Abbreviated Title: COVID vaccine allergy reaction (COVAAR)

NIH IRB #: 000460

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Title: A randomized, placebo-controlled crossover study to assess the safety of administering a second dose of a COVID-19 mRNA vaccine in individuals who experienced a systemic allergic reaction to an initial dose

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Investigational Agents:

Drug Name:	Comirnaty (COVID-19 Vaccine, mRNA); Pfizer-BioNTech COVID-19 Vaccine, Bivalent (Original and Omicron BA.4/BA.5).
Investigational New Drug (IND) Number:	27537
IND Sponsor:	Office of Clinical Research Policy and Regulatory Operations (OCRPRO), Division of Clinical Research (DCR), NIAID, NIH
Manufacturer:	Pfizer-BioNTech

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STATEMENT OF COMPLIANCE

The trial will be carried out in accordance with International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) Good Clinical Practice (GCP) and the following:

- United States (US) Code of Federal Regulations (CFR) applicable to clinical studies (45 CFR Part 46, 21 CFR Part 50, 21 CFR Part 56, 21 CFR Part 312, and/or 21 CFR Part 812)

NIH-funded investigators and clinical trial site staff who are responsible for the conduct, management, or oversight of NIH-funded clinical trials have completed Human Subjects Protection and ICH GCP Training.

The protocol, informed consent form(s), recruitment materials, and all participant materials will be submitted to the Institutional Review Board (IRB) for review and approval. Approval of both the protocol and the consent form must be obtained before any participant is enrolled. Any amendment to the protocol will require review and approval by the IRB before the changes are implemented to the study. In addition, all changes to the consent form will be IRB-approved; an IRB determination will be made regarding whether a new consent needs to be obtained from participants who provided consent using a previously approved consent form.

1 PROTOCOL SUMMARY

1.1 SYNOPSIS

Title: A randomized, placebo-controlled crossover study to assess the safety of administering a second dose of an mRNA-based COVID-19 vaccine in individuals who experienced a systemic allergic reaction to an initial dose

Study Description: This is a single-site study to determine the safety of administering a dose of the Pfizer-BioNTech mRNA coronavirus disease 2019 (COVID-19) vaccine (Comirnaty) to individuals who experienced a systemic allergic reaction to their first full dose of the same vaccine or the Moderna mRNA vaccine, and to investigate possible mechanisms underlying allergic reactions.

Objectives:

Primary Objective:

1. Assess the proportion of participants who develop a **systemic allergic reaction** (Consortium for Food Allergy Research [CoFAR; see Appendix A] grade 2 reaction and above regardless of tryptase, or CoFAR grade 1 with elevated tryptase [1.2 X baseline plus 2ng/mL]) to the Pfizer-BioNTech COVID-19 vaccine after previously demonstrating a systemic allergic reaction (CoFAR Grade 2 or 3 reaction regardless of tryptase OR Grade 1 reaction with elevated tryptase [1.2 X baseline plus 2ng/mL]) to their first full dose of the same vaccine or the Moderna COVID-19 vaccine.

Secondary Objectives:

1. Assess the proportion of participants who develop a **severe systemic allergic reaction** (CoFAR Grade 3 reaction or higher regardless of tryptase) to the Pfizer-BioNTech COVID-19 vaccine after previously demonstrating a systemic allergic reaction (CoFAR Grade 2 or 3 reaction regardless of tryptase OR Grade 1 reaction with elevated tryptase [1.2 X baseline plus 2ng/mL]) to their first full dose of the same vaccine or the Moderna COVID-19 vaccine.
2. Assess the proportion of participants who develop a **mild-moderate allergic reaction** (CoFAR Grade 1 or 2 reaction regardless of tryptase) to the Pfizer-BioNTech COVID-19 vaccine after previously demonstrating a systemic allergic reaction (CoFAR Grade 2 or 3 reaction regardless of tryptase OR Grade 1 reaction with elevated tryptase [1.2 X baseline plus 2ng/mL]) to their first full dose of the same vaccine or the Moderna COVID-19 vaccine.
3. Assess the proportion of participants with **anaphylactic reactions** (Levels 1-3) per Brighton Collaboration Criteria (see Appendix B) to the Pfizer-BioNTech COVID-19 vaccine after previously demonstrating a systemic allergic reaction (CoFAR Grade 2 or 3 reaction OR Grade 1 reaction with elevated tryptase [1.2 X baseline plus 2ng/mL]) to their first full dose of the same vaccine or the Moderna COVID-19 vaccine.
4. Assess the proportion of participants who develop a systemic allergic reaction (CoFAR Grade 2 reaction or higher regardless of tryptase OR Grade 1 reaction with elevated tryptase [1.2 X baseline

plus 2ng/mL]) to the Pfizer-BioNTech COVID-19 vaccine after previously demonstrating a systemic allergic reaction (CoFAR Grade 2 or 3 reaction regardless of tryptase OR Grade 1 reaction with elevated tryptase [1.2 X baseline plus 2ng/mL]) to their first full dose of the same vaccine or the Moderna COVID-19 vaccine compared to the rate of these reactions following placebo administration.

5. Compare the severity of allergic reactions to the first dose of the Pfizer-BioNTech or Moderna COVID-19 vaccine to the severity of the reaction following administration of a subsequent dose of the Pfizer-BioNTech vaccine in individuals who experienced a systemic allergic reaction (CoFAR grade 2 reaction and above regardless of tryptase, or CoFAR grade 1 with elevated tryptase [1.2 X baseline plus 2ng/mL]) to their first full dose of the Pfizer-BioNTech or Moderna COVID-19 vaccine.

Exploratory Objectives:

1. Assess the risk of having a systemic allergic reaction to a dose of the Pfizer-BioNTech COVID-19 vaccine in participants who previously demonstrated a systemic allergic reaction (CoFAR Grade 2 or 3 reaction regardless of tryptase OR Grade 1 reaction with elevated tryptase [1.2 X baseline plus 2ng/mL]) to their first full dose of the same vaccine or the Moderna COVID-19 according to baseline covariates.
2. Examine possible mechanisms of allergic reactions to mRNA-based COVID-19 vaccines.
3. Assess innate and adaptive immune responses, including functional antibody levels, to the Pfizer-BioNTech COVID-19 vaccine.
4. Investigate mental health characteristics of participants who experience an allergic reaction to the COVID-19 vaccine.
5. Assess psychological impact of allergic reactions to the COVID-19 vaccine and examine changes in stress levels over time.
6. Assess anxiety levels in participants and examine changes in anxiety over time.
7. Assess the proportion of participants who experience no reaction or only a mild reaction (CoFAR Grade 2 or below) to a booster dose of the Pfizer-BioNTech vaccine after previously demonstrating a systemic allergic reaction (CoFAR Grade 2 or 3 reaction regardless of tryptase OR Grade 1 reaction with elevated tryptase [1.2 X baseline plus 2 ng/mL]) to their first full dose of the Pfizer-BioNTech or Moderna COVID-19 vaccine and no allergic reaction or a mild reaction (CoFAR Grade 2 or below) to a subsequent (second) dose of the Pfizer-BioNTech vaccine.
8. Assess the development of autoantibodies after COVID-19 vaccination.

Endpoints:

Primary Endpoint:

1. The proportion of participants who previously demonstrated a systemic allergic reaction (CoFAR Grade 2 or 3 reaction regardless of tryptase OR Grade 1 reaction with elevated tryptase [1.2 X baseline plus 2ng/mL]) to their first full dose of the Pfizer-BioNTech or Moderna COVID-19 vaccine who experience a **systemic allergic reaction** (CoFAR Grade 2 and above reaction regardless of tryptase, or CoFAR grade 1 with elevated tryptase [1.2 X baseline plus 2ng/mL]) within the 3-hour post-vaccine observation period to a subsequent dose of the Pfizer-BioNTech vaccine.

Secondary Endpoints:

2. The proportion of participants who previously demonstrated a systemic allergic reaction (CoFAR Grade 2 or 3 reaction regardless of tryptase OR Grade 1 reaction with elevated tryptase [1.2 X baseline plus 2ng/mL]) to their first full dose of the Pfizer-BioNTech or Moderna COVID-19 vaccine who experience a **severe systemic allergic reaction** (CoFAR grade 3 reaction and above) within the 3-hour post-vaccine observation period to a subsequent dose of the Pfizer-BioNTech vaccine.
3. The proportion of participants who previously demonstrated a systemic allergic reaction (CoFAR Grade 2 or 3 reaction regardless of tryptase OR Grade 1 reaction with elevated tryptase [1.2 X baseline plus 2ng/mL]) to their first full dose of the Pfizer-BioNTech or Moderna COVID-19 vaccine who experience a **mild-moderate allergic reaction** (CoFAR grade 1 or 2 reaction regardless of tryptase) within the 3-hour post-vaccine observation period to a subsequent dose of the Pfizer-BioNTech vaccine.
4. The proportion of participants who previously demonstrated a systemic allergic reaction (CoFAR Grade 2 or 3 reaction regardless of tryptase OR Grade 1 reaction with elevated tryptase [1.2 X baseline plus 2ng/mL]) to their first full dose of the Pfizer-BioNTech or Moderna COVID-19 vaccine who experience an **anaphylactic reaction** (Levels 1-3) per Brighton Collaboration Criteria within the 3-hour post-vaccine observation period to a subsequent dose of the Pfizer-BioNTech vaccine.
5. The proportion of participants who previously demonstrated a systemic allergic reaction (CoFAR Grade 2 or 3 reaction regardless of tryptase OR Grade 1 reaction with elevated tryptase [1.2 X baseline plus 2ng/mL]) to their first full dose of the Pfizer-BioNTech or Moderna COVID-19 vaccine who experience a systemic allergic reaction (CoFAR grade 2 reaction and above regardless of tryptase, or CoFAR grade 1 with elevated tryptase [1.2 X baseline plus 2ng/mL]) within the 3-hour post-vaccine observation period to a subsequent dose of the Pfizer-BioNTech vaccine compared to the rate of these reactions following placebo administration.

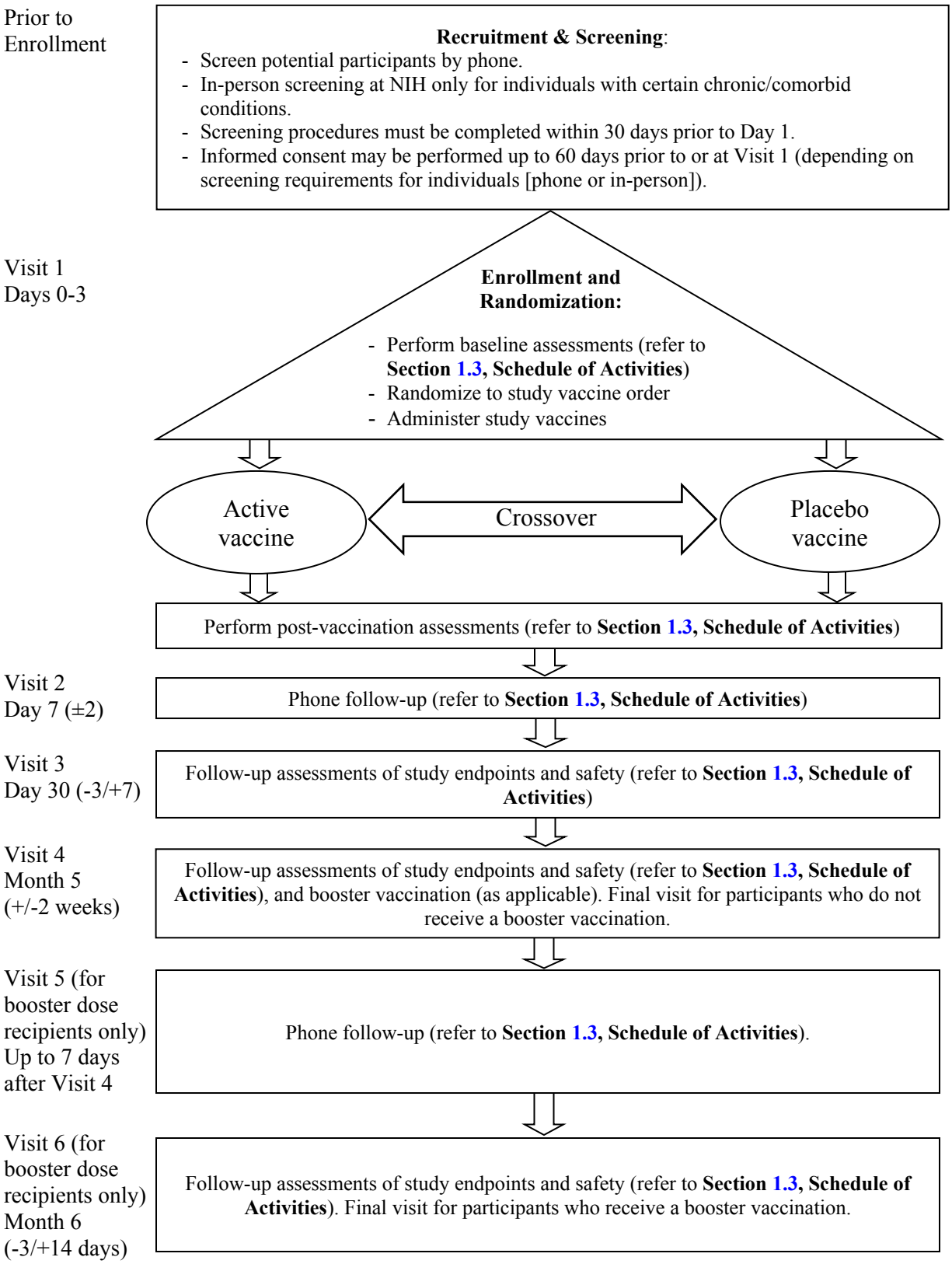
6. The proportion of participants who previously demonstrated a systemic allergic reaction (CoFAR Grade 2 or 3 reaction regardless of tryptase OR Grade 1 reaction with elevated tryptase [1.2 X baseline plus 2ng/mL]) to their first full dose of the Pfizer-BioNTech or Moderna COVID-19 vaccine who develop a lower or higher grade allergic reaction within the 3-hour post-vaccine observation period to a subsequent dose of the Pfizer-BioNTech vaccine.

Exploratory Endpoints:

7. Prevalence of polyethylene glycol (PEG) and severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) antibodies in each participant at baseline.
8. Changes in anti-PEG and SARS-CoV-2 antibodies in each participant approximately 1 and 5 months after receiving the dose of the Pfizer-BioNTech vaccine administered on study.
9. Prevalence of positive skin testing to the vaccine and/or vaccine components including PEG- and polysorbate 80-containing medications.
10. Changes in biomarkers from baseline to post-dose of the Pfizer-BioNTech vaccine administered on study (e.g., known mediators of systemic reactions due to mast cell activation, markers of inflammatory response, markers of basophil and neutrophil activation, markers associated with activation of the classical and alternative complement pathways or kinin system, proteomics, metabolomics).
11. Changes in blood transcriptomics after vaccination.
12. Changes in innate and adaptive immune responses including functional antibody levels after vaccination.
13. Mental health/anxiety questionnaire scores and anxiety level ratings at baseline.
14. Results of psychiatric consultation/mental health interview.
15. Changes in mental health/anxiety questionnaire scores and anxiety level ratings over the study period.
16. The proportion of participants who previously demonstrated a systemic allergic reaction (CoFAR Grade 2 or 3 reaction regardless of tryptase OR Grade 1 reaction with elevated tryptase [1.2 X baseline plus 2 ng/mL]) to their first full dose of the Pfizer-BioNTech or Moderna COVID-19 vaccine and no allergic reaction or a mild reaction (CoFAR Grade 2 or below) to a subsequent (second) dose of the Pfizer-BioNTech vaccine who experience no reaction or only a mild reaction (CoFAR Grade 2 or below) to a booster dose of the Pfizer-BioNTech vaccine administered approximately 5 months after the second dose.
17. The development of autoantibodies post-second and post-booster doses of the COVID-19 mRNA vaccines.

Study Population:	Up to 100 male and female participants ages 16-69 years of age who experienced a systemic allergic reaction (CoFAR Grade 2 or 3 reaction regardless of tryptase OR Grade 1 reaction with elevated tryptase [1.2 X baseline plus 2 ng/mL]) after receiving their first dose of the Pfizer-BioNTech or Moderna COVID-19 vaccine.
Phase:	N/A
Description of Sites/Facilities Enrolling Participants:	This is a single site study; all participants will be enrolled at the NIH Clinical Center.
Description of Study Intervention:	Participants who experienced a systemic allergic reaction after receiving their first full dose of the Pfizer-BioNTech or Moderna COVID-19 vaccine will be enrolled and receive a subsequent dose of the Pfizer-BioNTech vaccine and placebo in a randomized order on consecutive days.
Study Duration:	6 years
Participant Duration:	Approximately 5 or 6 months

1.2 SCHEMA


Prior to Enrollment
Recruitment & Screening:
- Screen potential participants by phone.
- In-person screening at NIH only for individuals with certain chronic/comorbid conditions.
- Screening procedures must be completed within 30 days prior to Day 1.
- Informed consent may be performed up to 60 days prior to or at Visit 1 (depending on screening requirements for individuals [phone or in-person]).
Visit 1 Days 0-3
Enrollment and Randomization:
- Perform baseline assessments (refer to Section 1.3, Schedule of Activities)
- Randomize to study vaccine order
- Administer study vaccines
Active vaccine
Crossover
Placebo vaccine
Perform post-vaccination assessments (refer to Section 1.3, Schedule of Activities)
Visit 2 Day 7 (±2)
Phone follow-up (refer to Section 1.3, Schedule of Activities)
Visit 3 Day 30 (-3/+7)
Follow-up assessments of study endpoints and safety (refer to Section 1.3, Schedule of Activities)
Visit 4 Month 5 (+/-2 weeks)
Follow-up assessments of study endpoints and safety (refer to Section 1.3, Schedule of Activities), and booster vaccination (as applicable). Final visit for participants who do not receive a booster vaccination.
Visit 5 (for booster dose recipients only) Up to 7 days after Visit 4
Phone follow-up (refer to Section 1.3, Schedule of Activities).
Visit 6 (for booster dose recipients only) Month 6 (-3/+14 days)
Follow-up assessments of study endpoints and safety (refer to Section 1.3, Schedule of Activities). Final visit for participants who receive a booster vaccination.

1.3 SCHEDULE OF ACTIVITIES (SOA)

Visit (location [c])	**Screening [a]** (remote/NIH)	**Visit 1** (NIH/ inpatient)			**Visit 2** (remote)	**Visit 3** (NIH)	**Visit 4** (NIH)	**Visit 5 (Booster recipients only)** (remote)	**Visit 6 (Booster recipients only)** (NIH)	**Unscheduled visits [b]** (remote/ NIH)
Timepoint (window)	Day -30-0	Enrollment/Day 0 [d]	Day 1	Day 2	Day 3 [e]	Day 7 (±2)	Day 30 (-3/+7)	Month 5 (±2 weeks)	Up to 7 days after Visit 4 (single timepoint unless additional safety follow-up needed)	Month 6 (-3/+14 days)	
Procedures											
Informed consent	X [f]	(X) [f]									
Consent addendum [x]								X			
Demographics	X										
Medical history	X	X	X	X	X	X	X	X	X	X	X
Additional screening procedures (per-participant basis) [g]	(X)										
Assessment of adverse events [h]		X	X	X	X	X	X	X	X	X	X
Randomization			X								
IV line insertions (1 or 2 sites)		X						X [i]			
Administer study vaccine [h]			X	X							

Visit (location [c])	**Screening [a]** (remote/NIH)	**Visit 1** (NIH/ inpatient)				**Visit 2** (remote)	**Visit 3** (NIH)	**Visit 4** (NIH)	**Visit 5 (Booster recipients only)** (remote)	**Visit 6 (Booster recipients only)** (NIH)	**Unscheduled visits [b]** (remote/ NIH)
Timepoint (window)	Day -30-0	Enrollment/Day 0 [d]	Day 1	Day 2	Day 3 [e]	Day 7 (±2)	Day 30 (-3/+7)	Month 5 (±2 weeks)	Up to 7 days after Visit 4 (single timepoint unless additional safety follow-up needed)	Month 6 (-3/+14 days)	
Administer Pfizer-BioNTech vaccine booster (optional) [j]								X			
Concomitant medication review	X	X	X	X	X	X	X	X	X	X	X
Targeted physical exam	(X)	X	X	X	X		X	X		X	(X)
Height and weight		X									
Vital signs [k]	(X)	X	X	X	X		X	X		X	
Peak flow measurement [l]			X	X	X		X	X		X	
Impulse oscillometry [l]			X	X	X		X	X		X	
Nasal swab [m]		X									
Serum pregnancy test [n]		X						X			
Mental health/anxiety questionnaires [o, p]	X						X	X			
Patient Health Questionnaire-9 [p]	X						X [q]	X [q]			

Visit (location [c])	**Screening [a]** (remote/NIH)	**Visit 1** (NIH/ inpatient)			**Visit 2** (remote)	**Visit 3** (NIH)	**Visit 4** (NIH)	**Visit 5 (Booster recipients only)** (remote)	**Visit 6 (Booster recipients only)** (NIH)	**Unscheduled visits [b]** (remote/ NIH)
Timepoint (window)	Day -30-0	Enrollment/Day 0 [d]	Day 1	Day 2	Day 3 [e]	Day 7 (±2)	Day 30 (-3/+7)	Month 5 (±2 weeks)	Up to 7 days after Visit 4 (single timepoint unless additional safety follow-up needed)	Month 6 (-3/+14 days)	
Anxiety rating scale	X	X	X [r]	X [r]	X	X	X	X	X	X	
Psychiatric consultation [s]	X										
Mental health interview benefit and burden questionnaire								X			
Urine collection [t]			X	X				X			
Blood collection [u]	(X)		X	X	X		X	X		X	(X)
Skin allergy testing							X [v]				
Clinical photography [w]			X	X	X		X	X		X	(X)

(X) = Only performed at the NIH/not included in a remote assessment.

Abbreviations: NIH, National Institutes of Health; IV, intravenous.

[a] All individuals will initially be screened remotely by phone/email/videoconference. If an individual has a co-morbid condition(s) that may increase their risk of having a severe allergic reaction, they may be asked to come to the NIH Clinical Center for additional studies, such as pulmonary function testing, echocardiogram, or blood laboratory testing, that are felt to be clinically necessary (based on the discretion of the study team) to ensure that they are medically stable to undergo the study intervention. These additional screening procedures may be performed on day 0 if logistically feasible.

[b] If a participant develops symptoms of a reaction post-visit or other concerns arise related to the vaccine administration, the participant will be instructed to contact study personnel and may be asked to return to the NIH for an unscheduled visit. If the participant presents with symptoms for a suspected allergic reaction, the investigator may elect to collect blood samples, at their discretion, for the assessment of serum biomarkers, complement activation, and other research studies. If the participant is not able to return to the NIH, they may be directed to receive medical care locally. Clinical care labs and research labs may be obtained.

[c] Some procedures/visits may be performed remotely via phone/email/videoconference.

[d] Depending on logistics and scheduling, some procedures indicated for Day 0 may be performed prior to study vaccination on Day 1 (e.g., IV insertion, pregnancy test). Subjects will be enrolled after eligibility is confirmed on Day 0.

[e] Participants will be discharged on day 3, unless they require additional treatment due to a reaction. The IV line(s) will be removed once participant is stable and before discharge.

[f] Informed consent may be obtained up to 60 days prior to Day 1. For individuals who can complete screening procedures remotely (i.e., those without comorbid conditions that would require additional in-person assessments), informed consent may be obtained remotely during screening or in person at Visit 1 prior to any study procedures. Individuals with comorbid conditions who need additional in-person screening assessments must provide informed consent remotely or in-person prior to undergoing the additional screening procedures.

[g] Testing necessary to assess stability of comorbid diseases that may affect reaction to the vaccine. May include pulmonary function testing, chest X-ray, electrocardiogram, echocardiogram, flexible nasolaryngoscopy, and/or blood laboratory testing.

[h] Study vaccinations will be administered in the Intensive Care Unit (ICU). The participant will be monitored for signs and symptoms of an allergic reaction and any other adverse event for a minimum of 3 hours after vaccination, and medications and equipment will be immediately available to treat possible reactions. The principal investigator and/or an experienced allergist associate investigator will be available. Day 2 dosing may be delayed to a later day (maximum delay of 7 days) at the discretion of the investigator if the participant experiences an allergic reaction on day 1 that has not resolved by day 2.

[i] Only for participants receiving booster vaccination.

[j] Participants (according to current FDA approvals/CDC guidelines) will be offered a booster dose of the Pfizer-BioNTech vaccine (monovalent or bivalent as described in section 4.1) at their 5-month visit (Visit 4) if they did not have a severe allergic reaction to the second dose in the ICU (ie, those who had CoFAR grade 2 reaction or less will be eligible for the booster). The booster vaccine will be administered in an outpatient clinic, and medications necessary to treat an allergic reaction, including epinephrine, will be readily available. Participants will be observed in the clinic for 2 hours after the booster dose is administered.

[k] Temperature, pulse rate, respiratory rate, oxygen saturation, and blood pressure will be measured within 30 minutes prior to each study vaccination, at the time of blood draws, and as clinically indicated following each study vaccination during Visit 1. Because participants will be in the ICU during Visit 1, many vital signs (e.g. pulse rate, respiratory rate) will be continuously monitored. At Visit 4, for participants who receive the booster dose, vital signs will be checked at baseline and prior to discharge, and as indicated in case of development of symptoms of an allergic reaction; otherwise, vital signs will be obtained per clinical routine at Visits 3 and 4.

[l] Performed prior to study vaccination on days 1 and 2. May be repeated during visit 1 and/or at follow-up visits at investigator discretion.

[m] A nasal swab (mid-turbinate or nasopharyngeal per current Clinical Center practice) will be collected for SARS-CoV-2 testing by polymerase chain reaction. If the result is positive, Visit 1 will be delayed until the participant has recovered and is outside of the quarantine window.

[n] For participants of childbearing potential only. Must be confirmed negative prior to proceeding with study vaccination (at Visit 1) and booster dose (as applicable at Visit 4).

[o] Mental health history questionnaire, Impact of Events Scale-6, State-Trait Anxiety Inventory, Generalized Anxiety Disorder-7 scale, and World Health Organization Disability Assessment Schedule-2. Participants may be given informational materials to help manage stress.

[p] To be completed by English or Spanish speakers only.

[q] May or may not be completed per investigator discretion.

[r] Completed pre- and post-dose.

[s] Mental health interview will take place via Clinical Center Microsoft Teams telehealth platform. Subjects will meet with a mental health clinician to review questionnaire results and be offered strategies to help manage distress. Subsequent sessions will be scheduled as needed based on ongoing reviews of mental health/anxiety assessments.

[t] During Visit 1, one void prior to each study vaccination and the first void after each study vaccination will be collected. If an allergic reaction occurs after the post-vaccination void is collected, an additional void may be collected. At Visit 4 (participants who opt in to booster dose only), one void prior to the booster dose and the first void post-dose prior to discharge will be collected, if possible.

[u] Blood samples will be collected from the IV line when possible; otherwise, venipuncture will be performed. During visit 1, samples will be collected before each study vaccination and approximately 35 minutes (+/-15 minutes), 2 hours (+/-30 minutes), 6 hours (+/-30 minutes), and 24 hours (+/-1 hour) after each study vaccination. The 24-hour blood draw for day 1 will also serve as the baseline blood draw for day 2. At Visit 4, participants who do not receive a booster vaccination will undergo a single blood draw, and those who receive a booster vaccination may undergo blood draws pre-dose, 35 minutes (+/-15 minutes) post-dose, and 2 hours (+/-30 minutes) post-dose.

[v] Will only be performed once, either at Visit 3 or Visit 4 per investigator discretion.

[w] If a participant develops a skin rash following vaccination, photographs of the affected area(s) may be taken with the participant's permission.

[x] If the bivalent formulation (Original and Omicron BA.4/BA.5) of the vaccine is available at the NIH, participants will be given the option of receiving the bivalent or monovalent version as the booster dose; if the bivalent version is not available, the monovalent will still be offered. A consent addendum will be discussed with the participants about this choice.

2 INTRODUCTION

2.1 STUDY RATIONALE

Widespread vaccination will be essential to overcome the public health crisis created by COVID-19. Although rare, the allergic reactions that have occurred as a result of COVID-19 mRNA vaccinations have created substantial fear and anxiety in the general population where allergic disease is common. This concern may translate to reduced willingness for patients with a history of severe allergies to receive the COVID-19 vaccine, and/or a hesitancy on the part of physicians to administer the vaccine to these patients out of concern that the vaccine will trigger an allergic reaction. Furthermore, the high rate of suspected allergic reactions following the initial doses of these vaccines may prevent a substantial fraction of the population from receiving a second dose, which may be necessary for the vaccine to be fully effective. Currently, the safety of administering a second dose of these vaccines to patients who experienced an allergic reaction to the first dose is unknown, as is the safety of administering a subsequent dose of an mRNA-based vaccine that is different than the one that caused a previous reaction (e.g., administering the Pfizer-BioNTech vaccine after a reaction to the Moderna vaccine). For these reasons, a better understanding of the mechanisms responsible for allergic reactions to the mRNA COVID vaccines is critically needed in order to reassure the public and to provide evidence-based guidance on who can safely receive these vaccines, including a second dose in those individuals who experienced a reaction to the first dose.

2.2 BACKGROUND

Prevalence and characteristics of allergic reactions to messenger RNA (mRNA) vaccines for COVID-19

The SARS-CoV-2 virus has incited a global pandemic leading to profound loss of human life and tremendous economic and social upheaval. In late 2020, the FDA issued emergency use authorizations (EUAs) for monovalent mRNA vaccines produced by Pfizer-BioNTech and Moderna that were found to be highly effective in Phase 3 trials at preventing COVID-19.[1, 2] The active ingredient in both vaccines is nucleoside-modified mRNA encoding the SARS-CoV-2 spike protein. The Pfizer-BioNTech monovalent COVID-19 vaccine, given as 2 doses 21 days apart, showed 95% efficacy at preventing symptomatic COVID-19 infection 7 days after administration of the second dose.[1] Protection from the vaccine was similar across different age, racial, and ethnic groups, and it received full FDA approval for individuals 16 years of age and older on August 23, 2021, under the brand name Comirnaty, with the eligible age group subsequently lowered to 12 years of age and older. As of September 16, 2022, it also continues to be available under EUA for individuals 6 months through 11 years of age and for the administration of a third primary dose in certain immunocompromised individuals. A previous authorization for use as a booster vaccination in individuals 12 years of age and older was removed when a new bivalent version was authorized for this use (see below). The Moderna monovalent COVID-19 vaccine, given as 2 doses 28 days apart, was 94% effective at preventing symptomatic infection 14 days after administration of the second dose.[2] The efficacy of the Moderna vaccine appeared to be slightly lower in people 65 years and older, but was equally effective across different racial and ethnic groups, and it received full FDA approval for people ages 18 years and older on January 31, 2022 under the brand name Spikevax, with authorizations for lower ages also anticipated. Both vaccines exhibited a favorable safety profile, with most side

effects being mild to moderate in severity and including pain, swelling, and redness at the injection site, headache, fatigue, fever, chills, nausea, myalgia, arthralgia, and lymphadenopathy.

However, within days of administration of the vaccines to healthcare workers in both the United States (US) and United Kingdom (UK), multiple reports of severe allergic reactions emerged. As of January 18, 2021, 47 cases of anaphylaxis (defined by Brighton Collaboration case definition criteria 1, 2, or 3 as assessed by Centers for Disease Control and Prevention [CDC] physicians)[3] attributed to the Pfizer-BioNTech vaccine, and 19 cases attributed to the Moderna vaccine, had been reported to the CDC/FDA Vaccine Adverse Event Reporting System (VAERS).[4] In nearly all cases (89% for both vaccines), symptoms appeared within 30 minutes of vaccine administration, and nearly all cases were in women (94% for Pfizer-BioNTech and 100% for Moderna). Most reactions (92%) were treated with epinephrine. Forty-eight percent of the individuals who experienced reactions were hospitalized (7 of whom required endotracheal intubation). The vast majority of individuals who experienced post-vaccine anaphylaxis had a documented history of severe allergies and/or allergic reactions to drugs, foods, insect stings, contrast media, and/or vaccines (77% of those who reacted to the Pfizer-BioNTech vaccine and 84% of those who reacted to the Moderna vaccine). As of January 18, 2021, the overall rate of anaphylaxis to the mRNA COVID-19 vaccines was estimated at 4.7 cases per million doses administered for the Pfizer-BioNTech vaccine and 2.5 cases per million doses administered for the Moderna vaccine.[4] This rate of anaphylaxis is higher than that reported for vaccines in general, which is 1.31 (95% CI, 0.90-1.84) cases per million vaccine doses, using similar criteria for defining anaphylaxis.[5] Of note, the rate of reported allergic reactions to the mRNA COVID-19 vaccines, including both mild-moderate and severe reactions, is significantly higher than 2.8-5.0 cases per million doses. However, the CDC found that nearly half of reported severe "allergic" reactions to the mRNA vaccines could not be confirmed after case review, emphasizing the importance of a thorough allergy evaluation in assessing vaccine reactions and the need for challenge studies to confirm and investigate the mechanisms responsible. As of January 2021, there were no fatalities associated with reported allergic reactions to either the Pfizer-BioNTech or Moderna vaccines.[4]

FDA granted an EUA on August 31, 2022, for the use of a bivalent version of the Pfizer-BioNTech vaccine (referred to as Pfizer-BioNTech COVID-19 Vaccine, Bivalent [Original and Omicron BA.4/BA.5]) as a booster vaccination. Subsequently, CDC updated their recommendation on September 1, 2022, as follows: "People ages 12 years and older are recommended to receive 1 age-appropriate bivalent mRNA booster dose after completion of any FDA-approved or FDA-authorized monovalent primary series or previously received monovalent booster dose(s). This new booster recommendation replaces all prior booster recommendations for this age group."[6] These recommendations were made after favorable vote from CDC's Advisory Committee on Immunization Practices (ACIP). The evidence was based primarily on the results of serological data against BA.1 variant and pre-clinical serological assessment using BA.4/BA.5 variant demonstrating superior neutralization antibody titers. These titers were noted to exceed pre-defined non-inferiority criteria against all Omicron variants, in particular BA.4/5, with the use of a bivalent booster dose including the original strain and Omicron BA.4/5. Reactogenicity was noted to be comparable to the original vaccine.[7, 8] These data were used to predict likely favorable outcomes by greater serological protection against Omicron variants. Other animal model studies noted similar enhancement in neutralizing

antibody titers after variant-based vaccination.[9, 10] Another study used a predictive model to estimate the benefit after use of variant-based modified vaccine and estimated that the efficacy of the modified vaccine was similar against severe disease while likely offering 4.6% more protection against symptomatic infection compared to the traditional vaccine's estimated protection at 85.6%, and hence was estimated to cause 8 less infections per 1000 population.[11, 12] At this time, the evidence is largely pre-clinical with extrapolation to the real-world scenario with assumption that the updated booster will likely offer improved protection against Omicron variants. Similarly, at this time it is unknown if there will be any difference in incidence of AEs such as anaphylaxis, myocarditis, and others, and the risk is presumed to be similar to the original (monovalent) vaccine. However, further clinical data is needed to confirm real-time efficacy and safety. While not in line with the current FDA authorizations and CDC guidelines, booster vaccination with the the monovalent version of the vaccine would still provide valuable scientific information for the purposes of this study, and will will likely continue to provide protection against severe COVID-19 illness if used as a booster vaccine in remaining subjects.

According to current FDA and CDC recommendations, individuals with a history of an anaphylactic reaction to any component of the mRNA COVID-19 vaccines should not receive these vaccines. Individuals with a history of an immediate allergic reaction to a vaccine or injectable or any history of anaphylaxis should be observed for 30 minutes post-vaccination, and all other individuals should be observed for 15 minutes. Additionally, patients who have an immediate (within 4 hours) or severe allergic reaction (e.g., anaphylaxis) to either mRNA COVID-19 vaccine are currently advised not to receive a second dose.

Immunologic and nonimmunologic mechanisms can trigger anaphylactic reactions

Anaphylaxis is a rapidly developing, life-threatening, systemic allergic reaction. Classically, anaphylaxis is caused by a type I hypersensitivity reaction where exposure to an antigen induces cross-linking of antigen-specific IgE bound to the high affinity IgE receptor, FcεRI, on the surface of mast cells and basophils. This triggers cellular degranulation and the release of pre-formed mediators, including histamine and preformed cytokines, tryptase, and other proteases, as well as synthesis and secretion of additional cytokines and lipid mediators including platelet activating factor (PAF), leukotrienes, and prostaglandins. The release of these mediators is responsible for the clinical signs and symptoms of an allergic reaction, including flushing, pruritus, rhinorrhea, tachycardia, bronchospasm, increased vascular permeability, and hypotension.

Although IgE-dependent mast cell degranulation is the most commonly recognized cause of anaphylaxis, multiple other mechanisms, both immunologic and non-immunologic in nature, can result in identical clinical presentations. In mice, IgG can also trigger anaphylaxis as a result of antigen-IgG binding to the FcγRIII on basophils, macrophages, and neutrophils, leading to the release of PAF that triggers an increase in vascular permeability and hypotension.[13] IgG-mediated anaphylaxis has not been definitively proven in humans, but some studies have found PAF to be an important mediator of anaphylaxis in patients.[14, 15] Activation of the complement pathway can also trigger symptoms of anaphylaxis through the release of C3a, C5a, and soluble C5b-C9 (sC5b-C9), which can bind to their receptors on mast cells, basophils, and other myeloid cells and stimulate degranulation.[16-18] Depletion of complement proteins and production of C3a and C5a have been seen both in murine models of anaphylaxis and in clinical

studies.[19] Additionally, mediators released from mast cells, including heparin, can also activate factor XII (FXII) and the pro-inflammatory kallikrein–kinin system that results in the release of the mediator bradykinin (BK). Some studies have found that levels of BK, which triggers vasodilation and an increase in vascular permeability, correlate with the severity of anaphylaxis.[20]

In some cases, anaphylaxis is induced via non-immunologic mechanisms as a result of the direct activation of mast cells.[21] For example, certain drugs, including opioids, dextrans, and quinolones, can directly stimulate mast cells and/or interact with the MAS-related G protein-coupled receptor-X2 (MRGPRX2) on mast cells, triggering their degranulation.

Finally, a number of conditions can masquerade as anaphylaxis, including vasovagal reactions, pain, and anxiety, which can be associated with flushing, pallor, shortness of breath, tachycardia, lightheadedness, and throat tightness.

Potential mechanisms underlying severe, immediate allergic reactions to COVID-19 mRNA vaccines

The Pfizer-BioNTech and Moderna vaccines utilize a liposomal-based drug delivery system to prevent degradation of the mRNA cargo and to facilitate delivery to target cells, although the lipids used in the 2 vaccines differ slightly (see Table 1). Both vaccine preparations also contain the excipient PEG 2000, which is conjugated to the lipids to improve water solubility, prevent aggregation, and provide increased stability and a longer half-life. While mRNA vaccines have been studied for some time, the current COVID-19 mRNA vaccines were the first to receive EUA, and the Pfizer-BioNTech product is the first to be licensed. These vaccines are also the first to use PEG. PEG has been hypothesized to be the antigenic trigger responsible for allergic reactions to the COVID-19 mRNA vaccines, but currently there is no direct evidence to support this.

Table 1: Composition of Pfizer-BioNTech COVID-19 Vaccine and Moderna COVID-19 Vaccine

	Pfizer-BioNTech COVID-19 Vaccine	Moderna COVID-19 Vaccine
Active	Nucleoside-modified messenger RNA (modRNA) encoding the viral spike (S) glycoprotein of SARS-CoV-2.	Nucleoside-modified mRNA encoding the prefusion stabilized spike (S) glycoprotein of SARS-CoV- 2
Inactive – lipids	((4-hydroxybutyl)azanediyl)bis(hexane-6,1diyl)bis(2-hexyldecanoate)	SM-102 (Proprietary to Moderna)
	2[(polyethylene glycol [PEG] 2000)]-N,N-ditetradecylacetamide	Polyethylene glycol (PEG) 2000 dimyristoyl glycerol (DMG)
	1,2-distearoyl-sn-glycero-3-phosphocholine	1,2-distearoyl-sn-glycero-3-phosphocholine
	Cholesterol	Cholesterol
Inactive – salts, sugars, buffers	Tromethamine, tromethamine hydrochloride	Tromethamine, Tromethamine hydrochloride, acetic acid, sodium acetate
	Sugar (sucrose)	Sugar (sucrose)

PEG is a very common ingredient found in a wide variety of commercial products including more than 1000 FDA-approved medications and over-the-counter products including skin creams, cosmetics, sunscreen, toothpaste, shampoo, and foods. PEG has notoriously been considered very safe, although rare allergic reactions have been reported. A review of FDA records from 2005 through 2017 identified an average of 4 cases (range, 2-8 cases) per year of PEG-associated anaphylaxis, typically following ingestion of PEG in colonoscopy preparations (Golytely) or treatments for constipation (Miralax).[22] A recent study found that at least some cases of PEG-associated anaphylaxis are caused by type I hypersensitivity reactions triggered by PEG-specific IgE. This study also screened for preexisting anti-PEG antibodies in sera from "normal" donors using a Dual Cytometric Bead Assay (DCBA). Up to 9% of samples were positive for anti-PEG IgG, 6% for anti-PEG IgM, and 0.1% for anti-PEG IgE.[23] In a separate study, Chang et al. discovered 7 single-nucleotide polymorphisms (SNPs) localized in the variable segment of immunoglobulin heavy chain gene that were significantly associated with high prevalence and concentrations of anti-PEG IgM in the general population in China.[24] This study therefore suggests that host genetic factors may contribute to PEG immunogenicity.

Although PEGylated liposomes are often considered nonimmunogenic, use of these preparations has been associated with the development of anti-PEG IgM and IgG antibodies that can instigate accelerated blood clearance of pegylated drugs (ABC phenomenon) and acute hypersensitivity reactions, termed Complement Activation Related Pseudoalleregy (CARPA).[25, 26] Both types of adverse reactions are thought to be driven by complement activation. In a pig model, delivery of 2-K-methoxy-PEGylated liposomes induced a strong anti-PEG IgM response that triggered fatal anaphylaxis as a result of complement pathway activation, as indicated by elevation of the

terminal complement complex (sC5b-9) in the blood of affected animals.[27] In the case of PEGylated liposomes harboring an mRNA payload, the immunostimulatory properties of the nucleic acid could further promote an immune response to PEG.

PEG shares significant structural similarity with polysorbate 80, which is found in several currently licensed vaccines and is a rare cause of vaccine allergic reactions.[22] Many of the COVID-19 vaccines currently under development that are using a platform distinct from the mRNA/PEGylated liposomes do not contain PEG but do contain polysorbate 80. Because of the potential cross-reactivity between PEG and polysorbate 80, individuals who have experienced a severe allergic reaction to the mRNA vaccines may be advised to avoid all polysorbate 80-containing injectables until a role for PEG in these reactions has been ruled out.

2.3 RISK/BENEFIT ASSESSMENT

2.3.1 Known Potential Risks

Vaccines: The risks of the Pfizer-BioNTech COVID-19 vaccines are detailed in the full FDA prescribing information documents.[28, 29] The most common adverse reactions following the Pfizer-BioNTech COVID-19 vaccines that have been reported in clinical trials include injection site pain, fatigue, headache, muscle pain, chills, joint pain, fever, injection site swelling, and injection site redness. Other side effects included nausea, malaise, lymphadenopathy, diarrhea, vomiting, and arm pain as well as asthenia, lethargy, decreased appetite, hyperhidrosis, night sweats, or rash. Fainting can happen from any vaccine given by injection.

Postmarketing data demonstrate increased risks of myocarditis and pericarditis, particularly within 7 days following the second dose. The observed risk is higher among males under 40 years of age than among females and older males. The observed risk is highest in males 12 through 17 years of age. Although some cases required intensive care support, available data from short-term follow-up suggest that most individuals have had resolution of symptoms with conservative management. Information is not yet available about potential long-term sequelae. The CDC has published considerations related to myocarditis and pericarditis after vaccination, including for vaccination of individuals with a history of myocarditis or pericarditis (https://www.cdc.gov/vaccines/covid-19/clinical-considerations/myocarditis.html). Individuals with a history of myocarditis or pericarditis may undergo additional screening procedures to assess for increased risk for these events following vaccination, and may be excluded from participation at the discretion of the investigator.

Severe allergic reactions have also been reported following the Pfizer-BioNTech monovalent vaccine during mass vaccination outside of clinical trials. Because the participants in this trial have previously experienced a systemic allergic reaction following administration of the Pfizer-BioNTech or Moderna vaccine, they are likely to be at higher risk for experiencing a second allergic reaction upon rechallenge with the mRNA-based Pfizer-BioNTech vaccine compared to the general population. Allergic reactions can range from mild to severe, with symptoms such as hives, and can include life-threatening anaphylactic reactions, with symptoms such as swelling of the airways, breathing difficulties, and low blood pressure. If a participant has an allergic reaction, they may need oral, intramuscular, or intravenous medications. Medications and equipment will be immediately available to treat possible reactions. Allergic reactions can also cause emotional distress.

The placebo vaccine does not contain active ingredients, and there are no known risks. However, since the participants in this study experienced allergic reactions to their first vaccine dose, they may experience increased anxiety, which may bring on symptoms similar to an allergic reaction.

Skin prick and intradermal testing: Skin testing is a standard medical procedure that rarely leads to complications. The procedure may lead to mild itching and swelling of the skin that is self-limited, subsides within 1-2 hours, and leaves no permanent mark on the skin. Anaphylaxis as a result of allergen skin testing is exceedingly rare and nearly always associated with intradermal testing **without** prior prick/puncture testing. For this reason, intradermal testing will only be performed after negative prick/puncture testing. Medications for treatment of anaphylaxis will be available on the unit and a physician in the hospital when skin testing is performed. Participants will stay in the clinic for 30 minutes after the skin testing to monitor for anaphylaxis.

Intravenous (IV) line insertion and venipuncture: Insertion of IV lines and venipuncture may cause pain, bruising, lightheadedness, dizziness, possible fainting, local discomfort, uncontrolled bleeding, and, rarely, infection at the site where the needle is inserted.

Nasal swab collection: Nasal swab collection may cause localized discomfort. Rarely, mild epistaxis may occur.

Clinical photography: Taking pictures of the face and body may be embarrassing to some people. These photographs may be published in medical journals, without identifying the participant. We will attempt to preserve the anonymity of the participant as much as possible, while providing the information needed to support the research being published. Participants may decline photographs or place any restrictions on their use. Participants will be given the opportunity to discuss this with the principal or associate investigators.

Pulmonary function testing: Risks of pulmonary function tests include possible fainting or light-headedness due to hyperventilation or, rarely, an asthmatic episode precipitated by deep inhalation exercises.

Peak flow measurement and impulse oscillometry: A sterile filter is used to prevent any risk associated with oral contact to the machines. Otherwise there are no associated risks with this testing procedure.

Chest X-ray: The risk associated with a single chest X-ray is exposure to a low level of radiation (<0.3 rem).

Echocardiogram and electrocardiogram (ECG): Other than possible minor skin irritation from the electrodes, there are no anticipated risks related to these procedures.

Flexible nasolaryngoscopy: Risks of flexible nasolaryngoscopy include a strange sensation as the scope is inserted into the nose and, rarely, nosebleed.

Urine collection: There are no risks associated with urine sample collection.

Mental health evaluations: The mental health evaluations will involve serial mental health questionnaires and surveys of topics such as anxiety and mood symptoms, as well as clinical interviews. The risks associated with the questionnaires and clinical interviews are minimal, beyond transient increases in stress associated with difficult topics. The questionnaires require a minimal amount of time to complete (up to 20 minutes total). Results will be maintained securely, but there is a slight risk of loss of confidentiality of information collected in the questionnaires. The interviews will be conducted by a mental health clinician who can follow-up on clinically significant issues. It is possible a participant may report high levels of mental health symptoms, onset of new symptoms, or show trends in worsening of symptoms that could indicate a need for mental health care. If there is a question of participant safety or a harm to others (e.g., suicidal ideation) a consultation to the National Institute of Mental Health Psychiatric Consultation Liaison Service will be requested. At a minimum, information about national and local mental health resources, such as hotlines, and guidance on steps to take to seek care in the community will be provided for any individual who could benefit.

2.3.2 Known Potential Benefits

Participants in this trial may benefit by receiving a second dose (and potentially a booster dose) of an mRNA-based COVID-19 vaccine (which they otherwise would not receive) in an environment where the investigators and staff are experienced in the care of patients with anaphylactic reactions, and all equipment and medications necessary to treat severe reactions will be readily available. The information gained in the study will improve the understanding of the mechanisms underlying allergic reactions to mRNA COVID-19 vaccines and support the creation of evidence-based guidance on who can safely receive these vaccines, including a second dose in individuals who experienced a reaction to the first dose, whether the second dose is the same or a discordant mRNA-based vaccine as that of the first dose, as well as a booster dose.

2.3.3 Assessment of Potential Risks and Benefits

In a case series study of 159 patients, including 19 who met anaphylaxis criteria after the first dose of an mRNA COVID-19 vaccine, all 159 patients tolerated the second dose.[30] Thirty-two (20%) reported immediate and potentially allergic symptoms that were associated with the second dose that were self-limited, mild, and/or resolved with antihistamines alone. However, individuals who experience a systemic allergic reaction following the first dose of the Pfizer-BioNTech or Moderna vaccines are advised not to receive a second dose according to current FDA and CDC guidelines. Although the efficacy of a single dose of these vaccines has not been systematically studied, 2 doses of the vaccine may be necessary for full efficacy. Whether a second dose with a discordant vaccine from that given as the first dose is as effective as receipt of the same vaccine for both doses is unknown. Because of the potential role for PEG as the culprit trigger for these allergic reactions, these individuals may also be advised to avoid any new COVID-19 vaccines under development that contain PEG or the cross-reactive polysorbate 80, limiting their access to vaccines that protect against COVID-19. Clarification of the mechanisms underlying allergic reactions in this population will facilitate risk factor analysis to improve patient selection for these vaccines and potentially improve the design of future vaccines using this platform.

To maximize safety, all participants will be admitted to the Intensive Care Unit (ICU) at the Clinical Center for visit 1, where experienced staff and all equipment and medications necessary to treat severe allergic reactions are readily available. Participants receiving a booster vaccination at visit 4 will be monitored closely for at least 2 hours after administration, with staff, equipment, and medications readily available in case of an allergic reaction. Patients will also be screened by phone prior to enrollment to ensure that other comorbid conditions (e.g. asthma) that may increase the risk of a severe reaction are stable. If sufficient information cannot be obtained over the phone, or additional testing (e.g., pulmonary function tests) is felt to be necessary to make this assessment, potential subjects may be asked to come to the NIH for additional testing before enrollment.

3 OBJECTIVES AND ENDPOINTS

OBJECTIVES	ENDPOINTS	JUSTIFICATION FOR ENDPOINTS
Primary		
1. Assess the proportion of participants who develop a **systemic allergic reaction** (Consortium for Food Allergy Research [CoFAR; see Appendix A] grade 2 reaction and above regardless of tryptase, or CoFAR grade 1 with elevated tryptase [1.2 X baseline plus 2ng/mL]) to the Pfizer-BioNTech COVID-19 vaccine after previously demonstrating a systemic allergic reaction (CoFAR Grade 2 or 3 reaction regardless of tryptase OR Grade 1 reaction with elevated tryptase [1.2 X baseline plus 2ng/mL]) to their first full dose of the same vaccine or the Moderna COVID-19 vaccine.	1. The proportion of participants who previously demonstrated a systemic allergic reaction (CoFAR Grade 2 or 3 reaction regardless of tryptase OR Grade 1 reaction with elevated tryptase [1.2 X baseline plus 2ng/mL]) to their first full dose of the Pfizer-BioNTech or Moderna COVID-19 vaccine who experience a **systemic allergic reaction** (CoFAR Grade 2 and above reaction regardless of tryptase, or CoFAR grade 1 with elevated tryptase [1.2 X baseline plus 2ng/mL]) within the 3-hour post-vaccine observation period to a subsequent dose of the Pfizer-BioNTech vaccine.	This endpoint was chosen since it will provide critical information regarding the safety of administering a dose of the Pfizer-BioNTech mRNA COVID-19 vaccine to individuals who experienced a previous allergic reaction to an mRNA-based COVID-19 vaccine. This information will be helpful in guiding public policies for these patients.
Secondary		
1. Assess the proportion of participants who develop a **severe systemic allergic reaction** (CoFAR Grade 3 reaction or higher regardless of tryptase) to the Pfizer-BioNTech COVID-19 vaccine after previously demonstrating a systemic allergic reaction (CoFAR Grade 2 or 3 reaction	1. The proportion of participants who previously demonstrated a systemic allergic reaction (CoFAR Grade 2 or 3 reaction regardless of tryptase OR Grade 1 reaction with elevated tryptase [1.2 X baseline plus 2ng/mL]) to their first full dose of the Pfizer-BioNTech or Moderna COVID-19 vaccine	These endpoints were chosen as they will inform the severity of allergic reactions that may be experienced following administration of a dose of the Pfizer-BioNTech mRNA COVID-19 vaccine to individuals who experienced an allergic

OBJECTIVES	ENDPOINTS	JUSTIFICATION FOR ENDPOINTS
regardless of tryptase OR Grade 1 reaction with elevated tryptase [1.2 X baseline plus 2ng/mL]) to their first full dose of the same vaccine or the Moderna COVID-19 vaccine.	who experience a **severe systemic allergic reaction** (CoFAR grade 3 reaction and above) within the 3-hour post-vaccine observation period to a subsequent dose of the Pfizer-BioNTech vaccine.	reaction to an initial dose of an mRNA-based COVID-19 vaccine, allow comparison of our data to other proposed and ongoing studies evaluating the safety of the Pfizer-BioNTech COVID-19 vaccine in individuals potentially at high risk for allergic reactions to the vaccine, as well as comparison to data collected by the CDC.
2. Assess the proportion of participants who develop a **mild-moderate allergic reaction** (CoFAR Grade 1 or 2 reaction regardless of tryptase) to the Pfizer-BioNTech COVID-19 vaccine after previously demonstrating a systemic allergic reaction (CoFAR Grade 2 or 3 reaction regardless of tryptase OR Grade 1 reaction with elevated tryptase [1.2 X baseline plus 2ng/mL]) to their first full dose of the same vaccine or the Moderna COVID-19 vaccine.	2. The proportion of participants who previously demonstrated a systemic allergic reaction (CoFAR Grade 2 or 3 reaction regardless of tryptase OR Grade 1 reaction with elevated tryptase [1.2 X baseline plus 2ng/mL]) to their first full dose of the Pfizer-BioNTech or Moderna COVID-19 vaccine who experience a **mild-moderate allergic reaction** (CoFAR grade 1 or 2 reaction regardless of tryptase) within the 3-hour post-vaccine observation period to a subsequent dose of the Pfizer-BioNTech vaccine.	
3. Assess the proportion of participants with **anaphylactic reactions** (Levels 1-3) per Brighton Collaboration Criteria (see Appendix B) to the Pfizer-BioNTech COVID-19 vaccine after previously demonstrating a systemic allergic reaction (CoFAR Grade 2 or 3 reaction OR Grade 1 reaction with elevated tryptase [1.2 X baseline plus 2ng/mL]) to their first full dose of the same vaccine or the Moderna COVID-19 vaccine.	3. The proportion of participants who previously demonstrated a systemic allergic reaction (CoFAR Grade 2 or 3 reaction regardless of tryptase OR Grade 1 reaction with elevated tryptase [1.2 X baseline plus 2ng/mL]) to their first full dose of the Pfizer-BioNTech or Moderna COVID-19 vaccine who experience an **anaphylactic reaction** (Levels 1-3) per Brighton Collaboration Criteria within the 3-hour post-vaccine observation period to a subsequent dose of the Pfizer-BioNTech vaccine.	

OBJECTIVES	ENDPOINTS	JUSTIFICATION FOR ENDPOINTS
4. Assess the proportion of participants who develop a systemic allergic reaction (CoFAR Grade 2 reaction or higher regardless of tryptase OR Grade 1 reaction with elevated tryptase [1.2 X baseline plus 2ng/mL]) to the Pfizer-BioNTech COVID-19 vaccine after previously demonstrating a systemic allergic reaction (CoFAR Grade 2 or 3 reaction regardless of tryptase OR Grade 1 reaction with elevated tryptase [1.2 X baseline plus 2ng/mL]) to their first full dose of the same vaccine or the Moderna COVID-19 vaccine compared to the rate of these reactions following placebo administration.	4. The proportion of participants who previously demonstrated a systemic allergic reaction (CoFAR Grade 2 or 3 reaction regardless of tryptase OR Grade 1 reaction with elevated tryptase [1.2 X baseline plus 2ng/mL]) to their first full dose of the Pfizer-BioNTech or Moderna COVID-19 vaccine who experience a systemic allergic reaction (CoFAR grade 2 reaction and above regardless of tryptase, or CoFAR grade 1 with elevated tryptase [1.2 X baseline plus 2ng/mL]) within the 3-hour post-vaccine observation period to a subsequent dose of the Pfizer-BioNTech vaccine compared to the rate of these reactions following placebo administration.	
5. Compare the severity of allergic reactions to the first dose of the Pfizer-BioNTech or Moderna COVID-19 vaccine to the severity of the reaction following administration of a subsequent dose of the Pfizer-BioNTech vaccine in individuals who experienced a systemic allergic reaction (CoFAR grade 2 reaction and above regardless of tryptase, or CoFAR grade 1 with elevated tryptase [1.2 X baseline plus 2ng/mL]) to their first full dose of the Pfizer-BioNTech or Moderna COVID-19 vaccine.	5. The proportion of participants who previously demonstrated a systemic allergic reaction (CoFAR Grade 2 or 3 reaction regardless of tryptase OR Grade 1 reaction with elevated tryptase [1.2 X baseline plus 2ng/mL]) to their first full dose of the Pfizer-BioNTech or Moderna COVID-19 vaccine who develop a lower or higher grade allergic reaction within the 3-hour post-vaccine observation period to a subsequent dose of the Pfizer-BioNTech vaccine.	
Tertiary/Exploratory		
1. Assess the risk of having a systemic allergic reaction to a dose of the Pfizer-BioNTech COVID-19 vaccine in	1. Prevalence of polyethylene glycol (PEG) and SARS-CoV-2 antibodies in each participant at baseline.	In order to determine which individuals are at high risk for having an allergic reaction to the mRNA

OBJECTIVES	ENDPOINTS	JUSTIFICATION FOR ENDPOINTS
participants who previously demonstrated a systemic allergic reaction (CoFAR Grade 2 or 3 reaction regardless of tryptase OR Grade 1 reaction with elevated tryptase [1.2 X baseline plus 2ng/mL]) to their first full dose of the same vaccine or the Moderna COVID-19 according to baseline covariates. 2. Examine possible mechanisms of allergic reactions to mRNA-based COVID-19 vaccines. 3. Assess innate and adaptive immune responses, including functional antibody levels, to the Pfizer-BioNTech COVID-19 vaccine. 4. Investigate mental health characteristics of participants who experience an allergic reaction to the COVID-19 vaccine. 5. Assess psychological impact of allergic reactions to the COVID-19 vaccine and examine changes in stress levels over time. 6. Assess anxiety levels in participants and examine changes in anxiety over time. 7. Assess the proportion of participants who experience no reaction or only a mild reaction (CoFAR Grade 2 or below) to a booster dose of the Pfizer-BioNTech vaccine after previously demonstrating a systemic allergic reaction (CoFAR Grade 2 or 3 reaction regardless of tryptase OR Grade	2. Changes in anti-PEG and SARS-CoV-2 antibodies in each participant approximately 1 and 5 months after receiving the dose of the Pfizer-BioNTech vaccine administered on study. 3. Prevalence of positive skin testing to the vaccine and/or vaccine components including PEG- and polysorbate 80-containing medications. 4. Changes in biomarkers from baseline to post-dose of the Pfizer-BioNTech vaccine administered on study (e.g., known mediators of systemic reactions due to mast cell activation, markers of inflammatory response, markers of basophil and neutrophil activation, markers associated with activation of the classical and alternative complement pathways or kinin system, proteomics, metabolomics). 5. Changes in blood transcriptomics after vaccination. 6. Changes in innate and adaptive immune responses, including functional antibody levels, after vaccination. 7. Mental health/anxiety questionnaire scores and anxiety level ratings at baseline. 8. Results of psychiatric consultation/mental health interview.	COVID-19 vaccines, and which patients who experienced an allergic reaction to the initial dose can safely receive a second dose, a better understanding of the mechanisms driving allergic reactions to these vaccines is needed. These endpoints will provide insight into the cellular and immunologic pathways that are driving allergic reactions to these vaccines. Additionally, a better understanding of the impact of administering a second dose of the vaccine to individuals who experienced an allergic reaction to the first dose on mental health and anxiety levels will provide valuable information to clinicians caring for these patients. Data collected from participants who receive a 5-month booster on this study will provide additional information regarding the safety of administering booster doses of the Pfizer-BioNTech vaccine to patients who experienced a systemic allergic reaction to the first dose of the COVID-19 mRNA vaccines but who tolerated the second dose with only mild or no allergic symptoms. This information will have important public health implications given the need for boosters now and in the future in order to achieve full protection against

OBJECTIVES	ENDPOINTS	JUSTIFICATION FOR ENDPOINTS
1 reaction with elevated tryptase [1.2 X baseline plus 2 ng/mL]) to their first full dose of the Pfizer-BioNTech or Moderna COVID-19 vaccine and no allergic reaction or a mild reaction (CoFAR Grade 2 or below) to a subsequent (second) dose of the Pfizer-BioNTech vaccine. 8. Assess the development of autoantibodies after COVID-19 vaccination.	9. Changes in mental health/anxiety questionnaire scores and anxiety level ratings over the study period. 10. The proportion of participants who previously demonstrated a systemic allergic reaction (CoFAR Grade 2 or 3 reaction regardless of tryptase OR Grade 1 reaction with elevated tryptase [1.2 X baseline plus 2 ng/mL]) to their first full dose of the Pfizer-BioNTech or Moderna COVID-19 vaccine and no allergic reaction or a mild reaction (CoFAR Grade 2 or below) to a subsequent (second) dose of the Pfizer-BioNTech vaccine who experience no reaction or only a mild reaction (CoFAR Grade 2 or below) to a booster dose of the Pfizer-BioNTech vaccine administered approximately 5 months after the second dose. 11. The development of autoantibodies post-second and post-booster doses of the COVID-19 mRNA vaccines.	COVID-19, and to provide reassurance to allergists and other medical professionals caring for these patients that they can safely administer booster doses to these patients in an outpatient setting.

4 STUDY DESIGN

4.1 OVERALL DESIGN

This will be a single-site study that will be performed at the NIH CC. The goal is to enroll up to 100 subjects, although we may not be able to reach that target. There is great uncertainty about the number of individuals with systemic allergic reactions, along with the acceptability of these individuals of repeat vaccination and a 4-day inpatient stay. The study will provide more precise estimates with greater enrollment; however, even if fewer than 100 subjects enroll, this will provide very valuable new information. The study population will consist of individuals 16-69 years of age who experienced a systemic allergic reaction, based on review of their history and medical records, to their first full dose of the Pfizer-BioNTech or Moderna COVID-19 vaccines.

Prior to enrollment, participants will be screened remotely by phone/email and/or videoconference (using an NIH-approved platform) to assess eligibility and to ensure co-morbid allergic and other diseases, including asthma and atopic dermatitis, are well-controlled and disease activity is stable. If individuals have co-morbid conditions that may increase their risk of having a severe allergic reaction, they may be asked to come to the NIH CC for additional studies, such as pulmonary function testing, echocardiogram, or blood laboratory testing, that are felt to be clinically necessary (based on the discretion of the study team) to ensure that they are medically stable to undergo the study intervention. After screening, eligible individuals will be enrolled and admitted to the NIH CC ICU on day 0. Two IV lines will be inserted prior to study vaccination to allow for frequent blood draws and rapid administration of fluids/medications if needed in the event of a reaction. We will aim to admit subjects so that their first study vaccination occurs as close as possible to when the second dose of vaccine is indicated (i.e., 21 days after the first dose of the Pfizer-BioNTech vaccine and 28 days after the first dose of the Moderna vaccine), but since the goal of this study is to assess allergic reactions and not provide clinical vaccinations as indicated, subjects may be enrolled regardless of time elapsed since their initial vaccination. On day 1, they will be randomized at a 1:1 ratio to receive either placebo or the Pfizer-BioNTech vaccine. A pre-vaccination blood and urine sample will be obtained, and then the first dose (per randomization) of study vaccine will be given. Serial blood draws and the first void (urine) after the dose will be collected. Another post-vaccination void may be collected if an allergic reaction occurs after the first collection. The participant will be monitored by the principal investigator and/or an experienced allergist associate investigator for signs and symptoms of an allergic reaction and any other AE for a minimum of 3 hours, and medications and equipment will be immediately available to treat possible reactions. If a reaction does develop, the participant will be treated according to our study MOP, and additional urine samples may be collected to assess the immune response. If a skin rash develops, photographs may be taken to document variations in presentation. In case of a reaction after the first dose, the participant will be counseled and the second study vaccination will proceed as scheduled if the participant is willing. On day 2, the participant will receive either placebo or the Pfizer-BioNTech vaccine (whichever was not given on day 1) using the same procedure, but in the opposite arm. The participant will be monitored and treated (as needed) for an allergic reaction or AEs and samples will be collected as described for day 1. Participants will be discharged on day 3, unless they require additional treatment due to a reaction. Additionally, day 2 dosing may be delayed to a later day at the discretion of the investigator if the participant experiences an allergic reaction on day 1 that has not resolved by day 2 and does not rise to the level of a pausing or halting criterion (see sections 8.4.5 and 8.4.6). Day 2 dosing may be delayed for a maximum of 1 week. If the participant experiences other AEs (nonallergic in nature) on day 2 that are expected from the vaccine based on data from the Phase 3 trials (e.g. fatigue, headache, fever, myalgia), then the second study vaccination may still be given on day 2 as scheduled. A participant may be moved to a general ward after the completion of their vaccinations and close monitoring, when safe to do so.

To assess potential effects of anxiety on the development of allergic reactions, and to support the psychological well-being of participants, each participant will complete standardized questionnaires assessing history of anxiety and past and current mental health within 30 days prior to or at Day 0. In addition, participants will have a mental health interview via a telehealth appointment before Day 0/admission to review questionnaire results and be offered strategies to

help manage distress. Vaccine-related anxiety will also be assessed at each timepoint, including before and after vaccine administration on Days 1 and 2. Responses will be assessed by a clinician, and a psychiatric evaluation and/or counseling or strategies to help with anxiety will be provided if indicated.

A member of the study team will perform videoconference, phone, and/or email follow-up with all participants within one week post-discharge to assess for any AEs from the study intervention. Participants will return to the NIH for follow-up visits approximately 1 and 5 months post-discharge to provide additional research samples, including samples to assess their response to the vaccine. At one of the follow-up visits (per investigator discretion), participants will undergo allergy skin testing to investigate the potential role of IgE in triggering allergic reactions to the vaccine. Mental health/anxiety questionnaires will be readministered at approximately day 30 and month 5, and anxiety levels will be assessed at each follow-up visit. Clinical follow-up will be provided as needed. At month 5, a brief questionnaire to garner participant feedback on the perceived benefits and burdens of the mental health assessments conducted on this study will also be administered. At the 5-month follow-up visit, we will offer participants the opportunity to receive a booster dose of the Pfizer-BioNTech vaccine if they did not have a severe allergic reaction when they received the second dose in the ICU (ie, those who had CoFAR grade 2 reaction or less will be eligible for the booster). For participants who decline the booster vaccination, the 5-month visit will be the final study visit.

All participants who opt in to receiving the booster dose at the 5-month visit will receive active vaccine; no placebo will be given. If the bivalent formulation (Original and Omicron BA.4/BA.5) of the vaccine is available at the NIH, participants will be given the option of receiving the bivalent or monovalent version as the booster dose; if the bivalent version is not available, the monovalent will still be offered. A consent addendum will be discussed with the participants about this choice. (Note: As of September 15, 2022, only 2 participants have yet to complete the 5-month visit, and it is unclear whether the NIH will have the bivalent version available for these 2 remaining visits.) A single IV line will be inserted prior to booster administration to allow for serial blood draws and rapid administration of fluids/medications if needed in the event of a reaction. The booster vaccine will be administered in an outpatient clinic, and medications necessary to treat an allergic reaction, including epinephrine, will be readily available. Participants will be observed in the clinic for at least 2 hours after the booster dose is administered. Vital signs will be checked at baseline and prior to discharge, and as indicated in case of development of symptoms, and we will draw blood pre-dose, 35 minutes post-dose, and 2 hours post-dose to assess for changes in biomarkers. A urinary specimen will be obtained pre-dose and the first void post-dose prior to discharge will be collected (if possible), to detect urinary biomarkers changes. A member of the study team will perform videoconference, phone, and/or email follow-up with participants who receive the booster vaccination within one week post-dose to assess for any AEs. Participants who received the booster dose will attend a final study visit at month 6 to undergo research procedures, including blood sample collection, to assess their response to the vaccine, and anxiety levels will be assessed. Clinical follow-up will be provided as needed. Information collected from participants receiving booster administration may provide additional reassurance that patients with a history of severe systemic allergic reactions to the first dose of the vaccine can safely receive a second dose and booster doses in an

outpatient setting, which is likely to be of significant public health interest. Unblinding will occur after the last participant has completed Visit 1.

4.2 Scientific Rationale for Study Design

The study population was chosen as they are unable to receive the recommended number of doses of the COVID-19 mRNA vaccines based on current FDA and CDC guidelines, and therefore information regarding the safety of administering a second dose (whether the same vaccine or a discordant vaccine) to this population is critically needed. They are also a group that has had a convincing allergic reaction to the initial dose of the vaccine, and therefore are likely to inform the mechanism of these reactions if they experience a reaction upon challenge with a subsequent dose of the Pfizer-BioNTech vaccine. Based on data reported to VAERS, the rate of allergic reactions to these vaccines is significantly more prevalent in women, and therefore we anticipate that our study population will be skewed towards a predominance of female participants. We will make every effort to ensure that any reactions that are observed can be tied to the active vaccine, and therefore are excluding participants who have received any allergen immunotherapy within the 24 hours prior to challenge or after the vaccination, or other vaccination within 14 days. We are including placebo controls as false positive reactions are not uncommonly observed in drug and other allergen challenges, as anxiety can lead to symptoms that clinically mimic anaphylaxis. We hope to begin to understand the mechanisms of allergic reactions to the vaccines, and so have excluded medication use that would be expected to interfere with analysis in mechanistic studies. This includes medications such as immunomodulators including systemic steroids, biologics including T helper 2 (Th2) cytokine (e.g. IL-4, IL-5, IL-13) inhibitors, as well as others such as rituximab and the 5-lipoxygenase inhibitor zileuton. Because of the risk that participants will experience an allergic reaction that may be severe, we are excluding pregnant women from enrolling.

4.3 Justification for Dose

Administration and dosing for the Pfizer-BioNTech vaccine will be in accordance with the FDA-approved dose as detailed in the prescribing information.

5 STUDY POPULATION

5.1 Inclusion Criteria

In order to be eligible to participate in this study, an individual must meet all of the following criteria:

1. Ability to provide informed consent.
2. Stated willingness to comply with all study procedures (including discontinuing medications as needed [section 5.5]) and availability for the duration of the study.
3. Aged 16-69 years.
4. Must have experienced a systemic allergic reaction (CoFAR Grade 2 or 3 reaction regardless of tryptase OR Grade 1 reaction with elevated tryptase [1.2 X baseline plus 2 ng/mL] per modified CoFAR Grading Scale for Systemic Allergic Reactions Version 3.0) to the first dose of the Pfizer-BioNTech or Moderna COVID-19 vaccine. Patients without documented hypoxia, hypotension, or evidence of end-organ damage who were treated with epinephrine infusion would be considered as CoFAR Grade 3 reaction and may be eligible per investigator discretion.

5. Must be at least 28 days out from their first dose of the Moderna vaccine or 21 days from their first dose of the Pfizer-BioNTech vaccine before proceeding with the placebo or vaccine challenge in this study.
6. Have a primary care physician or other health care provider who will manage their medical care outside of this study.

5.2 EXCLUSION CRITERIA

An individual who meets any of the following criteria will be excluded from participation in this study:

1. Experienced a Grade 4 systemic allergic reaction (per CoFAR Grading Scale for Systemic Allergic Reactions Version 3.0) to the first dose of the Pfizer-BioNTech or Moderna COVID-19 vaccine.
2. Known exposure to SARS-CoV-2 and still within the quarantine window.
3. Symptoms consistent with acute COVID-19 infection or known COVID-19 infection (positive reverse transcription-polymerase chain reaction [RT-PCR] or antigen test) and still within the quarantine window
4. Have an acute illness, including body temperature greater than 100.4 degrees F, within 14 days prior to enrollment.
5. History of autoimmune or other disorders requiring systemic immune modulators.
6. Are moderately or severely immunocompromised.
7. History of acute urticaria within 28 days prior to enrollment.
8. Pregnant.
9. Have received any vaccines within 14 days prior to enrollment.
10. Scheduled or planned receipt of approved or experimental vaccine prior to visit 3.
11. Had any allergen immunotherapy administration within 24 hours prior to the first study vaccination or plan to receive within 24 hours after the second study vaccination.
12. Have received a biological therapy within 6 months prior to enrollment.
13. Use of systemic steroids for any reason within 28 days prior to enrollment.
14. Use of zileuton within 14 days prior to enrollment.
15. Use of EUA monoclonal antibodies casirivimab and indevimab, or bamlanivimab, or any other antibody agent for treatment or prevention of COVID-19 within 3 months of randomization.
16. Presence of condition(s) that, in the judgment of the investigator or the referring physician, may put the participant at undue risk or make them unsuitable for participation in the study.

5.2.1 Exclusion of Special Populations

Children, adults who lack capacity to consent, and pregnant women: We are able to obtain knowledge about allergic responses to the mRNA COVID-19 vaccines without exposing children under age 16 (even though the Pfizer-BioNTech vaccine is authorized for emergency use in individuals 5 to 15 years of age, and authorizations for lower ages are anticipated), adults who lack capacity to consent, and pregnant women to the potential risk of severe allergic reactions.

5.3 INCLUSION OF VULNERABLE PARTICIPANTS

5.3.1 Participation of Children

Currently, the Pfizer-BioNTech vaccine is the only vaccine approved for use in children 16 to 17 years of age. They have no options for seeking another type of vaccine for full vaccination (unlike adults who can receive a non-mRNA vaccine), so there is an urgent public health need to determine the safety of giving a second dose in this population in order for them to be fully protected against COVID-19. Furthermore, there is likely to be significant hesitancy for practicing allergists to give a second dose to children who have had an anaphylactic reaction to the first dose unless evidence can be generated to support that it is safe to do so.

5.3.2 Adult Subjects Who Lack Capacity to Consent to Research Participation

Individuals who are unable to provide initial informed consent are excluded from the study. If participants lose the ability to provide ongoing consent subsequent to giving initial consent, they will be withdrawn from the study.

5.3.3 Participation of NIH Staff or Family Members of Study Team Members

NIH staff and family members of study team members may be enrolled in this study as this population meets the study entry criteria. Neither participation nor refusal to participate as a subject in the research will have an effect, either beneficial or adverse, on the participant's employment or position at NIH.

Every effort will be made to protect participant information, but such information may be available in medical records and may be available to authorized users outside of the study team in both an identifiable an unidentifiable manner.

The NIH investigator will provide and request that the NIH staff member review the Frequently Asked Questions (FAQs) for Staff Who are Considering Participation in NIH Research and the Leave Policy for NIH Employees Participating in NIH Medical Research Studies (NIH Policy Manual 2300-630-3). Please see section 10.1.3 for consent of NIH staff.

5.4 INCLUSION OF PREGNANT WOMEN, FETUSES OR NEONATES

Not applicable

5.5 LIFESTYLE CONSIDERATIONS

During this study, participants are asked to:

- Discontinue any medication (e.g., oral antihistamines) that might interfere with the vaccine challenge procedure, skin testing, or booster administration (as applicable) within an appropriate timeframe prior to the target procedure, depending on the medication.
- Refrain from taking multivitamins or supplements containing biotin (vitamin B7, which is commonly found in hair, skin, and nail supplements and multivitamins) for 12 hours before Visits 1, 3, 4, and 6.

5.6 SCREEN FAILURES

Screen failures are defined as participants who consent to participate in the clinical trial but are not subsequently assigned to the study intervention or entered in the study. A minimal set of screen failure information is required to ensure transparent reporting of screen failure

participants, to meet the Consolidated Standards of Reporting Trials (CONSORT) publishing requirements and to respond to queries from regulatory authorities. Minimal information includes demography, screen failure details, eligibility criteria, and any serious adverse event (SAE).

Individuals who do not meet the criteria for participation in this trial (screen failure) because of a modifiable factor may be rescreened. Rescreened participants should be assigned the same participant number as for the initial screening.

5.7 STRATEGIES FOR RECRUITMENT AND RETENTION

Participants will be recruited from referrals from outside physicians and from investigators or employees in the NIH Clinical Center. Letters and/or emails may be sent to physicians, allergists/immunologists, and other specialists, as well as directly to potential participants for subject recruitment. Fliers and other written information may be posted on the NIH campus, in newspapers and on news sites, and in public spaces including, but not limited to, libraries, listservs, and community events, advertising the study. Foundations and other patient advocacy groups may be contacted. The NIH media office may be contacted to issue a press release regarding the study. The study will be listed on clinicaltrials.gov. Information about the study may also be placed on social media sites.

5.7.1 Costs

No cost to participants is expected.

5.7.2 Compensation

Participant travel will be paid directly or reimbursed, per NIAID policy. However, participants will also be reimbursed for travel for their first in-person visit to the NIH (which would typically not be reimbursed per policy).

Participants will be compensated for study events and procedures after screening as summarized below:

Inpatient stay Day 0: $350

Inpatient stay Day 1: $350

Inpatient stay Day 2: $350

Inpatient stay Day 3: $300

Additional stay in ICU (e.g., if longer stay is needed to treat allergic reaction/AE): $300/day

Additional stay on ward (e.g., if longer stay is needed to treat allergic reaction/AE): $250/day

Blood draw (completion of all required per study team for study visit 1): $150

Nasal swab: $25

Urine collection (completion of all required for study visit 1): $25

Outpatient follow-up visit: $200

Outpatient blood draw: $50 ($150 at visit 4 only for participants who receive booster vaccination)

Outpatient skin testing: $75

Visits 2 and 5 (as applicable) phone call/videoconference: $20 each

Mental health/anxiety questionnaires: $10 for each timepoint. Questionnaires completed during screening/enrollment and visit 1 (day 0) will be compensated $10 total at the completion of visit 1. Participants must complete all questionnaires for the corresponding timepoint to receive the questionnaire compensation for that timepoint.

Psychiatric consultation (via telehealth appointment): $20

The total maximum compensation for the required study visits and procedures as scheduled is $2,195; however, compensation may be more than this if the participant receives a booster vaccination or if additional inpatient days or additional procedures are required. Screening procedures are not compensated. Subjects will receive remuneration after visit 2 (for visits 1 and 2) and after visit 4 or 6 (for visits 3, 4, 5, and 6, as applicable).

6 STUDY INTERVENTIONS

6.1 STUDY INTERVENTIONS ADMINISTRATION

6.1.1 Study Intervention Description

The Pfizer-BioNTech monovalent COVID-19 vaccine (Comirnaty) is approved by the FDA in individuals 12 years of age and older for active immunization to prevent COVID-19 caused by SARS-CoV-2. It is also authorized for use under EUA in additional populations, as described above (section 2.2). The bivalent formulation (Original and Omicron BA.4/BA.5) is authorized for use under EUA as a single booster dose for individuals 12 years of age and older who are at least 2 months removed from the completion of their completed primary vaccination or have received the most recent booster dose with any authorized or approved monovalent COVID-19 vaccine. The active ingredient in both formulations is nucleoside-modified mRNA encoding the SARS-CoV-2 spike protein. Additional details regarding the vaccine are provided below and in the prescribing information documents.[28, 29]

Commercially available sterile, preservative-free 0.9% Sodium Chloride Injection, USP will be used as placebo.

6.1.2 Dosing and Administration

A single dose of the Pfizer-BioNTech vaccines is 0.3 mL. Each dose of the placebo vaccine will match this volume (0.3 mL). All study vaccines will be administered intramuscularly in the deltoid in the order determined by the randomization schedule; the first and second vaccinations will be in opposite arms.

6.1.3 Booster Vaccination

Participants who receive a booster vaccination at visit 4 will receive a standard dose of the monovalent or bivalent Pfizer-BioNTech vaccine according to availability and participant choice.

6.2 PREPARATION/HANDLING/STORAGE/ACCOUNTABILITY

6.2.1 Acquisition and Accountability

The study drug will be distributed and accounted for by the NIH pharmacy according to standard pharmacy procedures.

6.2.2 Formulation, Appearance, Packaging, and Labeling

The Pfizer-BioNTech COVID-19 vaccines are supplied as frozen suspensions in vials with gray caps and labels with gray borders, and are not diluted prior to use. The label on the vials of the bivalent formulations are marked with the word "Bivalent." Each dose contains 30 mcg of a modified messenger RNA (modRNA) encoding the viral spike (S) glycoprotein of SARS-CoV-2. Each dose of the Pfizer-BioNTech COVID-19 vaccines also includes the following ingredients: lipids (0.43 mg ((4-hydroxybutyl)azanediyl)bis(hexane-6,1-diyl)bis(2-hexyldecanoate), 0.05 mg 2-[PEG-2000]-N,N-ditetradecylacetamide, 0.09 mg 1,2-distearoyl-sn-glycero-3-phosphocholine, and 0.19 mg cholesterol), 0.06 mg tromethamine, 0.4 mg tromethamine hydrochloride, and 31 mg sucrose. The Pfizer-BioNTech COVID-19 vaccines do not contain preservative. The vial stoppers are not made with natural rubber latex.

The Pfizer-BioNTech vaccines will be labeled per pharmacy procedures and in accordance with regulatory requirements.

Each mL of placebo vaccine contains sodium chloride 9 mg and water for injection, quantity sufficient. It contains no bacteriostat, antimicrobial agent, or added buffer. The solution may contain hydrochloric acid and/or sodium hydroxide for pH adjustment.

6.2.3 Product Storage and Stability

Pfizer-BioNTech COVID-19 vaccine will be supplied in cartons in thermal containers with dry ice. Once received, the vial cartons may be stored in an ultra-low temperature freezer between -90ºC to -60ºC (-130ºF to -76ºF) or immediately transferred to the refrigerator [2ºC to 8ºC (35ºF to 46ºF)], thawed, and stored for up to 10 weeks. The 10-week refrigerated expiry date should be recorded on the carton at the time of transfer. Once vials are thawed, they should not be refrozen. If cartons are received at 2°C to 8°C, they should be stored at 2°C to 8°C. The carton should be checked to ensure it has been updated to reflect the 10-week refrigerated expiry date. During storage, minimize exposure to room light, and avoid exposure to direct sunlight and ultraviolet light.

If not previously thawed at 2ºC to 8ºC (35ºF to 46ºF), vials should be thawed at room temperature [up to 25ºC (77ºF)] for 30 minutes prior to use. Vials may be stored at room temperature [8°C to 25°C (46°F to 77°F)] for a total of 12 hours prior to the first puncture. After first puncture, the vial should be held between 2ºC to 25°C (35°F to 77°F). Vials should be discarded 12 hours after first puncture.

Placebo should be stored at 20° C to 25°C (68° F to 77° F).

6.2.4 Preparation

The Pfizer-BioNTech COVID-19 vaccines are supplied as a frozen suspension that does not contain preservative. Each vial must be thawed prior to administration, and must not be diluted.

Detailed preparation instructions are provided in the full prescribing information documents.[28, 29]

The placebo vaccine will be prepared according to standard pharmacy procedures.

6.3 MEASURES TO MINIMIZE BIAS: RANDOMIZATION AND BLINDING

Participants will be randomized to receive either Pfizer-BioNTech vaccine or placebo on Day 1 in a 1:1 ratio at enrollment via block randomization. On Day 2, subjects will receive whichever of vaccine or placebo that was not given on Day 1. A randomization scheme will be generated by the NIAID Biostatistics Research Branch who will provide a randomized list of arm assignments to the NIH Clinical Center Pharmacy. The randomization code list will be maintained by the Investigational Drug Control Unit (IDCU) in the electronic Investigational Drug Management System (IDMS).

The participants, the clinical staff, and the study team will be blinded to the order of study vaccine assignments. The unblinded pharmacy staff is responsible for maintaining security of the assignments.

Intentional unblinding: Unblinding will occur after the last participant has completed Visit 1. There will be no emergency unblinding for CoFAR Grade 3 or lower reactions because allergic and anaphylactic reactions to the vaccine are expected and will be treated per standard clinical care, and it is not necessary to unblind to do so. However, if a CoFAR Grade 4 or higher systemic allergic reaction or other life-threatening AE occurs, then both the investigator and the participant will be unblinded. If a CoFAR Grade 4 or higher reaction occurs on day 1, then the participant will not receive the study intervention the next day (either placebo or vaccine).

To break a treatment blind, the principal investigator will request a participant's treatment assignment from the IDCU. If an emergency request for treatment assignment is made by an individual other than the principal investigator and the principal investigator is not immediately available, the request will be made to one of the physician associate investigators, who will then contact the IDCU or appropriate NIH pharmacy after-hours contact to obtain the treatment assignment. If a participant's study agent assignment is unblinded, the information will be provided to only the individuals needing it for treatment decisions; all attempts will be made to maintain the blind of the study team.

Unintentional unblinding: If the blind is unintentionally broken during the challenge, the study will proceed as normal, and the principal investigator will create a plan for preventing the recurrence of a similar incident, as appropriate. Since the active vaccine is known to have adverse side effects (e.g. fatigue, headache, fever, etc) based on data from the Phase 3 trials, patients who experience these symptoms may be unintentionally unblinded. However, if the participant experiences these symptoms on Day 2 (e.g. after receiving vaccine on Day 1), they may still receive the study vaccine on Day 2 without intentionally breaking the blind. Although these symptoms may indicate the participant received the active vaccine, this cannot be absolutely confirmed without direct knowledge of the participant's assignment; therefore, these instances will be evaluated by the principal investigator (without purposefully breaking the blind unless necessary) and will only be reported as outlined below if the assignment has been definitively revealed.

Intentional and unintentional unscheduled unblinding will be documented in the appropriate source and/or research record and will include the reason for the unscheduled unblinding, the date it occurred, who approved the unblinding, who was unblinded, who was notified of the unblinding, and the plan for the participant. The principal investigator will report all cases of intentional and unintentional unscheduled unblinding to the data and safety monitoring board (DSMB) in writing within 1 business day after site awareness via email to the DSMB mailbox (niaiddsmbia@niaid.nih.gov) outlining the reason for the unblinding and the date it occurred. The report will also be submitted to the sponsor medical monitor. If the unblinding meets the definition of a reportable event, it will be reported to the IRB according to NIH Human Research Protections Program (HRPP) Policy 801.

If an SAE has resulted in unblinding, this information will be included in the SAE report.

6.4 STUDY INTERVENTION COMPLIANCE

Participants in this study will receive the full dose of the assigned vaccine/placebo under direct observation.

6.5 CONCOMITANT THERAPY

The following medications are prohibited during participation in the study:

- Systemic immunomodulators including systemic steroids.
- Biologic agents that will interfere with the study objectives at the discretion of the investigator.
- EUA monoclonal antibodies casirivimab and indevimab, or bamlanivimab, or any COVID-19-specific biologic including antibodies or immunomodulator for at least 5 half-lives.
- Zileuton.
- Any investigational or approved vaccines administered outside of the study prior to visit 3 and, for participants receiving a booster vaccination at visit 4, at least 2 months before visit 4 through visit 6.
- Any allergen immunotherapy administration within 24 hours prior to the first study vaccination through 24 hours after the second study vaccination, and from 24 hours prior to through 24 hours after visit 4 (for participants receiving the booster vaccination).

Since one of the goals of this study is to understand the mechanisms of allergic reactions to the COVID-19 vaccines, these medications have been excluded since they may interfere with analysis in mechanistic studies.

Participants may be asked to discontinue use of antihistamines and drugs with antihistamine properties prior to the study intervention as well as prior to skin testing at visit 3 or 4 and booster vaccination at Visit 4 (as applicable) since these medications can mask signs and symptoms of an allergic reaction, thereby reducing the accuracy of skin testing and affecting results of research tests following the booster vaccination. Participants will be asked to discontinue these medications for a time period equal to at least 5 half-lives of the medication prior to the study

intervention.[31] These medications can be resumed once the participant is discharged at the end of Visit 1 and after Visit 3 or 4.

Unless medically contraindicated, participants may also be asked to discontinue use of beta blockers, ACE inhibitors, and NSAIDS that are thought to interfere with treatment of allergic reactions or that may act as eliciting factors that increase reactivity in susceptible individuals[31] for a time period of 5 half-lives of the medication prior to the vaccine challenge at Visit 1 and the booster vaccination at Visit 4 (as applicable). They can resume these medications once they complete the study interventions at the respective visit.

7 STUDY INTERVENTION DISCONTINUATION AND PARTICIPANT DISCONTINUATION/WITHDRAWAL

7.1 DISCONTINUATION OF STUDY INTERVENTION

Study intervention may be discontinued for an individual participant (i.e., pausing), or it may be discontinued for all participants and enrollment suspended (i.e., halting). Pausing and halting rules and procedures are described in sections 8.4.5 and 8.4.6.

7.2 PARTICIPANT DISCONTINUATION/WITHDRAWAL FROM THE STUDY

Participants are free to withdraw from participation in the study at any time upon request.

Criteria and procedures for withdrawal and replacement of a participant by the investigator are provided in section 8.4.3.

7.3 LOST TO FOLLOW-UP

A participant will be considered lost to follow-up if he or she fails to respond to the 1 week post-discharge phone and/or email contact. Study site staff will make at least 3 attempts to contact participants to complete this follow-up visit. These contact attempts should be documented in the participant's medical record or study file. Should the participant continue to be unreachable, they will be considered to have withdrawn from the study with a primary reason of lost to follow-up.

8 STUDY ASSESSMENTS AND PROCEDURES

The following study assessments and procedures will be performed according to the schedule provided in section 1.3.

8.1 SCREENING PROCEDURES

8.1.1 Screening Activities Performed Prior to Obtaining Informed Consent

Potential study participants may be identified by medical chart review, from referrals, or from a response to a study advertisement, and initially contacted by a member of the study team in person, by videoconference, over the phone, or by email. During this initial contact, the potential participant will be provided information on the study and asked to provide verbal permission for screening. If a participant provides verbal permission for screening, they may be screened remotely via an NIH-approved videoconferencing platform/by phone/by email. Medical records, including imaging and laboratory studies, may be requested and reviewed by the study team, demographic information will be collected, and concomitant medications will be reviewed. Participants will be asked about their reaction to the first dose of the COVID-19 vaccine as well as control of their other allergic diseases (e.g., asthma, atopic dermatitis) and other comorbidities

(if present) that may increase their risk of having a severe allergic reaction. Screened individuals who are found to be eligible or potentially eligible for enrollment and are interested in participating in the study will be scheduled for a Visit 1 at the study site. Individuals may be consented to the protocol up to Day -60 by phone/videoconference or in-person at Visit 1. In some cases, individuals with comorbidities that may increase study risks may be asked to come to the NIH Clinical Center for additional screening (see section 8.1.2).

8.1.2 Screening Activities Performed After a Consent for Screening Has Been Signed

Individuals with comorbidities identified at initial screening that may place them at higher risk for having a severe allergic reaction will be asked to come to the NIH for additional screening procedures (e.g., pulmonary function testing, chest X-ray, ECG, echocardiogram, flexible nasolaryngoscopy, and blood laboratory testing) to ensure the individual is stable to undergo the study intervention. Written informed consent to participate in this study will be obtained (either by phone or in person) before any of these additional screening procedures are performed. Individuals who are found not to be eligible to undergo the study intervention after these screening tests will be considered screen failures.

Screening procedures must be completed within 30 days prior to Day 1. Eligibility will be confirmed at Day 0 after pregnancy test (if applicable) and SARS-CoV-2 test results are known.

8.2 EFFICACY ASSESSMENTS

8.2.1 Clinical Evaluations

Demographics: Basic demographic information will be collected.

Medical and medication history/concomitant medication review: At screening (see above), we will review NIH and off-site medical records, as applicable, and interview participants to collect information about conditions or medications that may impact study eligibility or risk of developing severe allergic reactions to the study vaccinations. After enrollment, reviews will focus on updates since the last assessment, with a particular focus on new symptoms and AEs.

Targeted physical exam: Targeted physical exams will be conducted by a study physician or other qualified medical professional prior to each study vaccination and at other timepoint(s) as needed to assess new symptoms or AEs. A targeted physical exam may be conducted at screening for individuals asked to come to the NIH for additional screening procedures to assess the status of comorbid conditions.

Vital signs: Temperature, pulse rate, respiratory rate, oxygen saturation, and blood pressure will be measured within 30 minutes prior to each study vaccination, at the time of blood draws, and as clinically indicated after each study vaccination during Visit 1. At Visit 4, for participants who receive the booster dose, vital signs will be checked at baseline and prior to discharge, and as indicated in case of development of symptoms of an allergic reaction; otherwise, vital signs will be obtained per clinical routine at Visits 3, 4, and 6. Vital signs may be measured at screening for individuals asked to come to the NIH for additional screening procedures to assess the status of comorbid conditions.

Height and weight: Growth parameters (height and weight) will be measured prior to the first study vaccination.

Peak flow measurement and impulse oscillometry: Peak expiratory flow and respiratory resistance of the lungs will be measured by standard methods prior to each study vaccination on days 1 and 2. May be repeated during Visit 1 or at follow-up visits at investigator discretion.

Nasal swab: A nasal swab (mid-turbinate or nasopharyngeal per current Clinical Center practice) will be collected by standard methods prior to the first study vaccination for SARS-CoV-2 testing by PCR. If the SARS-CoV-2 result is positive, Visit 1 will be delayed until the participant has recovered and is outside of the quarantine window.

Pregnancy test: Serum pregnancy testing will be performed for participants of childbearing potential. The result must be confirmed negative prior to proceeding with the study vaccination (at Visit 1) and booster dose (as applicable at Visit 4).

Skin testing: Skin allergy testing to the vaccine, PEG-polysorbate 80-containing medications, and/or other allergens will be conducted according to practice parameters and recent guidelines[32, 33]. In general, all participants will undergo skin prick testing at Visit 3 or Visit 4. If skin prick testing is negative, intradermal allergen testing will be performed. However, the type and nature of the skin testing performed may change in accordance with standard practice, as guidelines are continually updated.

Urine collection: One void prior to each study vaccination and an additional void(s) after each study vaccination and/or the onset of an allergic reaction will be collected using standard methods at Visit 1. At Visit 4 (participants who opt in to booster dose only), one void prior to the booster dose and the first void post-dose will be collected, if possible. Urine samples will be used for laboratory testing (section 8.2.2) and storage.

IV insertion: Two peripheral IV lines will be inserted for Visit 1 by qualified personnel using standard methods to allow for frequent blood draws and rapid administration of fluids/medications if needed in the event of a reaction. For participants receiving a booster vaccination at Visit 4, a single IV line will be inserted prior to booster administration to allow for serial blood draws and rapid administration of fluids/medications if needed in the event of a reaction.

Blood collection: Blood samples will be collected from an IV line when possible, or via venipuncture using standard methods, for laboratory testing (section 8.2.2) and storage. During visit 1, samples will be collected before each study vaccination and approximately 35 minutes (+/-15 minutes), 2 hours (+/-30 minutes), 6 hours (+/-30 minutes), and 24 hours (+/-1 hour) after each study vaccination. The 24-hour blood draw for day 1 will also serve as the baseline blood draw for day 2. An additional blood draw will occur on day 3 of visit 1 prior to discharge. Approximately 464.5 mL of blood will be obtained at visit 1. Approximately 71 mL will be obtained at visit 3, at visit 4, and at visit 6; for participants who opt in to the booster vaccination at Visit 4, the total volume for that visit will be 245 mL. Additionally, up to 150 mL of blood may be collected from participants seen at unscheduled visits (due to AE development or other

study-related concern); however, blood collection would be restricted to only clinically indicated laboratory assessments if the maximum blood volume allowed for research purposes has been reached. Weight-based adjustments to blood volumes collected for research testing will be made as needed for minor participants to remain within the limits allowed at the CC.

Clinical photography: If a participant develops a skin rash after receiving a study vaccination, photographs of the affected area(s) may be taken with the participant's permission. Identifiable photographs will be kept in a secure database and will not be released without the permission of the study participant. Participants will sign the standard NIH photography consent form before photographs are taken.

Anxiety/mental health assessments: The following standardized questionnaires assessing past and current mental health and anxiety will be completed by the participant through the research electronic data capture (REDCap) system (on an electronic device during an in-person visit or by accessing a link sent by email) or on paper:

- Mental health history questionnaire
- Impact of Events Scale-6
- State-Trait Anxiety Inventory
- Generalized Anxiety Disorder-7 scale
- Patient Health Questionnaire-9
- World Health Organization Disability Assessment Schedule-2

Only participants who speak English or Spanish will complete these questionnaires, as these are the only languages in which they are available.

All participants will be asked to rate their current level of anxiety based on a 10-point scale at several different timepoints during the study.

Mental health questionnaire results will be reviewed and relaxation strategies and other recommendations will be provided by a National Institute of Mental Health mental health clinician via a brief telehealth appointment (prior to admission). Subsequent sessions will be scheduled as needed based on ongoing reviews of mental health/anxiety assessments. Participants may be given informational materials to help manage stress.

A brief questionnaire to garner participant feedback on the perceived benefits and burdens of the mental health assessments conducted on this study will be administered at month 5.

Pulmonary function testing: Pulmonary function tests will be performed using standard methods at screening, only for individuals with comorbidities that increase their risk of developing a severe allergic reaction to the study vaccination(s).

Chest X-ray: Chest X-ray will be performed using standard methods at screening, only for individuals with comorbidities that increase their risk of developing a severe allergic reaction to the study vaccination(s).

ECG: ECG will be performed using standard methods at screening, only for individuals with comorbidities that increase their risk of developing a severe allergic reaction to the study vaccination(s).

Echocardiogram: Echocardiogram will be performed using standard methods at screening, only for individuals with comorbidities that increase their risk of developing a severe allergic reaction to the study vaccination(s).

Flexible nasolaryngoscopy: Flexible nasolaryngoscopy will be performed using standard methods to allow assessment of the airway at screening, only if there is concern that a participant may have a difficult airway (e.g., due to history of laryngeal trauma, neck/thyroid surgery, etc).

8.2.2 Biospecimen Evaluations

Specimens (blood, urine, and nasal swabs) will be used to assess:

- Overall atopic status
- Pre-existing antibodies to polyethylene glycols (Sample analysis will not be completed prior to vaccination; therefore, results will not be used to assess eligibility.)
- Known mediators of systemic reactions due to mast cell activation (e.g., serum tryptase and urinary leukotrienes, prostaglandins, histamine)
- General markers of an acute inflammatory response (e.g., serum IL-6)
- Activation of the classical and alternative complement pathways
- Contact activation of the kinin system
- Activation of basophils and neutrophils
- Unsupervised proteomics, metabolomics, and whole blood transcriptomics
- Analysis of peripheral blood mononuclear cells (PBMCs) may also be explored to assess for new mechanisms
- Evidence of clonal mast cell disease and genetic variants that may increase the risk and/or severity of allergic reactions (e.g. hereditary alpha tryptasemia, clonal mast cell disease)
- Autoantibodies pre and post COVID-19 vaccination

Specific laboratory studies may include those listed below. Assays may be removed or added based on the number and character of reactions seen within the trial. In addition, the timepoints for the conduct of specific laboratory studies may change from what is specified below. Specimens will be processed at the Laboratory of Allergic Diseases (LAD), Laboratory of Viral Diseases (LVD), Laboratory of Immune System Biology (LISB), and the Laboratory of Bacteriology at the Rocky Mountain Laboratories at NIAID, the Department of Laboratory Medicine (DLM) at NIH (including send-outs to Mayo Clinic), and the Systemic Autoimmunity Branch at the National Institute of Arthritis and Musculoskeletal and Skin Diseases. Coded specimens will also be sent to collaborators at Yale University, Virginia Commonwealth University, Stanford University, University of North Carolina, University of Pittsburgh, KalVista Pharmaceuticals, Inc., and the US FDA for processing.

Blood/serum/plasma:

The following tests may be performed for all participants:

- Whole blood for genetic analysis of the tryptase locus, c-kit locus, and other genetic variants that might influence the risk and/or severity of allergic reactions (Visit 1, pre-dosing)
- Total IgE (Visit 1, pre-dosing Day 1 only)
- Allergen-specific IgE (Visit 1, pre-dosing)
- Anti-Polyethylene glycol (PEG) IgE, IgG, and IgM antibody measurements (Visit 1, pre-dosing, and Visits 3, 4, and 6)
- Anti-SARS-CoV-2 antibodies and functional antibodies (Visit 1, pre-dosing, and Visits 3, 4, and 6)
- Serum total and mature tryptase (Visit 1, pre-dosing, and post-dosing (placebo and vaccine) at 35 minutes and 2 hours)
- Complement activation testing (Visit 1, pre-dosing, and post-dosing (placebo and vaccine) at 35 minutes and 2 hours)
- Flow cytometry for immunophenotyping and assessment of immune activation (Visit 1, pre-dosing, and post-dosing (placebo and vaccine) at 35 minutes, 2 hours, 6 hours, and 24 hours; and Visits 3, 4, and 6)
- Flow cytometry and other assays to assess basophil and neutrophil activation (Visit 1, pre-dosing, and post-dosing (placebo and vaccine) at 35 minutes and 2 hours)
- CBC with differential (Visit 1, pre-dosing, and post-dosing (placebo and vaccine) at 35 minutes, 2 hours, 6 hours, and 24 hours; and Visits 3, 4, and 6)
- Whole blood and single cell transcriptomics and/or CITE-seq (Visit 1, pre-dosing, and post-dosing (placebo and vaccine) at 35 minutes, 2 hours, 6 hours, and 24 hours; and Visits 3, 4, and 6)
- Plasma proteomics and metabolomics (Visit 1, pre-dosing, and post-dosing (placebo and vaccine) at 35 minutes, 2 hours, 6 hours, and 24 hours; and Visits 3, 4, and 6)
- PBMC separation and storage (Visit 1, pre-dosing, and post-dosing (placebo and vaccine) at 35 minutes, 2 hours, 6 hours, and 24 hours; and Visits 3, 4, and 6)
- Plasma Kallikrein (Visit 1, pre-dosing, and post-dosing (placebo and vaccine) at 35 minutes and 2 hours)
- Measurement of platelet activating factor (PAF) and platelet activating factor acetylhydrolase (PAF-AH) as well as neutrophil nets (Visit 1, pre-dosing, and post-dosing (placebo and vaccine) at 35 minutes and 2 hours)
- Serum cortisol level (Visit 1, pre-dosing, and post-dosing (placebo and vaccine) at 35 minutes and 2 hours)
- Metanephrine/normetanephrine (Visit 1, pre-dosing, and post-dosing (placebo and vaccine) at 35 minutes and 2 hours)
- Autoantibody levels (Visit 1, pre-dosing; Visit 3, Visit 4, pre-dosing; and Visit 6)
- Serum pregnancy test for participants of childbearing potential (Visit 1 prior to study intervention)

For subjects who opt in to receiving the booster dose of vaccine at Visit 4, the following tests may be performed pre-dose, 35 minutes (+/-15 minutes) post-dose, and 2 hours (+/-30 minutes) post-dose. Assays may be removed or added based on the number and character of reactions seen within the trial. (Tests indicated for Visit 4 in the previous list will serve as the pre-dose assessment.)

- Serum total and mature tryptase
- CBC with differential
- Complement activation testing
- Plasma Kallikrein
- Measurement of platelet activating factor (PAF) and platelet activating factor acetylhydrolase (PAF-AH) as well as neutrophil nets
- PBMC separation and storage
- Flow cytometry for immunophenotyping and assessment of immune activation
- Flow cytometry and other assays to assess basophil and neutrophil activation
- Whole blood and single cell transcriptomics and/or CITE-seq
- Serum cortisol level
- Metanephrine/normetanephrine
- Serum pregnancy test for participants of childbearing potential (prior to booster vaccination)

Urine:

The following may be performed for all participants:

- Urinary LTE4, N-methyl histamine (NMH), Prostaglandin D2, and 2,3-Dinor-11Beta-Prostaglandin F2 Alpha (Visit 1, pre-dosing and first void(s) following vaccine administration and/or onset of reaction; Visit 4 (participants who opt in to booster dose only), pre-dose and first void post-dose prior to discharge, if possible)

Nasal swab:

The following will be performed for all participants:

- SARS-CoV-2 virus testing by RT-PCR using nasal swab (Visit 1 prior to study intervention)

8.2.3 Samples for Genetic/Genomic Analysis

8.2.3.1 Description of the scope of genetic/genomic analysis

Blood, plasma/serum, and/or urine may be used for:

- transcriptomic, proteomic, and metabolomic analyses to explore innate and adaptive immune cell activation, function, and metabolism
- genetic analysis of the tryptase locus and other genetic variants that might influence the risk and/or severity of allergic reactions
- D816V mutation testing as an indicator of clonal mast cell disease

Whole genome sequencing will not be performed under this study, but we will refer interested participants to the NIAID Central Sequencing Protocol (17-I-0122) for genomic sequencing. If a participant chooses to co-enroll on this protocol and 17-I-0122, we will have access to the identified findings from whole genome/exome sequencing via the Genomic Research Information System platform for use in this study to further inform our analyses. The informed consent document for 17-I-0122 describes the accessibility of the data generated to NIH researchers, and no additional samples outside of those required of each protocol will be obtained.

8.2.3.2 Description of how privacy and confidentiality of medical information/biological specimens will be maximized

Each participant will be assigned a unique identification number, and these numbers rather than names or other personally identifiable information will be used to collect, store, and report participant information. All biospecimens will be labeled with unique identification numbers. Only investigators or their designees will have access to the specimens and data. See section 10.3 for additional details regarding confidentiality and privacy protections.

8.2.3.3 Management of Results

Analyses under this protocol may identify results or incidental findings that are relevant to the health or medical care of participants. In general, a result would only be returned if the test is run in DLM or if confirmatory testing in a laboratory certified by the Clinical Laboratory Improvement Amendments (CLIA) is available. If needed, the study team will contact the participant and request a sample for confirmatory testing. After confirmation, the principal investigator or designee will contact the subject to inform them of the finding and counsel them on the result. The study team may also provide consultation with a participant's healthcare provider based on the result. Many research laboratory tests are not CLIA-certified, so generated data cannot be meaningfully interpreted outside the narrow focus of the study and will not be routinely returned to participants or their physicians. However, it is possible that the principal investigator may determine a result to be important to the participant's health even if a CLIA-certified test is not available or feasible; in this case, the result would be returned.

8.2.3.4 Genetic counseling

If a genetic analysis performed on this study generates an individually relevant finding that would be returned to a participant, the principal investigator will provide counseling or refer the participant for genetic counseling through the NIH at no cost.

8.3 Safety and Other Assessments

Safety screening assessment: As described above (section 8.1), some preexisting allergic diseases and other comorbidities that have the potential to increase a participant's risk of a severe allergic reaction or other adverse reaction (e.g., myocarditis or pericarditis) to the study vaccinations may be identified during the initial telephone/remote screening process. On a case-by-case basis, these participants may be asked to come to the NIH after the screening phone call to undergo additional clinical studies (e.g., pulmonary function testing, chest X-ray, ECG, echocardiogram, flexible nasolaryngoscopy, and/or blood laboratory tests) that are deemed necessary by the study team to make sure their comorbid diseases are stable before they undergo the study vaccinations. If there are concerns about the stability of any comorbid conditions, the participant may be removed from the study.

Assessment of AEs: Information about AEs will be assessed via appropriate questioning and examination at each study contact. Additional procedures may be performed (e.g., clinical laboratory evaluations) as needed to further characterize events.

The following specific local and systemic events, as well as symptoms of a potential allergic reaction, will be assessed during Visit 1 and Visit 2, and Visits 4 and 5 for participants receiving a booster dose. The following mild to moderate events are expected following vaccine administration:

- Local injection site reactions, with the exception of injection site reactions of erythema/redness and induration/swelling measuring <2.5 cm
- Fever
- Chills
- Myalgia
- Arthralgia
- Fatigue
- Headache
- Nausea
- Vomiting
- Diarrhea

8.4 SAFETY DEFINITIONS, MANAGEMENT, AND REPORTING

8.4.1 Definitions

Adverse Event: An AE is any untoward or unfavorable medical occurrence in a human subject, including any abnormal sign (e.g., abnormal physical exam or laboratory finding), symptom, or disease, temporally associated with the subject's participation in the research, whether or not considered related to the research.

Adverse Reaction (AR): An AR means any AE caused (see "Causality" below) by a study agent. ARs are a subset of all suspected adverse reactions (SARs; defined below) where there is reason to conclude that the study agent caused the event.

Suspected Adverse Reaction (SAR): SAR means any AE for which there is a reasonable possibility that the study agent caused the AE.

> Per US FDA guidance:
> For the purposes of IND safety reporting, "reasonable possibility" means there is evidence to suggest a causal (see "Causality" below) relationship between the study agent and the AE. A SAR implies a lesser degree of certainty about causality than an AR, which means any AE caused by a study agent.
>
> SARs are the subset of all AEs for which there is a reasonable possibility that the study agent caused (see "Causality" below) the event. Inherent in this definition, and in the requirement

to report SARs, is the need for the sponsor to evaluate the available evidence and make a judgment about the likelihood that the study agent actually caused the AE.

The sponsor is responsible for making the causality judgment.

Serious Adverse Event: An SAE:

- is an AE that results in death.
- is an AE that is a life-threatening event (places the subject at immediate risk of death from the event as it occurred).
- is an AE that requires inpatient hospitalization or prolongs an existing hospitalization.

  NOTE:
  - Hospitalization is considered required if outpatient treatment would generally be considered inappropriate.
  - Same-day surgical procedures that are required to address an AE are considered hospitalizations, even if they do not involve an overnight admission.
  - Hospitalization due to a condition that has not worsened and that pre-dates study participation (e.g., elective correction of an unchanged baseline skin lesion), or due to social circumstance (e.g., prolonged stay to arrange aftercare), or that is planned/required "per protocol" AND that proceeds without prolongation or complication, is NOT considered an SAE by this criterion.
- is, or results in, a persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions.
- is a congenital anomaly/birth defect/miscarriage/stillbirth.

  NOTE: This definition is more inclusive than some commonly published definitions. It includes an affected conceptus/neonate whose:
  - biological mother was exposed to a study agent at any point from conception through the end of the pregnancy, AND/OR, if breastfeeding, the 30-day neonatal period; or
  - biological father was exposed to a study agent at any point during the 90 days prior to conception.

  This is separate from, and in addition to, general reporting of pregnancy in a study participant or female partner of a male participant (see section 8.4.2.3.4 below).
- is a medically important event.

  NOTE: Medical and scientific judgment should be exercised. Events that significantly jeopardize the subject and/or require intervention to prevent one of the SAE outcomes listed above are generally considered medically important, and are thus SAEs.

Unexpected Adverse Event: An AE is unexpected if it is not listed in the investigator's brochure or package insert (for marketed products) at the frequency, AND specificity, AND severity that has been observed.

NOTE:

- Such events should also be evaluated for possible reporting as unanticipated problems (UPs) (see section 8.4.2.3.3 below).
- Unexpected, as used in this definition, also refers to AEs or SARs that are mentioned in the investigator's brochure as occurring with a class of drugs/biologics, or as anticipated from the pharmacological properties of the study agent but are not specifically mentioned as occurring with the particular study agent under investigation.

Serious and Unexpected Suspected Adverse Reaction (SUSAR): A SUSAR is an SAR (defined above) that is both serious and unexpected.

Unanticipated Problem: A UP is any event, incident, experience, or outcome that is:

1. **unexpected** in terms of nature, severity, or frequency in relation to:
    a. the research (including but not limited to risks) as described in the IRB-approved research protocol and informed consent document, investigator's brochure, or other study documents; **and**
    b. the characteristics of the subject population being studied; **and is**
2. possibly, probably, or definitely related (see "Causality" below) to participation in the research; **and**
3. suggests the research places subjects or others at a **greater risk** of harm (including physical, psychological, economic, or social harm) than was previously known or recognized, per the documents currently approved by the IRB.

    NOTE:

    - Per the sponsor, an SAE always meets this "greater risk" criterion.
    - An incident, experience, or outcome that meets the definition of a UP generally will warrant consideration of changes to the protocol or informed consent form, or to study procedures (e.g., the manual of procedures [MOP] for the study), in order to protect the safety, welfare, or rights of participants or others. Some UPs may warrant a corrective and preventive action plan (CAPA) at the discretion of the sponsor or other oversight entities.

Unanticipated Problem that is not an Adverse Event (UPnonAE): A UPnonAE belongs to a subset of UPs that:

- meets the definition of a UP, AND
- does NOT fit the definition of an AE or an SAE.

    NOTE: Examples of UPnonAEs include, but are not limited to:

    - a breach of confidentiality
    - prolonged shedding of a vaccine virus beyond the anticipated timeline
    - unexpectedly large number of pregnancies on a study
    - subject departure from an isolation unit prior to meeting all discharge criteria
    - accidental destruction of study records
    - unaccounted-for study agent

- overdosage, underdosage, or other significant error in administration or use of study agent or intervention, even if there is no AE/SAE
- development of an actual or possible concern for study agent purity, sterility, potency, dosage, etc

NOTE: A decision to temporarily quarantine, or to permanently not use all or part of study agent supply due to an unexpected finding or event (e.g., particulate, cloudiness, temperature excursion), even if there is no known or proven issue (i.e., out of an "abundance of caution"), is considered a UPnonAE.

Protocol Deviation: Any change, divergence, or departure from the IRB-approved research protocol.

1. **Major Deviations:** Deviations from the IRB-approved protocol that have, or may have the potential to, negatively impact the rights, welfare, or safety of the subject, or to substantially negatively impact the scientific integrity or validity of the study.
2. **Minor Deviations:** Deviations that do not have the potential to negatively impact the rights, safety, or welfare of subjects or others, or the scientific integrity or validity of the study.

Non-compliance: Failure of an investigator to follow the applicable laws, regulations, or institutional policies governing the protection of human subjects in research, or the requirements or determinations of the IRB, whether the failure is intentional or not.

- **Continuing non-compliance:** A pattern of recurring non-compliance that either has resulted, or, if continued, may result in harm to subjects or otherwise materially compromise the rights, welfare and/or safety of subjects, affect the scientific integrity of the study or validity of the results. The pattern may comprise repetition of the same non-compliant action(s), or different non-compliant events. Such non-compliance may be unintentional (e.g. due to lack of understanding, knowledge, or commitment), or intentional (e.g. due to deliberate choice to ignore or compromise the requirements of any applicable regulation, organizational policy, or determination of the IRB).
- **Serious non-compliance:** Non-compliance, whether intentional or not, that results in harm or otherwise materially compromises the rights, welfare and/or safety of the subject. Non-compliance that materially affects the scientific integrity or validity of the research may be considered serious non-compliance, even if it does not result in direct harm to research subjects.

Events of Special Interest (ESIs) and Protocol-Specified Exempt Events (PSEEs): ESIs and PSEEs are events, identified in detail within the protocol and specified in advance, that will be handled in a protocol/study-specific manner that **differs from statutory and general rules for reporting**. These events may include, but are not limited to:

- laboratory values
- clinical findings
- statutory AEs or SAEs per the standard definition

- hospitalizations and procedures

ESIs are protocol/study-specific rules for events that are of particular concern (due to population, study intervention, class effect, etc). Reporting is generally **enhanced/upgraded beyond the general standard**.

PSEEs are protocol/study-specific rules for events that are, to some degree, expected/anticipated (due to population or condition under study, concomitant treatments, etc). Reporting is generally **downgraded/diminished from the general standard**.

(See section 8.4.2 for special handling of ESIs and/or PSEEs in this protocol.)

8.4.2 Documenting, Assessing, Recording, and Reporting Events

All AEs, including those that may appear to have a non-study cause (see "Causality" below), will be documented (e.g., on the clinical chart/progress notes/clinical laboratory record), recorded (e.g., research database), and reported (e.g., cumulatively from the research database, or according to protocol-specified expedited reporting mechanism) to the sponsor from the time informed consent is obtained through the timeframe specified below. At each contact with the subject, information regarding AEs will be elicited by open-ended questioning and examinations.

AEs and SAEs will generally be recorded, assessed, and reported according to the timeframes stated in the protocol and followed up as outlined in Table 2. Descriptions of PSEEs and ESIs and timeframes for assessing and reporting these events are outlined in Table 3 and Table 4.

Table 2: Standard Times for Follow-up of Events

Event type	Follow events through
Related SAEs	End of subject participation in study, or if study personnel become aware thereafter
Unrelated SAEs	End of subject participation in study
Related non-serious AEs of grade 1 to 3	End of subject participation in study
All other related non-serious AEs	End of subject participation in study
Unrelated non-serious AEs	End of subject participation in study

Table 3: PSEE List and Reporting

Event description	How event will be reported
CoFAR Grade 1-3 allergic reaction	As an AE unless it meets statutory SAE criteria where it will be reported as an SAE. (All CoFAR Grade 4 events will be reported as SAEs.)

Table 4: ESI List and Reporting

Event description	How event will be reported
1. A single occurrence of an event that is uncommon and known to be strongly associated with vaccine exposure (e.g., Stevens-Johnson Syndrome, Guillain-Barré syndrome); 2. One or more occurrences of an event that is not commonly associated with vaccine exposure, but is otherwise uncommon in the population exposed to the drug (e.g., tendon rupture); 3. A single occurrence of myocarditis or pericarditis of any grade.	The ESI will be reported as an SAE/expeditedly even if it does NOT meet statutory SAE criteria.

8.4.2.1 Investigator Assessment of Adverse Events

The investigator will initially assess all AEs with respect to **seriousness** (according to SAE definition above), **severity** (intensity or grade, see below), and **causality** (relationship to study agent and relationship to participation in the research, see below). The Clinical Safety Office (CSO)/sponsor medical monitor (SMM) is responsible for final/regulatory determinations of expectedness and causality.

8.4.2.1.1 Severity Grading

The CoFAR scale will be used to grade the severity of allergic reactions. The investigator will grade the severity of other AEs, including laboratory and testing abnormalities and results, according to the "Common Terminology Criteria for Adverse Events (CTCAE)" (v 5.0) which can be found at: http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm

Events that are NOT gradable using the above specified table will be graded as follows:

- Mild = grade 1
- Moderate = grade 2
- Severe = grade 3
- Potentially life threatening = grade 4
- Death = grade 5

NOTE: A subject death should always be reported as grade 5.

8.4.2.1.1.1 Laboratory Value Assessment and Clinical Significance Criteria

Except as specified below, all abnormal lab values of grade 1 or above are reportable.

Grade 1 and 2 abnormal laboratory values are considered clinically significant, and are to be recorded in the research database, and reported, if they meet one or more of the following criteria:

- result in a study agent dosage adjustment, interruption, or discontinuation
- are accompanied by clinically abnormal signs or symptoms that are likely related to the laboratory abnormality (e.g., clinical jaundice)
- indicate a possible organ toxicity (e.g., elevated serum creatinine)
- result in additional/repeat testing or medical intervention (procedures/treatments) (e.g., EKG to evaluate arrhythmia potential with a high serum potassium; one or more EKGs to assess an elevated troponin level; potassium supplementation for hypokalemia)
- indicates possible over-dosage
- are considered clinically significant by the investigator or SMM

8.4.2.1.2 Causality

Causality (likelihood that the event is caused by the study agents) will be assessed by the principal investigator considering the factors listed under the following categories:

Definitely Related

- reasonable temporal relationship
- follows a known response pattern
- clear evidence to suggest a causal relationship
- there is no alternative etiology

Probably Related

- reasonable temporal relationship
- follows a suspected response pattern (based on similar agents)
- no evidence of a more likely alternative etiology

Possibly Related

- reasonable temporal relationship
- little evidence for a more likely alternative etiology

Unlikely Related

- does not have a reasonable temporal relationship

  AND/OR

- there is good evidence for a more likely alternative etiology

Not Related

- does not have a temporal relationship

AND/OR

- definitely due to an alternative etiology

Note: Other factors (e.g., dechallenge, rechallenge, if applicable) should also be considered for each causality category when appropriate. Causality assessment is based on available information at the time of the assessment of the AE. The investigator may revise the causality assessment as additional information becomes available.

Causality assessment will be reviewed by the sponsor. The sponsor may make a separate and final determination on the "reasonable possibility" that the event was "related" (comprising definitely, probably, and possibly related) or "unrelated" (comprising unlikely and not related) to the study agent, in keeping with applicable (US FDA) guidance on sponsor IND safety reporting.

8.4.2.2 Recording of Events

AEs will be promptly recorded in the research database, regardless of possible relationship to study interventions. If a diagnosis is clinically evident (or subsequently determined), the diagnosis rather than the individual signs and symptoms or laboratory abnormalities will be recorded as the AE. The investigator will review events regularly to ensure they have been captured correctly and to perform assessment of events individually and cumulatively to assess possible safety trends.

8.4.2.3 Investigator Reporting Responsibilities

The principal investigator and/or equally qualified designee will check daily for events that may require expedited reporting.

The principal investigator and/or equally qualified designee will also monitor all accumulating data no less than weekly, or according to superseding NIH or NIAID policy, whichever is more frequent.

Data will be reviewed by the principal investigator/designee on a regular basis for accuracy and completeness.

Data will be submitted to the sponsor in keeping with all applicable agreements and when requested, such as for periodic safety assessments, review of IND annual reports, review of IND safety reports, and preparation of final study reports.

The principal investigator will ensure prompt reporting to safety oversight bodies (e.g., CSO, DSMB), regulatory entities, and stakeholders as specified below, and according to any additional requirements or agreements.

8.4.2.3.1 Adverse Events

Unless otherwise specified above, AE data will be entered into the research database no less than every other week and will include all data through one week prior to database entry.

8.4.2.3.2 Serious Adverse Events (Expedited Reporting)

Unless otherwise specified above, all SAEs (regardless of relationship and whether or not they are also UPs) must be reported to the CSO as specified by the CSO (e.g., REDCap; use the Safety Expedited Report Form [SERF]/email if REDCap is not available). If the preferred/indicated mechanism for reporting is not available, the CSO/SMM should be contacted by telephone, fax, or other reasonable mechanism to avoid delays in reporting. ESIs will be reported in the same timeframe and mechanism as an SAE even though they do not meet the statutory SAE definition.

CSO CONTACT INFORMATION:

Clinical Safety Office
5705 Industry Lane
Frederick, MD 21704
Phone: 301-846-5301
Fax: 301-846-6224
Email: rchspsafety@mail.nih.gov
https://crimsonredcap.cc.nih.gov/redcap/index.php

Deaths and immediately life-threatening SAEs must be reported to the CSO promptly, and no later than the **first business day** following the day of study personnel awareness.

All other SAEs must be reported to the CSO no later than the **third business day** following the day of study personnel awareness.

If an individual subject experiences multiple SAEs in a closely timed/overlapping "cause-and-effect" (cascade) sequence, the principal investigator, after careful evaluation, will report ONLY primary/precipitating event(s) individually. SAEs that are determined to be definitely secondary to other SAEs will be detailed in the narrative portion of the report of the relevant primary/precipitating SAE. A clinical rationale and findings to support such reporting should be part of the narrative.

For each SAE report, the research database entry MUST match the corresponding entries on the SAE report (e.g., start and stop dates, event type, relationship, and grade), and **must be updated if necessary** (e.g., if the SAE report was generated after the corresponding AE was entered in the research database).

Unless otherwise specified above, SAEs that have not resolved by the end of the per-protocol follow-up period for the subject are to be followed until final outcome is known (to the degree permitted by the IRB-approved informed consent form). If it is not possible to obtain a final outcome for an SAE (e.g., the subject is lost to follow-up), and to update the CSO, the last known status and the reason a final outcome could not be obtained will be recorded by the investigator on an SAE report update and the CRF.

8.4.2.3.3 Unanticipated Problems

Unless otherwise specified above, UPs that are also AEs or SAEs, must be reported to the CSO (by REDCap, or by email and SERF if REDCap is not available) no later than when they are due to be reported to the IRB.

UPnonAEs are not reported to the CSO but must be reported to the clinical trials management (CTM) group and the IRB according to their requirements and preferred methods. If the UPnonAE raises a significant potential subject safety concern, the SMM should be consulted by email or phone no later than when the report is made to CTM.

8.4.2.3.4 Pregnancy

Pregnancy testing will be performed prior to the first study vaccination. If the result is positive, the participant will be withdrawn from the study, and no additional follow-up will be performed.

8.4.2.4 Sponsor's Reporting Responsibilities

Events reported to the sponsor will be promptly evaluated and will be reported as required according to FDA IND safety reporting guidance and regulations. IND safety reports will be sent to other investigators conducting research under the same IND and will be shared with other stakeholders according to applicable agreements (e.g., CRADAs and CTAs).

The sponsor will also submit an IND annual report of the progress of the investigation to the FDA as defined in 21 CFR 312.33.

All UPs will be evaluated by the sponsor, and a summary of the event, and any necessary (corrective/preventative) actions, will be distributed to investigators conducting research under the same IND as may be relevant and appropriate.

8.4.3 Withdrawal Criteria for an Individual Subject

An individual subject will be withdrawn from the study for any of the following:

- An individual subject's decision. (The investigator should attempt to determine the reason for the subject's decision.)
- Non-compliance with study procedures or requirements (see sections 5.5 and 6.5) to the extent that it is potentially harmful to the subject or to the integrity of the study data, per investigator discretion.
- The investigator determines that continued participation in the study would not be in the best interest of the subject.

The reason for participant withdrawal will be recorded in the research database.

8.4.3.1 Re-enrollment and Unplanned Procedure Repetition

Unless otherwise specified within this protocol, each person who is a subject in this study may be enrolled, and may pass through each step and process outlined in the protocol, only **ONCE** (i.e., subjects may not "go back" and repeat a protocol step already completed). On a case-by-case basis, a request for re-enrollment, or for repetition of a protocol step or procedure already

completed, may be submitted to, reviewed by, and approved by the SMM in writing. The SMM may also recommend or require consultation of the IRB and/or DSMB.

8.4.3.2 Replacement of Withdrawn Subjects or Subjects Who Discontinue Study Agent

Subjects who are withdrawn from the study or discontinue study vaccinations will not be replaced.

All subjects exposed to study agents must be included in the safety dataset.

8.4.4 Additional Safety Oversight

8.4.4.1 Safety Review and Communications Plan

A safety review and communication plan (SRCP) is required for this protocol. The SRCP is an internal communications document between the principal investigator and the CSO, as sponsor representative, which delineates key safety oversight responsibilities of the principal investigator, the CSO, and other stakeholders. The SRCP includes a plan for conducting periodic safety surveillance assessments by the CSO.

8.4.4.2 Sponsor Medical Monitor

A SMM, representing the sponsor, has been appointed for oversight of safety in this clinical study. The SMM will be responsible for performing safety assessments as outlined in the SRCP.

8.4.4.3 Oversight Committees

8.4.4.3.1 Data and Safety Monitoring Board

The NIAID intramural data and safety monitoring board (DSMB) includes independent experts that do not have direct involvement in the conduct of the study and have no significant conflicts of interest as defined by NIAID policy. The DSMB will review the study protocol, consent document, and investigator brochure prior to initiation and twice a year thereafter, or as may be determined by the DSMB. In addition, the DSMB will review unblinded data after the first 5 participants complete study vaccinations or after the first 4 months (whichever comes first), and every 4 months thereafter (or after every 20 participants completes study vaccinations, whichever comes first). The DSMB can make a variety of recommendations, such as: 1) stop the trial because of safety concerns, either based on the halting rules or based on results that are similarly concerning to those established in the halting rules, 2) stop the trial if there appears to be little value in proceeding (e.g., inability to recruit participants or if no reactions occur after, perhaps, 40 subjects have been evaluated), 3) continue the trial, but announce the topline interim results to the public, if timely release of the data will provide important guidance to the community, or 4) continue the trial without modification.

The DSMB may convene additional reviews as necessary or per principal investigator request to evaluate the safety, efficacy, study progress, and conduct of the study.

All deaths, SAEs, UPs, pregnancies, and IND safety reports will be reported to the DSMB at the same time they are submitted to the IRB and CSO unless otherwise specified herein. A summary of PSEEs will also be provided for review by the DSMB along with study documents submitted for periodic scheduled DSMB reviews.

All cases of intentional or unintentional unblinding will be reported to the DSMB not later than one business day from the time of study personnel awareness.

The principal investigator will notify the DSMB at the time pausing or halting criteria are met and obtain a recommendation concerning continuation, modification, or termination of the study. The principal investigator will submit the written DSMB summary reports with recommendations to the IRB.

8.4.5 Pausing Rules

"Pausing" is discontinuation of study vaccinations in a single subject. Other than discontinuation of study vaccinations, subjects will continue to be followed per protocol during a pause.

The pausing criterion for individual subjects in this study is the following:

- A subject experiences CoFAR Grade 4 allergic reaction to the first study vaccination.
- A subject experiences an ESI or an SAE assessed as being caused by the vaccine/placebo (unless the event is a PSEE).

The principal investigator or the CSO may also pause dosing/study interventions for one or more subjects for any safety issue. The DSMB may recommend a pause to the CSO.

8.4.5.1 Reporting a Pause

If a pausing criterion is met, a description of the AE(s) or safety issue must be reported by the principal investigator within 1 business day to the CSO and the IRB according to their requirements. The principal investigator will also notify the DSMB.

8.4.5.2 Resumption Following a Pause

If the pausing criterion is met following the first study vaccination, the second study vaccination will not be administered to that participant, but the other remaining study procedures will proceed as scheduled. The CSO, in collaboration with the principal investigator and DSMB or other safety review entity, will determine if any additional modifications or requirements may apply, for the impacted subject(s), or whether the events that triggered the pause require expansion to a study halt (see below).

The CSO or sponsor designee will notify the principal investigator of the decision. The principal investigator will notify the IRB of the decision according to the IRB's process.

8.4.5.3 Discontinuation of Study Agent

Discontinuation of study vaccination does not mean discontinuation from the study, and remaining study procedures may be completed as indicated by the study protocol if the subject is willing to remain on study.

8.4.6 Halting Rules for the Protocol

"Halting" is discontinuation of study vaccinations for all subjects in a study and suspension of enrollment until a decision is made to either resume or permanently discontinue such activity.

Subjects who have already received a study vaccination at the time of a halt will continue to be followed per protocol.

The halting rules are:

- More than 3 subjects experience a CoFAR Grade 4 allergic reaction and the frequency of Grade 4 allergic reactions is more than 10% of subjects enrolled to date;

  OR

- Any safety issue that the principal investigator or the CSO determines should halt the study. The DSMB may recommend a halt to the CSO;

  OR

- Any death.

In addition, the FDA or any regulatory body having oversight authority may halt the study at any time. The DSMB may recommend a study halt.

8.4.6.1 Reporting a Study Halt

If a halting criterion is met, a description of the AE(s) or safety issue must be reported by the principal investigator within 1 business day to the CSO and the IRB according to their requirements. The principal investigator will also notify the DSMB.

8.4.6.2 Resumption of a Halted Study

If a halting criterion is met, enrolled subjects who have received a study vaccination will continue with the remaining study procedures (other than study vaccinations) as scheduled. The principal investigator, CSO, and DSMB will discuss the option of continuing the study but administering the study agent in graded doses (10% of full dose, observe 30 minutes, and then administer remaining dose), or whether any other study modifications should be made.

The CSO or sponsor designee will notify the principal investigator of the decision. The principal investigator will notify the IRB of the decision according to the IRB's process.

8.4.6.3 Discontinuation of Study

The study will be stopped permanently for a single Grade 5 reaction. Subjects will continue to be followed for protocol-specified safety assessments or as clinically indicated, whichever is more conservative.

8.5 UNANTICIPATED PROBLEMS.

8.5.1 Definition of Unanticipated Problems

The definition of a UP is provided in section 8.4.1.

8.5.2 Unanticipated Problem Reporting

The investigator will report UPs to the NIH IRB as per Policy 801.

8.5.3 NIH Intramural IRB Reporting of IND Safety Reports

Only IND Safety Reports that meet the definition of a UP will need to be reported to the NIH Intramural IRB.

9 STATISTICAL CONSIDERATIONS

The analysis will be primarily aimed at estimation and description. There is considerable uncertainty about the number of subjects that can actually be enrolled, so both the sample size rationale and planned analysis are written to take that uncertainty into account. There is no intent to write a formal Statistical Analysis Plan.

9.1 STATISTICAL HYPOTHESIS

The primary goal is estimation of the primary and secondary endpoints, and estimation of the difference in endpoints between the active and placebo vaccines. Statistical hypothesis testing will be done to compare active versus placebo vaccines, but this is secondary to estimation.

The endpoints are as follows, and are defined in greater detail previously in the protocol (sections 1.1 and 3):

Primary Endpoint:

- Proportion with systemic allergic reaction to the active (ie, Pfizer-BioNTech) vaccine dose delivered in this study

Secondary Endpoints:

- Proportion with severe systemic allergic reaction to the COVID-19 vaccine dose delivered in this study
- Proportion with mild/moderate systemic allergic reaction to the COVID-19 vaccine dose delivered in this study
- Proportion with anaphylactic reaction to the COVID-19 vaccine dose delivered in this study
- Proportion with systemic allergic reaction to each study vaccination (i.e. active and placebo/pre- and post-crossover) by arm
- Proportion with more (or less) severe systemic allergic reaction to the COVID-19 vaccine dose delivered in this study than recorded reaction to the first full COVID-19 vaccine dose

9.2 SAMPLE SIZE DETERMINATION

The goal is to enroll up to 100 subjects; however, realistically, it may only be possible to enroll a smaller cohort. The ability to recruit for this study will depend on the rarity of such allergic reactions in the population and the acceptability of repeat vaccination and a 4-day hospital stay among individuals who have had these reactions. Current data indicates an extremely low systemic reaction rate, although it is likely that the overall rate may be higher since individuals recruited for this study have a history of a systemic allergic reaction following administration of the first dose of the vaccine. In sum, the study will provide more precise estimates with greater enrollment; however, even if fewer than 100 subjects enroll, the resulting data will provide valuable new information.

Since the primary goal of the study is estimation, we present the following example exact confidence intervals as a function of varying sample size and varying outcomes to illustrate achievable precision of estimates. We present such confidence intervals for the primary endpoint,

and also estimation of the risk difference of active vaccine versus placebo vaccine. The difference between active and placebo can be estimated in 2 different ways: one compares the results in the randomized arms at the first shot; and the other compares results within a subject across the 2 study vaccinations, exploiting the crossover design. Tables below present a subset of example scenarios in the study.

Table 5: Exact 95% Clopper Pearson Confidence Intervals for Primary Outcome, by Sample Size and Proportion with Systemic Allergic Reaction to Second Dose

Total **N**	Observed Reaction Rate					
	.00	.20	.40	.60	.80	1.00
5	0-0.522	0.005-0.716	0.053-0.853	0.147-0.947	0.284-0.995	0.478-1
10	0-0.308	0.025-0.556	0.122-0.738	0.262-0.878	0.444-0.975	0.692-1
15	0-0.218	0.043-0.481	0.163-0.677	0.323-0.837	0.519-0.957	0.782-1
20	0-0.168	0.057-0.437	0.191-0.639	0.361-0.809	0.563-0.943	0.832-1
40	0-0.088	0.091-0.356	0.249-0.567	0.433-0.751	0.644-0.909	0.912-1
60	0-0.06	0.108-0.323	0.276-0.535	0.465-0.724	0.677-0.892	0.94-1
80	0-0.045	0.119-0.304	0.292-0.516	0.484-0.708	0.696-0.881	0.955-1
100	0-0.036	0.127-0.292	0.303-0.503	0.497-0.697	0.708-0.873	0.964-1

As the sample size increases, precision in the estimate of the true proportion of subjects with a reaction to the second vaccine dose also increases. As an example, if the total N is 10, and there is .20 reaction rate (i.e., 2 react) to active vaccine, then the 95% CI for reaction rate is (.025, .556).

Table 6: Exact 95% Confidence Intervals for the Difference in Reaction Rate Between Vaccine and Placebo Arms on Day 1

Total N	Observed Reaction Rate per Arm on Day 1		
	1.00 Vaccine, .00 Placebo	.80 Vaccine, .20 Placebo	.60 Vaccine, .20 Placebo
6	0.082-1	-0.282-0.992	-0.554-0.889
10	0.383-1	-0.112-0.933	-0.268-0.858
20	0.663-1	0.127-0.862	-0.058-0.756
40	0.824-1	0.287-0.798	0.084-0.666
60	0.881-1	0.313-0.767	0.134-0.622
80	0.702-1	0.338-0.748	0.144-0.594
100	0.597-1	0.355-0.734	0.16-0.575

Note: If only 6 subjects are enrolled, a reaction rate of 80% in the vaccine arm corresponds to 3/3 reactions; a reaction rate of 60% in the vaccine arm corresponds to 2/3 reactions; and a reaction rate of 20% in the placebo arm corresponds 1/3 reactions.

Thus, if the total N is 20 with 10 on active vaccine on Day 1 and 10 on placebo, and 8 of those receiving active vaccine have a reaction and 2 of those receiving placebo have a rection, the 95% CI for the excess reaction in active vaccine minus placebo is (.127, .862). Since this particular confidence interval does not include zero, this is also a demonstration that vaccines lead to excess reactions.

Table 7: Exact 95% Confidence Intervals for the Risk Difference Comparing each Individual's Vaccine Phase to Placebo Phase Response Using McNemar's Approach

Total N	Observed Reaction Rate per Treatment Phase		
	1.00 Vaccine, .00 Placebo	.80 Vaccine, .20 Placebo	.60 Vaccine, .20 Placebo
5	-0.044-1	-0.275-0.947	-0.369-0.853
10	0.338-1	0.04-0.878	-0.088-0.738
15	0.517-1	0.162-0.837	0.026-0.677
20	0.621-1	0.235-0.809	0.084-0.639
40	0.797-1	0.369-0.751	0.192-0.567
60	0.861-1	0.423-0.724	0.238-0.535
80	0.895-1	0.453-0.708	0.264-0.516
100	0.915-1	0.473-0.697	0.281-0.503

The examples above assume that if a subject reacts to the placebo phase, they will also react to the active vaccine phase. For example, with 10 subjects, and a reaction rate of 80% in the active vaccine and 20% in the placebo, this means that 2 subjects react to both active vaccine and placebo, 6 subjects react only to active vaccine, and 2 subjects react to neither. And, in this example the 95% confidence interval for excess reaction rate in active vaccine minus placebo is (.04, .878). The confidence intervals in this table are narrower than the ones in the previous table, since they use information from both study vaccinations. (There are other scenarios to get the same reaction rates [i.e. in the previous example, 1 subject reacts to both active vaccine and placebo, 7 subjects react only to active vaccine, 1 subject reacts only to placebo, and 1 subject reacts to neither], but these alternatives are not presented here, and the confidence intervals would be slightly different.)

While hypothesis testing will be done comparing active vaccine and placebo reactions, this goal is viewed as secondary to estimation of the difference.

9.3 POPULATIONS FOR ANALYSES

The modified intention-to-treat analysis data set includes all subjects who receive the first injection in the study and are followed for the initial 3 hours for systemic allergic reaction.

9.3.1 Evaluable for Toxicity

All participants will be evaluable for toxicity from the time of their first study vaccination.

9.3.2 Evaluable Non-Target Disease Response

Not applicable

9.4 STATISTICAL ANALYSES

9.4.1 General Approach

Most analyses will result in estimates of binary endpoints and will be presented as proportions and corresponding exact 95% confidence intervals.

Exploratory endpoints, such as mechanistic endpoints, will be presented as means with standard deviations and/or as medians and interquartile ranges. Graphical presentation may be useful.

While hypothesis testing is likely to be limited in scope, two-sided p-values with alpha=.05 will be used, with appropriate cautions regarding interpretation due to multiplicity.

9.4.2 Analysis of the Primary Endpoints

The primary endpoint will be estimated using the proportion of reaction to the active vaccine, and presented with a 95% confidence interval, using the Clopper-Pearson method.

It is not anticipated that individuals randomized to receive placebo vaccination first will not get the active vaccine, but if this occurs, the following approaches will be used: i) analysis in the population with complete data (which will be the primary approach), and ii) analysis where missing data is imputed to be a vaccine reaction.

9.4.3 Analysis of the Secondary Endpoint(s)

Analyses to simple single proportion estimates will follow the same approach described for the primary endpoint.

Analysis of risk difference between active and placebo vaccines will be addressed in 2 separate ways. First, using only data from the first study vaccination, the risk difference will be estimated (proportion with systemic reaction in the active vaccine arm minus the analogue in the placebo arm) with an unconditional exact confidence interval. The second approach will exploit the crossover design (i.e., comparing each individual's active vaccine to placebo response), by estimating the risk difference using a McNemar's approach to an exact confidence interval.[34] While the McNemar's approach should be more precise than the group comparison approach at the first study vaccination, the potential for missing data on the second study vaccination, especially in the initial vaccine arm is not ideal. If there are such missing data, the second study vaccination will be imputed in the most extreme ways (i.e., smallest risk difference and largest risk difference) to see if the results are robust.

Hypothesis testing can be done for the active vaccine versus placebo risk difference. A Fishers exact test will be done when comparing the randomized arm at the first study vaccination. An exact McNemar's test will be done when comparing the within-subject differences. Approaches to missing data for McNemar's follow the estimation approaches.

A signed rank test will also be done to compare severity of reaction at the first vaccine (i.e., done prior to enrollment) to active study vaccine (where the difference in grade is the metric). Since that analysis assumes grade is linear, these data will also be analyzed using a purely ordinal approach to grade using a longitudinal GEE model with a cumulative logit link; this is effectively a generalization of McNemar's test, but for ordinal instead of binary data. Tabular data will also be presented.

9.4.4 Safety Analyses

The primary and secondary endpoints of this study are focused on safety and will be evaluated and analyzed as described above. Allergic reactions will be scored using the CoFAR Grading Scale for Systemic Allergic Reactions Version 3.0 and the Brighton Collaboration Criteria. Other

AEs following active vaccine administration are expected as described in Section 2.3.1, and will be assessed during the participant's ICU stay as well as the remote follow-up within 1 week post-discharge (Visit 2) and at the in-person follow-up visits (Visits 3, 4, and 6). Efforts will be made to record start and stop dates of all AEs (both allergic and nonallergic in nature), severity, relationship to the study intervention, and expectedness based on study intervention. If participants experience a CoFAR Grade 4 or higher reaction on Day 1, or any life-threatening adverse reaction related or unrelated to the study intervention, then no additional dosing will occur and participants will be followed per study protocol.

9.4.5 Baseline Descriptive Statistics

Baseline descriptive statistics will be presented in terms of proportions, means/medians, standard deviations/interquartile ranges. Unless enrollment is very limited in size, the baseline variables will also be presented by randomized arm.

9.4.6 Planned Interim Analyses

No formal interim analyses are planned.

9.4.7 Sub-Group Analyses

The baseline characteristics of the cohort will be described. If the number of enrolled patients is sufficient, endpoints will be analyzed in subgroups (e.g., gender; age; ethnicity; severity of reaction to original vaccine dose; menopausal status for female participants; presence, type, and severity of allergic diseases; laboratory assessments of allergic sensitization and inflammation; history of allergic reactions including cause, severity, and treatment required; presence, type, and levels of anti-PEG antibodies; history of prior SARS-CoV-2 infection; other medical comorbidities; medication use; presence of genetic variants that may influence response to vaccine including but not limited to hereditary alpha tryptasemia; level of inflammatory and immune markers at baseline).

9.4.8 Tabulation of individual Participant Data

Individual patient data may be listed, especially if enrollment is limited.

9.4.9 Exploratory Analyses

Baseline mechanistic endpoints and other potential risk factors including results of mental health and anxiety assessments as well as changes in these endpoints pre- and post-vaccine will be described and compared between those with and without systemic allergic reactions (and likewise for other allergic endpoints). Analyses will generally use Wilcoxon rank sum tests when comparing groups and signed rank tests when comparing within subjects (e.g., pre- versus post-vaccine), however parametric tests might be used if number of enrolled patients is sufficient.

The ability to do hypothesis testing and modelling of risk factors and changes in exploratory endpoints pre- and post-vaccine will depend on the number of subjects enrolled. The proportion of subjects who react to the study-administered booster dose (among those who receive it), will be computed along with the corresponding 95% confidence interval, using the Clopper-Pearson method.

10 REGULATORY AND OPERATIONAL CONSIDERATIONS

10.1 INFORMED CONSENT PROCESS

10.1.1 Consent/Assent Procedures and Documentation

Informed consent is a process where information is presented to enable persons to voluntarily decide whether or not to participate as a research subject. It is an ongoing conversation between the human research participant and the researchers which begins before consent is given and continues until the end of the participant's involvement in the research. Discussions about the research will provide essential information about the study and include purpose, duration, experimental procedures, alternatives, risks, and benefits. Coercion and undue influence will be minimized by informing participants that their decision to join the study will not affect any medical care they are currently receiving at the NIH, or their eligibility to participate in other research studies at the NIH. Participants will be given as much time as they need to read the consent form and ask questions of the investigators. Participants will also be given time to discuss their participation with family members, friends, and other healthcare providers.

Informed consent will be obtained in person or remotely via telephone or NIH-approved videoconference platform, by a study team member authorized to obtain consent. Whether in person or remote, the privacy of the subject will be maintained. Consenting investigators (and participant, when in person) will be located in a private area (e.g., clinic consult room). When consent is conducted remotely, the participant will be informed of the private nature of the discussion and will be encouraged to relocate to a more private setting if needed. When consent is obtained in person, participants and investigators may view the same approved consent document either as hard copy or by viewing an electronic version on a monitor, or they may view individual copies of the approved consent document as hard copies or on separate screens.

The participants will sign the informed consent document prior to undergoing any research procedures (except some screening procedures, as described in section 8.1). Consent will be documented with required signatures on the hard copy of the consent form or on the electronic document. When a document that is in electronic format is used for the documentation of consent, this study will use the iMed platform to obtain the required signatures. This platform is compliant with 21 CFR Part 11. The identity of the participant will be determined by a prompt which will require the provision of information from an official identification document, prior to obtaining the signature. Signatures can be captured via iMed either onsite at the CC via a signature pad, or remotely via the iMed Mobile Signature Capture that can be sent via email or text.

A copy of the informed consent document will be given to the participants for their records. The consenting investigator will document the signing of the consent form in the participant's medical record. The investigator will confirm that written legally effective consent has been obtained prior to initiating any study interventions.

The rights and welfare of the participants will be protected by emphasizing to them that the quality of their medical care will not be adversely affected if they decline to participate in this study. The participants may withdraw consent at any time throughout the course of the trial.

After September 15, 2022, a consent addendum will be reviewed with participants at Visit 4. This addendum will provide additional information about the potential for booster vaccination with the monovalent formulation or newly authorized bivalent formulation of the Pfizer-BioNTech vaccine. The process for review and signing of this consent addendum will be same as that described above for the informed consent document.

Remote consent process: When the informed consent process occurs remotely, the informed consent document will be sent via secure email, file transfer, or NIH-approved platform to the potential participant prior to the consent discussion. An explanation of the study will be provided over the telephone or NIH-approved videoconference method (e.g., Microsoft Teams) after the participant has had the opportunity to read the consent form. During the consent process, participants and investigators will view the same approved consent document simultaneously in their respective locations, or they will view individual copies of the approved consent document on screens or as hard copies at their respective locations. The participant can sign and date the hard copy, print the electronic form to sign and date in ink, or they can sign and date the electronic document as described above.

The participant will return the signed and dated consent form to the consenting investigator, who will sign and date it with the date it was received. The consent form can either be printed and signed and dated in ink, or the electronic document can be signed and dated as described above. A fully executed copy will be sent to the participant for their records.

Assent process: Minor participants will be included in all discussions about the study, and age-appropriate language will be used to describe the procedures and tests involved in this study, along with the risks, discomforts, and benefits of participation. Minor participants aged 16 or 17 years will provide assent by signing a hard copy or electronic copy of the informed consent document, as described above. The parent(s)/legal guardian(s) will provide permission for the minor participant to participate by signing a hard copy or electronic copy of the consent form, as described above. The consent/assent process will be documented in the minor participant's medical record.

10.1.2 Consent for Minors When They Reach the Age of Majority

When a pediatric subject reaches age 18, continued participation (including ongoing interactions with the subject or continued analysis of identifiable data) will require that consent be obtained from the now adult with the standard protocol consent document to ensure legally effective informed consent has been obtained.

If reconsent is not feasible, we request waiver of informed consent to continue to use data and/or specimens for those individuals who become lost to follow up or who have been taken off study prior to reaching the age of majority.

Requirements for Waiver of Consent consistent with 45 CFR 46.116(f)(3):

(1) The research involves no more than minimal risk to the subjects.
    a. Analysis of samples and data from this study involves no additional risks to subjects.
(2) The research could not practicably be carried out without the waiver or alteration.

a. Considering the length of time between the minor's last contact with the research team and their age of majority, it will likely be very difficult to locate them again. A significant reduction in the number of samples analyzed is likely to impact the quality of the research.

(3) As the research involves using identifiable private information or identifiable biospecimens, the research could not practicably be carried out without using such information or biospecimens in an identifiable format.

a. Though the purpose of future studies cannot yet be known, they often involve the correlation of clinical outcomes and clinical interventions with laboratory studies. Such information would be unavailable if access to medical record numbers was unavailable.

(4) The waiver or alteration will not adversely affect the rights and welfare of the subjects.

a. Retention of these samples or data does not affect the welfare of subjects.

(5) Whenever appropriate, the subjects will be provided with additional pertinent information after participation.

a. We only request a waiver of consent for those subjects who have been lost to follow-up or who have been taken off study prior to reaching the age of majority.

10.1.3 Considerations for Consent of NIH Staff, or Family Members of Study Team Members

Consent for NIH staff and family members of the study team will be obtained as detailed above and will comply with the requirements of NIH Human Research Protections Program (HRPP) Policy 404 *Research Involving NIH Staff as Subjects.*

Consent from NIH staff for whom this research is taking place within their own work unit or is conducted by any of their supervisors will, when possible, be obtained by an individual in a non-supervisory relationship with that staff member. Similarly, for family of the study team, a study team member unrelated to the participant will obtain their informed consent. When consent of that staff member or family member is conducted, a third party will be present to observe the consent process in order to minimize the risk of undue pressure on them.

10.2 STUDY DISCONTINUATION AND CLOSURE

This study may be temporarily suspended or prematurely terminated if there is sufficient reasonable cause. Written notification, documenting the reason for study suspension or termination, will be provided by the suspending or terminating party to the principal investigator, sponsor, and regulatory authorities. If the study is prematurely terminated or suspended, the principal investigator will promptly inform the study participants, IRB, sponsor, and FDA, as applicable, and will provide the reason(s) for the termination or suspension. Study participants will be informed of changes to the study visit schedule, if applicable.

Circumstances that may warrant termination or suspension include, but are not limited to:

- Determination of unexpected, significant, or unacceptable risk to participants

- Insufficient compliance to protocol requirements
- Data that are not sufficiently complete and/or evaluable
- Determination that the primary endpoint has been met
- Determination of futility

In the case of a temporary suspension, the study may resume once concerns about safety, protocol compliance, and data quality are addressed and satisfy the sponsor, IRB, and FDA, as applicable.

10.3 CONFIDENTIALITY AND PRIVACY

All records will be kept confidential to the extent provided by federal, state, and local law. Authorized representatives of the NIAID may inspect all documents and records required to be maintained by the investigator, including but not limited to, medical records. Records will be kept locked and data will be coded. Any personally identifiable information maintained for this study will be kept on restricted-access computers and networks. Personally identifiable information will only be shared with individuals authorized to receive it under this protocol. Individuals not authorized to receive personally identifiable information will be provided with coded information only, as needed. Clinical information will not be released without written permission of the participant, except as necessary for monitoring by the IRB, NIAID, Office for Human Research Protections (OHRP), the FDA, or the sponsor's designee.

To further protect the privacy of study participants, a Certificate of Confidentiality has been issued by the NIH. This certificate protects identifiable research information from forced disclosure. It allows the investigator and others who have access to research records to refuse to disclose identifying information on research participation in any civil, criminal, administrative, legislative, or other proceeding, whether at the federal, state, or local level. By protecting researchers and institutions from being compelled to disclose information that would identify research participants, Certificates of Confidentiality help achieve the research objectives and promote participation in studies by helping assure confidentiality and privacy to participants.

10.4 FUTURE USE OF STORED SPECIMENS AND DATA

Coded specimens and data will be stored at the NIH indefinitely for future research after the study is complete. Human genetic testing may be performed. Plans for future use of specimens and data will be described in the informed consent document. Specimens will be stored at the NIH Clinical Center in a locked facility with limited access. Data will be kept in password-protected computers. Only investigators or their designees will have access to the code key.

Other investigators (at NIH and elsewhere) may wish to study these specimens and data. If the planned research falls within the category of "human subjects research" on the part of the investigators, NIH IRB review and approval will be obtained. This includes the investigators sending out coded and linked specimens or data and getting results that they can link back to their participants.

10.5 SAFETY OVERSIGHT

Safety oversight for this study is described in section 8.4.4.

10.6 CLINICAL MONITORING

According to the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) E6(R2) Good Clinical Practice (GCP) guidelines, section 5.18, and FDA 21 CFR 312.50, clinical protocols are required to be adequately monitored by the study sponsor. This study monitoring will be conducted according to the "NIAID Intramural Clinical Monitoring Guidelines." Monitors under contract to the NIAID/OCRPRO will visit the clinical research site to monitor aspects of the study in accordance with the appropriate regulations and the approved protocol. The objectives of a monitoring visit will be: 1) to verify the existence of signed informed consent documents and documentation of the consent process for each monitored participant; 2) to verify the prompt and accurate recording of all monitored data points in CRIMSON and prompt reporting of all SAEs; 3) to compare abstracted information entered into CRIMSON with individual participants' records and source documents (participants' charts, laboratory analyses and test results, physicians' progress notes, nurses' notes, and any other relevant original participant information); and 4) to help ensure investigators are in compliance with the protocol. The monitors also will inspect the clinical site regulatory files to ensure that regulatory requirements (Office for Human Research Protections [OHRP], FDA) and applicable guidelines (ICH GCP) are being followed. During the monitoring visits, the investigator (and/or designee) and other study personnel will be available to discuss the study progress and monitoring visit.

The investigator (and/or designee) will make study documents (e.g., consent forms, CRIMSON data abstracts) and pertinent hospital or clinical records, including CRIMSON, readily available for inspection by the local IRB, FDA, the site monitors, and the NIAID staff for confirmation of the study data.

A specific protocol monitoring plan will be discussed with the principal investigator and study staff prior to enrollment. The plan will outline the frequency of monitoring visits based on such factors as study enrollment, data collection status, and regulatory obligations.

10.7 QUALITY ASSURANCE AND QUALITY CONTROL

To help ensure that NIH Office of Research Support and Compliance procedures and Good Clinical Practices (GCP) are being carried out, a Clinical Trials Management designee within the Office of Clinical Research Policy and Regulatory Operations, Regulatory Compliance and Human Subjects Protection Program will conduct a study initiation visit before study enrollment begins. The purpose of this meeting is to review with the principal investigator and study team designees the roles and responsibilities concerning their commitment to adhere to the requirements of the protocol, especially in terms of NIH OHSRP reporting requirements for reportable events. In addition, the quality management and data management plan for the study will be reviewed.

10.8 DATA HANDLING AND RECORD KEEPING

10.8.1 Data Collection and Management Responsibilities

Study data will be maintained in REDCap, CRIMSON, and CRIS and collected directly from participants during study visits and telephone calls or will be abstracted from participants' medical records. Source documents include all recordings of observations or notations of clinical activities and all reports and records necessary to confirm the data abstracted for this study. Data

entry into CRIMSON will be performed by authorized individuals. The investigator is responsible for assuring that the data collected are complete, accurate, and recorded in a timely manner. Study data, including cumulative participant accrual numbers, should be generated via the chosen data capture method and submitted to study oversight bodies as needed.

10.8.2 Study Records Retention

Study documents will be retained in accordance with regulatory and institutional requirements, ICH GCP guidelines, and the NIH Intramural Records Retention Schedule. No records will be destroyed without the written consent of the principal investigator and sponsor, as applicable.

Should the investigator wish to assign the study records to another party and/or move them to another location, the investigator will provide written notification of such intent to OCRPRO/NIAID with the name of the person who will accept responsibility for the transferred records and/or their new location. Relocation of research records will not proceed without written permission from OCRPRO/NIAID.

10.9 Protocol Deviations and Non-Compliance

It is the responsibility of the investigator to use continuous vigilance to identify and report deviations and/or non-compliance to the NIH IRB as per Policy 801. All deviations must be addressed in study source documents and reported as specified in the protocol quality management plan and/or monitoring plan. The investigator is responsible for knowing and adhering to the reviewing IRB requirements.

10.9.1 NIH Definition of Protocol Deviation

The definition of a protocol deviation is provided in section 8.4.1.

10.10 Reporting to the NIAID Clinical Director

The principal investigator will report UPs, major protocol deviations, and deaths to the NIAID clinical director according to institutional timelines.

10.11 Publication and Data Sharing Policy

10.11.1 Human Data Sharing Plan

We will comply with NIH policies on data access, sharing, and dissemination, and clinical trials registration, as applicable. Results information from this trial will be submitted to ClinicalTrials.gov. Human data generated in this study may be shared for future research as follows:

- De-identified data in an NIH-supported public data repository, such as the database of Genotypes and Phenotypes (dbGaP), the National Center for Biotechnology Information (NCBI) Gene Expression Omnibus (GEO), and the NCBI Sequence Read Archive (SRA).
- De-identified data in another public repository.
- Identified data in the Biomedical Translational Research Information System (BTRIS, automatic for activities in the Clinical Center).
- De-identified or identified data with approved outside collaborators under appropriate agreements.

- De-identified data in publications and/or public presentations.

Data will be shared at the time of or shortly after publication.

10.11.2 Genomic Data Sharing Compliance

Although the NIH Genomic Data Sharing Policy is not required for this research, we may want to share genomic data with an NIH-supported repository, as described in the previous section. We will comply with all requirements in order to do so, including creating a Data Sharing Plan and completing an Institutional Certification and seeking approval prior to beginning the research. Prior to sharing the data, we will de-identify it, code it, and maintain the code key linked to the data.

10.12 CONFLICT OF INTEREST POLICY

The independence of this study from any actual or perceived influence, such as by the pharmaceutical industry, is critical. Therefore, any actual conflict of interest of persons who have a role in the design, conduct, analysis, publication, or any aspect of this trial will be disclosed and managed. Furthermore, persons who have a perceived conflict of interest will be required to have such conflicts managed in a way that is appropriate to their participation in the design and conduct of this trial. The study leadership in conjunction with NIAID has established policies and procedures for all study group members to disclose all conflicts of interest and will establish a mechanism for the management of all reported dualities of interest.

11 ABBREVIATIONS

AE	Adverse Event
AR	Adverse Reaction
CAPA	Corrective and Preventive Action Plan
CARPA	Complement Activation Related Pseudoalleregy
CDC	Centers for Disease Control and Prevention
CFR	Code of Federal Regulations
CLIA	Clinical Laboratory Improvement Amendments
CMP	Clinical Monitoring Plan
COC	Certificate of Confidentiality
CoFAR	Consortium for Food Allergy Research
CONSORT	Consolidated Standards of Reporting Trials
CRF	Case Report Form
CSO	Clinical Safety Office
CTM	Clinical Trials Management
DLM	Department of Laboratory Medicine
DSMB	Data Safety Monitoring Board
ECG	Electrocardiogram
ESI	Event of Special Interest
EUA	Emergency Use Authorization
FDA	Food and Drug Administration
FXII	Factor XII
GCP	Good Clinical Practice
HRPP	Human Research Protections Program
ICH	International Conference on Harmonisation of Technical Requirements for Pharmaceuticals for Human Use
ICU	Intensive Care Unit
IDCU	Investigational Drug Control Unit
IND	Investigational New Drug application
IRB	Institutional Review Board
MOP	Manual of Procedures
MRGPRX2	MAS-Related G Protein-Coupled Receptor-X2
NIH	National Institutes of Health
OHRP	Office for Human Research Protections
PAF	Platelet Activating Factor
PAF-AH	Platelet Activating Factor Acetylhydrolase
PEG	Polyethylene Glycol
PI	Principal Investigator
PSEE	Protocol-Specified Exempt Event
REDCap	Research Electronic Data Capture
SAE	Serious Adverse Event
SAR	Suspected Adverse Reaction
SARS-CoV-2	Severe Acute Respiratory Syndrome Coronavirus 2

SERF	Safety Expedited Report Form
SMM	Sponsor Medical Monitor
SNP	Single-Nucleotide Polymorphism
SOA	Schedule of Activities
SRCP	Safety Review and Communication Plan
SUSAR	Serious and Unexpected Suspected Adverse Reaction
UP	Unanticipated Problem
UPnonAE	Unanticipated Problem that is not an Adverse Event
US	United States
VAERS	Vaccine Adverse Event Reporting System

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APPENDIX A. MODIFIED COFAR GRADING SCALE FOR SYSTEMIC ALLERGIC REACTIONS VERSION 3.0

Grade 1	Grade 2	Grade 3 [1]	Grade 4 [1]	Grade 5
Reaction involving **one of the following organ systems in which the symptoms are mild**: Cutaneous: Generalized pruritus, generalized urticaria, flushing, angioedema Upper respiratory: Rhinitis, cough unrelated to laryngeal edema or bronchospasm Conjunctival: Injection/redness, itching, tearing GI: Nausea, abdominal pain (no change in activity level), single episode of vomiting and/or single episode of diarrhea	Reaction involving **2 or more of the following organ systems in which the symptoms are mild**: Cutaneous: Generalized pruritus, generalized urticaria, flushing, angioedema Upper respiratory: Rhinitis, cough unrelated to laryngeal edema or bronchospasm Conjunctival: Injection/redness, itching, tearing GI: Nausea, abdominal pain (no change in activity level), single episode of vomiting, and/or single episode of diarrhea **OR** Reaction involving **at least one of the following organ systems in which the symptoms are moderate:** Cutaneous: Generalized pruritus, generalized urticaria, flushing, angioedema Upper respiratory: Rhinitis, cough unrelated to laryngeal edema or bronchospasm Conjunctival: Injection/redness, itching, tearing GI: Nausea, abdominal pain (with change in activity level), 2 episodes of vomiting and/or diarrhea	Reaction involving **one or more of the following organ systems**: Lower respiratory: Throat tightness, wheezing, chest tightness, dyspnea, cough that responds to treatment with or without supplemental oxygen GI: Severe abdominal pain, more than 2 episodes of vomiting and/or diarrhea Cardiovascular: Reduced BP without associated symptoms of end-organ dysfunction (e.g., hypotonia [collapse], syncope) defined as systolic BP of less than 90 mmHg or >30% decrease from baseline	**Life-threatening reaction** involving **one or more of the following organ systems** with or without other symptoms listed in Grades 1 to 3: Lower respiratory: Throat tightness with stridor, wheezing, chest tightness, dyspnea, or cough associated with a requirement for supplemental oxygen and refractoriness to short-acting bronchodilator treatment (including IM epinephrine) **OR** Respiratory compromise requiring mechanical support Cardiovascular: Reduced BP with associated symptoms of end-organ dysfunction (e.g., hypotonia [collapse], syncope) defined as: systolic BP of less than 90 mmHg or >30% decrease from baseline	Death

[1] Patients treated with epinephrine infusion without documented hypoxia, hypotension, or evidence of end-organ damage would be considered as COFAR grade 3 allergic reaction and may be eligible per investigator discretion.

APPENDIX B. BRIGHTON COLLABORATION CRITERIA

Case Definition of Anaphylaxis

For all levels of diagnostic certainty	Level 1 of diagnostic certainty	Level 2 of diagnostic certainty	Level 3 of diagnostic certainty
Anaphylaxis is a clinical syndrome characterized by sudden onset AND rapid progression of signs and symptoms AND involving multiple (≥2) organ systems	≥1 major dermatological AND ≥1 major cardiovascular AND/OR ≥1 major respiratory criterion	≥1 major cardiovascular AND ≥1 major respiratory criterion OR ≥1 major cardiovascular OR respiratory criterion AND ≥1 minor criterion involving ≥1 different system (*other than* cardiovascular or respiratory systems) OR (≥1 major dermatologic) AND (≥1 minor cardiovascular AND/OR minor respiratory criterion)	≥1 minor cardiovascular OR respiratory criterion AND ≥1 minor criterion from each of ≥2 different systems/ categories

The case definition should be applied when there is no clear alternative diagnosis for the reported event to account for the combination of symptoms.

Major and Minor Criteria Used in the Case Definition of Anaphylaxis

	Major Criteria	Minor Criteria
Dermatologic or mucosal	• generalized urticaria (hives) or generalized erythema • angioedema*, localized or generalized • generalized pruritus with skin rash	• generalized pruritus without skin rash • generalized prickle sensation • localized injection site urticaria • red and itchy eyes
Cardiovascular	• measured hypotension • clinical diagnosis of uncompensated shock, indicated by the combination of at least 3 of the following: - tachycardia - capillary refill time >3 s - reduced central pulse volume - decreased level of consciousness or loss of consciousness	• reduced peripheral circulation as indicated by the combination of at least 2 of - tachycardia - a capillary refill time of >3 s without hypotension - a decreased level of consciousness
Respiratory	• bilateral wheeze (bronchospasm) • stridor • upper airway swelling (lip, tongue, throat, uvula, or larynx) • respiratory distress—2 or more of the following: - tachypnoea - increased use of accessory respiratory muscles (sternocleidomastoid, intercostals, etc.) - recession - cyanosis - grunting	• persistent dry cough • hoarse voice • difficulty breathing without wheeze or stridor • sensation of throat closure • sneezing, rhinorrhea
Gastrointestinal		• diarrhoea • abdominal pain • nausea • vomiting
Laboratory		Mast cell tryptase elevation > upper normal limit

* Not hereditary angioedema