will be plotted on the \log_{10} scale, with plotting labels on the natural scale. As such, delta is \log_{10} fold-rise in the marker readout from baseline.

The following descriptive graphical analyses are done.

1. The distribution of each antibody marker readout at Day 1 and Day 57 will be described with plots of empirical reverse cumulative distribution functions (rcdfs) and boxplots (including individual data points) within each of the four groups defined by treatment arm (vaccine, placebo) and baseline serostratum (seronegative, seropositive). Inverse probability of sampling into the subcohort weights are used in the estimation of the rcdf curves; henceforth we refer to these weights as “inverse probability of sampling” (IPS) weights. Analyses of Day 1 markers always pool across vaccine and placebo recipients given that the two subgroups are the same at baseline.
2. Plots are arranged to compare each Day 57 marker readout between treatment arms within each of the baseline seropositive and baseline seronegative subgroups.
3. Plots are also arranged to compare each Day 57 marker readout between baseline serostatus groups within each treatment arm.
4. Barplots are used to report positive response rates.
5. The correlation of each antibody marker readout between Day 1 and Day 57, and between Day 1 and delta, is examined within each of the baseline strata subgroups, and within each treatment arm and baseline sero-

stratum. Pairs plots/scatterplots will be used, annotated with baseline strata-adjusted Spearman rank correlations, implemented in the PResiduals R package available on CRAN. For calculating the correlation within each treatment arm and baseline serostratum, because PResiduals does not currently handle sampling weights, the correlation estimates are computed as follows: For each re-sampled data set in the second approach to graphical plotting, the covariate-adjusted Spearman correlation is calculated. The average of the estimated correlations across re-sampled data sets is reported.

6. The correlation of each pair of Day 1 antibody marker readouts are compared within each of the baseline demographic subgroups and baseline serostratum, pooling over the two treatment arms. Pairs plots/scatterplots and baseline-strata adjusted Spearman rank correlations are used, with covariate-adjusted Spearman rank correlations computed as described above.
7. The correlation of each pair of Day 57 and delta antibody marker readouts are compared within each of the baseline demographic subgroups, treatment arm, and baseline serostratum. Pairs plots/scatterplots and baseline-strata adjusted Spearman rank correlations are used, with covariate-adjusted Spearman rank correlations computed as described above.
8. Point estimates of Day 57 marker positive response rates for the vaccine arm by the baseline demographic subgroups and the baseline serostrata are provided, as well as pooled over baseline demographic strata. The point and 95% CI estimates include all of the data and use IPS weights.

9.2 Methods for Positive Response Calls for bAb and nAb Assays

As noted above, binding antibody responders at each pre-defined timepoint are defined as participants who had baseline concentration values below the LLOQ with detectable concentration above the assay LLOQ (34 IU/ml), or as participants with baseline values above the LLOQ with a 4-fold increase in concentration. Therefore, for participants with binding antibody below the LLOQ, a positive response is defined by concentration > 34 IU/ml, and

a negative response as the complement (≤ 34), with value set to 17. This approach is used for each of the Spike and RBD and N protein antigen targets.

Pseudovirus neutralization responders at each pre-defined timepoint are defined as participants who had baseline ID50 values below the LLOD with detectable ID50 neutralization titer above the assay LLOD (value 20), or as participants with baseline values above the LLOD with a 4-fold increase in neutralizing antibody titer. Therefore, for participants with ID50 baseline values below the LLOD, a positive response is defined by serum ID50 titer $> 1 : 20$, and a negative response as the complement ($\leq 1 : 20$). The same approach is used based on ID80 titer, again based on the LLOD of 1:20 titer. Similarly, for the WT live virus-nAb MN50 marker, WT live virus neutralization responders at each pre-defined timepoint are defined as participants who had baseline MN50 values below the LLOD with detectable MN50 above the assay LLOD (value 62.16), or as participants with baseline values above the LLOD with a 4-fold increase in neutralizing antibody titer. Otherwise a value is negative for WT live virus neutralization.

9.3 SARS-CoV-2 Antigen Targets Used for bAb and nAb Markers

The homologous vaccine strain antigens are used for the immune correlates analyses.

9.4 Score Antibody Markers Combining Information Across Individual bAb and/or nAb Readouts

Depending on the number and features of antigens that are selected for defining antibody marker variables, feature extraction/selection techniques may be employed to determine score/synthesis marker variables that are optimized according to some criterion that would reflect maximum signal-relevant diversity (e.g., [He and Fong \(2019\)](#)). In addition, the unsupervised dimensionality reduction techniques such as principal components analysis (PCA) and non-linear extensions of PCA (e.g. FSDAM1 and FSDAM2; [Fong and Xu \(2020\)](#)) may also be used to define score variables that maximally capture the main immune response signal and to study whether there are more than one distinct signals that are associated with the outcome. If such synthesis features are

defined, then they will be included as input features in the machine learning (superlearning) prediction modeling (multivariable CoR objective).

9.4.1 Systematic ranking of Day 57 antibody markers by signal-to-noise ratio

The signal-to-noise ratio of each Day 57 antibody marker is defined as the ratio of biological variability over technical variability. The technical variability will be estimated as the median of the variances across two technical replicates for each test sample, and the biological variability will be estimated as the variance of the average of the two technical replicates across all test samples (without weighting for simplification) minus the technical variability (analysis done in the cohort of interest such as baseline negative vaccine recipients).

The ranking of the set of Day 57 antibody markers will be taken into account in the interpretation of results.

9.5 Decisions on Antibody Markers to Advance to Correlates or Risk and Correlates of Protection Analyses

The vaccine immunogenicity analysis characterizes SARS-CoV-2 directed antibody levels based on five antibody biomarkers measured at each blood storage time point: IgG concentration to Spike, IgG concentration to RBD, pseudovirus-nAb ID50, pseudovirus-nAb ID80, WT live virus-nAb MN50. It is likely that all five of the biomarkers at each of the Day 57 and Day 29 time points will be advanced to study as CoRs and CoPs for the initial correlates reports, given that all of the assays are validated. However, an objective of the unsupervised learning immunogenicity characterization is to determine if some markers should be prioritized (for example based on broader biologically-relevant dynamic range), such that p-values and multiplicity adjustment for tests of correlates of risk would only be done for the prioritized markers. In addition, it is possible that the unsupervised learning could lead to decisions to pare down the list of markers (e.g., eliminating markers that are very highly correlated with other markers, or eliminating markers that are revealed to have unexpected technical issues). Because the unsupervised learning is done based on random subcohort data and is thus independent

of case/non-case status, decisions made based on this learning do not compromise the validity of the CoR and CoP analyses. Decisions about the set of antibody makers to use in the CoR and CoP analyses – and their priority level – will be made and documented in the SAP prior to implementing the CoR and CoP analyses.

10 Baseline Risk Score (Proxy for SARS-CoV-2 Exposure)

A list of baseline covariates potentially relevant for SARS-CoV-2 exposure and infection risk will be specified. Based on these covariates, a baseline risk score is developed and controlled for in correlates analyses to adjust for potential confounding. The risk score is developed using placebo arm data only. The risk score is defined as the logit of the predicted outcome probability from a regression model estimated using the ensemble algorithm superlearner (i.e. stacking). The settings of superlearner (i.e., loss function, cross-validation technique, library of learners) that are used for implementation of superlearner for building a baseline risk score are described in [Section 12.5](#). A separate risk score will be developed for each study endpoint used in correlates analyses. If the risk score is highly similar across a set of endpoints, then a single risk score may be selected for application to each endpoint in the set.

Independent of the superlearner risk score, important individual risk factors will also be specified for inclusion as adjustment factors in correlates analyses, such as age, status of having a high-risk condition, and underrepresented minority status. For example, all of the baseline demographic strata used in the two-phase sampling design will be adjusted for, or a coarsened categorical variable derived from the baseline strata will be adjusted for, where the amount of coarsening may depend on the number of endpoints in the vaccine arm. Henceforth we refer to the baseline variables that are adjusted for in correlates analyses as “baseline factors” which, depending on the risk score results and performance, will consist of only the individual key risk factors, or key individual risk factors plus the baseline risk score.

11 Correlates Analysis Descriptive Tables by Case/Non-Case Status

The key tables summarizing the distribution of each of the five antibody markers are listed below. For each table, for each time point Day 1, Day 57 separately, the positive response rate with 95% CI, and the GMT or GMC with 95% CI, is reported for each of case and non-case groups. In addition, the point and 95% CI estimate of the difference in positive response rate (non-cases vs. cases) and the GMT or GMC ratio (non-cases/cases), is reported.

1. Antibody levels in the baseline SARS-CoV-2 negative per-protocol cohort (vaccine recipients). Cases are baseline negative per-protocol vaccine recipients with the symptomatic infection COVID-19 primary endpoint diagnosed starting 7 days after the Day 57 study visit. Non-cases/Controls are baseline negative per-protocol vaccine recipients sampled into the random subcohort with no evidence of SARS-CoV-2 infection up to the time of data cut.
2. Antibody levels in the baseline SARS-CoV-2 positive per-protocol cohort (vaccine recipients). Cases are baseline negative per-protocol vaccine recipients with the symptomatic infection COVID-19 primary endpoint diagnosed starting 7 days after the Day 57 study visit. Non-cases/Controls are baseline negative per-protocol vaccine recipients sampled into the random subcohort with no evidence of SARS-CoV-2 infection up to the time of data cut.
3. Antibody levels in baseline SARS-CoV-2 positive placebo recipients. Cases are baseline positive per-protocol placebo recipients with the symptomatic infection COVID-19 primary endpoint diagnosed any time after D1, after D29, or after D57 (by time of antibody measurement). Non-cases/Controls are baseline negative per-protocol vaccine recipients sampled into the random subcohort with no evidence of SARS-CoV-2 infection up to the time of data cut.
4. Repeat Table 2 above for fold-rise from baseline (of interest given the analysis cohort is baseline positive).

5. Repeat Table 3 above for fold-rise from baseline (of interest given the analysis cohort is baseline positive).

12 Correlates of Risk Analysis Plan

At first, this analysis plan for CoRs and CoPs as currently written focuses on the COVID primary endpoint, with its continuous failure times (failure time defined by the day of the event) and no competing risks. Later, it will be extended to handle the special issues with secondary endpoints.

12.1 CoR Objectives

The following CoR objectives are assessed in baseline seronegative vaccine recipients:

1. **Univariable CoR** To assess each individual Day 57 antibody marker as a CoR of outcome in vaccine recipients, adjusting for baseline factors
2. **Multivariable CoR** To build models predictive of outcome based on a set of Day 57 antibody marker readouts, adjusting for baseline factors

12.1.1 [With Day 29 markers]

If Day 29 markers are included, then a Univariable CoR objective is added, the same as above, except using the Day 29 versions of the markers instead of the Day 57 versions.

In addition, the Multivariable CoR objective is repeated to build models predictive of outcome based on a set of Day 29 antibody marker readouts. It is also repeated to build models predictive of outcome based on a set of Day 29 and 57 antibody marker readouts used together.

12.2 Outline of the Set of CoR Analyses

The univariable CoR objective is addressed by Cox proportional hazards regression and nonparametric threshold regression. The multivariable CoR objective is addressed by superlearning. All of these analyses are implemented in automated and reproducible press-button fashion.

In addition, supportive exploratory analyses of the univariable CoR objective are conducted using flexible parametric regression modeling: hinge/threshold regression and generalized additive model regression.

12.3 Day 57 Markers Assessed as CoRs and CoPs

The following five Day 57 markers are assessed as CoRs and CoPs, usually as quantitative variables and in some analyses as ordered trinary variables or binary variables, all of which do not subtract Day 1 (baseline) values:

1. binding Ab to Spike
2. binding Ab to RBD
3. pseudovirus neutralization ID50
4. pseudovirus neutralization ID80
5. live virus neutralization MN50

For all univariable CoR analyses (first objective), the non-baseline subtracted versions of the Day 57 antibody markers are studied; the baseline-subtracted versions are not studied given that the analyses are done in the baseline negative cohort for which Day 1 readouts will generally be negative. The multivariable machine learning CoR analyses include synthesis markers that combine information across the individual markers listed above, as well as including 2FR and 4FR versions of variables.

12.3.1 Inverse probability sampling weights used in CoR analyses

For CoR analyses that use inverse probability weighted complete-case (IP-WCC) methodology, only individuals in the case-cohort sample (i.e., with Day 1, 57 antibody markers measured) are included in the analysis. These methods incorporate inverse probability sampling (IPS) weights, defined based on the subcohort sampling strata (Table 5) and case/non-case status. Let $\pi(X) = P(R = 1|X, Y = 0)$, where R is the indicator that the phase-two marker data are available, X are the baseline demographic strata defining the stratified sampling, and $Y = 0$ means that the probability conditions on being a non-case. For sampling stratum x , the IPS weight w_x assigned

to a non-case participant in stratum x is defined by $\hat{w}_x = 1/\hat{\pi}(x) = N_x/n_x$, where N_x is the number of stratum x vaccine recipient non-cases in the Per-Protocol Baseline Negative Cohort and n_x is the number of these participants that also have Day 1, 57 marker data available. For non-case participant i in the random subcohort, $\hat{w}_i = 1/\hat{\pi}(X_i)$ denotes the weight \hat{w}_x for this individual's sampling stratum. All cases are assigned sampling weight N_1/n_1 where N_1 is the total number of vaccine recipient cases in the Per-Protocol Baseline Negative Cohort and n_1 is the number of these participants that also had the Day 1, 57 markers measured.

12.3.2 [With Day 29 markers]

If Day 29 markers are included, then the criterion for individuals being included in IPWCC analyses is expanded to also require that they have Day 29 markers measured (as well as Day 1, Day 57 markers measured). The same sampling weights are used for correlates analyses of Day 29 markers and of Day 57 markers.

12.3.3 Choice of regression methods

Time-to-event methods use the Day 57 visit date as the time origin.

The IPWCC Cox regression model designed for case-cohort sampling designs will be used for estimation and inference on hazard ratios of outcomes by Day 57 marker levels, and for estimation and inference on marginalized marker-conditional cumulative incidence over time. The models will be fit using the *survey* R package available on CRAN, and will adjust for the baseline factors. We use a method from the *survey* package that assumes without replacement two-phase sampling and not Bernoulli sampling, which matches the sampling design and approach to weight estimation (Lumley, 2010).

For models with a single antibody marker, a two-phase Cox model with improved efficiency through calibrated weights (Breslow et al., 2009b,a) will be used if there are baseline covariates that predict the antibody marker with $R^2 > 0.4$. Based on the phase-two sample, a superlearner model will be fit (using the library specified in Table 7) to predict the antibody marker

from the set of collected baseline covariates. The criterion $R^2 > 0.4$ will be checked based on the association of the fitted values from superlearner and the marker, where the association uses held-out marker values.

Based on the Cox model fit to all available data, a final time point t_F near the time of the last observed outcome will be defined. Let T be the failure time, S a Day 57 marker of interest, and X the vector of baseline factors that are adjusted for. With $S_1(t|s, x) = P(T > t|S = s, X = x, A = 1)$, the Cox model fit yields an estimate of $S_1(t|s, X_i)$ for each individual i in the phase-two sample. The marginalized conditional risk $risk_1(t|s) = E_X[P(T \leq t|s, X, A = 1)]$ through time t (for all times t through t_F simultaneously) is estimated based on the equation

$$risk_1(t|s) = \int (1 - S_1(t|s, x))dH(x) \quad (1)$$

where $H(\cdot)$ is the distribution of X in $A = 1$ individuals.

The function $risk_1(t|s)$ can be estimated by

$$\widehat{risk}_1(t|s) = \frac{\sum_{i=1}^n \frac{1}{\hat{\pi}(X_i)} (1 - \hat{S}_1(t|s, X_i))}{\sum_{i=1}^n \frac{1}{\hat{\pi}(X_i)}}, \quad (2)$$

where n is the number of participants with phase-two data.

The bootstrap is used to obtain 95% pointwise confidence intervals for $risk_1(t_F|s)$.

The bootstrap process will be performed by stratifying on the two-phase sampling strata and random subcohort. Across all bootstrap samples, the number of subjects in each stratum in the random subcohort remains fixed, but the number of cases does not stay the same.

The results of the above Cox modeling will be output in a variety of ways:

1. Plot $\widehat{risk}_1(t_F|s)$ vs. s with 95% CIs for continuous $S = s$ varying over its whole range. Include on the plot the estimate of $\widehat{risk}_0(t_F)$ with a 95% CI for the placebo arm (horizontal bands), computed by IPS weighted Kaplan-Meier analysis.

2. Based on a fit of the Cox model to a nominal categorical antibody marker defined as the tertiles of S , plot $\widehat{risk}_1(t|s)$ for each category of S values with 95% CIs, for all time points t from Day 57 through t_F . If more than 20% of vaccine recipients have S below the LLOQ of the assay, then the categories instead will be (1) values \leq LLOQ; (2) values below the median of values $>$ LLOQ; (3) values above the median of values $>$ LLOQ. Include on the plot the estimated curve $\widehat{risk}_0(t)$ with 95% CIs for the placebo arm, computed by IPS weighted Kaplan-Meier analysis.
3. Tabular reporting of the hazard ratio per 10-fold change in the quantitative Day 57 antibody marker with 95% confidence interval and 2-sided p-value
4. Tabular reporting of the hazard ratio for the Middle and Upper categories of the categorical Day 57 antibody marker vs. the Lower category, with 95% confidence interval and 2-sided p-value, as well as a global generalized Wald two-sided p-value for whether the hazard rate of the endpoint varies across the three categories. The table includes a point estimate and 95% CI for the cumulative incidence of the endpoint through t_F for the placebo arm.
5. Report point and 95% CI estimates for the hazard ratio per 10-fold change in the Day 57 antibody marker, for the entire baseline negative vaccine cohort and for each of the baseline demographic strata subgroups defined in Table 6 (reported via forest plotting).

Grambsch and Therneau (1994) tests are applied to test veracity of the proportional hazards assumption. This testing is done to aid interpretation of results, but not as a gateway to trigger the fitting of a more flexible version of the Cox model, as we seek to avoid computing new confidence intervals and p-values contingent on goodness-of-fit-testing, as they would not have their correct interpretations. Other correlates of risk methods explicitly model risk flexibly as a function of the marker.

The bootstrap is used to calculate 95% pointwise CIs for $risk_1(t_F|s)$ in s . The 2-sided Wald p-value for testing the regression coefficient of the marker in the Cox model provides a valid test of the null hypothesis $H_0 : risk_1(t_F|s) =$

$risk_1(t_F)$ for all s , and is reported.

In addition, the same Cox model analysis will be used to estimate the alternative marginalized conditional risk parameter defined by $risk_1(t|S \geq s)$ where $risk_1(t|S \geq s) = E_X[P(T \leq t|S \geq s, X, A = 1)]$, which can be estimated by

$$\widehat{risk}_1(t|S \geq s) = \frac{\sum_{i=1}^n \frac{1}{\hat{\pi}(X_i)} (1 - \hat{S}_1(t|S \geq s, X_i))}{\sum_{i=1}^n \frac{1}{\hat{\pi}(X_i)}}.$$

This parameter is useful because typically subgroups of interest are defined by having marker response above a threshold. We will plot $\widehat{risk}_1(t_F|S \geq s)$ vs. s with 95% CIs for continuous S with s varying over the range of S in which the number of cases to estimate $\hat{S}_1(t|S \geq s, X_i)$ is 5 or more. This type of analysis is also included because it analyzes the same parameter as the nonparametric threshold estimation method described below, providing a way to address the threshold question both by Cox modeling and by nonparametric analysis.

If the outcome under study is subject to competing risks, then the Cox model is fit in the same way, except counting the competing risk as right-censoring. Now the parameter being estimated is the marginal conditional cumulative incidence function $risk_1(t, 1|s) = E_X[P(T \leq t, J = 1|s, X, A = 1)]$ where $J = 1$ is the outcome of interest. Now, $risk_1(t, 1|s)$ can be written as

$$\begin{aligned} risk_1(t, 1|s) &= \int \int_0^t \lambda_1(t, 1|s, x) S_1(t'|s, x) dt' dF_1(x|s) \\ &= \frac{\int \int_0^t \lambda_1(t, 1|s, x) S_1(t'|s, x) dt' f_1(s|x) dH(x)}{\int f_1(s|x) dH(x)} \end{aligned} \quad (3)$$

where $\lambda_1(t, 1|s, x) = \lambda_{01}(t|A = 1)e^{\beta_1^T(s, x)}$ is the cause-specific hazard function (Prentice et al., 1978). Estimation of $risk_1(t, 1|s)$ could be done by nonparametric kernel smoothing for estimation of $\lambda_{01}(t|A = 1)$. To avoid kernel smoothing (and bandwidth dependence), we will instead base estimation on the equation

$$risk_1(t, 1|s) = \frac{\int risk_1(t, 1|s, x) f_1(s|x) dH(x)}{\int f_1(s|x) dH(x)} \quad (4)$$

where the Fine-Gray proportional subdistribution hazards model is used to estimate $risk_1(t, 1|s, x)$ (implemented in the *cmprsk* R package available on CRAN). While we prefer the interpretation of cause-specific hazards to subdistribution hazards as in Fine-Gray, in a (very) rare event situation such as in the COVID-19 VE trials (vaccine arm), the Fine-Gray method is expected to give similar answers, except it is more easily implemented. Therefore, we estimate $risk_1(t, 1|s)$ by

$$\widehat{risk_1}(t, 1|s) = \frac{\sum_{i=1}^n \frac{1}{\hat{\pi}(X_i)} \hat{risk}_1(t, 1|s, X_i) \hat{f}_1(s|X_i)}{\sum_{i=1}^n \frac{1}{\hat{\pi}(X_i)} \hat{f}_1(s|X_i)}. \quad (5)$$

As for the analyses without competing risks, the bootstrap is used for calculating 95% confidence intervals and for testing $H_0 : risk_1(t_F, 1|s) = risk_1(t_F, 1)$ for all s .

Similarly, point and 95% CIs for $risk_1(t_F, 1|S \geq s)$ are computed.

12.3.4 Univariate CoR: Nonparametric threshold regression modeling

The van der Laan et al. (2020) extension of the nonparametric CoR threshold estimation method of Donovan et al. (2019) is applied to each of the five non-baseline subtracted Day 57 antibody markers, using the version accounting for right-censoring of some follow-up times, assessing failure through the fixed time point t_F . The analyses adjust for the same baseline factors X as used in the Cox model CoR analyses.

The extension adjusts for baseline covariates by estimating the conditional mean function $E[I(T \leq t_F)|S \geq s, X, A = 1]$ using discrete-SuperLearner and then empirically averaging over the baseline covariates X to estimate the marginal risk $risk_1(t_F|S \geq s) = E_X[I(T \leq t_F)|S \geq s, X, A = 1]$ for each threshold s of the the antibody marker in a specified discrete set. We do not perform pooled regression across the thresholds s , which ensures we are totally nonparametric in estimating the threshold dependence of $E_X[I(T \leq t_F)|S \geq s, X, A = 1]$ on s . The SuperLearner library includes a range of increasingly flexible parametric learners including logistic regression (glm), bayesian logistic regression (bayesglm), and L1-penalized logistic regression

(glmnet). (Two of each learner is included in the library, one with only main-term variables and another with main-term and interaction variables.)

An advantage of the nonparametric CoR threshold method compared to Cox modeling that specifies a log linear hazard ratio with the marker is that it can potentially detect a threshold of very low risk.

The results are reported in the same way that Donovan et al. (2019) reports results in its Figure 2, where point estimates and simultaneous 95% confidence bands for $risk_1(t_F|S \geq s)$ are plotted for a range of threshold values (the simultaneous confidence bands cover the entire curve in s with at least 95% probability). The method uses the same empirical two-phase sampling estimated weights (IPS weights) as used for the other univariable IPWCC CoR analyses. In addition, for each pre-specified risk threshold c set to take values over a grid between 0 and the estimated outcome rate in placebo recipients, the method is applied to estimate the inverse function $s_c = \inf\{s : E_X[I(T \leq t_F)|S \geq s, A = 1, X] \leq c\}$, where s_c is estimated by substitution of the marginal risk function estimate. Note that the substitution estimator of s_c requires that the marginal risk function is estimated for all thresholds, which is computationally infeasible. Instead, we estimate the marginal risk function on a sufficiently large discrete set and linearly interpolate to obtain marginal risk estimates for all thresholds outside the discrete set. In order for this estimand to be well defined, we operate (for this estimand only) under the assumption that $s \mapsto E_X[I(T \leq t_F)|S \geq s, A = 1, X]$ is monotone. For the substitution-based estimator of the inverse function s_c to be well-defined, we require the estimate of $s \mapsto E_X[I(T \leq t_F)|S \geq s, A = 1, X]$ to be monotone as well. If there is evidence that the function estimate is not monotone then we replace the estimate with its monotone projection, which preserves its theoretical properties (Westling, van der Laan, Carone, 2020).

A plot of point and simultaneous 95% confidence interval estimates of s_c (over the grid of c values) is provided to help indicate marker thresholds defining subgroups with very low risk of outcome. The confidence interval estimates for s_c are derived directly from the simultaneous 95% confidence

band estimates for the marginal risk function $s \mapsto E_X[I(T \leq t_F)|S \geq s, A = 1, X]$, and therefore its estimates and inference are compatible with those of the marginal risk function. In particular, no multiple testing adjustments are needed.

The analysis is done using targeted maximum likelihood estimation (TMLE) as described in van der Laan, Zhang, and Gilbert (2020), and the simultaneous confidence bands are of the Wald-type, obtained from the asymptotic distribution of the TMLE.

12.4 Univariable CoR: Supportive Exploratory Flexible Parametric Risk Modeling

For each of the five non-baseline subtracted Day 57 antibody markers, flexible nonlinear modeling of outcome risk studied as a dichotomous outcome Y will be conducted, as exploratory supportive analyses. Again, the analyses adjust for the same baseline factors X as used in the Cox model CoR analyses.

The nonlinear relationship between the logit of risk and markers will be modeled using two-phase polynomial regression models (Son and Fong, 2020; Fong et al., 2017), e.g., hinge model, or three-phase segmented models (Chen, 2020). The mean function of a q^{th} order two-phase polynomial regression model can be expressed as follows:

$$\eta(s, X) = \alpha_1 + \alpha_2^X z + \beta_{1,-}(s - e)_- + \beta_{1,+}(s - e)_+ + \beta_{2,-}(s - e)_-^2 + \beta_{2,+}(s - e)_+^2 + \cdots + \beta_{q,-}(s - e)_-^q + \beta_{q,+}(s - e)_+^q,$$

where X is the baseline covariate vector, s is a fixed value of the immunologic marker of interest, e is the threshold parameter, $(s - e)_+ = s - e$ if $s > e$ and 0 otherwise, and $(s - e)_- = 0$ if $s > e$ and $s - e$ otherwise.

In addition, a generalized additive model with degree of smoothing estimated by cross-validation will be employed (Wood, 2017).

12.4.1 [With Day 29 markers]

For CoR analyses of Day 29 markers, the same analyses are done, except using the Day 29 visit date as the time origin and counting events starting 7

days after the Day 29 visit.

12.4.2 P-values and Multiple hypothesis testing adjustment for CoR analysis

In general, p-values are only reported from pre-specified and automated (press-button) analyses. For the CoR analyses, p-values are reported for the univariable Cox regression analyses of the five specified Day 57 antibody marker variables. Two-sided p-values for hypothesis testing of a Day 57 marker CoR are calculated both for the Cox regression of quantitative markers (two-sided Wald tests), and for the Cox regression of markers binned into tertiles (two-sided Generalized Wald tests). Therefore a total of ten 2-sided p-values for CoRs are calculated.

It is not completely clear whether to perform multiple hypothesis testing adjustment, given the expectation that the correlations among the markers are high, and possibly very high, meaning that multiplicity correction could incur a relatively high cost on the false negative error rate.

However, given that robust evidence supporting an antibody marker as a CoR will be required for qualifying a marker, we will conduct multiplicity adjustment for CoR analysis, as the ability to make an inference that a marker passed pre-specified multiplicity adjusted criteria should aid an overall evidence package for establishing a validated or non-validated surrogate endpoint. Therefore, multiplicity adjustment is performed across the ten 2-sided p-values.

A permutation-based method ([Westfall et al., 1993](#)) will be used for both family-wise error rate (Holm-Bonferroni) and false-discovery rate (q-values; Benjamini-Hochberg) correction. 10^4 replicates of the data under the null hypotheses will be created by randomly resampling the immunologic biomarkers with replacement. For each Cox regression CoR analysis the unadjusted p-value, the FWER-adjusted p-value, and the q-value is reported for whether there is a covariate-adjusted association, where all p-values and q-values are 2-sided. As a guideline for interpreting CoR findings, markers with FWER-adjusted p-value ≤ 0.05 are flagged as having statistical evidence for being a CoR. Additionally, markers with unadjusted p-value ≤ 0.05 and q-value \leq

0.10 are flagged as having a hypothesis generated for being a CoR.

As described in this SAP, the FWER adjustment is done for all advanced Day 57 markers along bAb Spike, bAb RBD PsV nAb ID50, PsV nAb ID80, and WT LV MN50. If the antibody data set available for correlates analysis does not yet contain the WT LV MN50 data (due to a longer time horizon on performing the assay), then no multiplicity adjustment will be performed in the report. That is, the first report with multiplicity adjustment will wait until data from all three assay types are included. This is done so as to avoid repeated multiplicity adjustment that would introduce complexity in interpretation.

12.4.3 [With Day 29 markers]

If Day 29 markers are included, the same multiplicity adjustment approach is used as for Day 57 markers. The multiplicity adjustment is done separately for Day 29 markers and for Day 57 markers, given the high degree of correlation of the analysis results (given that all endpoint cases starting 7 days post Day 57 are common among the two analyses). The results on Day 29 markers is considered to be a supportive analysis.

12.5 Multivariable CoR: Superlearning of Optimal Risk Prediction Models

12.5.1 Objectives

The multivariable CoR objective is addressed through two sub-objectives: first to build an ‘estimated optimal surrogate,’ (Price et al., 2018) a model that best predicts the outcome from Day 57 antibody markers and baseline factors. The second sub-objective is estimation and inference on variable importance measures for each Day 57 antibody marker, for ranking of antibody markers by their importance/influence on predicting risk. The analysis plan is patterned off of the analysis of the HVTN 505 HIV-1 vaccine efficacy trial (Neidich et al., 2019). For these analyses both baseline-subtracted and non-baseline subtracted versions of the Day 57 markers are used, in a broader unbiased analysis to build models most predictive of outcome.

12.5.2 Input variable sets

Day 57 antibody markers are classified into the following four antibody marker variable sets, with individual variables listed within categories:

1. Binding antibody anti-Spike (S-bAb)
 - a Day 57 anti-Spike IgG concentration
 - b delta anti-Spike IgG concentration
 - c indicator 2FR anti-Spike IgG concentration
 - d indicator 4FR anti-Spike IgG concentration
2. Binding antibody anti-RBD (RBD-bAb)
 - (a) Day 57 anti-RBD concentration
 - (b) delta anti-RBD concentration
 - (c) indicator 2FR anti-RBD concentration
 - (d) indicator 4FR anti-RBD concentration
3. Pseudovirus neutralizing antibody anti-Spike (pseudovirus-nAb)
 - a Day 57 anti-Spike ID50
 - b Day 57 anti-Spike ID80
 - c delta anti-Spike ID50
 - d delta anti-Spike ID80
 - e indicator 2FR anti-Spike ID50
 - f indicator 4FR anti-Spike ID50
 - g indicator 2FR anti-Spike ID80
 - h indicator 4FR anti-Spike ID80
4. Wild Type Live virus neutralizing antibody anti-Spike (WT live virus-nAb)
 - a Day 57 anti-Spike MN50

- b delta anti-Spike MN50
- c indicator 2FR anti-Spike MN50
- d indicator 4FR anti-Spike MN50

The baseline factors comprise a fifth set of variables to include in the super-learner modeling.

12.5.3 Missing data

We expect a very small amount of missing data from the five antibody marker types (bAb Spike, RBD; pseudovirus-nAb ID50, ID80; WT live virus-nAb MN50). However, there may be a small amount of missing data, with possibly different participants missing data for different markers. We take the following approach to handle any missing data that occurs.

First, we define the two-phase sampling indicator ϵ as taking value of one if a participant has Day 1 and Day 57 bAb data for both Spike and RBD, where here we assume that the MSD platform is highly robust such that it will have nearly 100% complete data for sampled participants. Second, for the other four marker types (pseudovirus-nAb ID50, ID80; WT live virus-nAb MN50), for participants with $\epsilon = 1$ but the Day 1 and/or Day 57 marker value is missing, we use single imputation to fill in any missing values, ignoring the uncertainty in the imputations in the analysis, because it should have negligible impact on results given the (very) small amount of missing data. Multiple linear regression will be used to impute missing values, separately for each antibody marker, based on the set of individuals with that antibody marker measured at Day 1 and 57. Accurate imputations are possible given the high correlations of the markers, especially between ID50 and ID80 within the same immunoassay. This process means that the two-phase data set has a simple ‘all-or-nothing’ missing data pattern where participants with $\epsilon = 1$ have all markers with Day 1 and Day 57 data, and are included in IPWCC analyses, and participants with $\epsilon = 0$ have some or all markers missing and are excluded from IPWCC analyses. This means that all IPWCC data analyses can use the same empirical frequency (IPS) sampling weights.

For analysis methods that use the whole cohort (phase-one plus phase-two data), the same phase-two data as described above are used. If some of the phase-one baseline factors that are adjusted for variables are missing with only a small amount of missing values, then single imputation will be used to fill in the values, and, as for the immunologic marker imputations, the uncertainty in the imputations will be ignored in the analyses. Simple average values will be used to fill in baseline covariate missing values of the baseline factors.

12.5.4 Implementation of superlearner

For risk score development, Superlearner is applied to the placebo arm only. For multivariable immune correlates of risk/estimated optimal surrogate development, Superlearner is applied to the vaccine arm only. The following details are used in the implementation of superlearner of the vaccine arm only:

- Pre-scale each quantitative and ordinal variable to have empirical mean 0 and standard deviation 1.
- For the immune correlates analysis, the final library of learners is selected accounting for the number of phase-two endpoint cases in the vaccine arm. If the number of cases is limited, at or near 25 evaluable endpoint cases, then the modeling will only allow learning algorithms to have a maximum of 5 Day 57 antibody marker variables, and will use leave-one-out cross-validation and the negative log-likelihood loss function, a combination that tends to provide good performance in small sample size settings. On the other hand, if there are larger numbers of endpoint cases in the vaccine arm, then 5-fold cross-validation will be used, and no more than $\text{floor}(n_v/6)$ input variables will be used in the model where n_v is the number of evaluable vaccine endpoint cases. The choices will be finalized prior to case/control unblinded analysis.
- Include learning algorithms with and without screening of variables. Screens used will be: 1) glmnet (lasso) pre-screening (with default tuning parameter selection), 2) logistic regression univariate 2-sided p-value

screening (at level $p < 0.10$), and 3) high-correlation variable screening (described below). The adaptive algorithms (SL.randomForest, SL.xgboost, SL.gam, SL.polymars) are only used with these screens, given that the limited number of endpoint cases may challenge use of these methods with no variable screening. Moreover, the adaptive algorithms are not used if there are only 25 (or close to it) endpoint cases. All of the selected learners are coded into the SuperLearner R package available on CRAN.

- Include high-correlation variable screening, not allowing any pair of input variables to have Spearman rank correlation $r > 0.9$. When a pair of variables has $r > 0.9$, the variable with the highest ranked signal-to-noise ratio (i.e., biological dynamic range) is selected; if these data are not available or there is a tie then variables are selected in the following order of priority: first WT live virus-nAb, second pseudovirus-nAb, third bAb.
- The superlearner is conducted averaging over 10 random seeds, to make results less dependent on random number generator seed.
- All of the learners are implemented with weighting (by the IPS weights $w_i = 1/\hat{\pi}(X_i)$ described in Section 9.1) to account for the two-phase sampling design.
- Two levels of cross-validation are used:
 - Outer level: CV-AUC computed over 5-fold cross-validation repeated 10 times to improve stability
 - Inner level: leave-one-out CV used to estimate ensemble weights (if n_v is near 25) and 5-fold CV if n_v is larger.
- Results for comparing classification accuracy of different models are based on point and 95% confidence interval estimates of cross-validated area under the ROC curve (CV-AUC) and difference in CV-AUC as a predictiveness metric (Hubbard et al., 2016; Williamson et al., 2020). Results are presented as forest plots of point and 95% confidence interval estimates similar to those used in Neidich et al. (2019) (Figure 3) and Magaret et al. (2019). CV-AUC is estimated using the R package *vimp*

available on CRAN, including the IPS weights that are used for other data analyses.

For the risk score SuperLearner analysis of the placebo arm, the same approach is used, with the following modifications: (1) 5-fold cross-validation will be used with no more than $\max(20, \text{floor}(n_p/20))$ input variables included in each model, where n_p is the number of evaluable placebo arm cases; (2) no IPS weighting is needed.

Table 7 lists the learning algorithms that are applied to estimate the conditional probability of the outcome based on the input variable sets considered above. Most of the algorithms are non-data-adaptive type learning algorithms, such as parametric regression models (e.g., glms), which are simple and stable and advantageous for an application with limited number of endpoint events. Data-adaptive type algorithms are also included, for increasing flexibility of modeling: SL.randomForest, SL.gam, SL.polymars, and SL.xgboost. All of the selected learners are coded into the SuperLearner R package.

Before fitting the superlearner models to the vaccine arm data, a decision will be made on how to define the “baseline risk factors” input variable set. To define this set, the performance of the SuperLearner model for all individual baseline demographic/potential exposure variables will be compared to that of the Superlearner model baseline risk score built as described above. Examining the results, the set of baseline risk factors will include a subset of individual risk factors and/or the baseline risk score itself.

For the immune correlates objective the superlearner model is fit to each of the following 12 variable sets, with immunological variables listed in Section 12.5.2:

1. Baseline risk factors
2. Baseline risk factors and the bAb anti-Spike markers
3. Baseline risk factors and the bAb anti-RBD markers
4. Baseline risk factors and the pseudovirus-nAb ID50 markers

5. Baseline risk factors and the pseudovirus-nAb ID80 markers
6. Baseline risk factors and the live virus-nAb MN50 markers
7. Baseline risk factors and the bAb markers and the pseudovirus-nAb ID50 markers
8. Baseline risk factors and the bAb markers and the pseudovirus-nAb ID80 markers
9. Baseline risk factors and the bAb markers and the live virus-nAb MN50 markers
10. Baseline risk factors and the bAb markers and the combination scores across the five markers [PCA1, PCA2, FSDAM1/FSDAM2 (the first two components of nonlinear PCA), and the maximum signal diversity score [He and Fong \(2019\)](#)].
11. Baseline risk factors and all individual marker variables
12. Baseline risk factors and all individual marker variables and all combination scores (full model)

Therefore in total, 12 variable sets are studied. The reason to include the first variable set is to investigate how much incremental improvement in predicting outcome is obtained by adding antibody marker variables on top of baseline demographic/exposure factors. The other variable sets are designed to compare the four immunoassay types by their predictiveness, to compare the two pseudovirus neutralization readouts ID50 and ID80 for their predictiveness, and to investigate incremental predictive value in using multiple immunoassays. The final variable set is included as the full model that considers all variables together, which serves as another reference model.

Table 7: Learning Algorithms in the Superlearner Library of Estimators of the Conditional Probability of Outcome¹.

Algorithms	Screens/ Tuning Parameters
SL.mean	None
SL.glm	Low-collinearity and (All, Lasso, LR)
SL.bayesglm	Low-collinearity and (All, Lasso, LR)
SL.glm.interaction	Low-collinearity and (All, Lasso, LR)
SL.glmnet	Low-collinearity and (alpha=0, 0.33, 0.67, 1; All, Lasso LR)
SL.gam	Low-collinearity and (Lasso, LR)
SL.nnet	Low-collinearity and (Lasso, LR)
SL.ksvm	Low-collinearity and (kernel="rbfdot", "polydot") and (Lasso, LR)
SL.polymars	Low-collinearity and (Lasso, LR)
SL.xgboost	Low-collinearity and (maxdepth, shrinkage)=(2,0.05),(2,0.1),(4,0.05),(4,0.1) and (Lasso, LR)
SL.randomForest	Low-collinearity and (Lasso, LR)

¹All continuous and ordinal covariates are pre-standardized to have empirical mean 0 and standard deviation 1.

²**All** = include all variables; **Lasso** = include variables with non-zero coefficients in the standard implementation of SL.glmnet that optimizes the lasso tuning parameter via cross-validation; **Low-collinearity** = do not allow any pairs of quantitative variables with Spearman rank correlation > 0.90; **LR** = Univariate logistic regression Wald test 2-sided p-value < 0.10.

In order to evaluate the relative performance of the superlearner estimated models for each of the 12 variable sets, derived using the learning algorithms specified in Table 7, the CV-AUC is estimated with a 95% confidence interval (Hubbard et al., 2016; Williamson et al., 2020). The point and 95% confidence interval estimates of CV-AUC are reported in a forest plot, which provide a way to discern which Day 57 antibody assays and readouts provide the most information in predicting COVID or other outcomes. The specified library of learners may be modified prior to SAP finalization (before breaking the blind of case/non-case status). As noted above CV-AUC is estimated using the R package *vimp* available on CRAN, which uses augmented inverse probability weighting to properly estimate CV-AUC accounting for the two-phase sampling design.

In addition, for selected variable sets, similar forest plots will be made com-

paring performance of the various estimated models (e.g., by individual learning algorithm types such as lasso), including discrete superlearner and superlearner models. The plot will be examined to determine which individual learning algorithm types are performing the best. If there is an interpretable algorithm that has performance close to the best-performing algorithm (which is most likely to be the superlearner), then it will be fit on the entire data set of vaccine recipients and the estimated model presented in a table.

Cross-validated ROC curves are plotted for the superlearner estimated models for each of the input variable sets. In addition, boxplots of cross-validated estimated probabilities of outcome by case-control status (as estimated from the superlearner models) are plotted.

12.5.5 [With Day 29 markers]

If Day 29 markers are included, then all multivariable CoR models continue to use the primary analysis cohort and to count events starting 7 days after the Day 57 visit. Therefore, participants identified to be cases anytime before 7 days after the Day 57 visit are excluded from all multivariable CoR superlearning analyses.

Regarding the 12 variable sets listed above, additional variable sets will be included to represent Day 29 markers as input variables. In particular, variable sets 2–12 will only include Day 57 markers. These variable sets will be cloned into 11 new sets (13–23) including Day 29 markers in place of Day 57 markers. Lastly, another 11 variable sets (24–34) will be formed with the same structure, where each input variable set includes both Day 29 and Day 57 markers. This allows the Superlearning modeling to address whether including markers from both time points improves prediction of outcome. Therefore, if Day 29 markers are included, the number of input variable sets is 34 instead of 12.

13 Correlates of Protection: Generalities

In general, for all of the correlate of protection analyses, the same antibody markers are assessed that were analysed as correlates of risk: the Day 57

antibody markers not subtracting for the Day 1 baseline readout are used. Each of the five Day 57 antibody biomarkers are separately studied as CoPs by the different analysis approaches summarized below.

13.0.1 [With Day 29 markers]

If Day 29 markers are included, then the same CoP analyses are done as for each of the five Day 57 markers, where, as for the CoR analyses, now the time origin is the Day 29 visit date and endpoint cases are counted starting 7 days after the Day 29 visit date.

14 Correlates of Protection: Correlates of Vaccine Efficacy Analysis Plan

For each of the five Day 57 antibody biomarkers, the method of Gilbert, Blette, Shepherd, and Hudgens (2020) will be used to estimate $VE(1)$, $VE(0)$, and $VE(1) - VE(0)$, each with a 95% confidence interval and a 95% estimated uncertainty interval (EUI), where $VE(1)$ is vaccine efficacy for the subgroup of vaccine recipients with Day 57 marker if assigned vaccine above a specified cut-point value s_{cut} , and $VE(0)$ is vaccine efficacy for the subgroup of vaccine recipients with Day 57 marker if assigned vaccine not greater than s_{cut} . The analysis will be done under the **NEE** assumption (“no early-effect”) of Gilbert et al. (2020), unless the data support positive vaccine efficacy before Day 57, in which case the analysis will be done under the **NEH** assumption (“no early harm”) of Gilbert et al. (2020). The cut point is defined as the percentile equal to one minus the estimated vaccine efficacy in the primary analysis, with logic that a maximally simple version of a perfect CoP would have binary marker with $S = 1$ corresponding to protection and $S = 0$ corresponding to no protection. We will repeat the analysis using two additional cut-points that creates greater balance in frequencies of $S = 1$ and $S = 0$ in the vaccine group random subcohort. This analysis method does not require closeout placebo vaccination (CPV) (Follmann, 2006) or a good baseline immunogenicity predictor of the Day 57 antibody marker. The method is implemented using Bryan Blette’s R package “psbinary” posted at

his Github repository.

A limitation of the Gilbert et al. method is that it only assesses a binary biomarker. Other analyses will be considered to estimate $VE(s)$ over biomarker values s over the entire range, treating S as a quantitative or categorical variable, and gaining efficiency by incorporating CPV and/or putative baseline immunogenicity predictors (BIPs). Because of the rarity of the COVID-19 event in vaccine recipients, simulations suggest limited ability of CPV to enhance statistical power (as seen in many simulation studies, e.g., [Follmann, 2006](#); [Huang et al., 2013](#)), such that the key for improving efficiency will be the availability of a BIP. VE curve analysis for continuous S will thus be conducted contingent on the availability of a BIP that satisfies the R^2 criterion outlined in Table 8.

Let $Y(a)$ denote the potential binary outcome of interest if receiving intervention a , with $a = 1, 0$ standing for assignment to vaccine and placebo, respectively. Let $S(a)$ denote the potential biomarker value if receiving intervention a . The vaccine efficacy curve ([Follmann, 2006](#); [Gilbert and Hudgens, 2008](#)) is defined as the curve of vaccine efficacy as a function of the immune response biomarker if assigned vaccination (i.e., $S(1)$): $VE(s) = 1 - P(Y(1) = 1 | S(1) = s) / P(Y(0) = 1 | S(1) = s)$. It characterizes the percentage reduction in clinical risk under vaccine assignment compared to under placebo assignment conditional on $S(1)$ and informs about the magnitude of potential immune response associated with certain levels of VE. Consider the existence of BIPs X correlated with $S(1)$ and/or a CPV component in the trial where a subset of placebo recipients free of the outcome are vaccinated and have their immune response biomarkers measured as substitutes for $S(1)$. Under the NEE assumption and assuming the set of participants with $S(1)$ available is nested within the set of participants with BIP measures, the pseudo-score estimation method ([Huang et al., 2013](#); [Zhuang et al., 2019](#)) based on discrete BIP measures allowing for adjustment of X will be adopted for estimating the risk model $P(Y(z) = 1 | S(1), X)$ and subsequently $VE(s) = 1 - \int P(Y(1) = 1 | S(1), x) dF_X(x | S(1)) / \int P(Y(0) = 1 | S(1), x) dF_X(x | S(1))$. Hypothesis testing will be conducted for testing the null hypothesis that the VE curve is constant ([Zhuang et al., 2019](#)). Estimated parametric ([Gilbert and Hudgens,](#)

2008), semiparametric (Huang and Gilbert, 2011), or nonparametric (Li and Luedtke, 2020) likelihood estimators of VE curves will be applied to continuous BIPs. In scenarios where some BIPs are not measured from all trial participants, VE curve estimators accounting for this monotone missingness in X and $S(1)$ will be adopted (Huang, 2018). If the data support positive vaccine efficacy before Day 57, sensitivity analysis approaches will be conducted for VE curve estimation under the NEH assumption. In the presence of multiple candidate biomarkers and when a CPV component is present, a multiple imputation approach as proposed in Dasgupta and Huang (2019) will be utilized to impute missing $S(1)$ data for selecting markers from multiple candidates and deriving a univariate marker score for VE curve estimation.

Finally, for scenarios with very rare events such that methods described above lack precision even with a CPV component but where the available BIP still satisfies the R^2 criterion outlined in Table 8, we will adopt sensitivity analysis methods that model the placebo risk conditional on the counterfactual $S(1)$ based on a sensitivity parameter that varies over some pre-specified range.

Among different strategies to identify BIPs, the following will be tried. First, for vector vaccines, we will study Day 1 bAb or nAb response to the vector as a BIP for the Day 57 markers of interest. Second, we will check whether Day 1 bAb or nAb to Nucleocapsid protein is a BIP for the anti-Spike/anti-RBD Day 57 markers of interest. The rationale for this latter analysis is that some studies have shown cross-reactive responses to Nucleocapsid protein and to common circulating human coronaviruses. We will also evaluate using a multivariate BIP that corresponds to all of these aforementioned candidate univariate BIPs, which may help to achieve the target R^2 (see Table 8).

15 Correlates of Protection: Interventional Effects

In these analyses, we seek to understand whether, how, and to what extent Day 57 antibody markers impact vaccine efficacy in causal ways. We describe three approaches to this problem. Each involves consideration of a binary counterfactual outcome $Y(a, s)$ (e.g., indicator of the COVID disease endpoint by a pre-specified time) under a hypothetical intervention that

both sets randomization assignment $A = a$ and sets the Day 57 immunologic marker S to a fixed value or based upon a random draw from a analyst-specified distribution. Below, we assume that S is scalar-valued, but some of the approaches below naturally extend to the case where a vector of immunologic markers are considered (currently such analyses are not planned). Given the central goal to develop a parsimonious surrogate endpoint based on a single immunoassay, the main analysis will use each of the methods to assess each of the five quantitative readouts (not baseline-subtracted) separately as CoPs, adjusting for the same set of baseline covariates as used in the CoR analyses previously described in Section 12.

15.1 CoP: Controlled Vaccine Efficacy

We first describe the controlled vaccine efficacy curve defined as

$$\text{CVE}(s) = 1 - \frac{P(Y(1, s) = 1)}{P(Y(0) = 1)}.$$

The value $\text{CVE}(s)$ takes represents the relative decrease in endpoint frequency achieved by administering vaccine and setting Day 57 immunologic marker level to s compared to the placebo control intervention. Under our approach, the value of $\text{CVE}(s)$ is assumed to be monotone non-decreasing in s ; in other words, vaccine efficacy can only potentially be improved by setting greater marker levels. The extent to which the marker plays a role in determining vaccine efficacy can be determined by the degree of flatness of the graph of $\text{CVE}(s)$ versus s .

In addition, because the primary study cohort for correlates analysis is naive to SARS-CoV-2, each of the Day 57 markers S has no variability in the placebo arm [all values are ‘negative,’ below the assay lower limit of detection (LLOD)]. Therefore, advantageously in this setting $\text{CVE}(s)$ has a special connection to the mediation literature, where $\text{CVE}(s = \text{LLOD})$ is the natural direct effect, and vaccine efficacy is 100% mediated through S if and only if $\text{CVE}(s = \text{LLOD}) = 0$. Thus inference on $\text{CVE}(s = \text{LLOD})$ evaluates full mediation.

Since $P(Y(0) = 1) = P(Y = 1 | A = 0)$ in view of vaccine versus placebo ran-

domization, the controlled vaccine efficacy $\text{CVE}(s)$ at level s can be identified using the fact that

$$P(Y(1, s) = 1) = E[P(Y = 1 \mid S = s, A = 1, X)]$$

whenever $Y(1, s)$ and S are independent given $A = 1$ and a vector X of covariates, and $P(S = s \mid A = 1, X) > 0$ almost surely. In other words, identification of the controlled vaccine efficacy requires that a rich enough set of covariates be available so that deconfounding of the relationship between endpoint Y and marker S is possible in the subpopulation of vaccine recipients, and that marker level $S = s$ may occur within each subpopulation defined by values of the covariates X (positivity).

For each s , the identified parameter corresponding to $\text{CVE}(s)$ is an irregular parameter within nonparametric models, making its estimation at root- n rate impossible; this significantly complicates estimation and inference on $\text{CVE}(s)$. Fortunately, the monotonicity of $s \mapsto \text{CVE}(s)$ provides an opportunity to circumvent these difficulties. Similarly to [Westling et al. \(2020a\)](#)'s approach for the causal dose-response function, we will use the general methodological template proposed in [Westling and Carone \(2020\)](#) to derive (i) a nonparametric Grenander-type estimator of $\text{CVE}(s)$ and (ii) a plug-in confidence interval for $\text{CVE}(s)$ based on an asymptotic Chernoff limit. This estimator will require, as an intermediate step, estimation of several nuisance functions, including the outcome regression $P(Y = 1 \mid S = s, A = 1, X = x)$ and the propensity score $P(S = s \mid X = x, A = 1)$. These nuisance functions will be estimated using the Superlearner ensembling algorithm with a rich library including both parametric regression methods as well as flexible machine learning tools.

The monotonicity-based procedure we will develop facilitates statistical inference for $\text{CVE}(s)$ for each s separately, where point estimates and 95% confidence intervals for $\text{CVE}(s)$ will be presented. However, it is also of interest to investigate whether the Day 57 marker plays a role in determining vaccine efficacy. To do so, we will formally test the null hypothesis

$$H_0 : \text{CVE}(s) \text{ is constant in } s$$

against various alternatives. We will first adapt the approach of [Westling \(2020\)](#) to devise a nonparametric omnibus test of this null hypothesis. We will also construct a nonparametric directional test of this hypothesis tailored to alternatives under which $CVE(s)$ is monotone in s , along the lines of [Hall and Heckman \(2000\)](#), for example. Leveraging the known monotonicity of the controlled vaccine efficacy will provide greater power than omnibus tests.

15.1.1 Conservative (upper bound) sensitivity analysis for the Cox model correlates of risk analysis

While the above nonparametric approach is considered to be the best scientific approach because it takes the greatest care to avoid the correctness of inferences depending on parametric modeling assumptions that cannot be fully verified, we also apply the same Cox modeling approach described in Section 12.3.3 augmented with a sensitivity analysis, with advantages of harmonization with the CoR analysis, sensitivity analysis that is generally warranted when a no unmeasured confounders assumption is made, and efficiency gain achieved via the added modeling assumptions. The sensitivity analysis quantifies the rigor of evidence for a controlled VE CoP after accounting for potential bias from unmeasured confounding.

[Gilbert et al. \(2020a\)](#) details the sensitivity analysis approach, which was applied to the CYD14 and CYD15 dengue phase 3 data sets ([Moodie et al., 2018](#)); we plan to apply it in the same way to the COVID-19 data sets (as the structure of the problem is the same). We summarize here the essential details needed for application to the COVID-19 data sets.

We define S to be a controlled risk CoP if $P(Y(1, s) = 1)$ is monotone non-increasing in s with $P(Y(1, s) = 1) > P(Y(1, s') = 1)$ for at least some $s < s'$, where point and 95% confidence interval estimates of $P(Y(1, s) = 1)$ versus s , with built in robustness to unmeasured confounding, describe the strength of the CoP in terms of the amount and nature of decrease. Suppose the CoR analysis based on the Cox model is conducted as described in Section 12.3.3.

Let marginalized conditional risk

$$r_M(s) = risk_1(t_F|s)$$

and controlled risk

$$r_C(s) = P(Y(1, s) = 1).$$

Given that CoR analysis is based on observational data — the biomarker value is not randomly assigned — a central concern is that unmeasured or uncontrolled confounding of the association between S and Y could render $r_M(s) \neq r_C(s)$, biasing estimates of the controlled risk curve $r_C(s)$ and of controlled risk ratios of interest

$$RR_C(s_1, s_2) = r_C(s_2)/r_C(s_1) .$$

Because we can never be certain that confounding is adequately adjusted for, sensitivity analysis is warranted, as considered in extensive literature — see, e.g., [VanderWeele and Ding \(2017\)](#) and references therein. Sensitivity analysis is useful to evaluate how strong unmeasured confounding would have to be to explain away an observed causal association, that is, to determine the strength of association of an unmeasured confounder between S and Y needed for the observed exposure-outcome association to not be causal, $r_M(s) \neq r_C(s)$. We follow the recommendation of VanderWeele and Ding (2017) to report the E-value as a summary measure of the evidence of causality, or, in our application, evidence of whether S is a controlled risk CoP based on variation in the controlled risk curve. We also include other closely related measures of sensitivity.

The E-value is the minimum strength of association, on the risk ratio scale, that an unmeasured confounder would need to have with both the exposure (S) and the outcome (Y) in order to fully explain away a specific observed exposure–outcome association, conditional on the measured covariates [[VanderWeele and Ding \(2017\)](#); [VanderWeele and Mathur \(2020\)](#)]. If, as in CoP analyses, the estimated marginalized risk ratio $\widehat{RR}_M(s_1, s_2) = \widehat{r}_M(s_2)/\widehat{r}_M(s_1)$ for $s_1 < s_2$ is less than one, then the E-value for $\widehat{RR}_M(s_1, s_2)$ is calculated as

$$e_{RR}(s_1, s_2) = \frac{1 + \sqrt{1 - \widehat{RR}_M(s_1, s_2)}}{\widehat{RR}_M(s_1, s_2)} . \tag{6}$$

We include the argument (s_1, s_2) in the notation, with $s_1 < s_2$ by convention, to be clear that the E-value depends on specification of two specific marker-level subgroups.

To illustrate the interpretation of an E-value, suppose S is binary and regression analysis yields an estimate $\widehat{RR}_M(0, 1) = \widehat{r}_M(1)/\widehat{r}_M(0) = 0.40$ with 95% confidence interval (CI) $(0.14, 0.78)$. An E-value $e(0, 1)$ of 4.4 means that a marginalized risk ratio $RR_M(0, 1)$ at the observed value 0.40 could be explained away (i.e., $RR_C(0, 1) = 1.0$) by an unmeasured confounder associated with both the exposure and the outcome by a marginalized risk ratio of 4.4-fold each, after accounting for the vector X of measured confounders, but that weaker confounding could not do so.

In addition, we follow the recommendation of VanderWeele and Ding (2017) to also report the E-value $e_{UL}(s_1, s_2)$ for the upper limit $\widehat{UL}(s_1, s_2)$ of the 95% CI for the observed marginalized risk ratio $\widehat{RR}_M(s_1, s_2)$, computed as 1 if $\widehat{UL}(s_1, s_2) \geq 1$ and, otherwise, as

$$\frac{1 + \sqrt{1 - \widehat{UL}(s_1, s_2)}}{\widehat{UL}(s_1, s_2)},$$

which in the example equals $e_{UL}(0, 1) = 1.88$. This E-value for the upper limit indicates, for given $s_1 < s_2$, the strength of unmeasured confounding at which statistical significance of the inference that $RR_C(s_1, s_2) < 1$ would be lost. The two E-values above are useful for judging how confident we can be that an immunologic biomarker is a controlled risk CoP, with E-values near one suggesting weak support and evidence increasing with greater E-values.

It is also useful to provide conservative estimates of controlled risk ratios and of the controlled risk curve, accounting for unmeasured confounding. We approach these tasks based on the sensitivity analysis, or bias analysis, approach of [Ding and VanderWeele \(2016\)](#). We give their main result and refer readers to the paper for details. We begin by defining two (possibly context-specific) fixed sensitivity parameters. First, we set $RR_{UD}(s_1, s_2)$ to be the maximum risk ratio for the outcome Y comparing any two categories of the unmeasured confounders U , within either exposure group $S = s_1$ or

$S = s_2$, conditional on the vector X of observed covariates. Second, we set $RR_{EU}(s_1, s_2)$ to be the maximum risk ratio for any specific level of the unmeasured confounder U comparing individuals with $S = s_1$ to those with $S = s_2$, with adjustment already made for the measured covariate vector X . Thus, $RR_{UD}(s_1, s_2)$ quantifies the importance of the unmeasured confounder U for the outcome, and $RR_{EU}(s_1, s_2)$ quantifies how imbalanced the exposure/marker subgroups $S = s_1$ and $S = s_2$ are in the unmeasured confounder U . The values $RR_{UD}(s_1, s_2)$ and $RR_{EU}(s_1, s_2)$ are always specified as greater than or equal to one. We suppose that $RR_M(s_1, s_2) < 1$ for the fixed values $s_1 < s_2$ — this is the case of interest for immune correlates. Defining the bias factor (or bounding factor)

$$B(s_1, s_2) = \frac{RR_{UD}(s_1, s_2)RR_{EU}(s_1, s_2)}{RR_{UD}(s_1, s_2) + RR_{EU}(s_1, s_2) - 1}$$

for $s_1 \leq s_2$, and letting $RR_M^U(s_1, s_2)$ be defined in the same way as $RR_M(s_1, s_2)$, except with marginalization over the distribution of the joint distribution of X and U , then the result of Ding and VanderWeele (2016) states that $RR_M^U(s_1, s_2) \leq RR_M(s_1, s_2) \times B(s_1, s_2)$, where

$$RR_M^U(s_1, s_2) = E\{r_1(t_F|s_2, X, U)\}/E\{r_1(t_F|s_1, X, U)\}$$

with $r_1(\cdot|\cdot)$ conditional risk $r_1(t|s_1, x, u) = P(T \leq t|S = s_1, X = x, U = u, A = 1)$, and the expectations are over the distribution of (X, U) .

Translating this result to our problem context, under the positivity assumption, we have that $RR_M^U(s_1, s_2) = RR_C(s_1, s_2)$ and so, it follows that

$$RR_C(s_1, s_2) \leq RR_M(s_1, s_2) \times B(s_1, s_2) . \quad (7)$$

This inequality states that the causal risk ratio is bounded above by the marginalized risk ratio multiplied by the bias factor. It follows that a conservative (upper bound) estimate of $RR_C(s_1, s_2)$ is obtained as $\widehat{RR}_M(s_1, s_2) \times B(s_1, s_2)$, and a conservative 95% CI is obtained by multiplying each confidence limit for $RR_M(s_1, s_2)$ by $B(s_1, s_2)$. These estimates for $RR_C(s_1, s_2)$ account for the presumed-maximum plausible amount of deviation from the

no unmeasured confounders assumption specified by $RR_{UD}(s_1, s_2)$ and $RR_{EU}(s_1, s_2)$. An appealing feature of this approach is that the bound (7) holds without making any assumption about the confounder vector X or the unmeasured confounder U .

The above approach does not directly provide a conservative estimate of the controlled risk curve $r_C(s)$, because additional information is needed for absolute versus relative risk estimation. We consider an approach that selects the value s^{cent} of S such that $\hat{r}_M(s^{cent})$ matches the proportion of vaccinees with outcome $Y = 1$. This value is a ‘central’ marker value at which the observed marginalized risk equals the observed overall risk. Then, the controlled risk curve is estimated by

$$\hat{r}_C(s) = \hat{r}_M(s) \left[B(s^{cent}, s) I(s \geq s^{cent}) + \frac{1}{B(s, s^{cent})} I(s < s^{cent}) \right]. \quad (8)$$

The bias factor $B(s_1, s_2)$ should have greater magnitude for a greater distance of s_1 from s_2 , as determined by specifying $RR_{UD}(s_1, s_2)$ and $RR_{EU}(s_1, s_2)$ each with magnitude increasing with $s_2 - s_1$ (for $s_1 \leq s_2$). The expression above uses bias $B(s^{cent}, s)$ for s above s^{cent} , pulling the observed risk $\hat{r}_M(s)$ up for subgroups with high biomarker values, and uses $1/B(s, s^{cent})$ for s below s^{cent} , pulling the observed risk $\hat{r}_M(s)$ down for subgroups with low biomarker values. In other words, it makes the controlled risk curve flatter, closer to the null curve.

We consider one simple specific approach, which we use in the illustrative application. First, we set $RR_{UD} = RR_{EU}$ and take RR_U to be the common value. Second, we set $RR_U(s_1, s_2)$ to be log-linear in the distance $s_2 - s_1$, that is, $\log RR_U(s_1, s_2) = \gamma(s_2 - s_1)$ for $s_1 \leq s_2$. Third, for any pair of values (s_1, s_2) , we fix a sensitivity parameter $RR_U(s_1^{fix}, s_2^{fix})$ to some value above one. It follows then that

$$\log RR_U(s_1, s_2) = \left(\frac{s_2 - s_1}{s_2^{fix} - s_1^{fix}} \right) \log RR_U(s_1^{fix}, s_2^{fix})$$

for all $s_1 \leq s_2$.

The sensitivity analysis is done for each of the two Cox model CoR analyses described in Section 12.3.3, first for tertiles of the Day 57 marker and second for the quantitative marker. For the former, E-values are reported for both the point estimate and the upper 95% confidence limit for $RR_C(0, 1)$, where category 1 is the upper tertile, category 0 is the lower tertile, and the intermediate middle tertile subgroup of vaccine recipients is excluded from the analysis. In addition, setting $RR_{UD}(0, 1) = RR_{EU}(0, 1) = 2$, such that $B(0, 1) = 4/3$, we report conservative estimation and inference on the causal risk ratio $RR_C(0, 1)$.

Next we repeat the analysis treating S as a quantitative variable, where $P(T \leq t | S = s, X, A = 1)$ is again estimated by two-phase Cox partial likelihood regression and now $RR_M(s_1, s_2)$ is the marginalized risk ratio between s_1 and s_2 . We will plot point and 95% confidence interval estimates of the observed marginalized risk and controlled risk curves, for the latter using the sensitivity analysis described in Section 15.1.1.

For validity the method requires the positivity assumption, and thus the method will only be applied if the data are reasonably supportive of the positivity assumption. To check positivity, we study the antibody marker distribution in vaccine recipients within each subgroup of the covariates X that are adjusted for. For the tertiles analysis we require evidence that within each subgroup some vaccine recipients have lower tertile responses and some vaccine recipients have upper tertile responses. For the quantitative S analysis, we look for evidence that S varies over its full range within each level of the potential confounders that are adjusted for.

15.2 CoP: Stochastic Interventional Effects on Risk and Vaccine Efficacy

Another approach to studying correlates of protection involves estimating the effect of shifting the immune response distribution in the vaccinated individuals (Hejazi et al., 2020a). Specifically, we can consider the effect on risk of a given endpoint of a controlled intervention that shifts the distribution of an immune response by δ units, where δ is an analyst-specified real number. Considering a counterfactual scenario in which we are able to intervene so as to modify the immune response induced by the vaccine (e.g., a hypothetical

change in dose or other re-formulation of the vaccine), we take this hypothetical intervention to lead to an improved (if $\delta > 0$) or lessened immune response (if $\delta < 0$) relative to the current vaccine (at $\delta = 0$). Using this framework, we can query the counterfactual risk of the endpoint under this hypothetical vaccine. Using notation established above, this quantity can be expressed as the mean of the counterfactual variable $Y(1, S(1) + \delta)$.

This approach is similar to the controlled effects approach described in Section 15.3, but with an important distinction. In the controlled effects approach, one assumes that it is possible to set $S = s$ for *all* individuals in the population. For high values of s , this assumption may be unrealistic if the vaccine fails to be strongly immunogenic for some subpopulations. On the other hand, with the interventional approach, it is only required that individuals' immune responses be shifted relative to their observed immune response, which may be more plausible for some vaccines.

Under assumptions (Hejazi et al., 2020a), the main two of which being no unmeasured confounders and positivity (forms of both are also required for the Controlled VE CoP analyses), the counterfactual risk of interest $E[Y(1, S(1) + \delta)]$ is identified by

$$E[P(Y = 1 \mid A = 1, S = S + \delta, X = x) \mid A = 1, X] .$$

Examining this quantity across a range of δ provides insight into the relative contribution of a given immune response marker in preventing the endpoint of interest.

Hejazi et al. (2020a) proposed nonparametric estimators that rely on estimates of the outcome regression (as described above) and the conditional density of the immune response marker in vaccinated participants. Their estimators efficiently account for two-phase sampling of immune responses and are implemented in the `txshift` package (Hejazi and Benkeser, 2020) for the R language and environment for statistical computing (R Core Team, 2020), available via both GitHub at <https://github.com/nhejazi/txshift> and the Comprehensive R Archive Network at <https://CRAN.R-project.org/package=txshift>.

These estimators will be applied to each of the five Day 57 antibody markers (without baseline adjustment) controlling for the same set of baseline risk factors controlled for in other analyses previously discussed. As with the mediation analysis approach described in Section 15.3, the procedure will leverage low-dimensional risk factors alongside parametric regression strategies and flexible conditional density estimators for endpoints with fewer than 100 observed cases (pooling over the treatment arms); however, more flexible learning techniques will be employed for modeling the outcome process for endpoints with a greater number of observed cases.

In particular, conditional density estimates of immune response markers will be principally based on a nonparametric estimation strategy that reconstructs the conditional density through estimates of the conditional hazard of the discretized immune response marker values (Hejazi et al., 2020a,d,c); this approach is an extension of the proposal of Díaz and van der Laan (2011). A Super Learner ensemble (van der Laan et al., 2007) of variants of this nonparametric conditional density estimator and semiparametric conditional density estimators based on Gaussinization of residuals will be constructed using the `s13` R package (Coyle et al., 2020). In settings with limited numbers of case endpoints, the outcome process will be modeled as a Super Learner ensemble of a library of parametric regression techniques (as recommended by Gruber and van der Laan, 2010), while the library will be augmented with flexible regression techniques — including lasso and ridge regression (Tibshirani, 1996; Tikhonov and Arsenin, 1977; Hoerl and Kennard, 1970), elastic net regression (Zou and Hastie, 2003; Friedman et al., 2009), random forests (Breiman, 2001; Wright et al., 2017), extreme gradient boosting (Chen and Guestrin, 2016), multivariate adaptive polynomial and regression splines (Friedman et al., 1991; Stone et al., 1994; Kooperberg et al., 1997), and the highly adaptive lasso (van der Laan, 2017; Benkeser and van der Laan, 2016; Hejazi et al., 2020b) — as the number of endpoint cases grows. These algorithm libraries will be coordinated to match those used in other CoP analyses.

Additionally, we note that $P(Y(0) = 1)$ is estimated in the same way as for the analysis of controlled vaccine efficacy, thus yielding an estimate of

stochastic intervention VE defined by

$$SVE(\delta) = 1 - \frac{E[P(Y = 1 \mid A = 1, S = S + \delta, X = x) \mid A = 1, X]}{P(Y(0) = 1)}.$$

Output of the analyses will be presented as point and 95% confidence interval estimates of $E[Y(1, S(1) + \delta)]$ and of $SVE(s)$ over the values of s for each of the Day 57 antibody markers, for each of a range of δ spanning -2 to 2.

Lastly, just as for the controlled VE CoP analyses, these analyses will only be performed if diagnostics support plausibility of the positivity assumption. Importantly, however, the positivity assumption for the stochastic interventional effects differs from that usually required. That is, where the positivity assumption for effects defined by static interventions requires a positive probability of treatment assignment across all strata defined by baseline factors (i.e., that a discretized immune response value be possible regardless of baseline factors), the positivity assumption of these effects is

$$s_i \in \mathcal{S} \implies s_i + \delta \in \mathcal{S} \mid A = 1, X = x$$

for all $x \in \mathcal{X}$ and $i = 1, \dots, n$. In particular, this positivity assumption does not require that the post-intervention exposure density, $q_{0,S}(S - \delta \mid A = 1, X)$, place mass across all strata defined by X . Instead, it requires that the post-intervention exposure mechanism be bounded, i.e.,

$$P\{q_{0,S}(S - \delta \mid A = 1, X)/q_{0,S}(S \mid A = 1, X) > 0\} = 1,$$

which may be readily satisfied by a suitable choice of δ .

More importantly, the static intervention approach may require consideration of counterfactual variables that are scientifically unrealistic. Namely, it may be inconceivable to imagine a world where every participant exhibits high immune responses, given the phenotypic variability of participants' immune systems. This too may be resolved by considering an intervention $\delta(X)$, allowing the choice of δ to be a function of baseline covariates X (Hejazi et al., 2020a; Díaz and van der Laan, 2012; Haneuse and Rotnitzky, 2013; Díaz and van der Laan, 2018).

15.3 CoP: Mediator of Vaccine Efficacy

A classic application of mediation is to decompose the overall VE into so-called *natural* direct and indirect effects. We will estimate this decomposition for each Day 57 antibody marker individually (focusing on the non-baseline subtracted markers as for the other CoP analyses described above), as well as when considering all antibody markers together (although this SAP currently restricts to analysis of the individual markers). For simplicity, as before, we describe this approach using a binary outcome, noting that extensions to time-to-event (with competing risks) are possible. The *total* effect of the vaccine can be represented by the risk ratio $RR = (1 - VE)$,

$$RR = \frac{P(Y(1, S(1)) = 1)}{P(Y(0, S(0)) = 1)} .$$

The natural direct and indirect effects are, respectively,

$$RR_{DE} = \frac{P(Y(1, S(0)) = 1)}{P(Y(0, S(0)) = 1)} \quad \text{and} \quad RR_{IDE} = \frac{P(Y(1, S(1)) = 1)}{P(Y(1, S(0)) = 1)} .$$

Note that $RR = RR_{DE}RR_{IDE}$, showing that the total effect decomposes into the direct times indirect effect. Another quantity of interest is the proportion mediated, which could be expressed as

$$PM = 1 - \frac{\log(RR_{DE})}{\log(RR)} .$$

We note that $PM=1$ if and only if $RR_{DE} = 1$, i.e., no direct effect means that the marker fully mediates VE. We will estimate PM defined in this way.

As above, we must assume all confounders X of S and Y have been measured. We also assume there is sufficient overlap of the immunologic marker distributions, and no confounders of the mediator-outcome relationship that are affected by treatment. Moreover, we require the assumption

$$P(S = s | A = 0, X = x) > 0 \text{ implies } P(S = s | A = 1, X = x) > 0 \quad (9)$$

for all subgroups $X = x$ (i.e., a.e.). Under these assumptions, $P(Y(a, S(a')) = 1)$ is identified by

$$E[P(Y = 1 | A = a, S, X) | A = a', X] .$$

In our immune CoP application it is expected that, for analyses restricting to baseline negative individuals, the conditional density of the immune response marker in the placebo arm will be a point mass at 0, that is with S below the LLOQ. In other words, we do not expect any placebo recipients to have a positive value of the immune response marker. This implies the identification result that for $a = 0, 1$, $P(Y(a, S(0)) = 1) = E[P(Y = 1 \mid A = a, S = 0, X)]$. Therefore, all of the needed terms $P(Y(a, S(a')) = 1)$ are identified; while $P(Y(0, S(1)) = 1)$ is not identified, it is not necessary to estimate this term in order for estimation of the parameters of interest (natural direct effect, natural indirect effect, PM).

[Zheng and van der Laan \(2012\)](#) provide a multiply robust targeted minimum loss-based plug-in estimator of natural direct and indirect effects. Their estimator will be consistent if either the estimated outcome regression or the estimated mediator distribution is consistent for their respective targets. We will utilize a modified version of this estimator that accounts for missing outcomes and for the two-phase sampling design. The estimator requires estimation of several regressions including:

- the outcome regression: a regression of Y onto A , S , and X amongst those with both Y and S recorded (the phase two sample);
- the propensity score: the known randomization probability for vaccine;
- the propensity for vaccination given the immune response marker: a regression of A onto X and S among those in the phase two sample;
- the probability of missing outcomes: a regression of the indicator of having missing endpoint status onto A , X , and S among those in the phase two sample; and
- a series of sequential regressions that de-confound the relationship between vaccine receipt, immune response, case-status, and sampling design.

The estimator provides a means of mapping these estimators into estimates of RR_{DE} , RR_{IDE} and PM. This estimator is robust, in that it only relies on consistently estimating certain combinations of the above regressions, and

efficient, in that it utilizes data on the entire trial population rather than only those included in the random sub-cohort.

For endpoints with fewer than 100 observed cases (pooled over the randomization arms), we will leverage logistic and linear regression models, as appropriate, to estimate each of the above regressions and only include a low-dimensional set of pre-specified characteristics in X . In these cases, 95% confidence intervals for RR_{DE} , RR_{IDE} and PM will be constructed using the percentile-based nonparametric bootstrap.

For endpoints with more than 100 observed cases (pooled over the randomization arms), we will instead employ super learning to estimate the above regression quantities and include a higher dimensional set of potential confounders in X ; the same set of baseline potential confounders adjusted for in other analyses will be included. In this case, the super learner library would include a diversity of pre-specified algorithms. In these cases, the nonparametric bootstrap cannot be used to construct confidence intervals, and we will instead rely on Wald-style confidence intervals with standard errors estimated based on the empirical variance of the estimators' estimated influence functions.

There may arise situations in which there is insufficient overlap of the immune response distribution. For example, if all individuals in the vaccinated arm have a non-zero response (i.e., response above the LLOQ), while all individuals in the placebo arm have no measured response (i.e., value below the LLOQ), then the assumption (9) of the above methods will be violated. In fact, the assumption (9) implies that within every subgroup $X = x$, there needs to be some vaccinated participants with S below the LLOQ. Therefore, for each Day 57 marker, the mediation analysis of the quantitative S will only be done if diagnostics support this assumption. We will also plan to conduct an analysis using a three-category ordinal version of S with the marker threshold defining the lowest category selected to ensure that some vaccine recipients have values in the lowest category, across the subgroups; the default for this categorical S will be tertiles and matched to the way that the three-category variable is analyzed for Cox model correlates of risk

analysis.

16 Summary of the Set of CoR and CoP Analyses and Their Requirements and Contingencies, and Synthesis of the Results, Including Reconciling Any Possible Contradictions in Results

Table 8 summarizes all of the Stage 1 / Day 57 marker correlates analyses that are done, including contingencies for whether and when each analysis is done. The quantitative version of each marker S , and the tertiles version of each marker S , is common across all of the analyses. All of the Day 57 markers are the versions that are not baseline subtracted, given that the cohort for analysis is baseline negative. Most of the analyses focus on univariate Day 57 markers. The primary reason to do this is the goal to identify a parsimonious correlate based on a single marker without needing to run the set of assays, and secondary reasons are: (1) the assay readouts are expected to be highly correlated, especially for the ID50 and ID80 readouts from the same pseudovirus neutralization assay, and (2) there is ample precedent for univariate markers being accepted as immunological surrogate endpoints for approved vaccines ([Plotkin, 2010](#)).

Table 8: Summary of Stage 1 Day 57 Marker CoR and CoP Analyses with Requirements/Contingencies for Conduct of the Analysis

Analysis	Structure of	Requirements/Contingencies	
	Day 57 Marker(s)	Min No. Vaccine Endpoints	Other
CoR Cox Model	Tertiles of S^1	25	None
	Quant. $S = s^2$	25	None
	Quant. $S \geq s^1$	25	None
CoR Nonpar. threshold	Quant. $S \geq s^1$	35	None
CoR GAM	Quant. $S = s^2$	35	None
CoR threshold log. regr.	Quant. $S = s^2$	25	None
CoR Superlearner ³	Quant. $S = s$, 2FR, 4FR	35	None
CoP: Correlates of VE	Binary S	50	None
	Quant. $S = s$	50	BIP with $R^2 \geq 0.25$
CoP: Controlled VE	Quant. $S = s$	50	Feasibility of positivity ⁴
	Tertiles of $S = s$	50	Feasibility of positivity ⁴
CoP: Stoch. Interv. VE	Quant. $S = s$	50	Feasibility of positivity ⁴
CoP: Mediators of VE	Quant. $S = s$	50	Feasibility of positivity ⁴
	Tertiles of S	50	Feasibility of positivity ⁴

¹These analyses are harmonized in addressing the same scientific question of how does endpoint risk vary over vaccinated subgroups defined by S above a threshold.

²These exploratory supportive analyses are harmonized in addressing the same scientific question of how does endpoint risk vary over vaccinated subgroups defined by S equal to a given marker value.

³Only this Superlearner analysis uses data from multiple assays and multiple readouts as input features; the other analyses consider one Day 57 biomarker at a time. ⁴The positivity assumptions are as follows. Controlled VE: $P(S = s | A = 1, X) > 0$ almost surely. Stochastic Interventional VE: $s_i \in \mathcal{S} \implies s_i + \delta \in \mathcal{S} | A = 1, X = x$ for all $x \in \mathcal{X}$ and $i = 1, \dots, n$. Mediators of VE: $P(S = s | A = 1, X) > 0$ almost surely and $P(S = s | A = 0, X = x) > 0$ implies $P(S = s | A = 1, X = x) > 0$. Graphical diagnostic analyses are used to assess feasibility of each positivity assumption, where the assumption may be more feasible for S as tertiles than as a quantitative variable. For quantitative S , the assumption is weaker for the Stochastic Interventional VE analysis, such that it is possible that only this analysis of the three will be done.

Some of the analyses include parametric assumptions for characterizing associations (Cox model and threshold analyses, Cox model versions of Controlled VE analyses) and others are nonparametric or approximately so (all other analyses). If parametric and nonparametric analyses of the same type (e.g.,

Cox model vs. nonparametric CoR analysis of the same association parameter; Controlled VE Cox model vs. nonparametric monotone dose-response) suggest contradictory results, then the interpretation from the nonparametric analysis will be prioritized, given it is more robust and less likely to be an incorrect result. The diagnostic testing of the parametric assumptions will aid this interpretation. As noted above, if the nonparametric analysis suggesting a contradictory result requires a positivity assumption, then its results will only be prioritized if diagnostics support feasibility of the positivity assumption.

16.1 Synthesis Interpretation of Results

To structure the interpretation of the whole set of CoR and CoP results, we consider the Bradford-Hill criteria for supporting causality assessments:

1. Temporal sequence of association (vaccination causes generation of antibodies, which precede occurrence of the clinical disease outcome)
2. Strength of association (CoR magnitude)
3. Consistency of association (across studies and methods)
4. Biological gradient (may be interpreted as dose-response with greater Day 57 antibody corresponding to lower risk and greater VE)
5. Specificity (that the antibody marker is induced by vaccination not natural infection, and the antibody impacts the particular clinical endpoint being analyzed)
6. Plausibility [(supported by other COVID vaccines through study in efficacy trials and challenge (animal or human) trials, and by other potential studies such as natural history re-infection studies and monoclonal antibody prevention efficacy studies that could be challenge (animal or human) or field trials)]
7. Coherence (the causality assumption does not appear to conflict with current knowledge)
8. Experimental reversibility (if VE wanes to a low level then the antibody

marker also wanes coincidentally; if the Day 57 marker is a strong correlate for outcome during the period of high VE, then it becomes a weaker correlate against endpoints occurring during the later period of low VE; also could be supported if vaccine breakthrough cases tend to occur early in follow-up when antibody levels are known to be relatively low)

9. Analogy (supported by other respiratory virus vaccines, and natural history studies or challenge studies of other respiratory virus vaccines)

On temporal sequence, because the analyses are done in baseline negative individuals, generally the Day 57 antibody responses must be generated by the vaccine, and if the outcome occurs well after Day 57, then there is clear temporal ordering of vaccination causing antibodies followed by outcome. The nuance is outcome cases with event times near 7 days post Day 57, some of which could have been infected with SARS-CoV-2 prior to Day 57 and have relatively long incubation periods, possibly perturbing temporal ordering by creating naturally-induced rather than vaccine-induced antibody. However, the knowledge about the distribution of the time period between SARS-CoV-2 acquisition and symptomatic COVID, and the time needed for an infection to create an adaptive immune response, suggests that this issue could only have a minor impact, and overall the temporal sequence criterion readily holds. Yet, the correlates analysis that stringently only includes cases with documented antigen negativity at both Day 29 and Day 57 may be helpful for evaluating the temporal sequence criterion.

On strength of association, this is directly quantified in all of the analyses as a core output of each method, quantified by point estimates and confidence interval estimates of covariate-adjusted association parameters or causal effect parameters.

On consistency of association, checking for similar estimates and inferences across the multiple vaccine efficacy trials will be relevant. The fact that all of the tested vaccines are designed to protect through induction of antibody to Spike protein suggest that consistency is plausible. The vaccine platform needs to be accounted for in this evaluation, where consistency may be expected for vaccines of a given type (e.g., mRNA vaccines, Spike protein

vaccines, viral vector vaccines with a similar vector), whereas across types a consistent body of evidence would be very helpful, but not a requirement. FDA guidance has stipulated that a surrogate endpoint for one vaccine platform is not necessarily expected to hold for another, and that evidence for one platform would not be seen on its own as support for a surrogate endpoint for another.

In addition, we will plan to study predictiveness of the estimated optimal surrogate built on each single trial data set applied to the other trial data sets, quantified by AUC on new data sets. Moreover, consistency of association may be assessed in another sense - by studying whether the different CoR methods tend to reveal a consistent directionality and pattern of an antibody marker correlated with risk, and whether the different CoP methods tend to reveal a consistent directionality and pattern of an antibody marker connected to vaccine efficacy (as measured by the various causal effect parameters) and with different versions of vaccine efficacy. A common core element of all of the CoR and CoP methods is covariate-adjusted estimation of marker-conditional risk in vaccine recipients, e.g. of marginal conditional risk $E_X[P(T \leq t_F | S = s, A = 1, X)]$ or $E_X[P(T \leq t_F | S \geq s, A = 1, X)]$. Generally, if an estimate of this function shows strongly decreasing risk with s , then likely all of the CoR analyses will detect such a decrease, and the CoP analyses will detect a version of vaccine efficacy increasing in s . A nuance in looking for consistency of results across methods stems from the fact that different methods have different power to detect the same effect; because of this consistency in magnitude (point estimate) and directionality are more important than consistency in inference/statistical significance.

The fact that all of the methods adjust for the same set of baseline covariates X will aid the ability to compare the results across methods in an interpretable manner. This discussion highlights the relevance of adjusting for the same set of baseline covariates across the different efficacy trials, although our choice to do covariate-adjustment through marginalization (rather than through conditional association parameters) lends some resilience to this issue.

Our comments on consistency of association have supposed a given study endpoint, such as COVID. Another dimension of consistency evaluation could include comparing results across endpoints. On the one hand, consistency in evidence across endpoints could strengthen the case for a CoP, especially for endpoints in the same ‘class’ such as moderate disease and severe disease. On the other hand, the greater the difference between endpoints, the less relevant consistency may be, because the vaccine may protect through different mechanisms against each endpoint (one potential example is prevention of asymptomatic infection vs. prevention of severe disease). Thus evidence for a CoP for a given endpoint should not necessarily be down-graded based on evidence that the same marker does not appear to be a CoP for another endpoint.

On biological gradient, many of the methods are flexible and designed to detect a dose-response pattern of antibody with risk or antibody with vaccine efficacy, with tabular and graphical output of point and confidence interval estimates designed to reveal dose-response.

On specificity, as noted above antibodies generally are almost surely vaccine-induced given the analysis is done in baseline negative individuals, although with nuance that care is needed to evaluate whether some vaccine breakthrough cases may have had SARS-CoV-2 acquisition unusually early in follow-up (e.g., prior to second vaccination). In addition, the assays are validated for measuring specific anti-SARS-CoV-2 antigen response. Moreover, the Day 57 antibody markers can be verified to be negative in all or almost all baseline negative placebo recipients. Therefore, the specificity criterion should readily hold, with the proviso of the complication of the possible inclusion of unusually early infections as vaccine breakthrough cases in some analyses.

On coherence, the results will be interpreted in the light of knowledge of immune correlates of protection for the same vaccine in animal challenge studies (and human challenge studies as available), where multiple studies have demonstrated that both binding and neutralizing antibodies are a correlate of protection.

The results will also be interpreted in light of any knowledge available on passively administered SARS-CoV-2 monoclonal antibodies for prevention of SARS-CoV-2 infection or COVID disease, either in challenge studies (animals or humans) or efficacy trials. In addition, the results will be interpreted in light of results on the antibody markers as correlates of re-infection in natural history studies. Note we are cautious to not use correlates studies in already-infected individuals, because the fact of infection may readily change the nature of a correlate of protection.

On experimental reversibility, we will evaluate whether the strength of association of the Day 57 CoRs and CoPs weakens when restricting to outcomes occurring more distal to vaccination. If the vaccine efficacy is found to wane over time, and the antibody marker wanes over time, then this decrease in the strength of association would be consistent with antibody as a correlate of protection. In contrast, if vaccine efficacy and antibody waned over time, but the strength of a Day 57 CoR and CoP was the same regardless of the timing of outcomes, it might call into question the role of the antibody marker as a CoP. The Stage 2 correlates analyses will also be helpful, where experimental reversibility could be supported simply by coincident waning of VE and waning antibody.

Experimental reversibility may also be supported by “population-level” correlates analyses, a term sometimes used in reference to meta-analysis that associates the level of VE with the population-level of a Day 57 marker across subgroups or trials; e.g. the population-level Day 57 marker response may be summarized by the geometric mean titer or geometric mean concentration.

On analogy, perhaps the most relevant vaccines are vaccines against other respiratory viruses, including influenza vaccine and RSV vaccines. The fact that neutralizing antibodies are a CoR and CoP for both inactivated and live virus vaccines supports that neutralizing antibodies can be a CoP for SARS-CoV-2. In addition, there is ongoing correlates of protection analysis of Novavax’s Phase 3 RSV vaccine efficacy trial, that is evaluating binding antibody and neutralizing antibody CoRs and CoP correlates for severe respiratory disease in infants of vaccinated pregnant mothers. Once those results

are available, they will aid in checking the analogy (and coherence) criterion. The univariate CoR analyses essentially assess four Day 57 antibody biomarkers (lumping ID50, ID80 within each neutralization assay into the same class based on very high correlation to arrive at the five total). The questions arise as to how do we select which biomarker seems to be the best-supported CoP, and do we need to be concerned about multiplicity adjustment issues? Given the multifactorial nature of the assessment involving biology and statistics, we for the most part avoid an approach that tries to pre-specify a quantitative ranking system; rather our approach presents the results of each marker side by side and allows human synthesis and interpretation. To guard against errors in this subjective process, we suggest that consistent results across analyses of a given trial, and consistent results (and predictive validation) across multiple trials, will provide particularly strong guidance for interpreting results. For example, if a particular Day 57 antibody marker shows remarkably consistent results in being a strong CoR and supported CoP but the other readouts do not, it may emerge as the best-supported CoP. In addition, the superlearning CoR estimated optimal surrogate objective has a special place of importance, because it includes variable importance quantification, providing some quantitative guidance on ranking the predictiveness of markers. This variable importance will be defined both internal to a given trial and based on external validation on the other efficacy trials. The metrics of CV-AUC and AUC on new trials quantifies evidence for signal in the data in a way that is protected from risk of false positive results, by virtue of having two layers of cross-validation used to estimate CV-AUC and hence avoid over-fitting. In addition, the CoR analyses use multiple hypothesis testing adjustment to help ensure clear signals and not false positive results (see Section [12.4.2](#)). We also need a plan for minimizing the risk of false positive results for CoP analyses, which we now address.

16.2 Multiple Hypothesis Testing Adjustment for CoP Analysis

For the univariable CoP analyses of the prioritized set of Day 57 antibody markers among the five specified marker variables, the analysis plan seeks evidence of a CoP through four different causal effect approaches. Because of

this looking for evidence through different lenses, for CoP analysis we do not focus on family-wise error rate adjustment, because FWER-adjustment aims to control the risk of making even a single false rejection. Rather, in an effort to build a body of consistent evidence and to ensure that a large fraction of that evidence is reliable, for CoP analysis we focus on false discovery rate correction. To do this, we use the same permutation-based method ([Westfall et al., 1993](#)) that is used for CoR analysis. The multiplicity adjustment is performed across the Day 57 markers and across the set of CoP methods that are applied, in a single suite of hypothesis tests with calculation of q-values. As a guideline for interpreting CoP findings (but not meant to be a rigid gateway), markers with unadjusted p-value ≤ 0.05 and q-value ≤ 0.10 are flagged as having statistical evidence for being a CoP.

17 CoP: Meta-Analysis Analysis Plan

We provide a brief summary of the overall plan, where the details will be developed closer to the time that data are available for meta-analysis of multiple phase 3 vaccine efficacy trials.

Once data sets are available from the set of OWS phase 3 trials, the data sets will be combined for additional analyses to support development of immune CoPs. Data analysis of the combined data sets provides interpretable results based on the standardization of the OWS phase 3 trial protocols – including harmonized study endpoints, follow-up, and blood storage time points – and on the common statistical analysis plan and laboratories/immunoassays (where the use of the Duke pseudovirus assay in some OWS trials and the Monogram pseudovirus assay in other OWS trials implies that the statistical analysis will make use of concordance testing data for making results interpretable referenced to one of the assays.) Meta-analysis surrogate endpoint evaluation methods will be applied to the combined data sets, both for assessing Day 29 and Day 57 antibody markers (Stage 1) as surrogate endpoints for COVID and for secondary outcomes, and for assessing the antibody markers over time (Stage 2) as surrogate endpoints for COVID and for secondary outcomes.

Both individual-level and trial-level meta-analysis will be applied, where the latter studies the association of vaccine effects on an antibody marker with vaccine effects on a study outcome, for example assessing how GMT nAb ID80 titer associates with the level of vaccine efficacy against COVID. Meta-analysis has a special role in being the only correlates approach that can potentially assess immunologic markers as CoPs that are measured using sampling types that were not stored from most trial participants (e.g., PBMC for measuring T cell responses). While the current statistical analysis plan focuses on assessing antibody markers as correlates, in the future plans may be devised to incorporate T cell response data (and potentially other data types) from phase 1-2 studies into meta-analysis evaluation.

In addition to applying formal meta-analysis surrogate endpoint evaluation methods, some of the CoR and CoP statistical methods applied to the individual phase 3 trial data sets will be adapted for application to the combined data sets. This will allow addressing the following objectives: (1) to assess consistency of CoRs and CoPs across trials, subpopulations, and vaccine platforms; (2) to evaluate how well an antibody marker CoR for an outcome developed in one phase 3 trial predicts the same outcome in the other phase 3 trials (cross-validation prediction accuracy); and (3) to provide data for prediction modeling of what would be the efficacy of a new vaccine based on its distribution of antibody markers. Objective (2) provides some empirical data for considering appropriateness of use of a CoP across vaccine platforms.

18 Estimating a Threshold for Predicting High Vaccine Efficacy Based on an Established/Putative CoP

For each antibody marker studied as a CoP, we will apply the Chang-Kohberger (2003) / Siber (2007) method to estimate a threshold of the antibody marker associated with the estimate of overall vaccine efficacy observed in the trial.

This method makes two simplifying assumptions: (1) that a high enough antibody marker value s^* implies that individuals with $S > s^*$ have essentially zero disease risk (protection) regardless of whether they were vaccinated; and (2) $P(Y = 1|S \leq s^*, A = 1)/P(Y = 1|S \leq s^*, A = 0) = 1$ (zero vaccine

efficacy if $S \leq s^*$). Based on these assumptions, s^* is calculated as the value equating $1 - \hat{P}(S \leq s^* | A = 1) / \hat{P}(S \leq s^* | A = 0)$ to the estimate of overall vaccine efficacy. This estimate is supplemented by estimating the reverse cumulative distribution function (RCDF) of S in baseline negative vaccine recipients and calculating a 95% confidence interval for the threshold value s^* as the points of intersection of the estimated RCDF curve with the 95% confidence interval for overall vaccine efficacy (as in the Figure in Andrews and Goldblatt, 2014).

This method essentially assumes that S has already been established as a coP, and under that assumption estimates a threshold that may be considered as a benchmark / study endpoint for future immunogenicity vaccine trial applications.

19 Considerations for Baseline Positive Study Participants

As stated above, if enough COVID cases in baseline positive vaccine and/or placebo recipients occur, then additional correlates analyses may be planned in baseline positive individuals. For example, the same or similar correlates of risk analysis plan that is used to analyze Day 57 marker correlates of risk in baseline negative vaccine recipients could be applied to assess Day 0 marker correlates of risk in baseline positive placebo recipients. In addition, analyses could be done to assess how vaccine efficacy in baseline positive participants varies with Day 0 markers. It is straightforward to make this analysis rigorous because Day 0 markers are a baseline covariate, such that regression analyses are valid based on the randomization.

20 Avoiding Bias with Pseudovirus Neutralization Analysis due to HIV Positivity

Because the lentivirus-based pseudovirus neutralization assay uses an HIV backbone, the presence of anti-retroviral drugs in serum will give a false positive neutralization signal. This can be easily screened for using an MuLV pseudotype control. Therefore, baseline samples of all infection endpoint

cases will be tested for HIV antibodies, as will a random sample of the OWS random subcohort that constitutes an approximate 5:1 non-cases:cases sampling for Stage 1 data analysis.

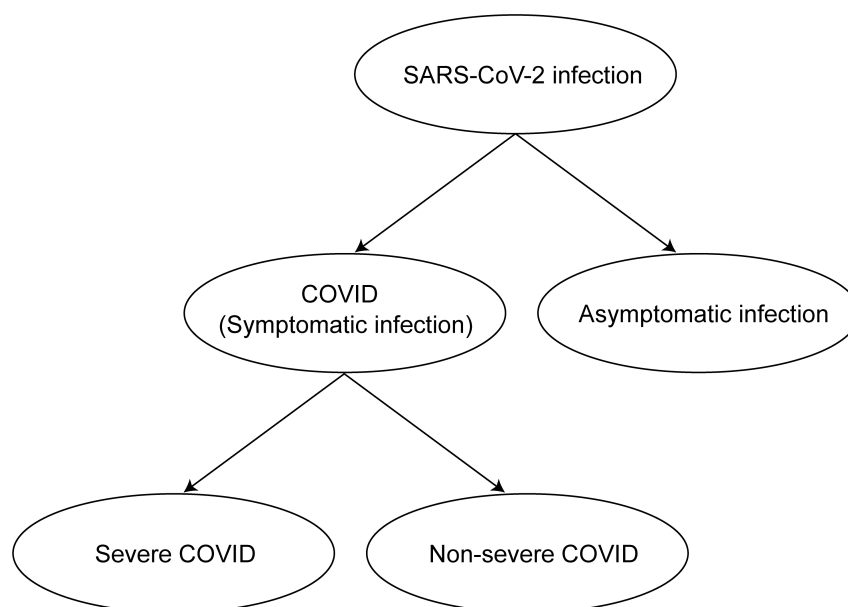
21 Accommodating Crossover of Placebo Recipients to the Vaccine Arm

We consider how the SAP would be impacted by a scenario where at some point most placebo recipients receive the study vaccine. The plan for assessing correlates of risk in vaccine recipients would be minimally affected, because the analysis is based on vaccine recipients alone. If crossed over placebo recipients have study visits and blood sample storage on the same schedule as if they had originally been assigned to the vaccine arm, then the new follow-up data from the crossed over placebo recipients will be included in correlates of risk analyses, which is expected to yield improved power and precision given the expanded sample size of vaccine recipients. The plan for assessing correlates of protection, on the other hand, would be altered based on crossover. The plan would be revised to only assess correlates of protection over follow-up through to the point that the size of the remaining placebo arm is small, for example when less than 20% of the original placebo arm remains.

22 COVID Correlates Analysis Report

This SAP is being implemented over time on a mock/practice COVID-19 vaccine efficacy trial data set, as discussed in the [Prelude](#). The report is provided at Dr. Youyi Fong's public GitHub repository CovidCorrSAP.

A



B

Clinical Endpoint	Definition
SARS-CoV-2 infection	Positive RNA PCR test or SARS-CoV-2 seroconversion*, whichever occurs first
COVID (Symptomatic infection)	Meeting a protocol-specified list of COVID-19 symptoms with virological confirmation of SARS-CoV-2 infection (symptom triggered)
Asymptomatic infection	SARS-CoV-2 seroconversion* without prior diagnosis of the COVID endpoint [†]
Severe COVID	COVID endpoint with at least one protocol-specified severe disease event
Non-severe COVID	COVID endpoint with zero protocol-specified severe disease events

*Seroconversion is assessed via a validated assay that distinguishes natural vs vaccine-induced SARS-CoV-2 antibodies

[†]Alternatively, the asymptomatic infection endpoint can also include an RNA PCR+ test result obtained through testing regardless of symptoms (e.g., as a requirement for travel, return to school or work, or elective medical procedures) and follow-up to confirm the participant remains asymptomatic

Figure 1: A) Structural relationships among study endpoints in a COVID-19 vaccine efficacy trial. B) Study endpoint definitions.

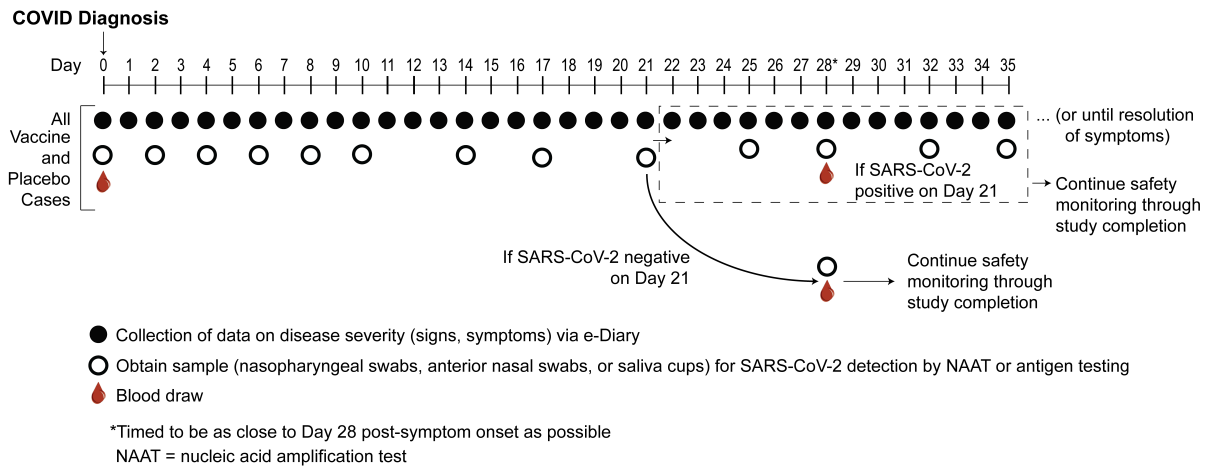


Figure 2: Example at-COVID diagnosis and post-COVID diagnosis disease severity and virologic sampling schedule, in a setting where frequent follow-up of confirmed cases can be assured. Participants diagnosed with virologically-confirmed symptomatic SARS-CoV-2 infection (COVID) enter a post-diagnosis sampling schedule to monitor viral load and COVID-related symptoms (types, severity levels, and durations).

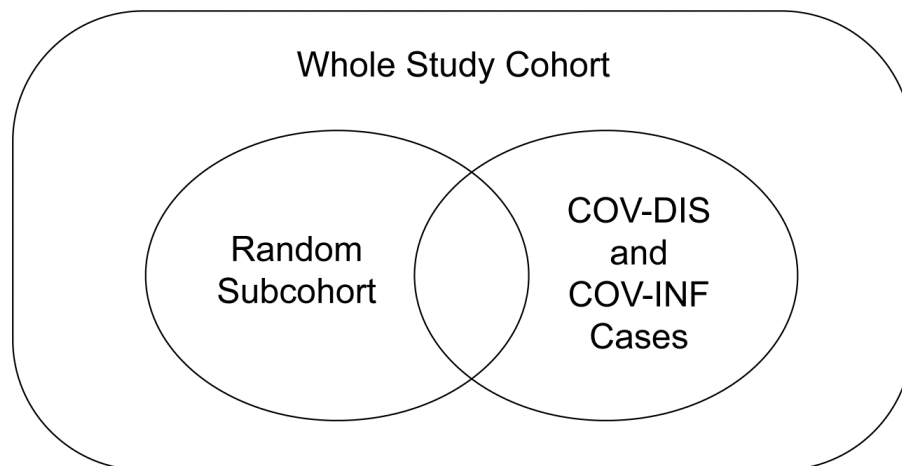


Figure 3: Case-cohort sampling design (Prentice, 1986) that measures Day 1, 57 antibody markers in all participants selected into the subcohort and in all COVID and COV-INF cases occurring outside of the subcohort.

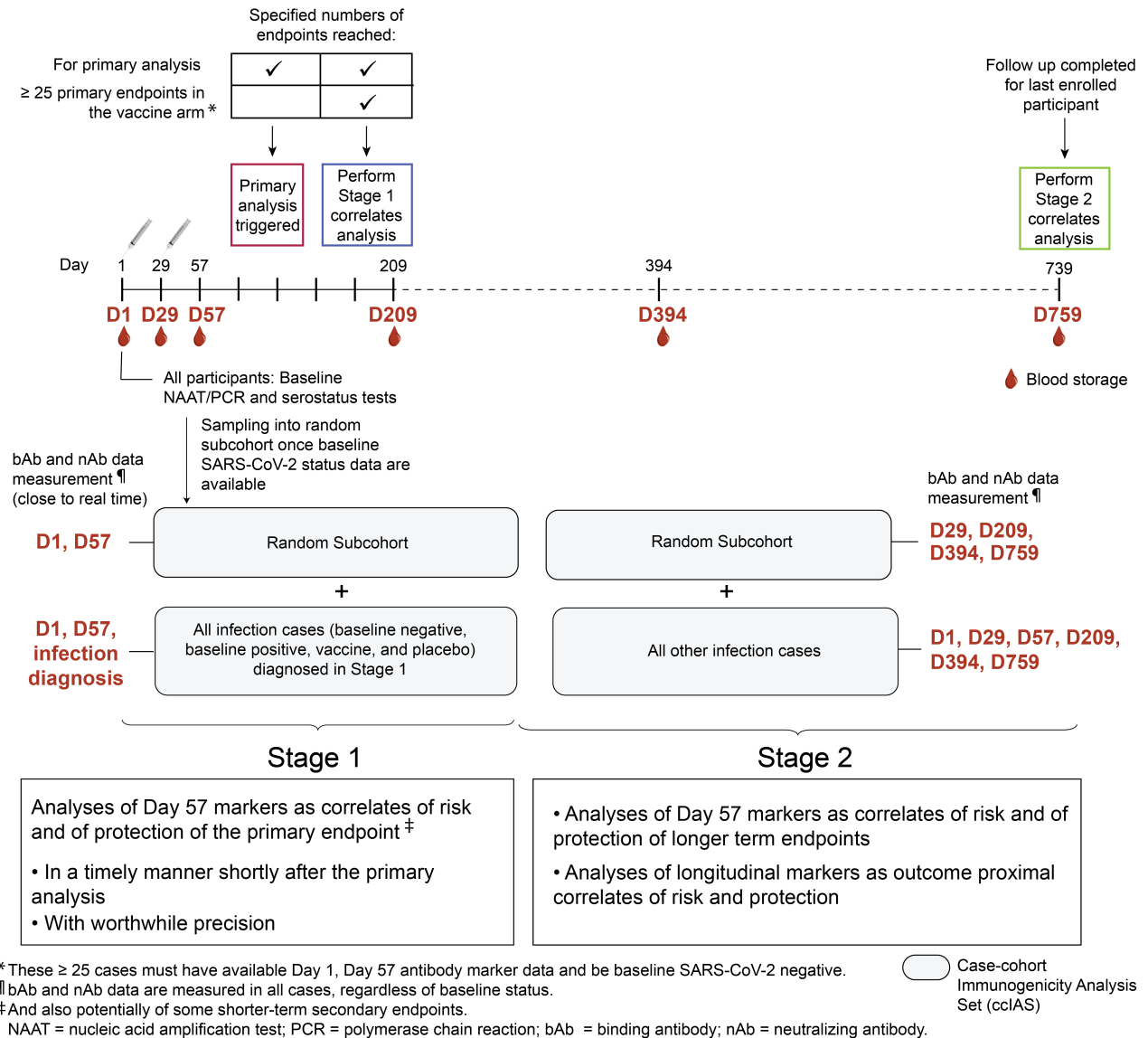


Figure 4: Two-stage correlates analysis. Stage 1 consists of analyses of Day 57 markers as correlates of risk and of protection of the primary endpoint and potentially also of some secondary endpoints, and includes antibody marker data from all COVID and SARS-CoV-2 infection cases (COV-INF) through to the time of the data lock for the first correlates analyses. Stage 2 consists of analyses of Day 57 markers as correlates of risk and of protection of longer term endpoints and analyses of longitudinal markers as outcome-proximal correlates of risk and of protection, and includes antibody marker data from all subsequent COVID and COV-INF cases. Stage 1 measures Day 1, 57 antibody markers and COV-INF and COVID diagnosis time point markers; Stage 2 measures antibody markers from all sampling time points and COV-INF plus COVID diagnosis sampling time points not yet assayed. The same random subcohort is used for both stages. If Day 29 markers are included, then Day 29 markers are included for the same participants with Day 57 markers measured.

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23 Appendix: Simulation of COVID-19 Vaccine Efficacy Trial Data Sets

24 Simulating COVID VE Trial Data Sets

24.1 Notation

1. A : randomization assignment to vaccine or placebo (1=vaccine, 0=placebo)
2. B : baseline SARS-CoV-2 status (0 if all SARS-CoV-2 diagnostic tests by Day 1 are negative and 1 if some are positive: 0=negative, 1=positive)
3. X : baseline covariate vector with components X_1, \dots, X_5
 - (a) X_1 : Indicator At-risk for COVID
 - (b) X_2 : Sex assigned at birth (1=female, 0=male)
 - (c) X_3 : Indicator of minority
 - (d) X_4 : Age in years (≥ 18)
 - (e) X_5 : BMI
4. S_1 : Vector of antibody markers measured at Day 1 (dose 1 visit), with components IgG Spike, IgG RBD, PsV ID50, PsV ID80, WT LV MN50
5. S_{29} : Vector of the same antibody markers measured at Day 29 (dose 2 visit)
6. S_{57} : Vector of the same antibody markers measured at Day 57 (\approx peak immunogenicity time point)

7. R : Indicator a participant is randomly sampled into the subcohort for measurement of (S_1, S_{29}, S_{57})
8. T_{29} : Number of days from Day 29 visit until COVID endpoint starting 7 days post Day 29 visit (failure time of interest for studying Day 29 markers as correlates)
9. C_{29} : Number of days from Day 29 visit until right-censoring
10. Δ_{29} : Indicator of $T_{29} \leq C_{29}$
11. $\tilde{T}_{29} = \min(T_{29}, C_{29})$
12. T_{57} : Number of days from Day 57 visit until COVID endpoint starting 7 days post Day 57 visit (failure time of interest for studying Day 57 markers as correlates)
13. C_{57} : Number of days from Day 57 visit until right-censoring
14. Δ_{57} : Indicator of $T_{57} \leq C_{57}$
15. $\tilde{T}_{57} = \min(T_{57}, C_{57})$

Next, in turn we describe the three steps for simulating a data set. First, we simulate the covariates in all participants, second we simulate the Day 57 onwards failure time information in all participants, third we fill in the Day 29 to Day 57 failure time information, and fourth we define R and set (S_1, S_{29}, S_{57}) values to NAs for those with $R = 0$ and $\Delta_{29} = 0$ (non-cases).

24.2 Simulation of the covariates

First, A and B are drawn as independent Bernoulli random draws with success probabilities $P_{a=1}$ and $P_{b=1}$, respectively specified by the user. Then, for each of the four baseline strata $A = a, B = b$ with $(a, b) \in \{0, 1\} \times \{0, 1\}$, the 20-vector

$$W = (X^T, S_1^T, S_{29}^T, S_{57}^T)^T$$

is simulated. As X_1, X_2 , and X_3 are the only binary variables, for simplicity we first simulate them as independent Bernoulli random variables.

1. X_1 is drawn from a Bernoulli distribution with specified success probability $P_1 = P(X_1 = 1)$
2. X_2 is drawn from a Bernoulli distribution with specified success probability $P_2 = P(X_2 = 1)$
3. X_3 is drawn from a Bernoulli distribution with specified success probability $P_3 = P(X_3 = 1)$

Next, we define a latent 17-vector variable W^L that has a multivariate normal distribution with mean vector.

$$\mu^L = (\mu_{X4}, \mu_{X5}, \mu_{S1}^T, \mu_{S29}^T, \mu_{S57}^T)^T$$

with variance elements Σ_{diag} = 17-vector of variances for the elements of W^L . The covariance elements are defined by specifying the correlation parameters $\rho[i, j]$ for all $i = 1, \dots, 17, j = 1, \dots, 17$ such that the $(i, j)^{th}$ element of the variance-covariance matrix Σ of W^L is

$$\rho[i, j] * \sqrt{\Sigma_{diag}[i] \Sigma_{diag}[j]}.$$

Based on specification of all of the input parameters, W^L is drawn. Then the following steps are done to attain W based on W^L :

1. X_4 is taken to be W_1^L rounded to the nearest year at enrollment
2. X_5 is taken to be W_2^L
3. S_{1j} is taken to be $W_{S1.j}^L$ for $j = 1, \dots, 5$, and in the analysis one follows the convention that values below the LLOQ are set to LLOQ/2 and values above the ULOQ are set to ULOQ.
4. $S_{29.j}$ and $S_{57.j}$ are also taken to be $W_{S29.j}^L$ and $W_{S57.j}^L$, respectively, for $j = 1, \dots, 5$, again following the convention that values below the LLOQ are set to LLOQ/2 and values above the ULOQ are set to ULOQ.

24.2.1 Input parameters for simulating covariates

The following lists the set of input parameters that are needed to simulate the covariate data, and indicates default values.

1. N = Total number of enrolled trial participants
2. $P_{a=1}$: $P(A = 1)$ = Probability an individual is randomized to vaccine A=1 (default 0.5)
3. $P_{b=1}$: $P(B = 1)$ = Probability an individual is baseline SARS-CoV-2 positive (default 0.10)
4. P_1 : $P(X_1 = 1)$ (default 0.3)
5. P_2 : $P(X_2 = 1)$ (default 0.5)
6. P_3 : $P(X_3 = 1)$ (default 0.3)
7. μ^L (defaults listed below)

Vaccine baseline negative ($a = 1, b = 0$):

$$\mu^L = c(55, 30, 0, 0, 0, 0, 0, 0, 4.4, 4.2, 2.2, 1.8, 2.2, 5.9, 5.7, 3.2, 2.8, 3.2)$$

Placebo baseline negative ($a = 0, b = 0$):

$$\mu^L = c(55, 30, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0)$$

Vaccine baseline positive ($a = 1, b = 1$):

$$\mu^L = c(55, 30, 3.9, 3.7, 1.8, 1.5, 1.7, 4.5, 4.3, 2.3, 1.9, 2.3, 6.0, 5.8, 3.3, 2.9, 3.3)$$

Placebo baseline positive ($a = 0, b = 1$):

$$\mu^L = c(55, 30, 3.5, 3.3, 1.4, 1.3, 1.5, 4.1, 4.0, 2.0, 1.8, 2.0, 5.6, 5.5, 3.0, 2.7, 3.1)$$

8. Σ_{diag} (defaults listed below)

Vaccine baseline negative ($a = 1, b = 0$):

$$\Sigma_{diag} = c(22.3^2, 7^2, 0.2, 0.2, 0.2, 0.2, 0.2, 0.2, 0.6^2, 0.7^2, 0.8^2, 0.8^2, 0.8^2, 0.7^2, 0.8^2, 0.98^2, 0.98^2, 0.94^2)$$

Placebo baseline negative ($a = 0, b = 0$):

$$\Sigma_{diag} = c(22.3^2, 7^2, 0.2, 0.2, 0.2, 0.2, 0.2, 0.2, 0.2, 0.2, 0.2, 0.2, 0.2, 0.2, 0.2, 0.2, 0.2, 0.2)$$

Vaccine baseline positive ($a = 1, b = 1$):

$$\Sigma_{diag} = c(22.3^2, 7^2, 0.6^2, 0.7^2, 0.8^2, 0.8^2, 0.8^2, 0.6^2, 0.7^2, 0.8^2, 0.8^2, 0.8^2, 0.7^2, 0.8^2, 0.98^2, 0.98^2, 0.94^2)$$

Placebo baseline positive ($a = 0, b = 1$):

$$\Sigma_{diag} = c(22.3^2, 7^2, 0.6^2, 0.7^2, 0.8^2, 0.8^2, 0.8^2, 0.6^2, 0.7^2, 0.8^2, 0.8^2, 0.8^2, 0.7^2, 0.8^2, 0.98^2, 0.98^2, 0.94^2)$$

9. Correlation parameters ρ (defaults listed below)

Vaccine baseline negative ($a = 1, b = 0$):

$$\begin{aligned}\rho[i, j] &= 0.25 + 0.1I(i = 3, j = 4) + 0.2 * I(i = 5, j = 6) : i = 1, \dots, 6, j > i \\ \rho[i, j] &= 0.6 + 0.1I(i = 8, j = 9) + 0.2 * I(i = 10, j = 11) : i = 7, \dots, 11, j > i \\ \rho[i, j] &= 0.7 + 0.1I(i = 13, j = 14) + 0.2 * I(i = 15, j = 16) : i = 12, \dots, 16, j > i\end{aligned}$$

Placebo baseline negative ($a = 0, b = 0$):

$$\begin{aligned}\rho[i, j] &= 0.15 + 0.05I(i = 3, j = 4) + 0.1 * I(i = 5, j = 6) : i = 1, \dots, 6, j > i \\ \rho[i, j] &= 0.2 + 0.05I(i = 8, j = 9) + 0.1 * I(i = 10, j = 11) : i = 7, \dots, 11, j > i \\ \rho[i, j] &= 0.25 + 0.05I(i = 13, j = 14) + 0.1 * I(i = 15, j = 16) : i = 12, \dots, 16, j > i\end{aligned}$$

Vaccine baseline positive ($a = 1, b = 1$):

$$\begin{aligned}\rho[i, j] &= 0.55 + 0.1I(i = 3, j = 4) + 0.2 * I(i = 5, j = 6) : i = 1, \dots, 6, j > i \\ \rho[i, j] &= 0.6 + 0.1I(i = 8, j = 9) + 0.2 * I(i = 10, j = 11) : i = 7, \dots, 11, j > i \\ \rho[i, j] &= 0.7 + 0.1I(i = 13, j = 14) + 0.2 * I(i = 15, j = 16) : i = 12, \dots, 16, j > i\end{aligned}$$

Placebo baseline positive ($a = 0, b = 1$):

$$\begin{aligned}\rho[i, j] &= 0.55 + 0.1I(i = 3, j = 4) + 0.2 * I(i = 5, j = 6) : i = 1, \dots, 6, j > i \\ \rho[i, j] &= 0.6 + 0.1I(i = 8, j = 9) + 0.2 * I(i = 10, j = 11) : i = 7, \dots, 11, j > i \\ \rho[i, j] &= 0.7 + 0.1I(i = 13, j = 14) + 0.2 * I(i = 15, j = 16) : i = 12, \dots, 16, j > i\end{aligned}$$

In the above correlation specification, note that extra correlation is added to IgG Spike and RBD readouts (same assay), as well as to PsV ID50 and ID80 (same assay). After $\rho[i, j]$ is define for all $i < j$, we set $\rho[j, i] = \rho[i, j]$ for all $j < i$.

24.3 Simulation of the failure time data

The failure time variables to simulate are $T_{29}, C_{29}, \tilde{T}_{29}, \Delta_{29}, T_{57}, C_{57}, \tilde{T}_{57}, \Delta_{57}$. First, in the placebo arm the event time T_{57} is simulated dependent on A, B, X_4^* , where X_4^* is a standardized version of age X_4 that has empirical mean 0 and empirical standard deviation 1. Second, in the vaccine arm T_{57} is simulated dependent on $A, B, X_4^*, S_{57.1}^*$, where $S_{57.1}^*$ is the first Day 57 marker (IgG Spike). The positive correlations among the different marker variables implies that T_{57} depends on the other Day 57 markers as well, but for a simple simulation model we only specify dependence on $S_{57.1}^*$. Alternatively, the simulations could be designed with the failure time connected to a latent variable that is an average of the markers.

Third and fourth, we implement a parallel approach to simulate T_{29} in the placebo arm and T_{29} in the vaccine arm, now using $S_{29.1}^*$ instead of $S_{57.1}^*$. These new simulations add intercurrent failures between the Day 29 visit and the Day 57 visit. Then calculations are made to enforce structural relationships between T_{29} and T_{57} .

24.3.1 Input parameters for simulating the failure time information

Let W^* be the vector W with each of the 17 normally distributed variables (elements 4 through 20) centered and scaled to have empirical mean zero and standard deviation one. The following parameters simulate failure time information starting from the Day 57 visit.

1. τ : final time point (in days) for analysis post Day 57 visit (default 180)
2. $P_{0b}(\tau)$: $P(T_{57} \leq \tau | A = 0, B = b, W^* = 0)$ = placebo arm baseline cumulative failure probability for baseline status group b (default $P_{00}(\tau) = 0.10, P_{01}(\tau) = 0.05$)
3. $VE_b = 1 - \frac{\lambda_{1b0}}{\lambda_{0b0}}$ for $b = 0, 1$, where $\lambda_{ab0} = \lambda(t | A = a, B = b, W^* = 0)$ for $a = 0, 1$ (default $VE_0 = VE_1 = 0.90$)
4. β_{0b} = Placebo group log hazard ratio per year increase in age X_4 for baseline stratum b . Once β_{0b} and $\Sigma_{diag}[1]$ are specified, the parameter β_{0b}^* in the Cox model (10) below is calculated as $\beta_{0b}^* = \beta_{0b} \sqrt{\Sigma_{diag}[1]}$.

- (default: $\beta_{0b} = \log_{10}(1.1)$ for each $b = 0, 1$)
5. β_{1b} = Vaccine group log hazard ratio per year increase in age X_4 for baseline stratum b . Once β_{1b} and $\Sigma_{diag}[1]$ are specified, the parameter β_{1b}^* in the Cox model (11) below is calculated as $\beta_{1b}^* = \beta_{1b}\sqrt{\Sigma_{diag}[1]}$. (default: $\beta_{1b} = \log_{10}(1.1)$ for each $b = 0, 1$)
 6. γ_{1b} = Vaccine group log hazard ratio per unit change in the marker $S_{57.1}$ (i.e., per 10-fold change in the marker on the natural/antilog10 scale). Once γ_{1b} and $\Sigma_{diag}[13]$ are specified, the parameter γ_{1b}^* in the Cox model (11) below is calculated as $\gamma_{1b}^* = \gamma_{1b}\sqrt{\Sigma_{diag}[13]}$. (default: $\gamma_{1b} = 0$ for each $b = 0, 1$ (null case))
 7. FollowupRange = range of days since enrollment until the date of data cut for the analysis, accounting for staggered enrollment (default FollowupRange = c(4*7, 6.5*7))
 8. $P_{LFU}(\tau)$: Probability loss to follow-up (prematurely) by τ (default $P_{LFU}(\tau) = 0.05$)

The following parameters simulate failure time information between the Day 29 and Day 57 visits (“intercurrent” failure defined in terms of T_{29}), specified in parallel fashion to the failure time information for T_{57} . With the exception of the intercurrent vaccine efficacy parameter, the following parameters are by default defined according to the Day 57 failure time simulation parameters.

1. $P_{0b.intcur}(57)$: $P(T_{29} \leq 28 | A = 0, B = b, W^* = 0)$ = placebo arm baseline cumulative intercurrent failure probability for baseline status group b (default $P_{0b.intcur}(57) = P_{0b}(\tau) * 28/\tau$, which specifies the same placebo arm incidence of failure from Day 29 to Day 57 as after Day 57)
2. $VE_{b.intcur} = 1 - \frac{\lambda_{1b0.intcur}}{\lambda_{0b0.intcur}}$ for $b = 0, 1$, where $\lambda_{ab0.intcur} = \lambda(t | A = a, B = b, W^* = 0)$ for $a = 0, 1$ (default $VE_{b.intcur} = 0.7 * VE_b$ for each $b = 0, 1$)
3. $\beta_{0b.intcur}$ = Placebo group log intercurrent hazard ratio per year increase in age X_4 for baseline stratum b . Once $\beta_{0b.intcur}$ and $\Sigma_{diag}[1]$ are specified, the parameter $\beta_{0b.intcur}^*$ in the Cox model (12) below is calculated as $\beta_{0b.intcur}^* = \beta_{0b.intcur}\sqrt{\Sigma_{diag}[1]}$. (default: $\beta_{0b.intcur} = \beta_{0b}$ for each $b = 0, 1$)

4. $\beta_{1b.intcur}$ = Vaccine group log intercurrent hazard ratio per year increase in age X_4 for baseline stratum b . Once $\beta_{1b.intcur}$ and $\Sigma_{diag}[1]$ are specified, the parameter $\beta_{1b.intcur}^*$ in the Cox model (13) below is calculated as $\beta_{1b.intcur}^* = \beta_{1b} \sqrt{\Sigma_{diag}[1]}$. (default: $\beta_{1b.intcur} = \beta_{1b}$ for each $b = 0, 1$)
5. $\gamma_{1b.intcur}$ = Vaccine group log intercurrent hazard ratio per unit change in the marker $S_{29.1}$ (i.e., per 10-fold change in the marker on the natural/antilog10 scale). Once $\gamma_{1b.intcur}$ and $\Sigma_{diag}[8]$ are specified, the parameter $\gamma_{1b.intcur}^*$ in the Cox model (13) below is calculated as $\gamma_{1b.intcur}^* = \gamma_{1b.intcur} \sqrt{\Sigma_{diag}[8]}$. (default: $\gamma_{1b.intcur} = \gamma_{1b}$ for each $b = 0, 1$)

24.3.2 Exponential/proportional hazards models for T_{57}

For the placebo arm, we assume the following simple proportional hazards models for T_{57} , separately by baseline status:

$$\lambda_{0b}(t|W) = \lambda_{0b0} e^{\beta_{0b}^* X_4^*} \quad (10)$$

where, assuming an exponential distribution, $\lambda_{0b0} = \lambda(t|A = 0, B = 0, W^* = 0)$ is determined by the equation

$$1 - e^{-\lambda_{0b0}\tau} = P_{0b}(\tau)$$

and the parameters β_{00}^* and β_{01}^* specify how strongly X_4^* (standardized age) associates with COVID.

For the vaccine arm, we assume the following proportional hazards models for T_{57} , again separately by baseline status:

$$\lambda_{1b}(t|W) = \lambda_{1b0} e^{\beta_{1b}^* X_4^* + \gamma_{1b}^* S_{571}^*} \quad (11)$$

where λ_{1b0} is determined by the equation

$$VE_b = 1 - \frac{\lambda_{1b0}}{\lambda_{0b0}},$$

where VE_b (proportional hazards vaccine efficacy at central covariate level $W^* = 0$) is input by the user.

24.3.3 Simulating T_{57}

Once a participant's values $A, B, X_4^*, S_{S57.1}^*$ are generated, then we simulate the participant's T_{57} value from an exponential distribution with rate parameter defined by the input parameters and the Cox model (10) or (11).

24.3.4 Simulating C_{57} and Δ_{57}

First, a random variable C_{157} is simulated from a Uniform distribution over the range FollowupRange. Second, an exponential random variable C_{257} is simulated with rate parameter λ_{cens} determined by

$$1 - e^{-\lambda_{cens}\tau} = P_{LFU}(\tau).$$

Then, we set $C_{57} = \min(C_{157}, C_{257})$, and next set $T_{57}^* = \min(T_{57}, C_{57})$ and $\Delta_{57} = I(T_{57} \leq C_{57})$. Note that, because in the analysis outcomes for Day 57 correlates analyses are only counted starting 7 days post Day 57 visit, cases with $T_{57} < 7$ are excluded from the analysis (this is handled in the data analysis code, not in the data set construction code).

24.3.5 Exponential/proportional hazards models for T_{29} intercurrent failure

For the placebo arm, we assume the following simple proportional hazards models for T_{29} , separately by baseline status:

$$\lambda_{0b.intcur}(t|W) = \lambda_{0b0.intcur} e^{\beta_{0b.intcur}^* X_4^*} \quad (12)$$

where, assuming an exponential distribution, $\lambda_{0b0.intcur} = \lambda(t|A = 0, B = b, W^* = 0)$ is determined by the equation

$$1 - e^{-\lambda_{0b0.intcur} * 57} = P_{0b.intcur}(57)$$

and the parameters $\beta_{00.intcur}^*$ and $\beta_{01.intcur}^*$ specify how strongly X_4^* (standardized age) associates with COVID. For the vaccine arm, we assume the following proportional hazards models for T_{29} intercurrently, again separately by baseline status:

$$\lambda_{1b.intcur}(t|W) = \lambda_{1b0.intcur} e^{\beta_{1b.intcur}^* X_4^* + \gamma_{1b.intcur}^* S_{291}^*} \quad (13)$$

where $\lambda_{1b0.intcur}$ is determined by the equation

$$VE_{b.intcur} = 1 - \frac{\lambda_{1b0.intcur}}{\lambda_{0b0.intcur}},$$

where $VE_{b.intcur}$ (proportional hazards vaccine efficacy at central covariate level $W^* = 0$) is input by the user.

24.3.6 Simulating T_{29}

Once a participant's values $A, B, X_4^*, S_{S29.1}^*$ are generated, then we initially simulate the participant's T_{29} value from an exponential distribution with rate parameter defined by the input parameters and the Cox model (12) or (13). If $T_{29} < T_{57} + 28$, then the value of T_{29} is kept. If $T_{29} \geq T_{57} + 28$, then we set the final value of $T_{29} = T_{57} + 28$ plus a draw from a random uniform distribution over -3 to 3 days rounded to the nearest day (to account for visit window variability).

24.3.7 Simulating C_{29} and Δ_{29}

For simplicity, we do not allow dropout between the Day 29 visit and the Day 57 visit. Therefore, we set $C_{29} = C_{57} + 28$. Then we set $\Delta_{29} = I(T_{29} \leq C_{29})$.

Note that, because in the analysis outcomes for Day 29 correlates analyses are only counted starting 7 days post Day 29 visit, cases with $T_{29} < 7$ are excluded from the analysis (this is handled in the data analysis code, not in the data set construction code).

24.4 Simulating the subcohort indicator R

The subcohort indicator R is one if a participant is sampled for measurement of (S_1, S_{29}, S_{57}) .

24.4.1 Input parameters for simulating R

We simulate R following Table 5 in this SAP, where the six baseline demographic strata are:

1. $X_4 \geq 65$ and $X_3 = 0$ (minority)

2. $X_4 \geq 65$ and $X_3 = 1$ (non-minority)
3. $X_4 < 65$ and $X_1 = 1$ (at-risk) and $X_3 = 0$
4. $X_4 < 65$ and $X_1 = 1$ (at-risk) and $X_3 = 1$
5. $X_4 < 65$ and $X_1 = 0$ (not at-risk) and $X_3 = 0$
6. $X_4 < 65$ and $X_1 = 0$ (not at-risk) and $X_3 = 1$

For each of the 24 subgroups/cells defined by (a, b) cross-classified with the above 6 demographic subgroups (as in Table 5), define the total numbers to be sampled into the random subcohort, $n2(a, b, c)$ for $a = 0, 1, b = 0, 1, c = 0, \dots, 6$. Then, for each subgroup (a, b, c) , R is set to 1 for a random sample of size $n2(a, b, c)$ without replacement. Lastly, all non-cases (with $\Delta = 0$) and $R = 0$ have all three values (S_1, S_{29}, S_{57}) set to NA.

The default settings for $n2(a, b, c)$ to match Table 5 in the SAP are as follows:

- $n2(1, 0, c) = 150$ for $c = 1, \dots, 6$
- $n2(0, 0, c) = 20$ for $c = 1, \dots, 6$
- $n2(1, 1, c) = 50$ for $c = 1, \dots, 6$
- $n2(0, 1, c) = 50$ for $c = 1, \dots, 6$

24.4.2 Per-protocol indicator

Lastly, we simulate the per-protocol (PP) indicator variable, which is 1 if both immunizations at Day 0, 29 were received and there were no specified protocol violations.

Input parameter:

$P_{PP=1}$ = Probability a participant is per-protocol (default = 0.99)

$P_{PP=1}$ is simulated from a Bernoulli random variable with success probability $P_{PP=1}$. The Day 57 marker correlates analyses are done in individuals with $P_{PP=1}$.

24.4.3 Variables output for the data set

The following collates all of the variables defined for the simulated data set.

- A
- B
- X_1, \dots, X_5
- PP
- R
- $S_{1.1}, \dots, S_{1.5}$
- $S_{29.1}, \dots, S_{29.5}$
- $S_{57.1}, \dots, S_{57.5}$
- $\tilde{T}_{29}, \Delta_{29}$
- $\tilde{T}_{57}, \Delta_{57}$

25 Appendix: Notes on Planning for Stage 2 Correlates Analyses

The paper Sun, Zhou, Gilbert (submitted) “Analysis of Cox model with Longitudinal Covariates with Measurement Errors and Partly Interval-Censored Failure Time, with Application to an AIDS Clinical Trial” may be a suitable method for assessing antibody markers over time as correlates of the SARS-CoV-2 infection endpoint, once there is follow-up data for more than a year with antibody markers measured at all time points up to at least a year. The paper extends Fu and Gilbert (2017) from right-censored failure time data to partly interval-censored failure time data, which means a composite endpoint is analyzed with one component subject to right-censoring and the other component subject to interval censoring. In our application, the COVID primary endpoint is subject to right-censoring, and seroconversion is subject to interval-censoring.