PROTOCOL NUMBER: CVIA 057

Title: A Phase 1, randomized, controlled, observer-blind study to assess the reactogenicity, safety, and immunogenicity of a live attenuated universal influenza vaccine (cH8/1N1 LAIV) administered as a single priming dose followed three months later by a single booster dose of an inactivated universal influenza vaccine (cH5/1N1 IIV) (adjuvanted with $AS03_A$ or unadjuvanted) in 18 through 39 year-old healthy subjects, contrasted with a two dose schedule of an inactivated universal influenza vaccine (cH8/1N1 IIV + $AS03_A$ followed three months later by cH5/1N1 IIV + $AS03_A$)

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Clinical Trials.gov NCT 03300050

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Pharmaceutical Support:
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Collaborating Partner:
Icahn School of Medicine
at Mount Sinai

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Funding:
[redacted]

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ABBREVIATIONS AND ACRONYMS

AE: adverse event
ADCC: antibody-dependent cell-mediated cytotoxicity
ADCP: antibody-dependent cellular phagocytosis
ALT: alanine aminotransferase
ARI: acute respiratory illness
ASC: antibody-secreting cells
AST: aspartate aminotransferase
BUN: blood urea nitrogen
CCHMC Cincinnati Children's Hospital Medical Center
CDC: Centers for Disease Control and Prevention, United States
cHA: chimeric hemagglutinin
CFR Code of Federal Regulations
CI: confidence interval
CMI: cell-mediated immune responses
CSR: clinical study report
eCRF: electronic case report form
ELISA: enzyme-linked immunosorbent assay
ELISPOT Enzyme-Linked ImmunoSpot
EoS: end of study
FDA: United States Food and Drug Administration
GMT: geometric mean titer
GSK: GlaxoSmithKline
HA: hemagglutinin
HI: hemagglutination inhibition
ICF: informed consent form
ICH-GCP: International Conference on Harmonisation Good Clinical Practices
ICMJE The International Committee of Medical Journal Editors
IDMC: Independent Data Monitoring Committee
IgA: immunoglobulin A
IgG: immunoglobulin G
IIV: inactivated influenza vaccine

ILI: influenza-like illness
IM: intramuscular
IN: intranasal
IND: Investigational New Drug
IRB: Institutional Review Board
ISMMS: Icahn School of Medicine at Mount Sinai
IWRS: interactive web response system
LAIV: live attenuated influenza vaccine
mAB: monoclonal antibody
MAE: medically attended event
MDCK: Madin Darby canine kidney
MedDRA: Medical Dictionary for Regulatory Activities
mg: milligram
MGI: mean geometric increase
mL: milliliter
MN: microneutralization
NA: neuraminidase
OP: oropharyngeal
PBMC: peripheral blood mononuclear cell
PBS: phosphate buffered saline
PI: principal investigator
pIMD: potential immune-mediated disease
PSRT: Protocol Safety Review Team
rHA: recombinant HA
RT-PCR: reverse transcription polymerase chain reaction
SAE: serious adverse event
SOP: standard operating procedure
TVC: total vaccinated cohort
VAERD: Vaccine-associated enhanced respiratory disease
WBC: white blood cell

GLOSSARY OF TERMS

Adequate contraception:	Adequate contraception is defined as a contraceptive method with a failure rate of less than 1% per year when used consistently and correctly and when applicable, in accordance with the product label for example: • Abstinence from penile-vaginal intercourse, when this is their preferred and usual lifestyle, • Combined estrogen and progesterone oral contraceptives, • Injectable progestogen, • Implants of etonogestrel or levonorgestrel, • Contraceptive vaginal ring, • Percutaneous contraceptive patches, • Intrauterine device or intrauterine system, • Male condom combined with a vaginal spermicide (foam, gel, film, cream or suppository), and/or progesterone alone oral contraceptive. Adequate contraception does not apply to subjects of child bearing potential with same sex partners or for subjects who are and will continue to be abstinent from penile-vaginal intercourse on a long term and persistent basis, when this is their preferred and usual lifestyle.
Adverse event:	Any untoward medical occurrence in a patient or clinical investigation subject, temporally associated with the use of a medicinal product, whether or not considered related to the medicinal product. An adverse event (AE) can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease (new or exacerbated) temporally associated with the use of a medicinal product. For marketed medicinal products, this also includes failure to produce expected benefits (i.e., lack of efficacy), abuse or misuse.

Blinding:	A procedure in which one or more parties to the trial are kept unaware of the treatment assignment in order to reduce the risk of biased study outcomes. The level of blinding is maintained throughout the conduct of the trial, and only when the data are cleaned to an acceptable level of quality will appropriate personnel be unblinded or when required in case of a serious adverse event. In an observer-blind study, the subject and the site and sponsor personnel involved in the clinical evaluation of the subjects are blinded while other study personnel may be aware of the treatment assignment.
Eligible:	Qualified for enrollment into the study based upon strict adherence to inclusion/exclusion criteria.
End of Study (EoS): (Synonym of End of Trial)	For studies with collection of Human Biologicals Samples or imaging data, EoS is defined as the date of the last testing/reading released of the Human Biological Samples or imaging data, related to primary and secondary endpoints. EoS must be achieved no later than 12 months after last subject last visit.
Epoch:	An epoch is a set of consecutive time points or a single time point from a single protocol. Epochs are defined to support a main purpose which is either to draw conclusions on participation or to draw a complete conclusion to define or refine the targeted label of the product. Supporting means that data collected at the time points included in an epoch must be sufficient to fulfill the purpose of the epoch. Typical examples of epochs are screening, primary vaccinations, boosters, yearly immunogenicity follow-ups, and surveillance periods for efficacy or safety.
Evaluable:	Meeting all eligibility criteria, complying with the procedures defined in the protocol, and, therefore, included in the per-protocol analysis.
Immunological correlate of protection:	The defined immune response above which there is a high likelihood of protection in the absence of any host factors that might increase susceptibility to the infectious agent.

Investigational vaccine/product: (Synonym of Investigational Medicinal Product)	A pharmaceutical form of an active ingredient being tested in a clinical trial, including a product with a marketing authorization when used in a way different from the approved form, or when used for an unapproved indication, or when used to gain further information about an approved use.
Mean Geometric Increase:	Geometric mean of the within-subject ratios of the post-vaccination reciprocal titer to the pre-vaccination reciprocal titer.
Medically attended event:	An event for which the subject received medical attention defined as hospitalization, an emergency room visit, or a visit to or from medical personnel (e.g., medical doctor) for any reason.
Menarche:	Menarche is the onset of menses for the first time in a young female and is preceded by several changes associated with puberty including breast development and pubic hair growth. Menarche usually occurs within 1-2 years of breast development, thelarche. However, a young female can become pregnant before her first menses. Thus, a conservative definition of non-childbearing potential in a pre-menarcheal female is a young female who has not yet entered puberty as evidenced by lack of breast development (palpable glandular breast tissue).
Menopause:	Menopause is the age associated with complete cessation of menstrual cycles, menses, and implies the loss of reproductive potential by ovarian failure. A practical definition accepts menopause after 1 year without menses with an appropriate clinical profile at the appropriate age e.g., > 45 years.
Potential Immune-Mediated Diseases:	Potential immune-mediated diseases are a subset of AEs that include autoimmune diseases and other inflammatory and/or neurologic disorders of interest which may or may not have an autoimmune etiology.
Primary completion date:	The date that the final subject was examined or received an intervention for the purpose of final collection of data for all primary outcomes, whether the clinical trial was concluded according to the pre-specified protocol or was terminated.
Randomization:	Process of random attribution of treatment to subjects in order to reduce bias of selection.

Self-contained study:	Study with objectives not linked to the data of another study.
Seroconversion rate:	The percentage of vaccinees with either a pre-vaccination hemagglutination inhibition (HI) titer < 1:10 and a post-vaccination HI titer ≥ 1:40 or a pre-vaccination HI titer ≥ 1:10 and at least 4-fold increase in post-vaccination HI titer.
Seropositivity rate:	The percentage of vaccinees with an antibody titer of at least the cut-off in assays where the cut-off is defined.
Seroprotection rate:	The percentage of vaccinees with serum HI titer ≥ 1:40; usually accepted as indicating protection in at least 50% of the vaccinees.
Solicited adverse event:	AEs to be recorded as endpoints in the clinical study. The presence/occurrence/intensity of these events is actively solicited from the subject or an observer during a specified post-vaccination follow-up period.
Study vaccine/product	Any investigational vaccine/product being tested and/or any authorized use of a vaccine/product/placebo as a reference or administered concomitantly, in a clinical trial that evaluates the use of an investigational vaccine/product.
Sub-cohort:	A group of subjects for whom specific study procedures are planned as compared to other subjects or a group of subjects who share a common characteristic (e.g., ages, vaccination schedule, etc.) at the time of enrollment.
Subject:	Term used throughout the protocol to denote an individual who has been contacted in order to participate or participates in the clinical study, either as a recipient of the vaccine(s)/product(s) or as a control.
Treatment:	Term used throughout the clinical study to denote a set of investigational product(s) or marketed product(s) or placebo intended to be administered to a subject.
Treatment number:	A number identifying a treatment to a subject, according to the treatment allocation.
Unsolicited adverse event:	Any AE reported in addition to those solicited during the clinical study. Also, any “solicited” AE with onset outside the specified period of follow-up for solicited AEs will be reported as an unsolicited AE.

KEY ROLES AND CONTACT INFORMATION

List names of individuals or organizations serving in key roles (or contributed to the development of the protocol). Including contact information, such as name of organization and address.

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PROTOCOL SUMMARY

Title	A Phase 1, randomized, controlled, observer-blind study to assess the reactogenicity, safety, and immunogenicity of a live attenuated universal influenza vaccine (cH8/1N1 LAIV) administered as a single priming dose followed three months later by a single booster dose of an inactivated universal influenza vaccine (cH5/1N1 IIV) (adjuvanted with $AS03_A$ or unadjuvanted) in 18 through 39 year-old healthy subjects, contrasted with a two dose schedule of an inactivated universal influenza vaccine (cH8/1N1 IIV + $AS03_A$ followed three months later by cH5/1N1 IIV + $AS03_A$)
Short Title	Reactogenicity, safety and immunogenicity study of an investigational live-attenuated universal influenza vaccine (prime) followed by an investigational inactivated universal influenza vaccine (boost)
Protocol Number	CVIA 057
Trial Phase	Phase 1
Investigational Products	• Investigational chimeric H8/1N1 live attenuated influenza vaccine (cH8/1N1 LAIV) • GlaxoSmithKline (GSK) Biologicals' investigational chimeric H8/1N1 inactivated influenza vaccine (cH8/1N1 IIV) and investigational chimeric H5/1N1 inactivated influenza vaccine (cH5/1N1 IIV) • $AS03_A$-like adjuvant ($AS03_A$-like will be obtained by dilution of the AS03 with PBS) • Normal saline • Phosphate buffered saline
Study Hypotheses	
Primary	• cH8/1N1 LAIV administered by intranasal route is acceptably safe and well-tolerated in healthy adults and cH5/1N1 IIV +/- $AS03_A$ administered by intramuscular route in those previously primed with cH8/1N1 LAIV or cH8/1N1 IIV + $AS03_A$ is acceptably safe and well-tolerated in healthy adults.
Secondary	• Prime-boost with cH8/1N1 LAIV followed by cH5/1N1 IIV +/- $AS03_A$ results in immune responses against conserved antigenic sites among influenza A Group 1 viruses that are acceptable.
Study Objectives	
Primary	To assess the reactogenicity and safety through 28 days after each priming dose of cH8/1N1 LAIV (or placebo) and the booster dose of cH5/1N1 IIV +/- $AS03_A$ (or placebo) and through 28 days after each dose of IIV (cH8/1N1 IIV + $AS03_A$ and cH5/1N1 IIV + $AS03_A$) (or placebo) in

	terms of rates of solicited local and general adverse events (AEs) through 7 days post-vaccination, unsolicited AEs through 28 days post-vaccination, hematological and biochemical laboratory abnormalities up to Visit 13, and medically attended event (MAEs), laboratory-confirmed influenza-like illness (LC-ILI), potential immune-mediated disease (pIMDs), and serious adverse events (SAEs) through Visit 13.
Secondary	To assess the safety of each treatment group during the entire study period in terms of rates of primary endpoints and additionally hematological and biochemical laboratory abnormalities up to Visit 15, and MAEs, LC-ILIs, pIMDs, and SAEs through Visit 16. **<u>Viral shedding – post-Dose 1</u>** To **describe the shedding of vaccine virus** through 5 days after administration of cH8/1N1 LAIV (Groups 1, 2, and 3 only) in terms of the proportions of subjects with influenza type A vaccine virus ribonucleic acid (RNA) detected by reverse transcription polymerase chain reaction (RT-PCR) in nasal and oropharyngeal (OP) swabs and the proportion of subjects with vaccine virus isolated in cell culture each day post-vaccination. **<u>Immunogenicity - descriptive, post-Dose 2</u>** To **describe the anti-H1 hemagglutinin (HA)-stalk humoral immune responses** (anti-H1 HA-stalk serum immunoglobulin G [IgG], anti-H1 HA-stalk serum neutralizing antibodies, anti-H1 HA-stalk serum immunoglobulin A [IgA], and antibody-dependent cell-mediated cytotoxicity [ADCC] activity) 28 days after the booster dose of cH5/1N1 IIV +/- $AS03_A$ (or placebo) ***(Groups 1, 2, and 3)*** and 28 days after the second dose of IIV (cH5/1N1 IIV + $AS03_A$) (or placebo) ***(Groups 4 and 5)*** in terms of seropositivity rates, geometric mean titers (GMTs), percentages of subjects with a 4-fold or greater increase in titer from Day 1, percentages of subjects with a 10-fold or greater increase in titer from Day 1, and mean geometric increases (MGIs) from Day 1. To **describe the anti-H1 HA-stalk mucosal immune responses** (anti-H1 HA-stalk salivary total IgA, anti-H1 HA-stalk secretory IgA in saliva, and anti-H1 HA-stalk salivary IgG) 28 days after the booster dose of cH5/1N1 IIV +/- $AS03_A$ (or placebo) ***(Groups 1, 2, and 3)*** and 28 days after the second dose of IIV (cH5/1N1 IIV + $AS03_A$) (or placebo) ***(Groups 4 and 5)*** in terms of seropositivity rates, GMTs, percentages of subjects with a 4-fold or greater increase in titer from Day 1, percentages of subjects with a 10-fold or greater increase in titer from Day 1, and MGIs from Day 1.

	Immunogenicity - descriptive, post-Dose 2 - breadth To **describe the breadth of the anti-H1 HA-stalk humoral immune responses** (anti-H2 HA-full length serum IgG, anti-H9 HA-full length serum IgG, anti-H18 HA-full length serum IgG, anti-H5N8 serum neutralizing antibodies, anti-avian swine H1N1 serum neutralizing antibodies, and anti-H1pdm09-like serum neutralizing antibodies) to Group 1 influenza A viruses 28 days after the booster dose of cH5/1N1 IIV +/- $AS03_A$ (or placebo) ***(Groups 1, 2, and 3)*** and 28 days after the second dose of IIV (cH5/1N1 IIV + $AS03_A$) (or placebo) ***(Groups 4 and 5)*** in terms of seropositivity rates, GMTs, percentages of subjects with a 4-fold or greater increase in titer from Day 1, percentages of subjects with a 10-fold or greater increase in titer from Day 1, and MGIs from Day 1. **Immunogenicity - descriptive, post-Dose 2 - persistence** To **describe the persistence of the anti-H1 HA-stalk humoral immune responses** (anti-H1 HA-stalk serum IgG, anti-H1 HA-stalk serum neutralizing antibodies, anti-H1 HA-stalk serum IgA, and ADCC activity) up to 12 months after the booster dose of cH5/1N1 IIV +/- $AS03_A$ (or placebo) ***(Groups 1, 2, and 3)*** and up to 12 months after the second dose of IIV (cH5/1N1 IIV + $AS03_A$) (or placebo) ***(Groups 4 and 5)*** in terms of seropositivity rates, GMTs, percentages of subjects with a 4-fold or greater increase in titer from Day 1, percentages of subjects with a 10-fold or greater increase in titer from Day 1, and MGIs from Day 1. To **describe the persistence of the anti-H1 HA-stalk mucosal immune responses** (anti-H1 HA-stalk salivary total IgA, anti-H1 HA-stalk secretory IgA in saliva and anti-H1 HA-stalk salivary IgG) up to 12 months after the booster dose of cH5/1N1 IIV +/- $AS03_A$ (or placebo) ***(Groups 1, 2, and 3)*** and up to 12 months after the second dose of IIV (cH5/1N1 IIV + $AS03_A$) (or placebo) ***(Groups 4 and 5)*** in terms of seropositivity rates, GMTs, percentages of subjects with a 4-fold or greater increase in titer from Day 1, percentages of subjects with a 10-fold or greater increase in titer from Day 1, and MGIs from Day 1. **Immunogenicity - descriptive, by vaccine regimen, post-Dose 1** To **describe the anti-H1 HA-stalk humoral immune responses** (anti-H1 HA-stalk serum IgG, anti-H1 HA-stalk serum neutralizing antibodies, anti-H1 HA-stalk serum IgA, and ADCC activity) 28 days after the prime dose of cH8/1N1 LAIV (or placebo) ***(Groups 1, 2, and 3)*** and 28 days after the first dose of IIV (cH8/1N1 IIV + $AS03_A$) (or placebo) ***(Groups 4 and 5)*** in terms of seropositivity rates, GMTs, percentages of subjects with a 4-fold or greater increase in titer from Day 1, percentages of subjects with a 10 fold or greater increase in titer from Day 1, and MGIs from Day 1.

	To **describe the anti-H1 HA-stalk mucosal immune responses** (anti-H1 HA-stalk salivary total IgA, anti-H1 HA-stalk secretory IgA in saliva and anti-H1 HA-stalk salivary IgG) 28 days after the prime dose of cH8/1N1 LAIV (or placebo) ***(Groups 1, 2, and 3)*** and 28 days after the first dose of IIV (cH8/1N1 IIV + $AS03_A$) (or placebo) ***(Groups 4 and 5)*** in terms of seropositivity rates, GMTs, percentages of subjects with a 4-fold or greater increase in titer from Day 1, percentages of subjects with a 10 fold or greater increase in titer from Day 1, and MGIs from Day 1. **<u>Immunogenicity - comparative, post-dose two</u>** To **compare the anti-H1 HA-stalk humoral immune responses** (anti-H1 HA-stalk serum IgG, anti-H1 HA-stalk serum neutralizing antibodies, anti-H1 HA-stalk serum IgA, and ADCC activity) 28 days after the booster dose with cH5/1N1 IIV +/- $AS03_A$ after previous receipt of cH8/1N1 LAIV ***(Groups 1 or 2)*** to that after priming with two doses of IIV (cH8/1N1 IIV + $AS03_A$ and cH5/1N1 IIV + $AS03_A$) ***(Group 4)*** in terms of the adjusted GMT ratio and the seroresponse (≥4-fold) rate difference. To **compare the anti-H1 stalk mucosal immune responses** (anti-H1 HA-stalk salivary IgA, anti-H1 HA-stalk secretory IgA in saliva, and anti-H1 HA-stalk salivary IgG) 28 days after the booster dose with cH5/1N1 IIV +/- $AS03_A$ after previous receipt of cH8/1N1 LAIV ***(Groups 1 or 2)*** to that after priming with two doses of IIV (cH8/1N1 IIV + $AS03_A$ and cH5/1N1 IIV + $AS03_A$) ***(Group 4)*** in terms of the adjusted GMT ratio and the seroresponse (≥4-fold) rate difference. To **evaluate the adjuvant effect of $AS03_A$ on the anti-H1 stalk humoral immune response** (anti-H1 HA-stalk serum IgG, anti-H1 HA-stalk serum neutralizing antibodies, anti-H1 HA-stalk serum IgA, and ADCC activity) after one booster dose of cH5/1N1 IIV + $AS03_A$ after priming with cH8/1N1 LAIV ***(Group 1)*** compared to boosting with cH5/1N1 IIV, unadjuvanted ***(Group 2)*** in terms of the adjusted GMT ratio and seroresponse (≥4-fold) rate difference.
Study Endpoints **Primary** **Secondary**	Endpoints are included in objective statements, above. For more details, see Protocol Section 11.
Study Design	This is a prospective, multi-center, randomized, controlled, observer-blind, Phase 1 trial in healthy male and female adults 18 through 39 years of age. Up to 65 eligible subjects will participate and will be randomized 4:3:1:3:2 to one of five groups to receive a first dose of study cH8/1N1 LAIV (or placebo) or study cH8/1N1 IIV + $AS03_A$ adjuvant (or placebo) followed three months later by study cH5/1N1 IIV +/- $AS03_A$ adjuvant (or placebo). See below table.

Study groups	Number of subjects	Dose 1		Dose 2	
		Treatment	Administration Route	Treatment	Administration Route
Group 1	20	cH8/1N1 LAIV	intranasal	cH5/1N1 IIV + AS03$_A$	intramuscular
Group 2	15	cH8/1N1 LAIV	intranasal	cH5/1N1 IIV	intramuscular
Group 3	5	Normal saline	intranasal	PBS	intramuscular
Group 4	15	cH8/1N1 IIV + AS03$_A$	intramuscular	cH5/1N1 IIV + AS03$_A$	intramuscular
Group 5	10	PBS	intramuscular	PBS	intramuscular

Two sites, Duke Early Phase Clinical Research at Duke University and the Gamble Program for Clinical Studies at Cincinnati Children's Hospital Medical Center, will participate and enroll 39 and 26 subjects, respectively. Prospective subjects will be carefully screened to determine eligibility. Subjects will be screened for eligibility through medical history review, physical examination, testing for serologic evidence of human immunodeficiency virus (HIV) infection, with proper pre- and post-test counseling, urine drug test, and routine biochemical and hematological blood tests. Women of childbearing potential will undergo pregnancy tests using urine samples or serum samples, as required. Screening may occur up to 8 weeks prior to receipt of Dose 1 of study vaccines or placebo. Subjects will be considered enrolled only after a subject has satisfied all eligibility criteria.

Eligible enrolled subjects will be randomized to any of the treatment arms (LAIV-IIV, Groups 1, 2, and 3; or IIV-IIV, Groups 4 and 5) under one allocation sequence, stratified by site, to allow comparability between study groups, such as LAIV-IIV vs IIV-IIV regimens (Groups 1 vs 4). While subjects will be blinded to their exact treatment group and whether they received active study vaccine versus placebo, subjects will certainly know if they received LAIV (or placebo) vs IIV (or placebo) at Dose 1, given the different treatment group requirements, presentations, and routes of administration of these products.

Enrolled subjects will be randomized to study groups as soon as eligibility is confirmed and at least 2 days (ideally at least 7 days) in advance of receipt of Dose 1 of study vaccines. This is because subjects randomized to LAIV-IIV treatment arms (Groups 1, 2, and 3) will receive Dose 1 of study cH8/1 LAIV (or placebo) in an inpatient clinical isolation unit and may need some time to plan for a specific admission date, to be scheduled by each site. Subjects randomized to IIV-IIV treatment arms (Groups 4 and 5) will receive Dose 1 in an outpatient research study clinic.

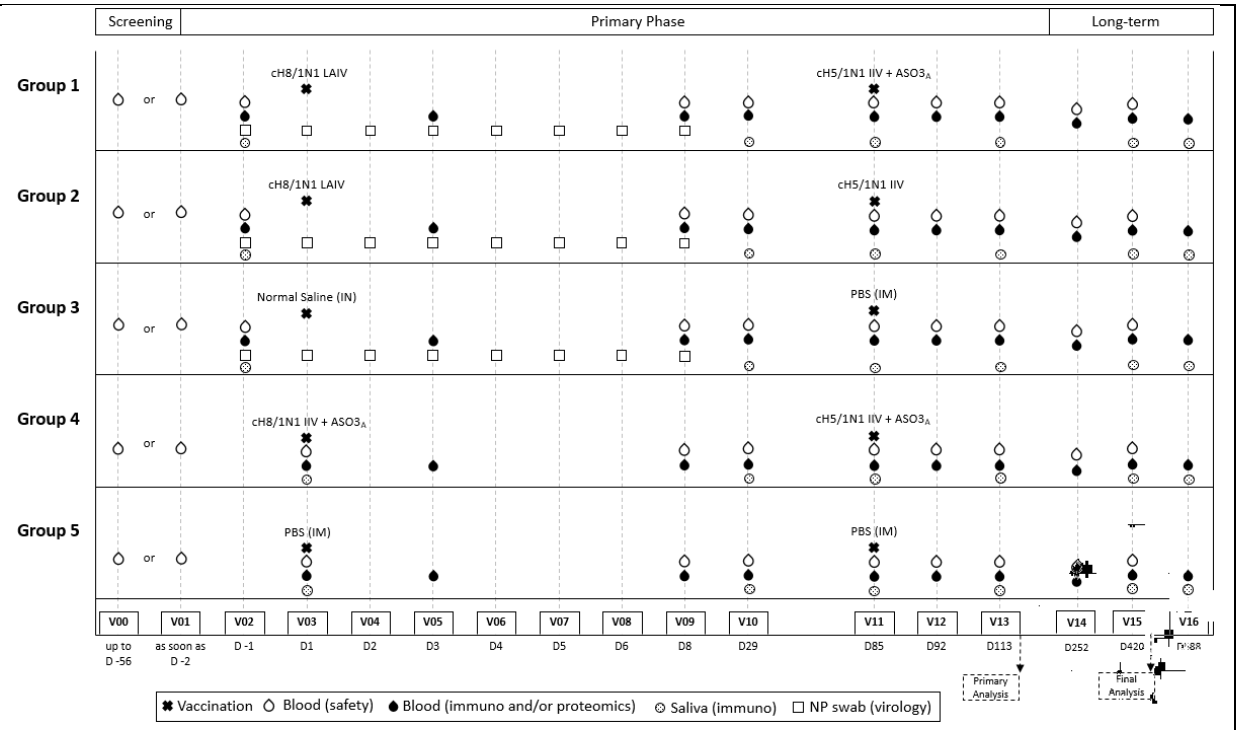
<u>LAIV-IIV treatment arms (Groups 1, 2, and 3):</u> Subjects in the LAIV-IIV treatment arms will be admitted into the inpatient clinical isolation unit the day before (at least 18 hours prior to) receipt of Dose 1 in order to observe the subject for acute onset of respiratory illness of some other etiology which the subject might be developing. At the first site to dose subjects with study LAIV (or placebo), only six subjects will be admitted initially. On Day 1 for these subjects, vaccination with Dose 1 (cH8/1 LAIV or

	placebo) will be staged such that each subject is dosed and observed for at least 60 minutes prior to the next subject being dosed. Consecutive dosing will proceed only if no adverse events meeting halting rules or other adverse events of concern to the site principal investigator (PI) are observed. Also on this day at the first site, the remaining subjects in LAIV-IIV treatment arms at this site will be admitted to the inpatient clinical isolation unit, again for observation of acute onset respiratory illness. After at least 18 hours since the sixth subject in the study to have received study LAIV (or placebo) have passed with no adverse events meeting halting rules or other adverse events of concern to the site PI, the remaining subjects in the LAIV-IIV treatment arms will be dosed. At the second site to dose subjects in the LAIV-IIV treatment arms, all subjects at this site will also be admitted into the inpatient clinical isolation unit the day before (at least 18 hours prior to) receipt of Dose 1 in order to observe subjects for acute onset of respiratory illness. Again, if no adverse events meeting halting rules or other adverse events of concern to the site PI were observed in the first six subjects to receive study LAIV (or placebo) in the trial at the first site, dosing of subjects at the second site will proceed. PATH or the Emmes Study Coordinating Center will coordinate communication between the two sites. The second site will not be allowed to dose any subjects with study LAIV (or placebo) until PATH has provided written approval to the site. IIV-IIV treatment arms (Groups 4 and 5): Vaccination with Dose 1 of cH8/1N1 IIV + $AS03_A$ (or placebo) in the IIV-IIV groups at each site will occur without limitation on the number of vaccinees per day or time between consecutive subjects, as initial cH8/1N1 IIV safety data (through 7 days post-vaccination) will have been collected and reviewed by an Independent Data Monitoring Committee (IDMC) for GSK's separate Phase 1 study of this IIV product prior to initiation of the current trial. All treatment arms (Groups 1, 2, 3, 4, and 5): Vaccination with Dose 2 (cH5/1 IIV +/- $AS03_A$ or placebo) at each site will occur in a staggered manner, with one half of the subjects at each site vaccinated initially and the remaining subjects vaccinated only after at least 5 days has passed since the initial half of subjects have all received their Dose 2. In addition, initial cH5/1 IIV safety data (through 7 days post-vaccination) will have been collected and reviewed by an IDMC for GSK's separate Phase 1 study of this IIV product prior to initiation of the current trial. Planned safety assessments will provide the data for active monitoring of vaccine safety during conduct of the trial and for the primary reactogenicity and safety endpoints. Immediate reactogenicity and vital signs will be assessed by a medically qualified study staff at 60 (+ 10) minutes following vaccination in all subjects. Immediate reactogenicity will include solicited local and general adverse events and unsolicited AEs, including AEs leading to withdrawal from the study and SAEs. During the 7-day follow-up period after each vaccination (day of vaccination and subsequent 6 days) subjects will complete diary cards

	for solicited local and general adverse events and unsolicited AEs, including scoring for severity, and for use of concomitant medications. Reactogenicity scoring will be reviewed by a research clinician prior to being entered into the electronic case report form (eCRF). Solicited local reactions will include runny nose/nasal congestion at the site of administration of study LAIV (or placebo) and pain/tenderness, erythema/redness, and induration/swelling at the site of injection of study IIV (or placebo). Solicited general reactions will be common for all study subjects and include arthralgia, cough, fatigue, fever (based on oral temperature), gastrointestinal symptoms (nausea, vomiting, diarrhea and/or abdominal pain), headache, myalgia, shivering, sore throat, and wheeze. For additional assessment of safety, both targeted physical examination and collection of blood specimens for analysis of biochemical (alanine aminotransferase, aspartate aminotransferase, creatinine, and urea nitrogen) and hematologic (leukocytes, neutrophils, lymphocytes, basophils, monocytes, eosinophils, hemoglobin, platelets, and erythrocytes) clinical laboratory parameters will also be done at each study visit. (Results for clinical laboratory tests conducted just prior to receipt of Dose 1 will serve to define baseline status for each subject prior to receipt of study vaccine or placebo but will not be used for screening purposes.) Clinical laboratory results assessed as AEs will be scored for severity using Food and Drug Administration Toxicity Tables (see Appendix 2). Subjects will be asked to further complete diary cards for unsolicited AEs and concomitant medications through the end of the study. At the study visits 7 days after each dose, medically qualified study staff will review diary cards and document AEs in the eCRF, along with severity, relatedness and duration. For each solicited and unsolicited AE the subject experiences, the subject will also be asked if he/she received medical attention defined as hospitalization, or an otherwise unscheduled visit to or from medical personnel for any reason, including emergency room visits. This information will also be recorded in the eCRF. Adverse events of specific interest for safety monitoring include pIMDs, a subset of AEs that include autoimmune diseases and other inflammatory and/or neurologic disorders of interest which may or may not have an autoimmune etiology. Both pIMDs (serious or non-serious) and SAEs will be documented in the eCRF and an Expedited Adverse Events Report will be completed. Additionally, occurrence of MAEs and ILIs (with laboratory confirmation) will be recorded and evaluated. All AEs will be coded using the Medical Dictionary for Regulatory Activities (version 15.1 or later). Any SAE ongoing at the time of the subject's end of study (EoS) visit will be attempted to be followed up until resolved, or assessed to be resolved with sequelae by the site PI, or his designee, until last subject last visit (LSLV) in the trial. If any SAE remains unresolved at the time of LSLV, it will be classified as ongoing. To

	facilitate rigorous safety monitoring, data captured at each visit will be entered into the electronic data capture (EDC) system within 72 hours from the date of the clinic visit. For ancillary data that may be obtained after a visit (e.g., laboratory test results, inpatient records) the goal of data entry will be to maintain data integrity and timely safety assessments. PATH will convene a Protocol Safety Review Team to regularly review blinded safety data and trial conduct. In addition, an IDMC will monitor vaccine safety. Prior to receipt of Dose 2 of study vaccines, the IDMC will perform a first interim safety review. The IDMC will review all AEs through Visit 10, including clinical laboratory evaluations pre- and post-vaccination, for all subjects. This will include a formal, unblinded review of all safety data accrued. The IDMC will also review all shedding data for LAIV-IIV treatment arms (see below). The IDMC will then advise if the participants may receive Dose 2 of study vaccines. The IDMC will also review all AEs through Visit 13 and through Visit 15 at separate safety reviews. The need for additional meetings to review unblinded safety data will be determined by the IDMC on an ad hoc basis, or as specifically requested by PATH. <u>Special safety monitoring during inpatient stay post-Dose 1 of cH8/1N1 LAIV or placebo:</u> To reduce the risk that subjects bring wild-type influenza into the inpatient clinical isolation unit or that subjects receiving study cH8/1N1 LAIV return to the community while shedding potentially viable vaccine virus, all subjects in Groups 1, 2, and 3 will be monitored for acute respiratory infection and for virologic evidence of influenza infection. Subjects will be admitted to the inpatient clinical isolation unit at least 18 hours prior to receipt of Dose 1 of study LAIV (or placebo) and monitored to ensure no subject is developing an acute respiratory illness. On day of entry to the unit, all subjects will have nasal and oropharyngeal (OP) swab specimens collected and tested for the presence of influenza type A viral RNA by RT-PCR. All subjects must be baseline influenza A negative prior to dosing of any subject. Subjects will also have nasal and OP swab specimens collected daily thereafter until day of discharge from the inpatient clinical isolation unit. Post-vaccination specimens will be used to determine discharge of subjects from the unit. All subjects will remain in the isolation unit for at least 5 days after receipt of study cH8/1N1 LAIV or placebo. Any subject exhibiting influenza A virus shedding, as determined by real-time RT-PCR positivity on a nasal and OP swab specimen, in the 2 days prior (D4 or D5) or on the day of planned discharge (D6) will be kept in the inpatient clinical isolation unit until 3 daily consecutive swab specimens taken over a 48 hours period all test influenza A negative by RT-PCR. RT-PCR testing of swab specimens collected on D6 (or later) will be completed on day of specimen collection. Any subject still exhibiting evidence of influenza virus shedding in a nasal and OP swab on Days 6 or later will be offered anti-neuraminidase treatment (e.g., oral oseltamivir, 75 milligrams [mg] twice a day for 5 days). To study virus infectivity, swab specimens that test influenza A positive by RT-PCR will be further tested for viability of

	virus in Madin Darby canine kidney cell culture and stained with monoclonal antibody specific to the cH8/1 LAIV virus to confirm detected virus is of vaccine origin. Nasal and OP swab specimens may also be tested by RT-PCR using primer-probe sets designed to detect specifically genes of A/Leningrad/134/17/1957, the master donor virus used to create study LAIV. For the evaluation of humoral and mucosal immune responses, sera and saliva will be collected pre-vaccination and on Visits 10, 11, 13, 14, and 15. Sera and saliva will also be collected at Visit 16 with testing for humoral and mucosal immune responses dependent upon the outcome analysis of primary and secondary objectives. Multiple assays (enzyme-linked immunosorbent assay, micro-neutralization, ADCC, antibody-dependent cellular phagocytosis, and hemagglutination inhibition) will be used to probe the presence, breadth and persistence of immune responses to study vaccines, including functional responses. Sera from Day 1 and Visits 13 and 15 will also be pooled for passive transfer to mice in an influenza challenge model. For evaluation of cell-mediated immune responses (CMI) whole blood will be collected. To assess vaccine induction of cytotoxic T lymphocytes and other cytokine indicators, as well as circulating T-follicular helper cells, whole blood for isolation of peripheral blood mononuclear cell will be collected pre-vaccination and on Visits 9, 11, 12, and 15. To assess vaccine induction of plasmablasts, whole blood will be collected on Visits 9 and 12. To assess vaccine induction of B memory cells, whole blood will be collected pre-vaccination and on Visits 10, 11, 13, and 15. Because of the need to process and ship whole blood for CMI in a timely manner, strong preference will be made to enroll all subjects in Groups 4 and 5 at each site on one day. Finally, for evaluation of blood biomarkers associated with vaccination and immune responses whole blood will be collected pre-vaccination and on Visits 5, 9, 10, 11, 12, 13, 14, and 15. A summary of study visits is shown in the below figure. A more detailed listing of study procedures by Visit is contained in the tables in Appendix 1.



Study population	65 healthy male and female adults 18 through 39 years of age in metropolitan areas of Cincinnati, Ohio and Durham, North Carolina
Number of participating sites	Two sites [redacted]
Study Duration	Each participant will participate from 21 to 23 months. Two months (8 weeks) are planned for screening to accrue necessary sample size.

1. BACKGROUND AND RATIONALE

1.1 Background

Influenza is a contagious disease caused by influenza viruses that infect the upper and lower respiratory tract of humans. The overall public health impact (e.g., infections, hospitalizations, and deaths) of an influenza season varies from year to year. Worldwide seasonal influenza virus epidemics cause between 3-5 million cases of severe illness and up to 250,000-500,000 deaths per year [1].

Influenza virus of humans is mainly caused by two different influenza A subtypes (H1N1 and H3N2) and two lineages of influenza B virus (Yamagata-like and Victoria-like) [2]. The main reservoir of influenza A virus is wild aquatic birds where all the influenza A virus subtypes exist in different combinations. There are 16 different hemagglutinins (HAs) and 9 different neuraminidases (NAs), with two more HA-like and two more NA-like proteins found in bats [3-6].

The HA and NA are the main glycoproteins found on the viral membrane. Their primary roles are to facilitate viral entry into and egress out of the host cell. Since these glycoproteins are the main components located on the viral membrane, the immune system recognizes them and generates a protective antibody response. This is primarily true for HA and to a lesser extent for NA. However, these glycoproteins, specifically the HA globular head domain, are very plastic [7,8] and change from season to season causing a phenomenon known as antigenic drift. As a result, currently licensed influenza virus vaccines need to be updated annually to incorporate changes that accumulate in circulating strains [8].

Influenza virus infection can be prevented by vaccination. However, seasonal influenza virus vaccines must be reformulated every year because of antigenic changes that affect vaccine efficacy [8]. The traditional influenza inactivated vaccine (IIV) consists of reassortants of those strains with laboratory donor strains that result in vaccine strains with internal genes/proteins from the laboratory donor strains and the surface antigens of the circulating viruses. Those reassortant viruses are usually grown in embryonated eggs, and are then inactivated and split with detergents and administered through intramuscular injection. Live attenuated influenza vaccines (LAIV) have been used in Russia since the 1970s. Using a cold adapted/temperature sensitive/attenuated influenza strain as donor (A/Leningrad/134/17/1957), reassortant viruses containing the surface antigens of the circulating strains are generated. This vaccine is then administered intranasally. The vaccine viruses are able to replicate in the upper respiratory tract (low temperature) inducing an immune response but are unable to replicate in the lower respiratory (high temperature) tract. Since 2003, a similar strategy using a different donor strain was approved in the US and marketed under the name FluMist.

In certain instances, adjuvants are added to vaccine formulations to create stronger immune responses. Pandemrix™(H1N1), an antigen-sparing pandemic influenza vaccine containing only 3.75 µg of HA (as compared with 15 µg in the conventional vaccines) but adjuvanted with $AS03_A$ (squalene, DL-α-tocopherol and polysorbate 80), was developed by GlaxoSmithKline (GSK) and licensed for use in the 2009 H1N1 influenza pandemic. GSK estimates that more than 90 million

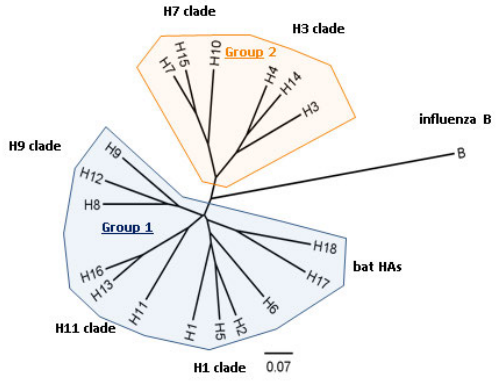
persons have received Pandemrix™ (H1N1). Other pandemic influenza vaccines have also been tested in clinical trials with adjuvants, after initial trials with unadjuvanted vaccines failed to induce strong immune responses. Currently, an H5N1 monovalent pandemic vaccine adjuvanted with AS03 is approved in the US [9], Europe, and multiple countries in Asia and Latin America.

1.1.1 Universal influenza virus vaccine based on chimeric HAs

As mentioned above, influenza virus vaccines must be reformulated every year because of antigenic changes that affect vaccine efficacy [8]. These antigenic changes occur predominantly in the globular head region of the HA molecule, in so-called “antigenic sites” that are the target of neutralizing antibodies. The current correlate of protection for influenza virus vaccines is the hemagglutination inhibition (HI) titer, as measured in an HI assay, which measures immune responses in terms of antibodies directed to the HA head [10,11]. Antibodies that target other, more conserved regions of HA, such as the stalk domain, are potentially protective but since they are immunosubdominant, are not efficiently induced by current influenza virus vaccines. Stalk reactive antibodies do not show activity in the HI assay. As a result, novel assays and new correlates of protection might need to be developed for HA stalk based vaccines. Anti-HA stalk antibodies have been isolated from humans and are broadly neutralizing [12-15]. Unfortunately, they are usually only present at low titers. Therapeutic monoclonal antibodies that target the conserved stalk domain of the influenza virus hemagglutinin and stalk-based universal influenza virus vaccine strategies are being developed as promising countermeasures for influenza virus infections. The epitopes recognized by these antibodies are conformational and involve HA1 as well as HA2 residues [16].

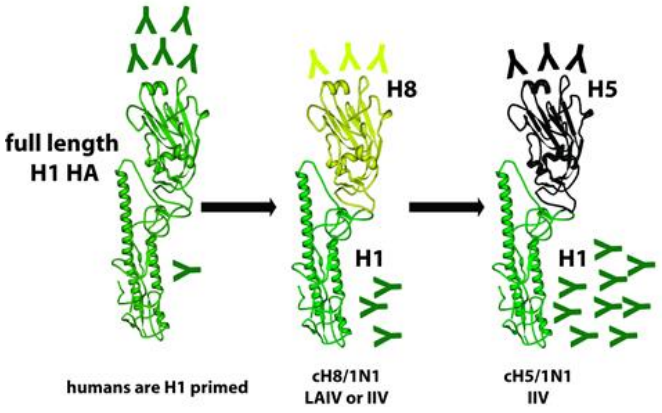
Due to the conservation of the stalk domain these antibodies are able to broadly neutralize a wide spectrum of influenza virus strains and subtypes. Influenza A HAs can be divided into two phylogenetic groups (Group 1 and Group 2) with the influenza B HA more distantly related (Figure 1) [3]. Within groups, the stalk domain of the HAs is relatively conserved while the head domains are more divergent and responsible for different antigenicities. For instance, the stalk of H1 HA is very similar to the stalk of H5 HA (both are group 1 HAs) and antibodies elicited against the H1 stalk will likely cross-react with the H5 stalk and stalks of other group 1 HAs. Similar cross-reactivity exists within group 2 HAs.

Figure 1 Phylogenetic diagram showing Group 1 and 2 HAs for influenza A and influenza B



Based on these observations, a novel approach to induce broadly protective antibody responses against influenza viruses, developed at the Icahn School of Medicine at Mount Sinai (ISMMS) and funded by the Bill & Melinda Gates Foundation in collaboration with GSK, utilizes chimeric HA (cHA) molecules that pair the globular head region of an exotic (for example, avian) HA with the HA stalk region of a currently circulating seasonal influenza virus [17,18]. These chimeric HAs are then used in vaccination regimens (Figure 2) in which the same stalk domain but different head domains are sequentially presented to the immune system.

Figure 2 Vaccination regimen to induce anti-stalk antibodies for group 1 in humans.



The purpose of this approach is to boost pre-existing cross-reactive memory responses (or de novo-induced in infants lacking such immunity) to the HA stalk without further boosting strain-specific responses against the head region of HA. This approach has been shown to provide effective protection in mice and ferrets [19-24] but has not yet been tested in humans.

1.1.2 Summary results of preclinical trials

A preclinical experiment was performed in ferrets using a similar approach. Ferrets were used as the animal model since they mimic influenza disease in humans, including clinical signs, pathogenesis and transmission. As the ferrets used in the study were naïve for influenza, animals were first immunized with an influenza B virus carrying a chimeric HA containing the H9 head and the H1 stalk (fluB-cH9/1). The ferrets then received regimens of heterologous-head chimeric HA LAIV followed by heterologous chimeric IIV or heterologous chimeric HA IIV followed by heterologous-head chimeric IIV. IIVs were given with or without adjuvant. As control, a group of ferrets was vaccinated with the standard of care (two human doses of trivalent vaccine). Another control group included unvaccinated animals. After vaccination, all ferrets were challenged with pandemic H1N1 virus. All vaccines were safe and well tolerated. All chimeric approaches provided good protection against challenge as determined by the absence of viral replication in the lung. Importantly, LAIV-IIV plus adjuvant induced a strong stalk antibody response as measured by enzyme-linked immunosorbent assay (ELISA) (Nachbagauer, *et al*. npj Vaccines 2, Article number: 26 (2017); doi: 10.1038/s41541-017-0026-4). The observations of the ferret challenge

experiment generated the hypothesis that is to be explored in this clinical trial. The hypothesis is that administration of a live-attenuated cHA vaccine via the intranasal route as the first dose in a prime boost immunization sequence, followed by an inactivated cHA vaccine boost via the intramuscular route, offers superior active immunization relative to 2 intramuscular doses of inactivated cHA vaccine.

A preclinical repeat-dose toxicology study has been completed in ferrets with the current study vaccines (please see IB for further details). In this study, ferrets were administered two doses of intranasal LAIV (or placebo) two weeks apart followed four weeks later by two doses of intramuscular IIV with or without $AS03_A$ adjuvant (or saline) two weeks apart. Twenty ferrets per group underwent necropsy two days after the second IIV dose and ten ferrets per group after a 28-day recovery period. In seven of twenty ferrets in the $AS03_A$ adjuvant group, following the second IIV dose, at the site of the second injection, despite an absence of any gross observations, there was an observation on microscopy of either moderate (in six) or severe (in one) necrosis; however, there was no evidence of any local necrosis on microscopy in any of the ferrets, including the ten in the $AS03_A$ adjuvant group, at the 28-day recovery time-point.

1.1.3 Summary results of related clinical trials

Indirect evidence that this approach could work has been obtained from clinical trials with H5N1 vaccines. Although these vaccines were originally designed to induce anti-head neutralizing antibodies, retrospective analysis of sera from vaccinees confirmed an increase in anti-stalk antibodies post-H5N1 vaccination [25-27]. The immune systems of these human subjects had never previously seen the exotic novel H5 HA component, but the H5 stalk is very similar to other stalks in Group 1, particularly the H1 stalk. As a result, anti-stalk antibody responses were boosted since the vaccines induced proliferation of pre-existing memory B cells, with specificities in the Group 1 HA stalk. While there is not data for *in vivo* protection of humans, it has been shown that these human antibodies are able to provide protection in passive transfer experiments in mice against viral challenge [25]. This evidence, plus evidence of stalk-specific neutralizing titers post-vaccination, confirms the functionality of the induced anti-stalk antibodies [25]. In the clinical trial design, the H1 stalk will be kept constant in all vaccines while the head domain will vary from prime to boost (H8 and H5 respectively). This approach is expected to boost anti-Group 1 stalk antibodies which are already present in adult humans (Figure 2). It is expected that these antibodies will confer protection against H1 viruses circulating in humans as well as other Group 1 viruses.

1.1.4 Rationale for the LAIV-IIV prime-boost

It has previously been shown in animal models and humans that priming with either live-attenuated influenza vaccines or DNA vaccines followed by boosting with inactivated influenza virus vaccines induces broader and possibly longer lasting immune responses [28-34]. Hence, for this initial clinical trial, the study team will test an LAIV as prime followed by an IIV booster vaccination with or without adjuvant. LAIV with an HA having the head domain from H8 and the stalk domain from H1 influenza (termed a cH8/1 chimera) has been produced for human clinical studies in an ongoing collaboration between PATH and ISMMS. This LAIV also incorporates the

N1 neuraminidase and uses the Leningrad cold-adapted backbone to provide attenuation. A booster immunization will be given with IIV comprising a different chimeric HA, with head domain from another novel influenza virus such as H5 and the same stalk domain as used previously. In this trial, IIV will be adjuvanted with $AS03_A$ or administered unadjuvanted. The proposed vaccines are the following:

- **cH8/1N1 LAIV** A/mallard/Sweden/24/2002-A/California/04/2009 (cH8/1N1)-A/Leningrad/134/17/1957)
- **cH8/1N1 IIV** A/mallard/Sweden/24/2002-A/California/04/2009 (cH8/1N1)-(PR8 Mt Sinai)
- **cH5/1N1 IIV** A/Vietnam/1203/04-A/California/04/2009 (cH5/1N1)-(PR8 Mt Sinai)

Antibody responses against the stalk domain will be measured in novel assays (ELISA and antibody-dependent cell-mediated cytotoxicity [ADCC]). Neutralizing antibody responses and neuraminidase inhibiting antibodies will also be measured. However, this study will not assess the ability of the vaccine to protect against live influenza infection and will not assess the functional role of NA in the protective response to the candidate vaccine. If this Phase 1 study demonstrates that one or more vaccine combinations is both safe and immunogenic, further Phase 2 work may be considered including a challenge study to determine protection against a live, influenza virus.

The inactivated vaccines to be used in this study are split inactivated cH8/1N1 and cH5/1N1 monovalent antigens produced by GSK Biologicals at its Dresden facility. The bulk manufacturing process for the candidate vaccines follow the Fluarix® process that was licensed until influenza season Northern Hemisphere 2016-17 (Submission Track Number: Biologics License number 125127). GSK will be initiating a Phase 1/2 trial of these chimeric IIVs prior to PATH initiating this trial. For this trial, PATH will be cross-referencing GSK's IND for its Phase 1/2 trial of these chimeric influenza vaccine candidates. The Investigator's Brochure (IB) for the chimeric IIVs will be provided by GSK to PATH for this trial. Information on the AS03 adjuvant to be used in this trial will also be contained in that IB.

1.1.5 Dose rationale

For this trial, one of the study vaccines, live-attenuated influenza vaccine (cH8/1N1 LAIV, manufactured by Meridian Life Science, Memphis, Tennessee) will be tested for the first time in humans. The two inactivated influenza vaccines (cH8/1N1 IIV and cH5/1N1 IIV, manufactured by GSK) with and without $AS03_A$ adjuvant, will be tested for the first time in humans in a trial initiated by GSK prior to initiation of our trial. Although the main focus of this trial is the LAIV-IIV approach, an IIV-IIV adjuvanted arm has been included as a bridging group to the trial conducted by GSK in parallel and as a comparator for the LAIV-IIV approach. For this study, the vaccine will use a dose of $10^{7.5}$ EID_{50} for the LAIV (a dose similar to the doses used in Russia with the Leningrad backbone) and 15 µg of split HA for the IIV (conventional dose) with or without $AS03_A$ adjuvant.

1.1.6 Rationale for use of adjuvants

The use of adjuvants enhanced the induction of stalk-reactive antibodies in animal models [23,35]. For this study, the trial will include use of $AS03_A$, a proprietary GSK adjuvant. $AS03_A$ has been shown to act through two mechanisms for seasonal influenza vaccination in humans: 1) the adjuvant stimulated increased activation of naïve B cells, thus reducing immune interference with previous vaccine responses; 2) the adjuvant was able to increase the adaptability of the recalled memory cells to give improved specificity to the new vaccine antigen [36]. Therefore, the use of $AS03_A$ in the cHA vaccination experiments is expected to assist in the induction of high titers of broadly protective antibodies.

1.2 Rationale for the study design

Sixty-five subjects 18-39 years of age will be enrolled and randomized to five different treatment groups. All subjects will receive two doses of study vaccines (or placebo) three months apart. Subjects receiving active study vaccine will receive vaccines that are based on an influenza virus expressing a chimeric HA with the same stalk domain (H1 stalk) at each dose but which have a different exotic group A_1 head domain. Three groups totaling 40 subjects will be randomized 4:3:1 to receive a priming LAIV dose (Groups 1, 2) or placebo, (Group 3) on study Day 1 followed by a booster IIV dose with $AS03_A$ (Group 1) or without (Group 2) or placebo (Group 3) at Visit 11 (about 3 months later). Two groups totaling 25 subjects will be randomized 3:2 to receive a first priming IIV dose with $AS03_A$ (Group 4) or placebo (Group 5) on study Day 1 followed by a second priming IIV dose with $AS03_A$ (Group 4) or placebo (Group 5) at Visit 11 (about 3 months later). All eligible enrolled subjects will be randomized to any of the treatment arms (LAIV-IIV, Groups 1, 2, and 3; or IIV-IIV, Groups 4 and 5) under one allocation sequence, stratified by site, to preserve comparability between study groups, particularly LAIV-IIV vs IIV-IIV regimens (e.g., Groups 1 vs 4).

The main purpose of this study is to assess the safety and reactogenicity of each dose of LAIV or IIV. The study will also evaluate 1) acceptability of the immune responses of the LAIV-IIV prime-boost regimen contrasted with those of the IIV-IIV two-dose prime regimen and 2) the adjuvant effect of $AS03_A$ on the immune response to the IIV booster dose in the LAIV-IIV regimen when compared to the non-adjuvanted formulation. Because influenza vaccines based on influenza virus containing a chimeric HA have not been studied in humans previously, the study will evaluate both humoral and secretory immune responses, as well as cell-mediated immune responses. To ensure comparability of group level data across study regimens, one cohort of subjects will be recruited, enrolled, and randomized, stratified by site, to the five study arms.

1.3 Risk/Benefit assessment

The Investigator Brochures for the study products summarize the potential risks and benefits of the investigational LAIV and IIV. Neither the investigational LAIV nor the investigational IIVs to be used in this study have been administered previously to humans. However, this trial will not be initiated until a letter of approval to proceed is received from GSK following its Independent Data

Monitoring Committee's (IDMC) review of safety and reactogenicity data for the seven days post-Dose 1 from all 80 subjects in GSK's Phase 1/2 trial of IIVs with chimeric HAs.

1.3.1 Risk assessment

1.3.1.1 Risks to subjects

Intranasal administration of LAIV or placebo may cause the subject only mild discomfort or a tickling sensation in the nasal passages. The live, attenuated study vaccine to be used for this trial is based on the same master donor influenza virus upon which seasonal LAIV currently approved for use in Russia is based; therefore, adverse events may be similar. These may include, minimally, mild flu-like symptoms [37,38]. Intramuscular administration of IIV or placebo may cause the subject immediate mild pain in the deltoid region. The inactivated study vaccine to be used for this trial is similar to inactivated split virion pandemic vaccine (i.e., H5N1/AS03) manufactured by GSK and currently approved for use in the US, although the HA content of that product is lower (3.75 µg HA). Therefore, adverse events may also be similar. These may include pain and inflammation at the injection site or systemic symptoms such as fever and body aches. However, for either LAIV or IIV serious or allergic reactions may be possible. Study subjects will be observed closely by qualified clinicians, and emergency care will be immediately available to subjects. Clinical units administering vaccines will have all the necessary resources onsite to provide emergency care.

Despite the absence of a local reactogenicity safety concern with the use of licensed $AS03_A$-adjuvanted inactivated influenza vaccines with non-chimeric hemagglutinin in human populations, in light of the microscopic observation of reversible local necrosis at the injection site in some ferrets in the preclinical toxicology study, the administration of the second dose of inactivated influenza vaccine in the study population will be staggered so that at least 5 days will have passed after one half of the subjects are vaccinated before the remaining half shall be vaccinated.

Other important potential or known risks to subjects for receipt of study vaccines or participation in this trial are listed in Table 1

Table 1 Important risks to subjects

Important Potential/Identified Risk	Data/Rationale for Risk	Mitigation Strategy
study LAIV		
Risk of coinfection with wild-type seasonal influenza	Reassortment between vaccine virus and wild type-influenza could result in a new strain with epidemic potential.	See 1.3.1.2.
Anaphylaxis	Anaphylaxis reactions have been reported in people who had influenza vaccination. People allergic to any ingredients in the study vaccines could have an allergic reaction to the vaccine. The viruses for the vaccine are grown in eggs; therefore, people allergic to eggs could have an allergic reaction to study vaccines.	The investigators are advised of possible anaphylaxis following study vaccine administration by information included in the Investigator Brochures. As with all vaccines, appropriate medical treatment and supervision should always be readily available in case of an anaphylactic event following the administration of the vaccine. Subjects will be closely monitored for at least 60 minutes after vaccination. Subjects with history of any reaction or hypersensitivity likely to be exacerbated by any component of the vaccine, or anaphylaxis following the administration of influenza vaccine(s) will be excluded from the study.
$AS03_A$-adjuvanted study IIV		
Theoretical risk of acquiring a vaccine-induced autoimmune disease after vaccination	Theoretical safety concerns have arisen from studies in which adjuvants have induced autoimmune diseases in various animal models and literature reports that diverse compounds with "adjuvant" activity could be associated with autoimmunity [39]. However, a pooled safety analysis of data *from prospective clinical trials* with AS03-adjuvanted pandemic IIVs (both H5N1 and H1N1pdm09) found no increased incidence of autoimmunity induced by vaccination with these products [40].	Close monitoring of pIMDs (see Table 20) in clinical development programs using adjuvants systems. The potential risk of events of possible autoimmune etiology to occur is mentioned in the Informed Consent Form (ICF). Subjects with a history or with a current diagnosis of an auto-immune disease will be excluded from this study.

Important Potential/Identified Risk	Data/Rationale for Risk	Mitigation Strategy
study IIV with or without AS03$_A$ adjuvant		
Anaphylaxis	Anaphylaxis reactions have been reported in people who had influenza vaccination. People allergic to any ingredients in the study vaccines could have an allergic reaction to the vaccine. The viruses for the vaccine are grown in eggs; therefore, people allergic to eggs could have an allergic reaction to study vaccines.	The investigators are advised of possible anaphylaxis following study vaccine administration by information included in the Investigator Brochures. As with all vaccines, appropriate medical treatment and supervision should always be readily available in case of an anaphylactic event following the administration of the vaccine. Subjects will be closely monitored for at least 60 minutes after vaccination. Subjects with history of any reaction or hypersensitivity likely to be exacerbated by any component of the vaccine, or anaphylaxis following the administration of influenza vaccine(s) will be excluded from the study.
Narcolepsy	Epidemiological data developed by organizations not associated with GSK suggest an increased risk of narcolepsy following vaccination with Pandemrix™ H1N1 in children and adolescents. Due to the methodological limitations of the studies, which are retrospective observational studies, further research is needed to determine whether the observed risk is related to the vaccine, environmental effects, genetic factors, other factors, or a combination of factors. Arepanrix, another AS03-adjuvanted vaccine produced in Quebec, Canada, with a slightly different H1N1 viral antigen manufacturing process, has not been associated with an increased risk of narcolepsy comparable to Pandemrix [41]. GSK considers narcolepsy to be a clear signal that requires further investigation, and continues to support both epidemiological and mechanistic studies. No such risk has been identified in clinical trials of AS03-adjuvanted vaccines against other pandemic influenza antigens.	Close monitoring of pIMDs in clinical development programs using adjuvants systems. The potential risk of events of possible autoimmune etiology to occur (like narcolepsy) is mentioned in the ICF. Subjects with a history or with a current auto-immune disease will be excluded from this study.

Important Potential/Identified Risk	Data/Rationale for Risk	Mitigation Strategy
Other study procedures		
Risk from blood sampling	Blood sampling-associated risk of discomfort, syncope, dizziness, infection at the site after or during venipuncture.	Blood samples will be obtained by a trained professional and medical assistance will be available. The potential risk of feeling faint, or experiencing mild local pain, bruising, irritation or redness at the site where blood was taken, will be mentioned in the ICF. The amount of blood to be taken for sampling will not be harmful to the subject's health.
Other risks of study participation		
Risk of influenza	Because study subjects will be required to forego seasonal influenza vaccination for the duration of the trial, they will be at risk of infection with influenza.	Subjects will be informed that immediately upon development of an influenza-like illness i.e., fever or muscle aches and cough or sore throat, they should present to the study outpatient clinic free of charge for nasal and throat swab collection, influenza diagnostic testing and physician's assessment. If they test positive for influenza, or if in the case of a negative diagnostic test, signs and symptoms (and local influenza activity) indicate a high likelihood of influenza, they will be offered anti-neuraminidase treatment free of charge.

Vaccine-associated enhanced respiratory disease (VAERD) has been described in influenza-naïve pigs vaccinated with inactivated H1 influenza vaccine and subsequently challenged with an antigenically mismatched H1 virus [42,43]. Although VAERD is a reproducible observation under well-controlled laboratory conditions with pigs, it has never been observed in natural conditions or in other laboratory animal model used to study influenza viruses (i.e. mice and ferrets). VAERD has not been described with GSK Biologicals' AS03-adjuvanted H5N1-pandemic vaccination. The latter is a relevant surrogate for the SUIV candidate since it elicits a robust immune response to the H1 HA stalk domain without inducing a strong serum neutralizing antibody response to A/H1N1pdm09 virus. Although VAERD is not considered as a potential safety risk in the current study, surveillance will be performed to capture all influenza like illnesses for the duration of the study.

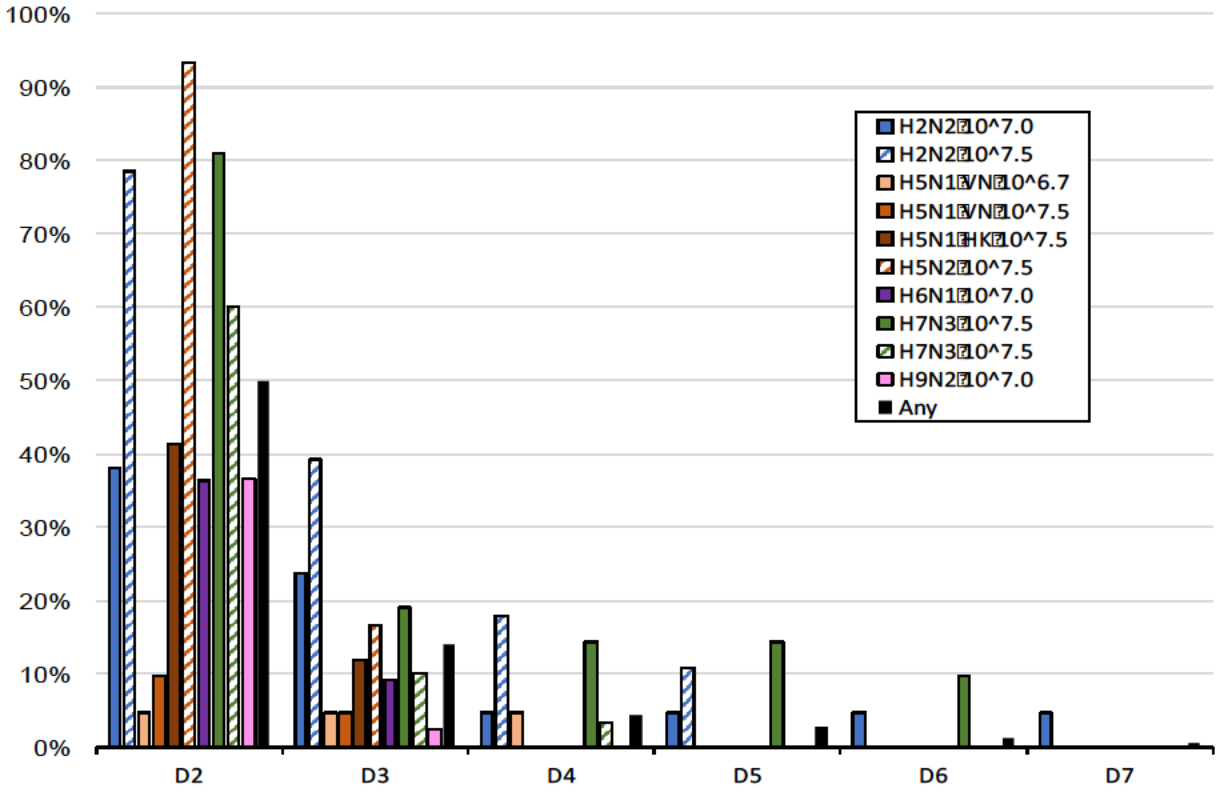
In addition, all cases of respiratory illness, as any safety data, will be closely monitored by the Independent Data Monitoring Committee (IDMC). The theoretical risk of VAERD will be stated in the ICF.

1.3.1.2 Risks to the community

The influenza virus A/mallard/Sweden/24/2002 (H8N4) was used to construct the chimeric H8/1 HA in the LAIV to be used in this study. It is a low pathogenicity influenza virus found to infect poultry. It does *not* contain the multi-basic cleavage site of H5 and H7 influenza viruses which facilitates systemic virus replication and results in switching from a low-pathogenic avian influenza phenotype to a highly pathogenic avian influenza phenotype. Moreover, H8 influenza virus infections have never been detected in humans [44]. Nonetheless, it is theoretically possible that live study vaccine virus and a wild-type seasonal human influenza virus could co-infect and re-assort in cells of the upper respiratory tract of a single human, resulting in a new strain of influenza of epidemic potential.

In previous trials of pre-pandemic monovalent candidate LAIVs based on A/Ann Arbor and A/Leningrad master donor viruses with avian full-length HAs (not chimeric HAs) administered to healthy adults, shedding has been generally low, with most detections by reverse transcription polymerase chain reaction (RT-PCR) occurring in the first two days post-vaccination [37,45-51] (Figure 3).

Figure 3 LAIV virus detections by RT-PCR post-administration of a single dose of pre-pandemic monovalent LAIV candidates containing avian full-length HAs.*



*Solid bars represent data from NIH-sponsored trials in the US of A/Ann Arbor-based pre-pandemic LAIV candidates while cross-hatched bars represent data from Microgen-sponsored trials in the Russian Federation of A/Leningrad-based pre-pandemic LAIV candidates.

Nonetheless, to reduce the risk of any study LAIV-wild type influenza virus coinfection in a study subject or among any contact of study subjects (including study staff), administration of study LAIV will occur within an inpatient clinical unit with self-contained ventilation, and all staff will follow appropriate precautions for contact with persons potentially infected with novel influenza A viruses. Subjects receiving study LAIV will remain in the inpatient clinical unit and be monitored for at least 5 days post-vaccination before being discharged to the community. Moreover, discharge will be contingent on absence of detectable vaccine virus (by RT-PCR) in the subject's nasal and oropharyngeal passages for at least 48 hours prior to discharge or an additional 24 hours in the inpatient clinical isolation unit after initiation of anti-neuraminidase treatment with the last nasal and OP swab specimen prior to discharge testing influenza A negative by RT-PCR. A set of study related policies and procedures will be implemented to monitor circulation of wild-type influenza and reduce the risk of transmission of live vaccine virus to study staff and the community as well as reduce the risk of transmission of wild-type influenza to subjects dosed with study LAIV (Table 2).

Table 2 Community risk mitigation policies and procedures

Site/Community-level mitigation procedures
• Study site will not administer study LAIV from December 15 through March 31, the period indicating usual influenza circulation in prospective sites. Influenza activity will be monitored through virologic surveillance from November 1 or through May 31 if the study will have active study LAIV dosing prior to December 15 or after March 31.
• Study site will coordinate with local virologic influenza surveillance, or will establish local virologic influenza surveillance, and will closely monitor local influenza circulation during at least the four-week period prior to planned study initiation and during study conduct until at least one week after discharge from inpatient clinical unit of final subject receiving study LAIV. The site will provide timely reporting on all influenza-positive specimens (including type and subtype of influenza and number of specimens tested locally per week) to PATH.
• In case of identification of at least 2 laboratory-confirmed influenza A cases in each of the previous 2 weeks at a study site, subjects will not be enrolled to receive study LAIV.
• Study site will use an inpatient clinical unit with self-contained ventilation system which exhausts air to the outside or if air is recirculated which returns air filtered through a high-efficiency particulate air (HEPA) filter.
• Study site inpatient clinical unit will be staffed 24 hours each day for the duration of the inpatient portion of the trial and unit exits will be monitored 24 hours each day to ensure unauthorized persons do not enter and study subjects not cleared for discharge do not leave the unit unless the subject withdraws from the trial.
• The study site Principal Investigator (PI) will inform the local city and/or county public health officials of planned conduct of the study and will inform officials in real time when the study initiates.
• The inpatient clinical unit will follow standard infection control guidelines, including environmental management practices such as laundry, cleaning, and disinfection of the facility and subject care equipment, and proper waste management. All unit procedures will be documented in a manual prior to study initiation.
• The site PI will ensure the study is reviewed by the site Institutional Biosafety Committee and follow all guidelines/procedures required by the Institutional Biosafety Committee.
Site staff-level mitigation procedures
• Unit staff will agree to "annual" seasonal influenza vaccination with available inactivated vaccine containing latest World Health Organization-recommended strains at least two weeks prior to site initiation.
• Unit staff will be trained on and monitored for adherence to infection control procedures.
• Unit staff will sign an agreement to adhere to infection control and other study procedures.
• Unit staff will agree to monitor any household members for acute respiratory illness (ARI), record oral body temperature daily for any such ARI, and report occurrence of the ARI to the site PI. Staff reporting such illness in the household may be barred from working in the inpatient clinical unit at the discretion of the site PI.
• Unit staff will agree to provide nasal and/or oropharyngeal swab specimens for testing for influenza virus (and other respiratory pathogens) for any ARI occurring in the staff member from one week prior to admission of subjects to the inpatient clinical unit until one week after discharge of the last subject from the inpatient clinical unit for each sub-cohort. Staff reporting such illness may be barred from working in the inpatient clinical unit at the discretion of the site PI.
• Unit staff will agree to immediately begin anti-neuraminidase treatment—oseltamivir (75 mg taken orally twice daily for 5 days) or zanamivir (10 mg taken by oral inhalation twice daily for 5 days)—for any febrile acute respiratory illness, at least until time that first nasal/oropharyngeal swab specimen test returns influenza negative by RT-PCR. Staff who test influenza positive by RT-PCR for influenza and have other persons sharing his/her household may be offered complimentary single occupancy stay in a hotel for up to 72 hours.
• Unit staff will agree to possible prophylactic use of anti-neuraminidase drugs—oseltamivir (75 mg taken orally once daily) or zanamivir (10 mg taken by oral inhalation once daily) for 10 days—if influenza is detected in the community (single influenza positive specimen detected through established site surveillance), either starting from day of detection or from day prior to study LAIV dosing in subjects until after discharge from the inpatient clinical unit (second sub-cohort) of last subject receiving study LAIV. The decision to initiate prophylaxis of all staff shall be the responsibility of the site PI.

• Unit staff will agree to wear a surgical face mask, eye protection, fluid-resistant gown, and gloves when conducting intranasal administration of study LAIV to any subject.
• Unit staff will agree to wear gloves while working with healthy subjects and perform hand hygiene a) *before* touching any subject, b) *before* performing any aseptic procedure, c) after exposure to any body-fluid of any subject, d) *after* touching any subject, and e) after touching any subject surroundings.
• Unit staff will agree to a surgical face mask, eye protection, fluid-resistant gown, and gloves when in close proximity (within 2 meters) of any subject exhibiting any signs or symptoms of acute respiratory illness.
Subject-level mitigation procedures
• Subjects will consent to enter the inpatient clinical unit one day (at least 18 hours) prior to study LAIV dosing to allow for orientation and monitoring for acute respiratory illness caused by a pathogen for which they might already be infected.
• Subjects will consent to daily nasal and OP swabbing for RT-PCR testing for influenza virus (and other respiratory pathogens) as part of protocol-specified study procedures.
• Subjects will consent to remain in the inpatient clinical unit for at least 5 days post-administration of study LAIV. Protocol-allowed discharge begins on study Day 6. Only those subjects whose nasal and OP swab specimens from Day 4, Day 5, and Day 6 all test influenza negative by RT-PCR (3 negative tests over 48 elapsed hours) may be discharged on Day 6. Any subject exhibiting influenza A virus shedding, as determined by real-time RT-PCR positivity on a nasal and OP swab specimen, in the 2 days prior (Day 4 or Day 5) or on the day of planned discharge (Day 6) will be kept in the inpatient clinical isolation unit until either a) three daily consecutive swab specimens taken over a 48 hour period all test influenza A negative by RT-PCR or b) the subject agrees to initiation of anti-neuraminidase treatment—oseltamivir (75 mg taken orally twice daily for 5 days) or zanamivir (10 mg taken by oral inhalation twice daily for 5 days)—24 hours after which point the subject may be discharged as long as the last nasal and OP swab specimen prior to discharge tested influenza A negative by RT-PCR. Subjects whose nasal and OP swab specimen from Day 6 (or thereafter) tests positive but who decline anti-neuraminidase treatment may be discharged only after three subsequent negative nasal and OP swab specimens (3 negative tests over 48 elapsed hours). Subjects who agree to initiate anti-neuraminidase treatment will still be requested to return to the clinical daily for nasal and OP swab collection until 3 nasal and OP swab specimens test negative over 48 elapsed hours in order to document loss of detectable virus.
• Subjects will consent to remain in the inpatient clinical unit until Day 6 if they develop any acute respiratory illness regardless of cause. This is to ensure that no subject is discharged prematurely and then begins to shed study LAIV in the community. Such subjects will be informed that they might be confined to their room to reduce spread of the agent to other subjects in the unit.
• Subjects exiting the inpatient clinical unit prior to meeting discharge criteria will be informed that they may be requested to begin anti-neuraminidase treatment—oseltamivir (75 mg taken orally twice daily for 5 days) or zanamivir (10 mg taken by oral inhalation twice daily for 5 days)—and will be counseled on the theoretical risks to their household contacts and the community.

1.3.2 Benefit assessment

Known benefits include:

- Medical evaluation/assessments associated with this study, i.e., physical examination for all subjects and blood testing (hematology, biochemistry, and virologic data).

Potential benefits include:

- Subjects receiving one of the investigational universal influenza vaccines may potentially have the benefit of being protected against influenza A Group 1 viruses. However, since the efficacy of these investigational vaccines has not yet been assessed, it is not known whether they are effective in protecting against influenza A Group 1 infection in humans.
- Contribution to the process of developing a universal influenza vaccine that might provide protection against all influenza A or B strains in the future.

1.3.3 Overall Risk/Benefit conclusion

It has been demonstrated in mice that the chimeric HA-based vaccination regimen with investigational inactivated influenza vaccines similar to those being used in this study induced higher stalk antibody titers than the seasonal inactivated influenza vaccine. These stalk antibody responses were long lasting, cross-reactive to distantly related HAs and provided protection in vivo in a serum passive transfer challenge mouse model [52]. As already discussed, the LAIV-IIV approach may also offer superior immune responses to the IIV-IIV approach. The investigational universal influenza vaccines being used in this study are currently in a very early stage of clinical development and no vaccine efficacy has been demonstrated in humans. Taking into account the measures taken to minimize risk to subjects participating in this study and the measures taken to minimize risk of co-infection with the study LAIV and wild-type influenza virus in a subject or community member, the potential risks identified in association with these universal influenza vaccine products are justified by the potential benefits linked to the development of such an influenza vaccine.

2 HYPOTHESES, OBJECTIVES, AND ENDPOINTS

2.1 Study hypotheses

Primary hypothesis, safety: cH8/1N1 LAIV administered by intranasal route is acceptably safe and well-tolerated in healthy adults and cH5/1N1 IIV +/- $AS03_A$ administered by intramuscular route in those previously primed with cH8/1N1 LAIV or cH8/1N1 IIV + $AS03_A$ is acceptably safe and well-tolerated in healthy adults.

Secondary hypothesis, immunogenicity: Prime-boost with cH8/1N1 LAIV followed by cH5/1N1 IIV +/- $AS03_A$ results in immune responses against conserved antigenic sites among influenza A Group 1 viruses that are acceptable and at least comparable to those elicited by one dose of cH8/1N1 IIV + $AS03_A$ followed by cH5/1N1 IIV + $AS03_A$.

2.2 Study objectives

2.2.1 Primary objective

- To assess the reactogenicity and safety through 28 days after each priming dose of cH8/1N1 LAIV (or placebo) and the booster dose of cH5/1N1 IIV +/- $AS03_A$ (or placebo) and through 28 days after each dose of IIV (cH8/1N1 IIV + $AS03_A$ and cH5/1N1 IIV + $AS03_A$) (or placebo) in terms of rates of solicited local and general AEs through 7 days post-vaccination, unsolicited AEs through 28 days post-vaccination, hematological and biochemical laboratory abnormalities up to Visit 13, and medically attended event (MAEs), influenza-like illnesses (ILIs), potential immune-mediated disease (pIMDs), and serious adverse events (SAEs) through Visit 13.

2.2.2 Secondary objectives

2.2.2.1 Safety

- To assess the safety of each treatment group during the entire study period in terms of rates of primary endpoints and additionally hematological and biochemical laboratory abnormalities up to Visit 15, and MAEs, ILIs, pIMDs and SAEs through Visit 16.

2.2.2.2 Viral shedding - post-Dose 1

- To **describe the shedding of vaccine virus** through 5 days after administration of cH8/1N1 LAIV (Groups 1, 2, and 3 only) in terms of the proportions of subjects with influenza type A vaccine virus RNA detected by RT-PCR in nasal and OP swabs and the proportion of subjects with vaccine virus isolated in cell culture each day post-vaccination.

2.2.2.3 Immunogenicity - descriptive, post-Dose 2

- To **describe the anti-H1 HA-stalk humoral immune responses** (anti-H1 HA-stalk serum immunoglobulin G [IgG], anti-H1 HA-stalk serum neutralizing antibodies, anti-H1 HA-stalk serum immunoglobulin A [IgA], and ADCC activity 28 days after the booster dose of cH5/1N1 IIV +/- $AS03_A$ (or placebo) ***(Groups 1, 2, and 3)*** and 28 days after the second dose of IIV (cH5/1N1 IIV + $AS03_A$) (or placebo) ***(Groups 4 and 5)*** in terms of seropositivity rates, geometric mean titers (GMTs), percentages of subjects with a 4-fold or greater increase in titer from Day 1, percentages of subjects with a 10-fold or greater increase in titer from Day 1, and mean geometric increases (MGIs) from Day 1.

- To **describe the anti-H1 HA-stalk mucosal immune responses** (anti-H1 HA-stalk salivary total IgA, anti-H1 HA-stalk secretory IgA in saliva, and anti-H1 HA-stalk salivary IgG) 28 days after the booster dose of cH5/1N1 IIV +/- $AS03_A$ (or placebo) ***(Groups 1, 2, and 3)*** and 28 days after the second dose of IIV (cH5/1N1 IIV + $AS03_A$) (or placebo) ***(Groups 4 and 5)*** in terms of seropositivity rates, GMTs, percentages of subjects with a 4-fold or greater increase in titer from Day 1, percentages of subjects with a 10-fold or greater increase in titer from Day 1, and MGIs from Day 1.

2.2.2.4 Immunogenicity - descriptive, post-Dose 2 - breadth

- To **describe the breadth of the anti-H1 HA-stalk humoral immune responses** (anti-H2 HA-full length serum IgG, anti-H9 HA-full length serum IgG, anti-H18 HA-full length serum IgG, anti-H5N8 serum neutralizing antibodies, anti-avian-swine H1N1 serum neutralizing antibodies, and anti-H1pdm09-like serum neutralizing antibodies) to Group 1 influenza A viruses 28 days after the booster dose of cH5/1N1 IIV +/- $AS03_A$ (or placebo) ***(Groups 1, 2, and 3)*** and 28 days after the second dose of IIV (cH5/1N1 IIV + $AS03_A$) (or placebo) ***(Groups 4 and 5)*** in terms of seropositivity rates, GMTs, percentages of subjects with a 4-fold or greater increase in titer from Day 1, percentages of subjects with a 10-fold or greater increase in titer from Day 1, and MGIs from Day 1.

2.2.2.5 Immunogenicity - descriptive, post-Dose 2 - persistence

- To **describe the persistence of the anti-H1 HA-stalk humoral immune responses** (anti-H1 HA-stalk serum IgG, anti-H1 HA-stalk serum neutralizing antibodies, anti-H1 HA-stalk serum IgA, and ADCC activity) up to 12 months after the booster dose of cH5/1N1 IIV +/- $AS03_A$ (or placebo) ***(Groups 1, 2, and 3)*** and up to 12 months after the second dose of IIV (cH5/1N1 IIV + $AS03_A$) (or placebo) ***(Groups 4 and 5)*** in terms of seropositivity rates, GMTs, percentages of subjects with a 4-fold or greater increase in titer from Day 1, percentages of subjects with a 10-fold or greater increase in titer from Day 1, and MGIs from Day 1.

- To **describe the persistence of the anti-H1 HA-stalk mucosal immune responses** (anti-H1 HA-stalk salivary total IgA, anti-H1 HA-stalk secretory IgA in saliva and anti-H1 HA-stalk salivary IgG) up to 12 months after the booster dose of cH5/1N1 IIV +/- $AS03_A$ (or placebo) ***(Groups 1, 2, and 3)*** and up to 12 months after the second dose of IIV (cH5/1N1 IIV + $AS03_A$) (or placebo) ***(Groups 4 and 5)*** in terms of seropositivity rates, GMTs, percentages of subjects with a 4-fold or greater increase in titer from Day 1, percentages of subjects with a 10-fold or greater increase in titer from Day 1, and MGIs from Day 1.

2.2.2.6 Immunogenicity - descriptive, by vaccine regimen, post-Dose 1

- To **describe the anti-H1 HA-stalk humoral immune responses** (anti-H1 HA-stalk serum IgG, anti-H1 HA-stalk serum neutralizing antibodies, anti-H1 HA-stalk serum IgA, and ADCC activity) 28 days after the prime dose of cH8/1N1 LAIV (or placebo) ***(Groups 1, 2, and 3)*** and 28 days after the first dose of IIV (cH8/1N1 IIV + $AS03_A$) (or placebo) ***(Groups 4 and 5)*** in terms of seropositivity rates, GMTs, percentages of subjects with a 4-fold or greater increase in titer from Day 1, percentages of subjects with a 10 fold or greater increase in titer from Day 1, and MGIs from Day 1.

- To **describe the anti-H1 HA-stalk mucosal immune responses** (anti-H1 HA-stalk salivary IgA, anti-H1 HA-stalk secretory IgA in saliva and anti-H1 HA-stalk salivary IgG) 28 days after the prime dose of cH8/1N1 LAIV (or placebo) ***(Groups 1, 2, and 3)*** and 28 days after the first dose of IIV (cH8/1N1 IIV + $AS03_A$) (or placebo) ***(Groups 4 and 5)*** in terms of seropositivity rates, GMTs, percentages of subjects with a 4-fold or greater increase in titer from Day 1, percentages of subjects with a 10 fold or greater increase in titer from Day 1, and MGIs from Day 1.

2.2.2.7 Immunogenicity - comparative, post-Dose 2

- To **compare the anti-H1 HA-stalk humoral immune responses** (anti-H1 HA-stalk serum IgG, anti-H1 HA-stalk serum neutralizing antibodies, anti-H1 HA-stalk serum IgA, and ADCC activity) 28 days after the booster dose with cH5/1N1 IIV +/- $AS03_A$ after previous receipt of cH8/1N1 LAIV ***(Groups 1 or 2)*** to that after priming with two doses of IIV (cH8/1N1 IIV + $AS03_A$ and cH5/1N1 IIV + $AS03_A$) ***(Group 4)*** in terms of the adjusted GMT ratio and the seroresponse (≥4-fold) rate difference.

- To **compare the anti-H1 stalk mucosal immune responses** (anti-H1 HA-stalk salivary IgA, anti-H1 HA-stalk secretory IgA in saliva, and anti-H1 HA-stalk salivary IgG) 28 days after the booster dose with cH5/1N1 IIV +/- $AS03_A$ after previous receipt of cH8/1N1 LAIV ***(Groups 1 or 2)*** to that after priming with two doses of IIV (cH8/1N1 IIV + $AS03_A$ and cH5/1N1 IIV + $AS03_A$) ***(Group 4)*** in terms of the adjusted GMT ratio and the seroresponse (≥4-fold) rate difference.

- To **evaluate the adjuvant effect of $AS03_A$ on the anti-H1 stalk humoral immune response** (anti-H1 HA-stalk serum IgG, anti-H1 HA-stalk serum neutralizing antibodies, anti-H1 HA-stalk serum IgA, and ADCC activity) after one booster dose of cH5/1N1 IIV + $AS03_A$ after priming with cH8/1N1 LAIV ***(Group 1)*** compared to boosting with cH5/1N1 IIV, unadjuvanted ***(Group 2)*** in terms of the adjusted GMT ratio and seroresponse (≥4-fold) rate difference.

2.2.3 Tertiary objectives

- To explore the cell-mediated immune responses (B-cells and T-cells) after each vaccination.

- To explore blood biomarkers associated with different vaccination regimens (transcriptomic analysis).

- To explore the immune response against the HA head of cH8/1N1, cH5/1N1, cH6/1N5, and H1N1pdm2009-like virus after each vaccination using a HI assay against these viruses.

- To explore the anti-H3 stalk response (i.e. influenza A Group 2).

- To explore the immune response in terms of anti-NA antibodies after each vaccination.

- To explore the protective effect of the stalk-reactive antibodies induced by vaccination in a passive transfer challenge experiment in mice.

- To develop assays for evaluation/characterization of the humoral and cellular immune responses.

- To explore the anti-stalk antibodies' activity (e.g. antibody-dependent cellular phagocytosis [ADCP].

For a detailed listing of study endpoints, see Section 11.

3 STUDY DESIGN

This is a prospective, multi-center, randomized, controlled, observer-blind, Phase 1 trial in healthy male and female adults 18 through 39 years of age. Up to 65 eligible subjects will participate and will be randomized 4:3:1:3:2 to one of five groups (Table 3) to receive a first dose of study cH8/1N1 LAIV (or placebo) or study cH8/1N1 IIV + $AS03_A$ adjuvant (or placebo) followed three months later by study cH5/1N1 IIV +/- $AS03_A$ adjuvant (or placebo). Two sites, Duke Early Phase Clinical Research at Duke University and the Gamble Program for Clinical Studies at Cincinnati

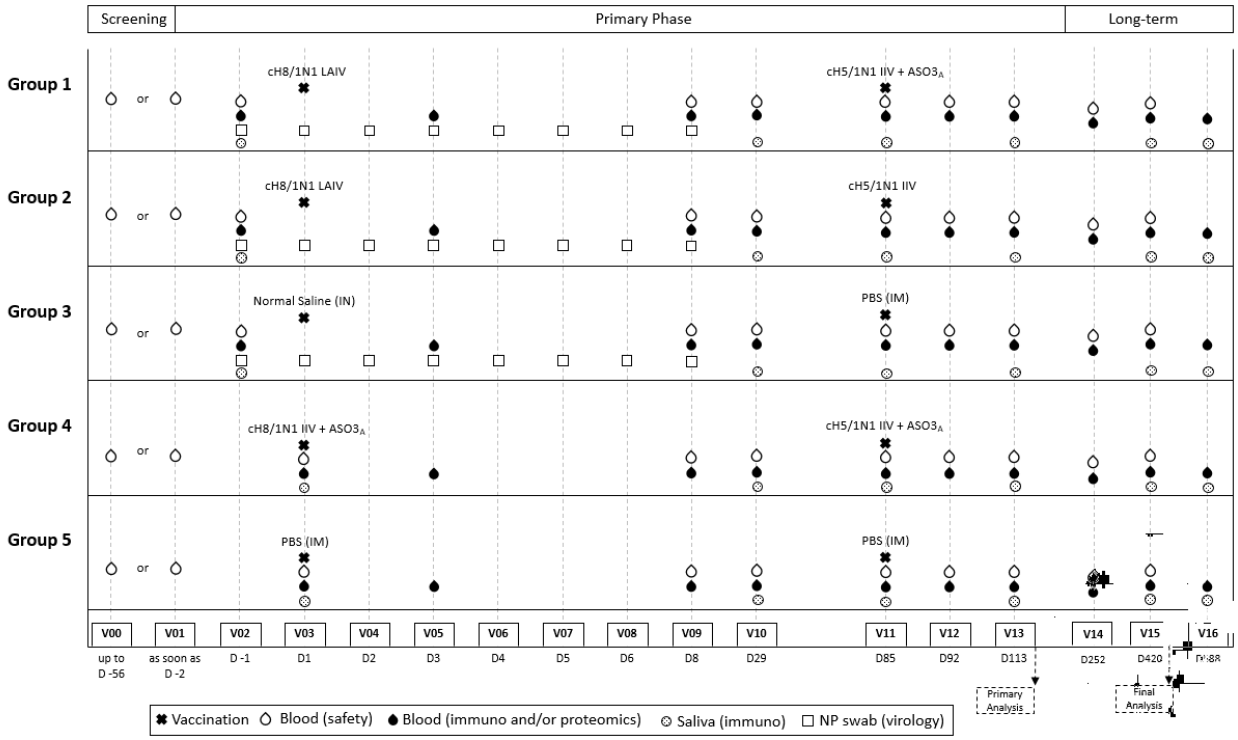
Children's Hospital Medical Center, will participate and enroll 39 and 26 subjects, respectively. Eligible enrolled subjects will be randomized to any of the treatment arms (LAIV-IIV, Groups 1, 2, and 3; or IIV-IIV, Groups 4 and 5) under one allocation sequence, stratified by site, to allow comparability between study groups, such as LAIV-IIV vs IIV-IIV regimens (Groups 1 vs 4). While subjects will be blinded to their exact treatment group and whether they received active study vaccine versus placebo, subjects will certainly know if they received LAIV (or placebo) vs IIV (or placebo) at Dose 1, given the different presentations and routes of administration of these products.

Table 3 Study groups and treatment and route of administration by dose

Study groups	Number of subjects	Dose 1		Dose 2	
		Treatment	Administration Route	Treatment	Administration Route
Group 1	20	cH8/1N1 LAIV	intranasal	cH5/1N1 IIV + AS03$_A$	intramuscular
Group 2	15	cH8/1N1 LAIV	intranasal	cH5/1N1 IIV	intramuscular
Group 3	5	Normal saline	intranasal	PBS	intramuscular
Group 4	15	cH8/1N1 IIV + AS03$_A$	intramuscular	cH5/1N1 IIV + AS03$_A$	intramuscular
Group 5	10	PBS	intramuscular	PBS	intramuscular

The study design is summarized in Figure 4. The following sections provide details of study procedures by visit and these are summarized in the tables in Appendix 1.

Figure 4 Study design overview



Protocol waivers or exemptions are not allowed unless necessary for the management of immediate safety concerns. Therefore, adherence to the study design requirements, including those specified in the outline of study procedures (Section 6), are essential and required for study conduct.

3.1 Screening

Prospective subjects will be carefully screened to determine eligibility. Subjects will be screened for eligibility through medical history review, physical examination, testing for serologic evidence of human immunodeficiency virus (HIV) infection, with proper pre- and post-test counseling, urine drug test, and routine biochemical and hematological blood tests. Women of childbearing potential will undergo pregnancy tests using urine samples or serum samples, as applicable. Screening may occur up to 8 weeks prior to receipt of Dose 1 of study vaccines or placebo. Subjects will be considered enrolled only after a subject has satisfied all eligibility criteria.

3.2 Randomization and dosing

Enrolled subjects will be randomized to study groups as soon as eligibility is confirmed and at least 2 days (ideally at least 7 days) in advance of receipt of Dose 1 of study vaccines. This is because subjects randomized to LAIV-IIV treatment arms (Groups 1, 2, and 3) will receive Dose 1 of study cH8/1 LAIV (or placebo) in an inpatient clinical isolation unit and may need some time to plan for a specific admission date, to be scheduled by each site. Subjects randomized to IIV-IIV treatment arms (Groups 4 and 5) will receive Dose 1 in an outpatient research study clinic.

3.2.1 Dose 1 LAIV-IIV treatment arms (Groups 1, 2, and 3)

Subjects in the LAIV-IIV treatment arms will be admitted into the inpatient clinical isolation unit the day before (at least 18 hours) prior to receipt of Dose 1 in order to observe the subject for acute onset of respiratory illness of some other etiology which the subject might be developing.

At the first site to dose subjects with study LAIV (or placebo), only six subjects will be admitted initially. On Day 1 for these subjects, vaccination with Dose 1 (cH8/1 LAIV or placebo) will be staged such that each subject is dosed and observed for at least 60 minutes prior to the next subject being dosed. Consecutive dosing will proceed only if no adverse events meeting halting rules or other adverse events of concern to the site principal investigator (PI) are observed. Also on this day at the first site, the remaining subjects in LAIV-IIV treatment arms at this site will be admitted to the inpatient clinical isolation unit, again for observation of acute onset respiratory illness. After at least 18 hours since the sixth subject in the study to have received study LAIV (or placebo) have passed with no adverse events meeting halting rules or other adverse events of concern to the site PI, the remaining subjects in the LAIV-IIV treatment arms will be dosed.

At the second site to dose subjects in the LAIV-IIV treatment arms, all subjects at this site will also be admitted into the inpatient clinical isolation unit the day before (at least 18 hours prior to) receipt of Dose 1 in order to observe subjects for acute onset of respiratory illness. Again, if no adverse

events meeting halting rules or other adverse events of concern to the site PI were observed in the first six subjects to receive study LAIV (or placebo) in the trial at the first site, dosing of subjects at the second site will proceed.

PATH or the Emmes Study Coordinating Center will coordinate communication between the two sites. The second site will not be allowed to dose any subjects with study LAIV (or placebo) until PATH has provided written approval to the site.

3.2.2 Dose 1 IIV-IIV treatment arms (Groups 4 and 5)

Vaccination with Dose 1 of (cH8/1N1 IIV + $AS03_A$ or placebo) in the IIV-IIV groups at each site will occur without limitation on the number of vaccinees per day or time between consecutive subjects, as initial cH8/1N1 IIV safety data (through 7 days post-vaccination) will have been collected and reviewed by an IDMC for GSK's separate Phase 1 study of this IIV product prior to initiation of the current trial.

3.2.3 Dose 2 all treatment arms (Groups 1, 2, 3, 4, and 5)

Vaccination with Dose 2 (cH5/1 IIV +/- $AS03_A$ or placebo) at each site will occur in a staggered manner, with one half of the subjects at each site vaccinated initially and the remaining subjects vaccinated only after at least 5 days has passed since the initial half of subjects have all received their Dose 2. In addition, initial cH5/1 IIV safety data (through 7 days post-vaccination) will have been collected and reviewed by an IDMC for GSK's separate Phase 1 study of this IIV product prior to initiation of the current trial.

3.3 Safety monitoring

3.3.1 Safety follow-up for all subjects

Planned safety assessments will provide the data for active monitoring of vaccine safety during conduct of the trial and for the primary reactogenicity and safety endpoints (see Section 8).

Immediate reactogenicity and vital signs will be assessed by medically qualified study staff at 60 (+ 10) minutes following vaccination in all subjects. Immediate reactogenicity will include solicited local and general adverse events and unsolicited AEs, including AEs leading to withdrawal from the study and SAEs.

During the 7-day follow-up period after each vaccination (day of vaccination and subsequent 6 days) subjects will complete diary cards for solicited local and general adverse events and unsolicited AEs, including scoring for severity, and for use of concomitant medications. Reactogenicity scoring will be reviewed by a research clinician prior to being entered into the electronic case report form (eCRF). Solicited local reactions will include runny nose/nasal congestion at the site of administration of study LAIV (or placebo) and pain/tenderness, erythema/redness, and induration/swelling at the site of injection of study IIV (or placebo). Solicited general reactions will be common for all study subjects and include arthralgia, cough,

fatigue, fever (based on oral temperature), gastrointestinal symptoms (nausea, vomiting, diarrhea and/or abdominal pain), headache, myalgia, shivering, sore throat, and wheeze.

For additional assessment of safety, both targeted physical examination and collection of blood specimens for analysis of biochemical (alanine aminotransferase [ALT], aspartate aminotransferase [AST], creatinine, and urea nitrogen) and hematologic (leukocytes, neutrophils, lymphocytes, basophils, monocytes, eosinophils, hemoglobin, platelets, and erythrocytes) clinical laboratory parameters will also be done at each study visit. (Results for clinical laboratory tests conducted just prior to receipt of Dose 1 will serve to define baseline status for each subject prior to receipt of study vaccine or placebo but will not be used for screening purposes.) Clinical laboratory results assessed as AEs will be scored for severity using FDA Toxicity Tables.

Subjects will be asked to further complete diary cards for unsolicited AEs and concomitant meds through the end of the study. At the study visits 7 days after each dose, medically qualified study staff will review diary cards and document AEs in the eCRF, along with severity, relatedness and duration. For each solicited and unsolicited AE the subject experiences, the subject will also be asked if he/she received medical attention defined as hospitalization, or an otherwise unscheduled visit to or from medical personnel for any reason, including emergency room visits. This information will also be recorded in the eCRF. Adverse events of specific interest for safety monitoring include pIMDs, a subset of AEs that include autoimmune diseases and other inflammatory and/or neurologic disorders of interest which may or may not have an autoimmune etiology. AEs that need to be recorded and reported as pIMDs include those listed in Table 20. Both pIMDs (serious or non-serious) and SAEs will be documented in the eCRF and an Expedited Adverse Events Report will be completed. Additionally, occurrence of MAEs and LC-ILI will be recorded and evaluated.

All adverse events will be coded using the Medical Dictionary for Regulatory Activities (MedDRA) (version 15.1 or later). Any SAEs ongoing at the time of the subject's EoS visit will be attempted to be followed up until resolved, or assessed to be resolved with sequelae by the site PI or his designee, until last subject last visit (LSLV) in the trial. If any SAE remains unresolved at the time of LSLV, it will be classified as ongoing. To facilitate rigorous safety monitoring, data captured at each visit will be entered into the electronic data capture (EDC) system within 72 hours from the date of the clinic visit. For ancillary data that may be obtained after a visit (e.g., laboratory test results, inpatient records) the goal of data entry will be to maintain data integrity and timely safety assessments.

PATH will convene a Protocol Safety Review Team (PSRT) to regularly review blinded safety data and trial conduct (see Section 9). In addition, an IDMC will monitor vaccine safety. Prior to receipt of Dose 2 of study vaccines, the IDMC will perform a first interim safety review. The IDMC will review all AEs through Visit 10, including clinical laboratory evaluations pre- and post-vaccination, for all subjects. This will include a formal, unblinded review of all safety data accrued. The IDMC will also review all shedding data for LAIV-IIV treatment arms (see below). The IDMC will then advise if the subjects may receive Dose 2 of study vaccines. The IDMC will also review all AEs through Visit 13 and through Visit 15 at separate safety reviews. The need for additional

meetings to review unblinded safety data will be determined by the IDMC on an ad hoc basis, or specifically requested by PATH.

3.3.2 Special safety monitoring during inpatient stay post-Dose 1 of cH8/1N1 LAIV (or placebo)

To reduce the risk that subjects do not bring wild-type influenza into the inpatient clinical isolation unit or that subjects receiving study cH8/1N1 LAIV do not return to the community while shedding potentially viable vaccine virus, all subjects in Groups 1, 2, and 3 will be monitored for acute respiratory infection and for virologic evidence of influenza infection. Subjects will be admitted to the inpatient clinical isolation unit at least 18 hours prior to receipt of Dose 1 of study LAIV (or placebo) and monitored to ensure no subject is developing an acute respiratory illness. On day of entry to the unit, all subjects will have nasal and OP swab specimens collected and tested for the presence of influenza type A viral RNA by RT-PCR. All subjects must be baseline influenza A negative prior to dosing of any subject. Subjects will also have nasal and OP swab specimens collected daily thereafter until day of discharge from the inpatient clinical isolation unit. Post-vaccination specimens will be used to determine discharge of subjects from the unit. All subjects will remain in the isolation unit for at least 5 days after receipt of study cH8/1 LAIV or placebo. Any subject exhibiting influenza A virus shedding, as determined by real-time RT-PCR positivity on a nasal and OP swab specimen, in the 2 days prior (Day 4 or Day 5) to or on the day of planned discharge (Day 6) will be kept in the inpatient clinical isolation unit until three daily consecutive swab specimens taken over a 48 period all test influenza A negative by RT-PCR. RT-PCR testing of nasal and OP swab specimens collected on Day 6 (or later) will be completed on day of specimen collection. Any subject still exhibiting evidence of influenza virus shedding in a nasal and OP swab on Day 6 or later will be offered anti-neuraminidase treatment (e.g., oral oseltamivir, 75 milligrams [mg] twice a day for 5 days), 24 hours after which point the subject may be discharged as long as the last nasal and OP swab specimen prior to discharge tested influenza A negative by RT-PCR. To study virus infectivity, nasal and OP swab specimens that test influenza A positive by RT-PCR will be further tested for viability of virus in Madin Darby canine kidney (MDCK) cell culture and stained with monoclonal antibody specific to the cH8/1N1 LAIV virus to confirm detected virus is of vaccine origin. Nasal and OP swab specimens may also be tested by RT-PCR using primer-probe sets designed to detect specifically genes of A/Leningrad/134/17/1957, the master donor virus used to create study LAIV, or of the cH8/1 HA.

3.4 Immunogenicity / Transcriptomic testing

For the evaluation of humoral and mucosal immune responses, sera and saliva will be collected on pre-vaccination and on Visits 10, 11, 13, 14, and 15. Sera and saliva will also be collected at Visit 16 with testing for humoral and mucosal immune responses dependent upon the outcome of primary and secondary analyses. . Multiple assays [ELISA, microneutralization (MN), ADCC, ADCP, HI] will be used to probe the presence, breadth and persistence of immune responses to study vaccines, including functional responses. Sera from pre-vaccination or Visit 13 or Visit 15 will also be pooled for passive transfer to mice in an influenza challenge model. For evaluation of cell-mediated immune responses (CMI) whole blood will be collected. To assess vaccine

induction of cytotoxic T lymphocytes, whole blood for isolation of peripheral blood mononuclear cells (PBMCs) will be collected on pre-vaccination and on Visits 9, 11, 12, and 15. To assess vaccine induction of plasmablasts, whole blood will be collected on Visits 9 and 12. To assess vaccine induction of B memory cells, whole blood will be collected on pre-vaccination and on Visits 10, 11, 13, and 15. Because of the need to process and ship whole blood for CMI in a timely manner, strong preference will be made to enroll all subjects in Groups 4 and 5 at each site on one day. For transcriptomics, whole blood will be collected pre-vaccination and on Visits 5, 9, 10, 11, 12, 13, 14, and 15.

4 STUDY POPULATION

4.1 Description of study population

This study will be conducted at two major medical research centers in the US. They are the Gamble Program for Clinical Studies at Cincinnati Children's Hospital Medical Center (CCHMC) located in Cincinnati, Ohio, and the Duke Early Phase Clinical Research Unit at Duke Clinical Research Institute in Durham, North Carolina. A total of 65 subjects aged 18 through 39 years old will be enrolled in this study.

4.2 Inclusion criteria for enrollment

Deviations from inclusion criteria are not allowed because they can potentially jeopardize the scientific integrity, regulatory acceptability of the study or subject safety. Therefore, adherence to the criteria as specified in the protocol is essential.

All subjects must satisfy ALL the following criteria at study entry:

- Able to understand planned study procedures and demonstrate comprehension of the protocol procedures and knowledge of study by passing a written examination* prior to vaccination
  *Passing grade ≥70%

- In the opinion of the investigator, can and will comply with the requirements of the protocol (e.g., completion of the diary cards, return for follow-up visits).

- Written informed consent obtained from the subject prior to performance of any study specific procedure.

- Male or non-pregnant female between, and including, 18 and 39 years of age at the time of the first vaccination.

- Healthy subjects without acute or chronic, clinically significant pulmonary, cardiovascular, hepatic or renal functional abnormality*.
  **As determined by medical history, physical examination or laboratory screening tests.*

- Female subjects of non-childbearing potential may be enrolled in the study.
  - Non-childbearing potential is defined as pre-menarche, current bilateral tubal ligation or occlusion, hysterectomy, bilateral ovariectomy or post-menopause. Please refer to the glossary of terms for the definition of menarche and menopause.
- Female subjects of childbearing potential must have a negative pregnancy test within 24 hours of vaccination.
- Female subjects of childbearing potential must have practiced adequate contraception for 30 days prior to first vaccination and agree to continue adequate contraception until 2 months after completion of the vaccination series (Month 5). Please refer to the glossary of terms for the definition of adequate contraception.
- Male subjects must be surgically sterile (e.g., vasectomy) or agree to practice adequate contraception from the first vaccination until 2 months after completion of the vaccination series (Month 5). Please refer to the glossary of terms for the definition of adequate contraception.

4.3 Exclusion criteria for enrollment

Deviations from exclusion criteria are not allowed because they can potentially jeopardize the scientific integrity, regulatory acceptability of the study, or subject safety. Therefore, adherence to the criteria as specified in the protocol is essential.

The following criteria should be checked at the time of study entry. If ANY exclusion criterion applies, the subject must not be included in the study:

- Use of any investigational or non-registered product (drug or vaccine) other than the study vaccines†
  †*During the period starting 30 days before the first dose of study vaccines (Day -29 to Day 1), or planned use during the study period Between Visit 15 and Visit 16, the subject may participate in other clinical studies except for those in which investigational influenza vaccines would be administered..*
- Concurrently participating in another clinical study, at any time during the study period, in which the subject has been or will be exposed to an investigational or a non-investigational vaccine/product‡. Between Visit 15 and Visit 16, the subject may participate in other clinical studies except for those in which investigational influenza vaccines would be administered.
  ‡*Pharmaceutical product or device.*
- Any medical condition that in the judgment of the investigator would make study participation (including intramuscular injection) unsafe.
- Medically diagnosed deviated nasal septum or nasal obstruction.

- Chronic administration (defined as more than 14 days in total) of immunosuppressants or other immune-modifying drugs within 6 months before the first dose.
  - For corticosteroids, this will mean prednisone ≥ 20 mg/day, or equivalent.
  - For high-dose inhaled corticosteroids this will mean beclomethasone dipropionate chlorofluorocarbon ≥ 840 mcg/day, or equivalent.
  - Topical steroids are allowed.
- Administration of long-acting immune-modifying drugs (e.g., infliximab, rituximab) within 6 months before the first dose (Visit 03), or planned administration any time during the study period.
- Planned administration/administration of a vaccine not foreseen by the study protocol in the period starting 30 days before the first dose (Visit 03) up to Month 15 (Visit 15)[§].

  [§]*30 days before the first the first dose (Visit 03) up to the blood sampling at Day 113 (Visit 13) and in the period starting 30 days before blood sampling at Month 9 (Visit 14) and Month 15 (Visit 15)*
- Persons who should be annually vaccinated against influenza, if they themselves are or they live with or care for persons at high risk for influenza-related complications.[‖]

  [‖]*including:*
  - *health care personnel such as physicians, nurses, and other workers in inpatient and outpatient-care settings, medical emergency-response workers, such as paramedics and emergency medical technicians) employees of nursing home and long-term care facilities who have contact with patients or residents, and students in these professions who will have contact with patients, and*
  - *household contacts and caregivers of persons not eligible for seasonal influenza vaccination (e.g. children aged <6 months) or among whom seasonal influenza vaccine effectiveness is diminished (e.g. the elderly ≥65 years).*
  - [Medical conditions that put persons at high risk for severe complications from influenza are identified by the Advisory Committee on Immunization Practices (see https://www.cdc.gov/mmwr/volumes/65/rr/rr6505a1.htm) and include the following:
    - Persons who have chronic pulmonary (including asthma) or cardiovascular (except isolated hypertension), renal, hepatic, neurologic, hematologic, or metabolic disorders (including diabetes mellitus);
    - Persons who have immunosuppression (including immunosuppression caused by medications or by HIV infection);
    - Persons who have extreme obesity (body mass index [BMI] ≥40)]
- History of influenza vaccination within 6 months prior to study enrollment or unwillingness to forego seasonal influenza vaccination during the entire study period.

- History of vaccination with an investigational pandemic influenza vaccine other than an H1N1pdm09 vaccine.

- Any confirmed or suspected immunosuppressive or immunodeficient condition, based on medical history and physical examination.

- Infection with human immunodeficiency virus regardless of clinical stage of immunodeficiency.

- History of current infection with hepatitis B virus or hepatitis C virus regardless of clinical presentation.

- History of or current autoimmune disease.

- Subjects diagnosed with excessive daytime sleepiness¶ or narcolepsy; or history of narcolepsy in a subject's parent or sibling.
  ¶*Unintended sleep episodes during the day present almost daily for at least one month*

- History of Guillain-Barré syndrome.

- History of any reaction or hypersensitivity likely to be exacerbated by any component of the vaccines (including egg proteins); a history of anaphylactic-type reaction to consumption of eggs; or a history of severe adverse reaction to a previous influenza vaccine.

- Hypersensitivity to latex.

- Administration of immunoglobulins and/or any blood products during the period starting 3 months before the first dose of study vaccines or planned administration during the study period.

- Pregnant or lactating female.

- Female planning to become pregnant or male planning to father a child or either planning to discontinue contraceptive precautions.

- Current smoker.

- During screening, have a positive test for opiates without a prescription.

- History of chronic alcohol consumption and/or drug abuse as deemed by the investigator to render the potential subject unable/unlikely to provide accurate safety reports.

- Have a history of convulsions or encephalomyelitis within 90 days prior to study vaccination.

- Have any diagnosis, current or past, of schizophrenia, bipolar disease, or other psychiatric diagnosis that may interfere with subject compliance or safety evaluations.

- Have been hospitalized for psychiatric illness, history of suicide attempt, or confinement for danger to self or others within 10 years prior to study vaccination.

- Blood donation or planned blood donation within 30 days prior to the study vaccination through 30 days after the last blood drawn for this study.

- Have signs or symptoms that could confound or confuse assessment of study vaccine reactogenicity.

- Any hematological[#] or biochemical[**] parameter that is out of range of normal[††], and is considered clinically significant by the investigator.

  [#]*Complete blood cell count [red blood cells, white blood cells], white blood cells differential count [lymphocytes, neutrophils and eosinophils], platelet count or hemoglobin level*

  [**]*Creatinine, blood urea nitrogen [BUN], ALT or AST*

  [††]*Per the site clinical laboratory's reference ranges. All tests with out of range results must be repeated before any subject is allowed to be enrolled.*

- The following hematological or biochemical laboratory results will be considered exclusionary, irrespective of assessment of clinical significance:

Hemoglobin (Male)	< 13.0 g/dL
Hemoglobin (Female)	< 11.7 g/dL
White Blood Cell count	< 3,000 cells/mm^3
Neutrophil count	< 1,500 cells/mm^3
Eosinophil count	> 600 cells/mm^3
Platelet count	< 130,000 cells/mm^3
Creatinine	> 1.4 mg/dL
ALT	> Upper limit of the normal range (ULN) [††]
AST	> Upper limit of the normal range (ULN) [††]

[††]*Per the site clinical laboratory's reference ranges.*

4.4 Temporary exclusion criteria for receipt of Dose 1

- Travel within 7 days of planned receipt of Dose 1 of study LAIV (or placebo) to a region with known or suspected ongoing influenza circulation (Groups 1, 2, and 3 only).

- Acute disease and/or fever[¶¶] at the time of planned receipt of Dose 1 of study vaccine.

  [¶¶]*Fever for this purpose is defined as temperature ≥ 38.0°C or 100.4°F. The preferred location for measuring temperature in this study will be the oral cavity.*

5 STUDY PRODUCTS

The quality control standards and requirements for each study vaccine are described in separate quality assurance documents (e.g., release protocols, certificate of analysis) and the required approvals will be obtained prior to study initiation. The study vaccines are labelled and packed according to applicable regulatory requirements. Commercial sterile normal saline will be

purchased for use as intranasal placebo and phosphate buffered saline (PBS) will be provided by GSK for use as intramuscular placebo. Table 4 provides an overview of the study vaccines/products.

Table 4 Study Vaccines/Products

Treatment	Product name	Formulation	Presentation	Volume to be administered	Number of doses
cH8/1N1 LAIV	cH8/1N1 LAIV	HA head, A/mallard/Sweden/24/2002 (H8N4); HA stalk, A/California/04/2009 (H1N1); NA,A/California/04/2009 (H1N1) containing the backbone of the cold adapted/temperature sensitive of the Russian LAIV A/Leningrad/134/17/1957 (Len17 IDCDC-RG46D); LAIV dose=$10^{7.5 +/- 0.5}$ EID_{50}	Frozen liquid	0.5 mL**	1
	Normal saline	0.9% Sodium chloride solution	Liquid in vial		
cH8/1N1 IIV + $AS03_A$-like*	cH8/1N1	HA head, A/mallard/Sweden/24/2002 (H8N4); HA stalk, A/California/04/2009 (H1N1); NA, A/California/04/2009 (H1N1); HA=15µg	Freeze-dried pellet	0.5 mL**	1
	AS03	Emulsion containing tocopherol, tocopherol=47.44mg/ml	Whitish to yellowish homogenous milky liquid emulsion in multi-dose vial		
	PBS	Na_2HPO_4=1.3mg; KH_2PO_4=186µg; NaCl=3.85mg; KCl=100µg; $MgCl_2$=50µg	Liquid in vial		
cH5/1N1 IIV + $AS03_A$-like*	cH5/1N1	HA head, A/Vietnam/1203/2004 (H5N1); HA stalk, A/California/04/2009 (H1N1); NA, A/California/04/2009 (H1N1); HA=15µg	Freeze-dried pellet	0.5 mL**	1
	AS03	Emulsion containing tocopherol, tocopherol=47.44mg/ml	Whitish to yellowish homogenous milky liquid emulsion in multi-dose vial		
	PBS	Na_2HPO_4=1.3mg; KH_2PO_4=186µg; NaCl=3.85mg; KCl=100µg; $MgCl_2$=50µg	Liquid in vial		
cH5/1N1 IIV	cH5/1N1	HA head, A/Vietnam/1203/2004 (H5N1); HA stalk, A/California/04/2009 (H1N1); NA, A/California/04/2009 (H1N1); HA=15µg	Freeze-dried pellet	0.5 mL***	1
	PBS	Na_2HPO_4=1.3mg; KH_2PO_4=186µg; NaCl=3.85mg; KCl=100µg; $MgCl_2$=50µg	Liquid in vial		
Normal saline	Normal saline	0.9% Sodium chloride solution	Liquid in vial	0.5 mL	1
PBS	PBS	Na_2HPO_4=1.3mg; KH_2PO_4=186µg; NaCl=3.85mg; KCl=100µg; $MgCl_2$=50µg	Liquid in vial	0.5 mL	1

*$AS03_A$-like will be obtained by dilution of the AS03 with PBS

**After dilution and reconstitution

***After reconstitution

5.1 cH8/1N1 LAIV

5.1.1 Product description

The cH8/1N1 LAIV was developed utilizing chimeric HA molecules that pair the globular head region of an exotic avian HA (A/mallard/Sweden/24/2002 [H8N4]) with the HA stalk region and the complete sequence of the NA molecule of a currently circulating seasonal influenza virus (A/California/04/2009 [H1N1]), and the remaining genes from the cold adapted, attenuated A/Leningrad/134/17/1957 (Len17 IDCDC-RG46D).

The LAIV was generated from plasmid DNA by reversed genetics in Vero cells and passaged in Specific Pathogen Free (SPF) embryonated chicken eggs to generate a Master Virus Bank (MVB). For GMP manufacturing of the vaccine, the master virus was further expanded in SPF eggs, and the allantoic fluid was harvested, formulated with SPG buffer, sterile filtered and filled in cryovials (SPG: 5 mM Glutamic acid, 10% sucrose in PBS, pH 7.2). Each vial contains 0.75 milliliter (mL) of a sterile solution of LAIV in allantoic fluid (approx. 20% v/v) and SPG buffer (approx. 80% v/v). Filled vials were snap frozen in liquid nitrogen and are stored at ≤-65°C (part number: 88135, batch number: 16072281). The sterile LAIV contained in the filled vials will be diluted before use in saline, following a procedure provided to the pharmacy, to generate the final product for intranasal delivery at the target titer of $10^{7.5 +/- 0.5}$ EID50 per dose (0.5 mL total).

5.1.2 Manufacturer

The cH8/1N1 LAIV was manufactured by Meridian Life Science, Memphis, Tennessee. Meridian is an ISO 9001:2008 certified, USDA licensed (9 Code of Federal Regulations [CFR]), FDA registered facility that follows all applicable portions of the Quality System Regulations (21 CFR 820) and cGMP regulations (21 CFR 210 and 211). In addition, Meridian is EC1774/2002 registered and follows Export Administration Regulations and Department of Transportation/ International Air Transport Association regulations.

5.1.3 Presentation and formulation

The cH8/1 LAIV is presented as 0.75 mL frozen concentrated liquid of IDCDC-RG46D cH8/1 in a 2 mL cryovial. Prior to administration, the vaccine will be diluted per the procedures set forth in the Pharmacy Manual. Briefly, four vials of LAIV will be thawed in a water bath and placed within a biosafety cabinet (BSC). Inside the BSC, 0.5 mL of vaccine from each vial will be removed, pooled, and mixed in a 15 mL conical tube. The study vaccine will be diluted with 9 mL of sterile saline solution. After dilution with sterile saline, one 0.5 mL dose contains cH8/1N1 LAIV titer of $10^{7.5 +/- 0.5}$ EID50. The prepared vaccine may be stored between 2°C to 8°C for up to 8 hours. For administration of the vaccine, a needle-less syringe will be prepared with 0.25 mL administered per nostril (0.5 mL total).

5.1.4 Stability and storage

cH8/1N1 LAIV must be stored at ≤-65°C prior to dilution in a safe and locked place. Access to the storage space should be limited to authorized study personnel. The storage conditions will be assessed during pre-trial monitoring visits. The storage temperature should be continuously monitored with calibrated temperature monitoring device(s) and recorded. Temperature excursions must be reported in degrees Celsius. Once the dilution process is completed, the prepared vaccine must be stored between 2°C to 8°C and administered within 8 hours.

Any temperature excursion outside the ranges specified in the Pharmacy Manual must be reported within 24 hours of site awareness. The impacted study vaccines must not be used and must be stored in quarantine at label temperature conditions until usage approval has been obtained from PATH. Refer to the Pharmacy Manual for more details on storage of the study vaccines and instructions on the temperature excursion reporting and usage decision process, packaging and accountability of the study vaccines/products.

5.2 cH8/1N1 IIV

5.2.1 Product description

The cH8/1N1 IIV is an inactivated split virion influenza A virus vaccine bearing chimeric HA molecules that pair the globular head region of an exotic avian HA (A/mallard/Sweden/24/2002 [H8N4]) with the HA stalk region of a currently circulating seasonal influenza virus (A/California/04/2009 [H1N1]), and the relevant NA from the seasonal influenza virus (A/California/04/09 [H1N1]). After filling 3mL vials with 0.5 mL of formulated vaccine, the vaccine is freeze dried, sealed, and stored at 2°C to 8°C.

5.2.2 Manufacturer

The cH8/1N1 IIV is manufactured by GSK in Dresden, Germany.

5.2.3 Presentation and formulation

cH8/1N1 IIV for intramuscular injection, will be supplied in a 0.5mL dose as a sterile, lyophilized powder in 3mL glass vials. At the time of use, the vaccine will be reconstituted with either PBS for the unadjuvanted group or $AS03_A$-like for the adjuvanted group. Each reconstituted 0.5 mL dose contains 15µg split HA.

5.2.4 Stability and storage

cH8/1N1 IIV must be stored at 2°C to 8°C prior to reconstitution in a safe and locked place. Access to the storage space should be limited to authorized study personnel. The storage conditions will be assessed during pre-trial monitoring visits. The storage temperature should be continuously monitored with calibrated temperature monitoring device(s) and recorded. Temperature excursions must be reported in degree Celsius. Once the reconstitution process is completed, the

prepared vaccine should be kept at room temperature for at least 15 minutes. cH8/1N1 IIV must be administered within one hour of reconstitution/preparation.

Any temperature excursion outside the ranges specified in the Pharmacy Manual must be reported within 24 hours of site awareness. The impacted study vaccines must not be used and must be stored in quarantine at label temperature conditions until usage approval has been obtained from PATH. Refer to the Pharmacy Manual for more details on storage of the study vaccines and instructions on the temperature excursion reporting and usage decision process, packaging and accountability of the study vaccines/products.

5.3 cH5/1N1 IIV

5.3.1 Product description

The cH5/1N1 IIV is an inactivated split virion influenza A virus vaccine bearing chimeric HA molecules that pair the globular head region of an exotic avian HA (A/Vietnam/1203/2004 [H5N1]) with the HA stalk region of a currently circulating seasonal influenza virus (A/California/04/2009 [H1N1]), and the relevant NA from the seasonal influenza virus (A/California/04/09 [H1N1]). After filling 3 mL vials with 0.5 mL of formulated vaccine, the vaccine is freeze dried, sealed, and stored at 2°C -8°C.

5.3.2 Manufacturer

The cH5/1N1 IIV is manufactured by GSK in Dresden, Germany.

5.3.3 Presentation and formulation

cH5/1N1 for intramuscular injection, will be supplied in a 0.5 mL dose as a sterile, lyophilized powder in 3 mL glass vials. At the time of use, the vaccine will be reconstituted with either PBS for the unadjuvanted group or $AS03_A$-like for the adjuvanted group. Each reconstituted 0.5 mL dose contains 15µg split HA.

5.3.4 Stability and storage

cH5/1N1 IIV must be stored at 2°C-8°C prior to reconstitution in a safe and locked place. Access to the storage space should be limited to authorized study personnel. The storage conditions will be assessed during pre-trial monitoring visits. The storage temperature should be continuously monitored with calibrated temperature monitoring device(s) and recorded. Temperature excursions must be reported in degree Celsius. Once the reconstitution process is completed, the prepared vaccine should be kept at room temperature for at least 15 minutes. cH5/1N1 IIV must be administered within one hour of reconstitution/preparation.

Any temperature excursion outside the ranges specified in the Pharmacy Manual must be reported within 24 hours of site awareness. The impacted study vaccines must not be used and must be stored in quarantine at label temperature conditions until usage approval has been

obtained from PATH. Refer to the Pharmacy Manual for more details on storage of the study vaccines and instructions on the temperature excursion reporting and usage decision process, packaging and accountability of the study vaccines/products.

5.4 AS03

For study vaccines adjuvanted with AS03, preparation of the $AS03_A$-like adjuvant through dilution of AS03 (presented in multi-dose vials) with the accompanying PBS diluent is required prior to mixing with the investigational flu freeze-dried cake for reconstitution. After reconstitution (as described in the Pharmacy Manual), the individual dose of the appropriate study vaccine will be withdrawn into a syringe using aseptic technique. The needles used for vial withdrawal are to be disposed of and replaced by new needles for intramuscular (IM) injection.

5.5 Dose preparation and administration

Dose preparation, as described in the Pharmacy Manual, will be carried out by a qualified unblinded research pharmacist and witnessed by another un-blinded study staff member. Investigational product will be dispensed in a masked syringe, labeled with subject number, and administered by an unblinded study staff member.

Investigational product is prepared at the research pharmacy at each of the trial sites. The prepared LAIV vaccine may be stored in the research pharmacy refrigerator (2°C to 8°C) for up to 8 hours after preparation prior to administration. The prepared IIV must be used within 1 hour of preparation.

Dosage and administration of study vaccines by study group and visit are shown in Table 5.

Table 5 Dosage and administration

Type of contact and time point	Study group	Treatment name	Volume to be administered	Route	Site	
					Location	Laterality
Visit 03 (Day 1)	Group 1	cH8/1N1 LAIV	0.25 mL* (0.5 mL total)	(Intranasal) IN	Nares	Bilateral
	Group 2					
	Group 3	Normal Saline	0.25 mL* (0.5 mL total)	IN	Nares	Bilateral
	Group 4	cH8/1N1 IIV + $AS03_A$-like	0.5 mL*	IM	Deltoid	Non-Dominant**
	Group 5	PBS	0.5 mL	IM	Deltoid	Non-Dominant**
Visit 11	Group 1	cH5/1N1 IIV + $AS03_A$-like	0.5 mL*	IM	Deltoid	Non-Dominant**
	Group 2	cH5/1N1 IIV	0.5 mL*	IM	Deltoid	Non-Dominant**
	Group 3	PBS	0.5 mL	IM	Deltoid	Non-Dominant**
	Group 4	cH5/1N1 IIV + $AS03_A$-like	0.5 mL*	IM	Deltoid	Non-Dominant**
	Group 5	PBS	0.5 mL	IM	Deltoid	Non-Dominant**

*After reconstitution

**The non-dominant arm is the preferred arm of injection. In case it is not possible to administer the vaccine in the non-dominant arm, an injection in the dominant arm may be performed.

5.6 Contraindications to subsequent vaccination

The following events constitute absolute contraindications to further administration of any study vaccines. If any of these events occur during the study, the subject must not receive additional doses of vaccine but may continue other study procedures at the discretion of the investigator:

- Anaphylaxis following the administration of vaccine(s).
- Pregnancy.
- Any condition that in the judgment of the investigator would make intramuscular injection unsafe.
- Ineligibility (either arising during the trial or retrospectively having been overlooked at screening).
- Significant non-compliance with treatment regimen or trial requirements.
- Occurrence of a new pIMD or the exacerbation of an existing pIMD. Refer to Table 20 for the definition of pIMDs.
- Discovery of any health condition which, in the investigator's opinion, places the subject at increased risk from receiving further study vaccines dose(s); or discovery of a change in the subject's health status which make him/her unable to comply with protocol-mandated safety follow-up.
- Hypersensitivity to the active substances or to any of the excipients or to any component that may be present as traces.

The following events constitute contraindications to administration of any study vaccine at that point in time; if any of these events occur at the time scheduled for vaccination, the subject may be vaccinated at a later date, within the time window specified in the protocol, or withdrawn at the discretion of the investigator:

- Acute disease and/or fever at the time of vaccination.
    - Fever for this purpose is defined as temperature ≥ 38.0°C or 100.4°F. The preferred location for measuring temperature in this study will be the oral cavity.

5.7 Warnings and precautions

None.

5.8 Accountability and disposal

The site pharmacist is required to maintain complete records of all study products received from the Sponsor and will be responsible for maintaining an accurate record of the randomization codes, inventory, and an accountability record of vaccine/s, adjuvant, and placebo supplies for this study. The site pharmacist will also be responsible for ensuring the security of these documents. Partially used vials will not be used for human administration or for in vitro experimental studies. At the end of the study, the site will receive instruction from the Sponsor regarding the final disposition of any remaining study products.

5.9 Concomitant medications/products/vaccines that may lead to the elimination of a subject from Per-Protocol analyses

The use of the following concomitant medications/products/vaccines will not require withdrawal of the subject from the study but may determine a subject's evaluability in the Per-Protocol analysis:

Any investigational or non-registered product (drug or vaccine) other than the study vaccine(s)/product(s) used during the study period.

- Immunosuppressants or other immune-modifying drugs administered chronically (i.e., more than 14 days in total) during the study period. For corticosteroids, this will mean prednisone ≥ 20 mg/day, or equivalent. For high-dose inhaled corticosteroids this will mean beclomethasone dipropionate chlorofluorocarbon ≥ 840 mcg/day, or equivalent. Topical steroids are allowed.

- Long-acting immune-modifying drugs administered at any time during the study period (e.g., infliximab, rituximab).

- A vaccine not foreseen by the study protocol administered during the period starting 30 days before the first dose (Visit 03) up to the blood sampling at Visit 13. In case an emergency mass vaccination for an unforeseen public health threat (e.g., a pandemic) is organized by the public health authorities, outside the routine immunization program, the time period described above can be reduced if necessary for that vaccine provided it is licensed and used according to its Prescribing Information and according to the local governmental recommendations and provided a written approval of PATH is obtained.

- Seasonal influenza vaccine administered at any time during the study period.

- Immunoglobulins and/or any blood products administered during the study period.

- Drug and/or alcohol abuse.

5.10 Intercurrent medical conditions that may lead to the elimination of a subject from Per-Protocol analyses

Subjects may be eliminated from the Per-Protocol cohort for immunogenicity if, during the study, they incur a condition that has the capability of altering their immune response or are confirmed to have an alteration of their initial immune status (i.e., if any confirmed or suspected immunosuppressive or immunodeficient condition appears during the study period). These conditions would include any cases of lab-confirmed influenza-like illnesses.

6 STUDY SCHEDULE AND PROCEDURES

It is the responsibility of the investigator to ensure only medically qualified study staff perform medical assessments and procedures which require medical training. Results of assessments should be reviewed with the subject, as appropriate, in a timely manner. It is also the responsibility of the investigator to ensure strict observance of the intervals between visits/procedures. These intervals are precisely defined for subjects in accordance with the protocol and are reflected in the study schedule outlined below. Where not specified, time windows (for example, plus or minus a certain number of days) are not allowed.

Table 6 Intervals between study visits

Interval	Optimal length of interval	Allowed interval
Visit 00 to Visit 03	7-56	2*-56
Visit 03 → Visit 05	2 days	2 days
Visit 03 → Visit 09	7 days	7-10 days
Visit 03 → Visit 10	28 days	28-38 days
Visit 03 → Visit 11	84 days	84-94 days
Visit 11 → Visit 12	7 days	7-10 days
Visit 11 → Visit 13	28 days	28-38 days**
Visit 11 → Visit 14	168 days	168-196 days**
Visit 11 → Visit 15	336 days	336-364 days**
Visit 15 → Visit 16	504 days	504-532 days**

*Subjects should be screened no later than 2 days prior to Day 1 to allow time to complete screening.

**Subjects may not be eligible for inclusion in the Per-Protocol set for post-booster immunogenicity analysis for the specified interval if blood samples are collected outside this interval.

6.1 Study visits

Prior to any screening activities, study staff will review the informed consent process with the volunteer. The investigator, or a person designated by the investigator, will fully inform the volunteer of all pertinent aspects of the study and individual consent will be documented by a signature. The subject will be required to pass a written exam about the study prior to any study

vaccination. Subjects will be screened through multiple procedures, including medical history review, physical examination, and clinical laboratory testing.

6.1.1 Visit 00: Screening visit (up to 56 days prior to Dose 1)

After subject consents, the below activities will occur. Sites may complete screening procedures over two visits (completed at Enrollment Visit, Visit 01, as is standard practice for each site). Final confirmation of eligibility, enrollment, and randomization may only occur at the Enrollment Visit.

1. The subject will be assigned a screening identification number.

2. The subject will be interviewed to collect demographic data.

3. The subject will be interviewed and any available medical records will be reviewed to collect a comprehensive medical history, including details of previous vaccinations and reaction to such vaccinations, and history of any chronic or recurrent medical and psychiatric conditions. Females will be interviewed to determine childbearing potential, and if of childbearing potential, will be interviewed as to use of adequate contraception. Males will be interviewed as to surgical sterility, and if not, as to the use of adequate contraception.

4. Collected information will be reviewed to confirm eligibility prior to conduct of further procedures.

5. Blood (serum and whole) specimens will be collected for clinical laboratory testing of biochemical and hematological parameters. For any subject with a clinical laboratory parameter out of local reference range, one retest may be performed for that parameter. Results of clinical laboratory tests should be reviewed with the subject.

6. Serum specimens will also be collected for testing of HIV infection. Results of serologic testing for HIV infection will also be reviewed with the subject with appropriate post-test counseling (and referral provided to any subjects testing HIV positive).

7. A urine specimen will be collected for drug screening (opioids).

8. A urine (or serum, as required by site) specimen will be collected for a pregnancy test if the subject is a female of childbearing potential.

9. A targeted physical examination of the subject will be performed for any signs or symptoms experienced by the subject.

10. Vital signs, height and weight will be measured.

6.1.2 Visit 01: Enrollment Visit (up to 2 days prior to Dose 1)

1. At the second screening visit, the subject will be queried regarding any new medical events since medical histories were recorded and eligibility will be confirmed prior to conduct of

further procedures, including a complete physical examination. If the subject is a female of childbearing potential or non-sterile male, they will be interviewed as to continued use of adequate contraception.

2. Results of all assessments will be reviewed to confirm eligibility. If the subject is eligible to continue in the study, the subject will be assigned a subject identification number and considered enrolled.

3. The subject will be randomized per the pre-defined procedure and informed as to whether he/she will be an inpatient or outpatient subject for Dose 1.

4. The subject will be informed as to when he/she must return to the inpatient clinical isolation unit for Visit 02 or to the outpatient clinic for Visit 03.

6.1.3 Initial Visits for LAIV-IIV (or placebo) arms (Groups 1, 2, & 3)

The following Visits 02-08 and accompanying procedures apply only to those subjects randomized to receive study LAIV (or placebo) at Dose 1 (Groups 1, 2, & 3)

Subjects randomized to receive study IIV (or placebo) at Dose 1 (Groups 4 & 5) will have a similar Visit 03 (Section 6.1.4). Subsequent visits will be common to subjects in all study groups.

At the first site to begin dosing subjects in Groups 1, 2, & 3, six subjects will enter the unit together one day prior to the remaining subjects in the LAIV-IIV treatment arms. Thus, the following schedule of events will be staggered by a day for a portion of the subjects in these three study groups at that site. At the second site to begin dosing subjects in Groups 1, 2 & 3, all subjects will enter the unit a day prior to receipt of Dose 1.

6.1.3.1 Visit 02: Day of admission into the isolation unit (Day -1)

1. The subject will be admitted to the inpatient clinical isolation unit at least 18 hours prior to receipt of Dose 1 of study LAIV (or placebo).

2. A targeted physical examination (including vital signs) will be performed for any signs or symptoms experienced by the subject.

3. A nasal and OP swab specimen will be collected for testing for virologic evidence of influenza A infection (to ensure the subject is not incubating wild-type influenza virus). Specimen collection information must be documented in the eCRF and in specimen collection logs.

4. Blood specimens will also be collected for biochemistry and hematology. Results need not be obtained and reviewed prior to administration of study vaccine or placebo; these results will only serve to define baseline status for subjects.

5. Serum will be collected for humoral immunologic assays, saliva will be collected for mucosal immunologic assays, and whole blood will be collected for cell-mediated immunologic and transcriptomic assays.

6. If the subject is a female of childbearing potential, she will be interviewed as to continued use of adequate contraception, and a urine specimen will be collected for a pregnancy test. If the subject is a non-sterile male, he will be interviewed as to continued use of adequate contraception.

7. The subject will be observed for at least 18 hours for the development of acute respiratory illness (ARI) for an infection the subject may already have.

8. If the subject develops signs or symptoms of ARI, a nasal and OP specimen will be collected for testing of etiology by multiplex PCR and the subject will be discharged from the inpatient unit and excluded from receipt of Dose 1 of study LAIV (or placebo).

6.1.3.2 Visit 03: Day of receipt of study LAIV (Day 1)

Prior to dosing of any subject, results of virologic testing of nasal and oropharyngeal (OP) swab specimens for influenza A must be reviewed by the investigator. No subject may be dosed if any subject tests influenza A positive by RT-PCR. If any subject tests influenza A positive, the Sponsor should be alerted immediately and the study will be terminated for all subjects in Groups 1, 2, and 3 at the site.

For the first 6 subjects at the first site to begin dosing, the next subject cannot be administered study LAIV (or placebo) until the previous subject has been observed for at least 60 minutes post-vaccination. The following day at this first site, after the first 6 subjects have received study LAIV (or placebo), the remaining subjects receiving study LAIV (or placebo) will be vaccinated as convenient. At the second site to begin dosing, subjects will be vaccinated with study LAIV (or placebo), also as convenient.

1. Vital signs will be collected from the subject. A targeted physical examination will be performed for any signs or symptoms experienced by the subject.

2. The subject will be administered one dose of study vaccine or placebo per the allocation sequence.

3. The subject will be observed for at least 60 minutes after administration in case of any immediate adverse reactions. If the subject experiences an immediate adverse reaction, he/she will be treated and the event will be recorded in the eCRF. ***If the event meets a pause rule event definition 1a-1d, dosing of remaining subjects may not proceed.***

4. The subject will be instructed on the use of a diary card for solicited and unsolicited reactions and requested to document signs and symptoms, including oral temperature, he/she may be experiencing each day on the diary card.

5. If the subject develops an MAE, pIMD, SAE, or pregnancy, the event will be documented on the case report form (CRF), and an expedited reporting form must be completed, if required. ***If the event meets a pause rule event definition 1a-1d, dosing of remaining subjects may not proceed.***

6.1.3.3 Visit 04: First day after receipt of study LAIV (Day 2)

1. Each morning a nasal and OP swab specimen will be collected. Specimen collection information must be documented on the CRF and in specimen collection logs.

2. Subject diary card completion will be reviewed.

3. A targeted physical examination (including vital signs) will be performed for any signs or symptoms experienced by the subject. Subjects will be encouraged to spontaneously report any concern or symptom they are experiencing.

4. If the subject develops an MAE, pIMD, SAE, or pregnancy, the event will be documented on the CRF, and an appropriate expedited reporting form must be completed, if required. ***If the event meets a pause rule event definition 1a-1d, dosing of remaining subjects may not proceed.***

6.1.3.4 Visit 05: Second day after receipt of study LAIV (Day 3)

1. Each morning a nasal and OP swab specimen will be collected. Specimen collection information must be documented on the CRF and in specimen collection logs.

2. Subject diary card completion will be reviewed.

3. A targeted physical examination (including vital signs) will be performed for any signs or symptoms experienced by the subject. Subjects will be encouraged to spontaneously report any concern or symptom they are experiencing.

4. Whole blood will be collected for transcriptomic assays.

5. If the subject develops an MAE, pIMD, SAE, or pregnancy, the event will be documented on the CRF, and an appropriate expedited reporting form must be completed, if required. ***If the event meets a pause rule event definition 1a-1d, dosing of remaining subjects may not proceed.***

6.1.3.5 Visit 06: Third day after receipt of study LAIV (Day 4)

1. Each morning a nasal and OP swab specimen will be collected. Specimen collection information must be documented on the CRF and in specimen collection logs.

2. Subject diary card completion will be reviewed.

3. A targeted physical examination (including vital signs) will be performed for any signs or symptoms experienced by the subject. Subjects will be encouraged to spontaneously report any concern or symptom they are experiencing.

4. If the subject develops an MAE, pIMD, SAE, or pregnancy, the event will be documented on the CRF, and an appropriate expedited reporting form must be completed, if required. ***If the event meets a pause rule event definition 1a-1d, dosing of remaining subjects may not proceed.***

6.1.3.6 Visit 07: Fourth day after receipt of study LAIV (Day 5)

1. Each morning a nasal and OP swab specimen will be collected. Specimen collection information must be documented on the CRF and in specimen collection logs.

2. Subject diary card completion will be reviewed.

3. A targeted physical examination (including vital signs) will be performed for any signs or symptoms experienced by the subject. Subjects will be encouraged to spontaneously report any concern or symptom they are experiencing.

4. If the subject develops an MAE, pIMD, SAE, or pregnancy, the event will be documented on the CRF, and an appropriate expedited reporting form must be completed, if required. ***If the event meets a pause rule event definition 1a-1d, dosing of remaining subjects may not proceed.***

6.1.3.7 Visit 08: Day of discharge after receipt of study LAIV (Day 6)

Only subjects influenza A negative by RT-PCR on Days 4, 5, and 6 may be discharged on Day 6. Subjects testing influenza A positive on Days 4, 5 or 6 will be offered anti-neuraminidase treatment on Day 6. Any subject influenza A positive by RT-PCR on Days 4, 5, or 6 must remain in the inpatient clinical isolation unit until 3 consecutive daily nasal and OP swab specimens collected over 48 hours (3 daily time points) all test influenza negative or until 24 hours after the subject initiates anti-neuraminidase treatment and the last nasal and OP swab specimen prior to discharge tests influenza A negative by RT-PCR.

1. In the morning, a nasal and OP swab specimen will be collected. Specimen collection information must be documented on the CRF and in specimen collection logs.

2. Subject diary card completion will be reviewed.

3. A targeted physical examination (including vital signs) will be performed for any signs or symptoms experienced by the subject. Subjects will be encouraged to spontaneously report any concern or symptom they are experiencing.

4. Any MAE, pIMD, SAE, or pregnancy will be documented on the CRF and an appropriate expedited reporting form must be completed, if required. ***If the event meets a pause rule event definition 1a-1d, dosing of remaining subjects may not proceed.***

5. The subject will be asked to continue diary card completion until their next study visit.

6. The subject will be instructed that if he/she later experiences any illness for which he/she seeks medical care, the subject should inform the health care provider(s) of participation in this study, provide the health care provider(s) with contact information for the investigator, and separately contact the investigator to inform the investigator of the medically attended illness.

7. Day 4, 5, and 6 influenza A virologic testing results for the subject will be reviewed by a designated unblinded member of the site team. If all results are negative, the designated member of the site team will inform the clinical team that the subject may be discharged.

   If any result in the 2 days prior (Day 4 or Day 5) to or on the day of planned discharge (Day 6) is positive, the subject will be requested to remain in the inpatient clinical isolation unit until either a) three daily consecutive swab specimens taken over a 48 hour period all test influenza A negative by RT-PCR or b) the subject agrees to initiation of anti-neuraminidase treatment—oseltamivir (75 mg taken orally twice daily for 5 days) or zanamivir (10 mg taken by oral inhalation twice daily for 5 days)—on Day 6, 24 hours after which point the subject may be discharged, as long as the last nasal and OP swab specimen prior to discharge tested influenza A negative by RT-PCR. Subjects whose nasal and OP swab specimen from Day 6 (or thereafter) tests positive but who decline anti-neuraminidase treatment may be discharged only after three subsequent negative nasal and OP swab specimens (3 negative tests over 48 elapsed hours). Subjects who agree to initiate anti-neuraminidase treatment will still be requested to return to the clinical daily for nasal and OP swab collection until 3 daily nasal and OP swab specimens test negative over 48 elapsed hours in order to document loss of detectable virus.

8. For subjects testing negative for influenza A, the subject will be discharged from the isolation unit and informed of the scheduled date of return to the outpatient clinic for Visit 09.

6.1.4 Initial Visits for IIV-IIV (or placebo) arms (Groups 4 & 5)

The following visits and accompanying procedures apply only to those subjects randomized to receive IIV (or placebo) at Dose 1 (Groups 4 & 5).

Subsequent visits will be common to subjects in all study groups.

6.1.4.1 Visit 03: Day of receipt of study IIV (Day 1)

At each site, preference will be to schedule administration of Dose 1 to all subjects in IIV-IIV arms on the same day.

1. Vital signs will be collected from the subject. A targeted physical examination will be performed for any signs or symptoms experienced by the subject.

2. Blood specimens will be collected for biochemistry and hematology. Results need not be obtained and reviewed prior to administration of study vaccine or placebo; these results will only serve to define baseline status for subjects.

3. Serum will be collected for humoral immunologic assays, saliva will be collected for mucosal immunologic assays, and whole blood will be collected for cell-mediated immunologic and transcriptomic assays.

4. If the subject is a female of childbearing potential, she will be interviewed as to continued use of adequate contraception, and a urine specimen will be collected for a pregnancy test. If the subject is a non-sterile male, he will be interviewed as to continued use of adequate contraception.

5. The subject will be administered one dose of study vaccine or placebo per the allocation sequence.

6. The subject will be observed for at least 60 minutes after administration in case of any immediate reactions. If the subject experiences an immediate adverse reaction, he/she will be treated and the event will be recorded on the eCRF. **If the event meets a pause rule event definition 1a-1d, dosing of remaining subjects may not proceed.**

7. The subject will be instructed on the use of a diary card for solicited and unsolicited reactions and requested to document signs and symptoms, including oral temperature, he/she may be experiencing each day on the diary card.

8. The subject will be instructed that if the subject later experiences any illness for which they seek medical care, the subject should inform the health care provider(s) of participation in this study, provide the health care provider(s) with contact information for the investigator, and separately contact the investigator to inform the investigator of the medically attended illness.

9. The subject will be informed of the scheduled date of return to the outpatient clinic for Visit 05.

6.1.4.2 Visit 05: Second day after receipt of study IIV (Day 3)

1. Subject diary card completion will be reviewed.

2. A targeted physical examination (including vital signs) will be performed for any signs or symptoms experienced by the subject. Subjects will be encouraged to spontaneously report any concern or symptom they are experiencing.

3. Whole blood will be collected for transcriptomic assays.

4. If the subject develops an MAE, pIMD, SAE, or pregnancy, the event will be documented on the CRF, and an appropriate expedited reporting form must be completed, if required. ***If the event meets a pause rule event definition 1a-1d, dosing of remaining subjects may not proceed.***

5. The subject will be asked to continue diary card completion until their next study visit.

6. The subject will be instructed that if the subject later experiences any illness for which they seek medical care, the subject should inform the health care provider(s) of participation in this study, provide the health care provider(s) with contact information for the investigator, and separately contact the investigator to inform the investigator of the medically attended illness.

7. The subject will be informed of the scheduled date of return to the outpatient clinic for Visit 09.

6.1.5 Subsequent Visits for all study subjects (Groups 1, 2, 3, 4, & 5)

This and all following Visits and accompanying procedures apply to all study subjects (in any of the 5 treatment arms.

6.1.5.1 Visit 09: 7 + 2 days post-Dose 1 (post-Visit 03)

1. The subject will be interviewed regarding any recent medical events since the last visit, including study vaccine administration site reactions. If the subject is a female of childbearing potential, she will be interviewed as to continued use of adequate contraception and occurrence of pregnancy. If the subject is a non-sterile male, he will be interviewed as to continued use of adequate contraception and occurrence of pregnancy in a female partner.

2. Any MAE, pIMD, SAE, or pregnancy will be documented on the CRF, and an expedited reporting form must be completed, if required.

3. The subject diary card will be collected and reviewed with the subject. Diary card information will be transferred to the eCRF. The subject will be given an unsolicited AE diary card to complete and return at the next visit.

4. A targeted physical examination (including vital signs) will be performed for any signs or symptoms experienced by the subject.

5. Blood (serum and whole) specimens will be collected for biochemistry and hematology.

6. Whole blood will be collected for cell-mediated immunologic and transcriptomic assays.

7. The subject will be instructed that if he/she later experiences any illness for which he/she seeks medical care, he/she should inform the health care provider(s) of participation in this study, provide the health care provider(s) with contact information for the investigator, and separately contact the investigator to inform the investigator of the medically attended illness.

8. The subject will also be instructed that if he/she later experiences symptoms of ILI (fever [oral temperature, ≥ 37.8°C or 100°F] or myalgia AND cough or sore throat), he/she should contact the investigator. The subject will be requested to present to the study outpatient

clinic as soon as possible for an unscheduled ILI visit at which a nasal and OP swab specimen will be collected for testing for seasonal influenza virus by RT-PCR.

9. The subject will be informed of the scheduled date of return to the outpatient clinic for Visit 10.

6.1.5.2 Visit 10: 28 + 10 days post-Dose 1 (post-Visit 03)

1. The subject will be interviewed regarding any recent medical events since the last visit. If the subject is a female of childbearing potential, she will be interviewed as to continued use of adequate contraception and occurrence of pregnancy. If the subject is a non-sterile male, he will be interviewed as to continued use of adequate contraception and occurrence of pregnancy in a female partner.

2. Any MAE, pIMD, SAE, or pregnancy will be documented on the CRF, and an appropriate expedited reporting form must be completed, if required.

3. Subject diary card will be collected and reviewed with the subject. Diary card information will be transferred to the eCRF by the study clinician. The subject will be given an unsolicited AE diary card to complete and return at the next visit.

4. A targeted physical examination (including vital signs) will be performed for any signs or symptoms experienced by the subject.

5. Blood (serum and whole) specimens will be collected for biochemistry and hematology.

6. Serum will be collected for humoral immunologic assays, saliva will be collected for mucosal immunologic assays, and whole blood will be collected for cell-mediated immunologic and transcriptomic assays.

7. The subject will be instructed that if he/she later experiences any illness for which he/she seeks medical care, the subject should inform the health care provider(s) of participation in this study, provide the health care provider(s) with contact information for the investigator, and separately contact the investigator to inform the investigator of the medically attended illness.

8. The subject will also be instructed that if he/she later experiences symptoms of ILI (fever [oral temperature, ≥ 37.8°C or 100°F] or myalgia AND cough or sore throat), he/she should contact the investigator. The subject will be requested to present to the study outpatient clinic as soon as possible for an unscheduled ILI visit at which a nasal and OP swab specimen will be collected for testing for seasonal influenza virus by RT-PCR.

9. The subject will be informed of the scheduled date of return to the outpatient clinic for Visit 11.

6.1.5.3 Visit 11: 84 + 10 days post-Dose 1 (post-Visit 03)

The following procedures apply to all subjects in all treatment arms. At each site, Dose 2 will be administered in a staggered manner. Initially, at each site, one half of the subjects from those who received LAIV or from those who received IIV on Day 1 may be vaccinated. Once 5 days have passed following the vaccination of the initial half of subjects at each site and documentation that there has been no significant Investigator clinical concerns with regard to any unexpected severity of solicited adverse events having been reported from any of these subjects to the site, then the remaining subjects may be vaccinated. This would imply that one half of the subjects at each site could not be scheduled any earlier than study Day 91 for their Visit 11.

1. The subject will be interviewed regarding any recent medical events since the last visit. If the subject is a female of childbearing potential, she will be interviewed as to continued use of adequate contraception and occurrence of pregnancy. If the subject is a non-sterile male, he will be interviewed as to continued use of adequate contraception and occurrence of pregnancy in a female partner.

2. Any MAE, pIMD, SAE, or pregnancy will be documented on the CRF, and an appropriate expedited reporting form must be completed, if required.

3. Subject diary card will be collected and reviewed with the subject. Diary card information will be transferred to the eCRF by the study clinician.

4. Vital signs will be collected from the subject. A targeted physical examination will be performed for any signs or symptoms experienced by the subject.

5. Blood specimens will be collected for biochemistry and hematology. Results need not be obtained and reviewed prior to administration of study vaccine or placebo; these results will only serve to define baseline status for subjects pre-Dose 2.

6. Serum will be collected for humoral immunologic assays, saliva will be collected for mucosal immunologic assays, and whole blood will be collected for cell-mediated immunologic and transcriptomic assays.

7. If the subject is a female of childbearing potential, a urine specimen will be collected for a pregnancy test.

8. The subject will be administered one dose of study vaccine or placebo per the allocation sequence.

9. The subject will be observed for at least 60 minutes after administration in case of any immediate reactions. If the subject experiences an immediate adverse reaction, he/she will be treated and the event will be recorded on the CRF. ***If the event meets a pause rule event definition 1a-1d, dosing of remaining subjects may not proceed.***

10. The subject will be instructed on the use of a diary card for solicited and unsolicited reactions and requested to document signs and symptoms, including oral temperature, he/she may be experiencing each day on the diary card.

11. The subject will be instructed that if the subject later experiences any illness for which they seek medical care, he/she should inform the health care provider(s) of participation in this study, provide the health care provider(s) with contact information for the investigator, and separately contact the investigator to inform the investigator of the medically attended illness.

12. The subject will also be instructed that if he/she later experiences symptoms of ILI (fever [oral temperature, ≥ 37.8°C or 100°F] or myalgia AND cough or sore throat), he/she should contact the investigator. The subject will be requested to present to the study outpatient clinic as soon as possible for an unscheduled ILI visit at which a nasal and OP swab specimen will be collected for testing for seasonal influenza virus by RT-PCR.

13. The subject will be informed of the scheduled date of return to the outpatient clinic for Visit 12.

6.1.5.4 Visit 12: 7 + 3 days post-Dose 2 (post-Visit 11)

1. The subject will be interviewed regarding any recent medical events since the last visit. If the subject is a female of childbearing potential, she will be interviewed as to continued use of adequate contraception and occurrence of pregnancy. If the subject is a non-sterile male, he will be interviewed as to continued use of adequate contraception and occurrence of pregnancy in a female partner.

2. Any MAE, pIMD, SAE, or pregnancy will be documented on the CRF, and an appropriate expedited reporting form must be completed, if required.

3. The subject diary card will be collected and reviewed with the subject. Diary card information will be transferred to the eCRF. The subject will be given an unsolicited AE diary card to complete and return at the next visit.

4. A targeted physical examination (including vital signs) will be performed for any signs or symptoms experienced by the subject.

5. Blood (serum and whole) specimens will be collected for biochemistry and hematology.

6. Whole blood will be collected for cell-mediated immunologic and transcriptomic assays.

7. The subject will be instructed that if he/she later experiences any illness for which he/she seeks medical care, he/she should inform the health care provider(s) of participation in this study, provide the health care provider(s) with contact information for the investigator, and separately contact the investigator to inform the investigator of the medically attended illness.

8. The subject will also be instructed that if he/she later experiences symptoms of ILI (fever [oral temperature, ≥ 37.8°C or 100°F] or myalgia AND cough or sore throat), he/she should contact the investigator. The subject will be requested to present to the study outpatient clinic as soon as possible for an unscheduled ILI visit at which a nasal and OP swab specimen will be collected for testing for seasonal influenza virus by RT-PCR.

9. The subject will be informed of the scheduled date of return to the outpatient clinic for Visit 13.

6.1.5.5 Visit 13: 28 + 10 days post-Dose 2 (post-Visit 11)

1. The subject will be interviewed regarding any recent medical events since the last visit. If the subject is a female of childbearing potential, she will be interviewed as to continued use of adequate contraception and occurrence of pregnancy. If the subject is a non-sterile male, he will be interviewed as to continued use of adequate contraception and occurrence of pregnancy in a female partner.

2. Any MAE, pIMD, SAE, or pregnancy will be documented on the CRF, and an appropriate expedited reporting form must be completed, if required.

3. The subject diary card will be collected and reviewed with the subject. Diary card information will be transferred to the eCRF. The subject will be given an unsolicited AE diary card to complete and return at the next visit.

4. A targeted physical examination (including vital signs) will be performed for any signs or symptoms experienced by the subject.

5. Blood (serum and whole) specimens will be collected for biochemistry and hematology.

6. Serum will be collected for humoral immunologic assays, saliva will be collected for mucosal immunologic assays, and whole blood will be collected for cell-mediated immunologic and transcriptomic assays.

7. The subject will be instructed that if he/she later experiences any illness for which he/she seeks medical care, he/she should inform the health care provider(s) of participation in this study, provide the health care provider(s) with contact information for the investigator, and separately contact the investigator to inform the investigator of the medically attended illness.

8. The subject will also be instructed that if he/she later experiences symptoms of ILI (fever [oral temperature, ≥ 37.8°C or 100°F] or myalgia AND cough or sore throat), he/she should contact the investigator. The subject will be requested to present to the study outpatient clinic as soon as possible for an unscheduled ILI visit at which a nasal and OP swab specimen will be collected for testing for seasonal influenza virus by RT-PCR.

9. The subject will be informed of the scheduled date of return to the outpatient clinic for Visit 14.

6.1.5.6 Visit 14: 168 + 28 days post-Dose 2 (post-Visit 11)

1. The subject will be interviewed regarding any recent medical events since the last visit. If the subject is a female of childbearing potential, she will be interviewed as to continued use of adequate contraception (protocol requirement until Month 5) and occurrence of pregnancy. If the subject is a non-sterile male, he will be interviewed as to continued use of adequate contraception (protocol requirement until Month 5) and occurrence of pregnancy in a female partner.
2. Any MAE, pIMD, SAE, or pregnancy will be documented on the CRF, and an appropriate expedited reporting form must be completed, if required.
3. The subject diary card will be collected and reviewed with the subject. Diary card information will be transferred to the eCRF. The subject will be given an unsolicited AE diary card to complete and return at the next visit.
4. A targeted physical examination (including vital signs) will be performed for any signs or symptoms experienced by the subject.
5. Blood (serum and whole) specimens will be collected for biochemistry and hematology.
6. Serum will be collected for humoral immunologic assays and whole blood will be collected for transcriptomic assays.
7. The subject will be instructed that if he/she later experiences any illness for which he/she seeks medical care, he/she should inform the health care provider(s) of participation in this study, provide the health care provider(s) with contact information for the investigator, and separately contact the investigator to inform the investigator of the medically attended illness.
8. The subject will also be instructed that if he/she later experiences symptoms of ILI (fever [oral temperature, ≥ 37.8°C or 100°F] or myalgia AND cough or sore throat), he/she should contact the investigator. The subject will be requested to present to the study outpatient clinic as soon as possible for an unscheduled ILI visit at which a nasal and OP swab specimen will be collected for testing for seasonal influenza virus by RT-PCR.
9. The subject will be informed of the scheduled date of return to the outpatient clinic for Visit 15.

6.1.5.7 Visit 15: 336 + 28 days post-Dose 2 (post-Visit 11)

1. The subject will be interviewed regarding any recent medical events since the last visit. If the subject is a female of childbearing potential, she will be interviewed as to continued use of adequate contraception (protocol requirement until Month 5) and occurrence of pregnancy. If the subject is a non-sterile male, he will be interviewed as to continued use of adequate contraception (protocol requirement until Month 5) and as to occurrence of pregnancy in a female partner.

2. Any MAE, pIMD, SAE, or pregnancy will be documented on the CRF, and an appropriate expedited reporting form must be completed, if required.

3. The subject diary card will be collected and reviewed with the subject. Diary card information will be transferred to the eCRF.

4. A targeted physical examination (including vital signs) will be performed for any signs or symptoms experienced by the subject.

5. Blood (serum and whole) specimens will be collected for biochemistry and hematology.

6. Serum will be collected for humoral immunologic assays, saliva will be collected for mucosal immunologic assays, and whole blood will be collected for cell-mediated immunologic and transcriptomic assays.

7. The investigator, or a person designated by the investigator, will fully inform the volunteer of the study extension to include an additional visit (Visit 16). If the subject agrees to continued participation in the study, individual consent will be documented by a signature and the subject will be informed of the scheduled date of return to the outpatient clinic for Visit 16.

8. If the subject does not consent to continued participation in the study, study staff will complete the final visit CRF and the subject will be exited from the study.

6.1.5.8 Visit 16: 504 + 28 days post-Dose 2 (post-Visit 11)

1. The subject will be interviewed regarding any recent medical events since the last visit. If the subject is a female of childbearing potential, she will be interviewed as to continued use of adequate contraception (protocol requirement until Month 5) and occurrence of pregnancy. If the subject is a non-sterile male, he will be interviewed as to continued use of adequate contraception (protocol requirement until Month 5) and as to occurrence of pregnancy in a female partner.

2. Any MAE, pIMD, SAE, or pregnancy will be documented on the CRF, and an appropriate expedited reporting form must be completed, if required.

3. The subject diary card will be collected and reviewed with the subject. Diary card information will be transferred to the eCRF.

4. A targeted physical examination (including vital signs) will be performed for any signs or symptoms experienced by the subject.

5. Serum and saliva will be collected for humoral immunologic and mucosal immunologic assays, with testing dependent upon the outcome of primary and secondary analyses.

6. Study staff will complete the final visit CRF and the subject will be exited from the study.

6.1.6 ILI Visits

Beginning from Visit 09 (Day 8), all subjects will be monitored for the occurrence of laboratory-confirmed influenza like illness (see Section 8.1.6). During the typical influenza season period (November 1 through April 30) during the study, subjects will also be reminded biweekly by phone, email, or text messages to notify the investigator in case of ILI symptoms: at least 1 systemic symptom (fever [oral temperature ≥ 37.8°C or 100°F] or myalgia) AND at least 1 respiratory symptom (cough or sore throat). Subjects will be provided with a calibrated thermometer to measure temperature and an ILI diary card to record temperatures and symptoms during any ILI episode. After notifying the investigator, the subject will be asked to present for collection of a nasal and OP swab as soon as possible and no later than 5 days following the ILI onset. At this visit, the investigator will also document signs and symptoms of ILI on the eCRF. Any physician-diagnosed pneumonia, lower respiratory tract infection, bronchiolitis, bronchitis, or laryngotracheitis will also be documented. A nasal and OP swab specimen will be collected for testing for virologic evidence of seasonal influenza infection by RT-PCR. Specimen collection information must be documented in the eCRF and in specimen collection logs. Fourteen days after ILI onset, subjects will be contacted by phone to document outcome of their ILI.

6.1.7 Unscheduled visits

Subjects may present to the study center during operating hours for an unscheduled visit should they experience any other AE of concern to the subject. Data for any examinations performed on the subject at an unscheduled visit must be recorded in the CRF. If an unscheduled visit is performed, the procedures for the next following visit should not be made earlier than scheduled above.

6.2 Discontinuation of vaccination or study procedures

Participants have the right to decline study vaccinations or procedures for any reason and at any time during the study. If a subject declines further vaccination or study procedures this will be recorded as a study deviation and the reason will be clearly documented in the source document. The subject will be encouraged to complete all remaining safety related follow-up visits and procedures through Visit 16, including unscheduled ILI visits. Participants refusing to receive Dose 2 will be encouraged to complete all immunogenicity blood draws through Visit 10 if notification of such refusal occurs prior to Visit 10. (Participants refusing to receive Dose 1 will be immediately withdrawn from the study.)

Subjects will be discontinued from further vaccination if they experience an event which qualifies as a contraindication to subsequent vaccination (see Section 5.6). At each study visit subsequent to the first vaccination visit, it must be verified if the subject has experienced or is experiencing any intercurrent medical condition. Any intercurrent medical condition(s) must be recorded in the eCRF. Acute intercurrent febrile illness may delay receipt of Dose 2. Discontinuation from further vaccination may also be at the discretion of the investigator if it is in the interest of the subject or is recommended by the PSRT or IDMC based on safety reviews.

After administration of Dose 1 of study LAIV (or placebo), procedures may be discontinued for a subject who is scheduled to be in the inpatient clinical isolation unit and the subject may be removed from the unit by the investigator if the subject develops a reaction to study vaccine which the investigator believes threatens the subject's well-being and requires the subject to be transferred to a separate facility for treatment.

The reason for all discontinuations will be documented in source documents and the eCRF.

6.3 Subject withdrawal (premature termination)

After enrollment, a subject might be withdrawn from participation for various reasons, including:

- The investigator requests withdrawal of participation of the subject because the subject develops a contraindication to subsequent vaccination or is non-compliant with study procedures;
- A subject voluntarily withdraws participation at his/her own request;
- A subject is lost to follow-up; or
- The trial is terminated prematurely by PATH.

Participation in the study is strictly voluntary. Participants have the right to withdraw from the study at any time and for any reason, without penalty. Withdrawal of a subject by the investigator should occur only if the subject is identified to have a contraindication to subsequent vaccination or if continued participation of the subject threatens the subject's well-being or the integrity of the study (or the well-being of other subjects in the isolation unit).

6.3.1 Handling of withdrawals

In case of premature withdrawal for any reason, the investigator should exert his/her best effort to:

1. Review the subject's diary card if it is still in use at the time of withdrawal.
2. Update any ongoing AE/SAEs that remained ongoing at the time of the subject's last visit prior to withdrawal.
3. Conduct an interview to determine if the subject has had any reaction or AE (serious or non-serious) since the last visit. Where possible, the investigator should visibly or physically assess any reported adverse reaction or AE and document whether it led to the withdrawal.
4. Conduct a physical examination.
5. Collect blood for biochemical and hematological clinical laboratory parameters if the previous collection for these safety evaluations was greater than 21 days prior.
6. Collect blood and saliva from the subject for Visit 13 immunologic evaluations if the withdrawal occurs between 28 and 38 days post-Dose 2.

7. Document the reason for premature withdrawal on the CRF.
8. Update the subject's contact information.

PATH must be informed within 24 hours of awareness about all instances of the premature termination of a subject's participation in the trial. Any withdrawal that occurs while a subject is scheduled to be in the isolation unit requires immediate notification to the Sponsor. While participation in the trial is voluntary and subjects are free to withdraw consent at any time, subjects will be told that local public health authorities might be notified of any subject's premature exiting from the isolation unit. A standard operating procedure (SOP) for the isolation unit will detail procedures to be followed for any withdrawal that occurs while a subject is scheduled to be in the isolation unit.

6.3.2 Withdrawals at specific time-points

6.3.2.1 During screening

If after the subject signs the informed consent form, the investigator ascertains that the subject does not meet the inclusion/exclusion criteria, the investigator only needs to note the reason for subject exclusion on the screening log. The subject should not be assigned a subject identification number, as only eligible subjects will be assigned an identification number.

6.3.2.2 As a result of erroneous inclusion

If a subject who does not meet the inclusion/exclusion criteria is inadvertently included in the trial, the investigator must terminate the subject's participation in the trial and the Sponsor should be informed immediately prior to administration of Dose 1 and a protocol deviation must be completed. In the event, study vaccine (or placebo) has already been given, the subject will be encouraged to complete all remaining safety related follow-up visits and procedures through Visit 15, but a protocol deviation must be completed. However, the subject's data will be excluded from the analysis.

If determination of ineligibility occurs while the subject is in the inpatient clinical isolation unit after receipt of Dose 1, the subject allocation will be unblinded, and if the subject was administered study LAIV, the subject should be maintained in the unit for the specified period, if possible.

6.3.2.3 For violation of the protocol

A subject will be considered as largely not in compliance with the requirements of the protocol and may be withdrawn if any of the following conditions apply:

- The subject egregiously violates the rules of the inpatient clinical isolation unit per the opinion of the investigator;
- The subject refuses to complete the self-observation diary;
- The subject skips entirely a scheduled visit, especially Visits 11 and 13;

- Non-compliance at a scheduled visit for planned procedures; or
- Failure to inform the investigator of the appearance of any AE or SAE in the subject.

6.3.2.4 As a result of withdrawal of informed consent

If a subject wishes to withdraw consent for participation in the trial, the subject should indicate the reasons for the termination of participation, if possible. If such withdrawal occurs after the subject has been admitted to the inpatient clinical isolation unit and has been administered study LAIV, the subject allocation will be unblinded, and if the subject was administered study LAIV, the subject will be asked to remain in the unit for the specified period, if possible. The subject will also be requested to initiate anti-neuraminidase treatment—oseltamivir (75 mg taken orally twice daily for 5 days) or zanamivir (10 mg taken by oral inhalation twice daily for 5 days) and to return to the clinic daily for collection of nasal and OP swab specimens until 3 specimens collected over a 48-hour period all test influenza A negative by RT-PCR. The subject will be informed that the public health authorities may be notified of the subject's premature discharge from the isolation unit, as well as of the results of RT-PCR testing of the subject's nasal and OP swab specimens for detection of viral shedding. For any subject leaving the unit prematurely, the subject will be advised to remain at home and avoid close contact with other persons until 7 days post-administration of study LAIV have passed unless they have agreed to initiation anti-neuraminidase treatment.

6.3.2.5 As a result of occurrence of an adverse event/serious adverse event

If any subject develops an AE/SAE leading to premature withdrawal, the event will be fully followed-up to resolution of the problem, to acknowledgement of the diagnosis of the AE/SAE, or to change in status of the acute AE/SAE as chronic or stable or as long as such a change is justified from a clinical point of view. However, for premature withdrawal in connection with the emergence of an AE/SAE that is considered to be clinically relatively favorable (for example, the diagnosis is known, and it is expected to be resolved completely within a week), intermittent follow-up may be accepted as long as the plan for follow-up of the event is fully described in the notes section of the subject's CRF. Follow-up visits will be carried out for all subjects who are withdrawn due to occurrence of an AE or in connection with a change in any other safety indicator (vital sign or clinical laboratory result).

6.4 Loss to follow-up

To prevent lost to follow-up, subjects will be reminded by phone, email, or text message of their next study visit. In the event of a missed visit, subject will be contacted by phone within 1 day. A subject who misses two consecutive visits and cannot be reached / located after 5 attempts will be considered lost to follow-up. Efforts to contact the subject will be documented in source documents. Any subject who fails to attend the final study visit will also be classified as lost to follow-up. There will be no replacement for subjects who are lost to follow-up.

6.5 Concomitant medications/products/vaccines

At each study visit, subjects will be queried about use of concomitant medications, products and vaccinations. The following concomitant medication(s)/product(s)/vaccine(s) must be recorded in the eCRF:

- All concomitant medications/products, except vitamins and dietary supplements, administered during the period starting on the day of administration of the first dose of study vaccines (Day 1) and ending at the last study visit (Visit 16).

- Any concomitant vaccination administered in the period starting 30 days before the first dose of study vaccines and ending at the last study visit (Day -29 to Visit 16).

- Prophylactic medication (i.e., medication administered in the absence of ANY symptom and in anticipation of a reaction to the vaccination). E.g., an anti-pyretic is considered to be prophylactic when it is given in the absence of fever and any other symptom, to prevent fever from occurring (fever for this purpose is defined as an oral temperature ≥38.0°C or 100.4°F).

- Any concomitant medications/products/vaccines relevant to an SAE/pIMD or administered during the study period for the treatment of an SAE/pIMD. In addition, concomitant medications relevant to SAEs and pIMDs need to be recorded on the expedited reporting form.

6.6 Unblinding procedure

In the event of a medical emergency, the site Investigator may require that the blind be broken for the subject experiencing the emergency when knowledge of the subject's treatment assignment may influence the subject's clinical care. Unblinding will occur through the secure interactive web response system (IWRS) to which there will be 24-hour access.

Details and documentation surrounding such unblinding will be described in the relevant operations manual and will be trained on during the site initiation visit. Documentation of the unblinding event (including the rationale and requestor) will be captured by the IWRS. Every effort will be made not to unblind the subject unless it is considered necessary for the welfare of the subject. Prior to unblinding, the site Investigator is encouraged (to the extent possible, without jeopardizing the subject's health) to contact the Sponsor (or designee) to discuss the decision to break the blind. The site PI will be expected to provide a rationale for the necessity of unblinding based on the expectation that knowledge of the subject's treatment assignment will have a meaningful impact on the subject's medical care in the short term. If a subject's treatment assignment is unblinded, the subject will remain in the study and continue with protocol-defined study visits, but not receive further study vaccines. The decision to unblind will be communicated to any regulatory bodies (e.g., institutional review boards [IRBs]) as required. At the end of the study, documentation of all unblinded subjects (and the rationale for unblinding) will be incorporated into the Trial Master File.

6.7 Management of birth control and pregnancy during study

All females of childbearing potential and males who are not surgically sterile will be monitored for use of adequate contraception and pregnancy (in female subject or female partner of male subject) during the trial.

Adequate contraception will be assessed at each study visit during the entire study period. Adequate contraception is defined as a contraceptive method with a failure rate of less than 1% per year when used consistently and correctly and when applicable, in accordance with the product label, for example:

- Abstinence from penile-vaginal intercourse, when this is their preferred and usual lifestyle;
- Combined estrogen and progesterone oral contraceptives;
- Injectable progestogen;
- Implants of etonogestrel or levonorgestrel;
- Contraceptive vaginal ring;
- Percutaneous contraceptive patches;
- Intrauterine device or intrauterine system;
- Male condom combined with a vaginal spermicide (foam, gel, film, cream or suppository), and/or progesterone alone oral contraceptive.

Adequate contraception does not apply to subjects of child bearing potential with same sex partners or for subjects who are and will continue to be abstinent from penile-vaginal intercourse on a long term and persistent basis, when this is their preferred and usual lifestyle.

If any female subject becomes pregnant after randomization, study vaccination will be discontinued and the subject will be encouraged to complete all safety-related study visits and procedures to the end of the trial. The investigator should fully follow the subject to term and document the outcome of the pregnancy, even if birth occurs after the scheduled end of the trial for the subject.

If the pregnancy occurs *prior to* receipt of Dose 2 of study vaccine, the subject will discontinue participation in all remaining immunogenicity blood draws. If the pregnancy occurs *after* receipt of Dose 2 of study vaccine, the subject will be requested to participate in all remaining immunogenicity related study visits and procedures.

6.8 Clinical procedures

6.8.1 Medical history

At enrollment, medical histories must be thoroughly reviewed through interview with the subject. The following medical conditions, especially, will be assessed:

- Current or recent (within two weeks of enrollment) acute respiratory illness with or without fever;
- Recent vaccination history and history of influenza vaccination since the 2014/2015 season;
- Practice of nasal irrigation on a regular basis within the past six months;
- Recent history of frequent nose bleeds (>5 within the past year);
- Clinically relevant abnormal paranasal anatomy;
- Recent history (within the past month) of rhino or sinus surgery, or surgery for any traumatic injury of the nose;
- Recent receipt of immune globulin or other blood products, or injected or oral corticosteroids or other immune modulator therapy within 6 months (before) of enrollment;
- Hypersensitivity of any kind;
- Asthma;
- Tuberculosis;
- Clinically relevant history of renal, gastrointestinal, cardiovascular, hematological, dermatological, endocrine, neurological or immunological diseases;
- History of excessive daytime sleepiness or narcolepsy; familial history of narcolepsy;
- Seizures, including history of febrile seizures, or any other neurologic disorder;
- Known or suspected immunologic function impairment of any kind and/or known HIV infection;
- Known Hepatitis B or Hepatitis C infection;
- Mental illness;
- Tobacco, alcohol, or drug use;
- Medications taken (in the past 6 months, for screening), including trade name, dosing or change in dosing, indications, start date, and termination date;
- For women, pregnancy and menstrual status, and for both men and women, contraceptive use and/or history of surgical sterility.

6.8.2 Physical examination

A qualified clinician (study physician or differently qualified person) will conduct a complete physical examination at the V01 screening visit and targeted physical examinations (including vital signs) directed to any subject symptoms or signs at all other study visits. Vital signs will be measured at the initial screening visit and prior to each scheduled vaccination at the V03 and V11 study visits. Site SOPs will provide instruction on the details for procedures for measurement of routine clinical parameters to ensure standardized measurement. These will include the following:

6.8.2.1 Vital signs

- Body temperature will be measured in degrees Celsius or Fahrenheit as per site SOPs (recorded to the nearest 0.1 degree) by oral thermometer.
- Respiratory rate will be measured in breaths per minute.
- Pulse rate will be measured in beats per minute.
- Blood pressure will be measured in millimeters of mercury (Hg).

6.8.2.2 Height and weight

- Height will be measured to the nearest centimeter.
- Weight will be measured in kilograms and recorded to the nearest 0.1 kilogram.

6.8.2.3 Included systems

The complete physical examination will include an assessment of general appearance and examination of the head, eyes, ears, nose, oropharynx, neck, chest (by auscultation), lymph nodes (neck, supraclavicular, axillary, inguinal), abdomen (auscultation and palpation), skin and musculoskeletal system, and nervous system.

6.8.3 Assessment of local and general adverse events

Study staff will train subjects on use of diary cards for self-assessment of solicited local and general adverse events through 7 days (day of vaccination and subsequent 6 days) post-administration of study vaccine (or placebo) and for self-assessment of unsolicited adverse events and concomitant medications through the end of the study. Timing and severity grading of adverse events will be according to protocol Section 8 (Safety Assessment and Reporting). Study staff will review and discuss with the subject all recorded events to ensure appropriate documentation and scoring prior to transferring of the diary card record to the eCRF.

7 LABORATORY EVALUATIONS / REQUIREMENTS

The time points and amounts of blood drawn for clinical laboratory and immunologic / transcriptomic evaluations are shown in the Schedule of Events tables (Appendix 1). Good Clinical Laboratory Practice audits may be conducted by the Sponsor, PATH, to ensure safety clinical laboratory testing meets state-required quality standards (e.g., Clinical Laboratory Improvement Amendments). Laboratories conducting immunologic evaluations may also be audited for Good Clinical Laboratory Practice compliance.

7.1 Sample collection, processing, storage

7.1.1 Nasal and oropharyngeal swab specimens

7.1.1.1 Collection of nasal and oropharyngeal swab specimens

Nasal and oropharyngeal swab specimens are the upper respiratory tract specimens that are to be collected for the detection of influenza virus RNA by RT-PCR assays and for virus isolation in MDCK cell culture. Collection instructions will be contained in site SOPs. All specimen collection will be conducted by trained individuals.

7.1.1.2 Conditions for transport, processing and storage of swab specimens

Collected nasal and OP swab specimens will be stored at refrigerator temperatures until transport to the site's research laboratory. Specimens will be securely transported on ice each day. In the laboratory, vials/tubes containing swab specimens will be opened and swab specimen applicator sticks will be agitated to remove collected material, fluid will be expressed, and swabs discarded. Each swab specimen will be divided into two aliquots of approximately 0.5 mL, one tube for RT-PCR testing and one tube for backup for virus isolation for specimens influenza A positive by RT-PCR. Day 6 specimens must be tested by RT-PCR within 6 hours of collection. Specimen material not used for virus identification will be stored at –65˚C or lower in each laboratory. Handling and storage of specimens will emphasize avoidance of freeze-thaw cycles.

7.1.2 Blood specimens

Blood will be collected for testing in multiple standard clinical (biochemical and hematological) and research immunologic and transcriptomic assays. Therefore, attention must be given to proper collection and processing per the requirements of each assay.

7.1.2.1 Collection of blood

Following universal precautions, blood will be collected by venipuncture into vacutainer tubes. If collection is from the subject’s forearm, preference will be for blood collection to occur from forearm contralateral to arm of study IIV injection. Blood specimens will be affixed with coded labels that link the specimen to the subject, specimen type, specimen collection date, and specimen number and time-point. Specimen collection tube must be appropriate for type of specimen required. See Laboratory Manual for further specifications.

Blood should be held at room temperature (+18°C to +25°C) until processing. Volumes of blood required for the different categories of assays at different time-points and total to be collected are shown in the below table. No subject will have more than 400 mL of blood drawn in any 8 week period of the study. The cumulative blood draw for each subject over the entire course of study participation is approximately 906 mL.

Table 7 Volume Estimates of whole blood drawn for clinical and immunologic assays at each visit.

Assay	V00 or V01	V03*	V05	V09	V10	V11	V12	V13	V14	V15	V16***
		D1	D3	D8	D29	D85	D92	D113	M9	M15	M21
Biochemistry	3.5	3.5	--	3.5	3.5	3.5	3.5	3.5	3.5	3.5	--
Hematology	3	3	--	3	3	3	3	3	3	3	--
HIV screening	3.5	--	--	--	--	--	--	--	--	--	--
Humoral immunologic testing**	--	40	--	--	20	20	--	40	1	40	40
ELISA	--	1.4	--	--	1.4	1.4	--	1.4	1.0	1.4	
Microneutralization	--	1.2	--	--	1.2	1.2	--	1.2	--	1.2	
Hemagglutination inhibition	--	1.2	--	--	1.2	1.2	--	1.2	--	1.2	
Serum ADCC activity	--	0.5	--	--	0.5	0.5	--	0.5	--	0.5	
Serum ADCP activity	--	0.5	--	--	0.5	0.5	--	0.5	--	0.5	
Passive Transfer	--	20	--	--	--	--	--	20	--	20	
Backup sera	--	15	--	--	15	15	--	15	--	15	
Cell-mediated immunologic testing**	--	118	--	58	86	118	58	86	--	110	--
Stimulated plasmablasts	--	--	--	26	--	--	26	--	--	0	--
Stimulated B memory cells	--	86	--	--	86	86	--	86	--	86	--
Stimulated T cells	--	32	--	32	--	32	32	--	--	24	--
Transcriptomics (using extracted RNA)	--	5	5	5	5	5	5	5	5	5	--
Total Blood Volume**	**10**	**170**	**5**	**70**	**118**	**150**	**70**	**138**	**13**	**162**	**40**

*Will occur at Visit 02 (one day prior to Visit 03) for subjects in LAIV-IIV treatment arms (Groups 1, 2, & 3)
**Rounded up to nearest whole mL
***Assays performed at Visit 16 will be determined following analysis of primary and secondary endpoints

7.1.2.2 Processing, storage, and transport of blood

7.1.2.2.1 Processing of sera for biochemistry, HIV screening, and serum pregnancy testing (as applicable)

Immediately after collection, the serum separator tubes will be inverted 6-10 times, stood upright, and transferred immediately to the site clinical laboratory for further processing and testing.

7.1.2.2.2 Processing of whole blood for hematology

Immediately after collection, the tube of blood for hematology will be inverted 6-10 times, stood upright, and transferred immediately to the site clinical laboratory for further processing and testing. Whole blood for hematology will not be divided.

7.1.2.2.3 Processing of sera for humoral immunology

Immediately after collection, the serum separator tubes for humoral immunologic testing will be inverted 6-10 times and stood upright to clot for at least 30 minutes at room temperature. Tubes will then be transferred to the site research laboratory for processing. At the laboratory, specimens will be centrifuged before division and storage. Centrifuging and aliquoting procedures will be defined in specimen handling SOPs.

Serum specimens for humoral immunology will be immediately frozen to –65˚C or lower after division. Frozen sera will be at all times maintained in a frozen state during transport between the sites and the designated immunology laboratories. All handling will be done to prevent unnecessary freeze-thaw cycles.

7.1.2.2.4 Processing of whole blood for plasmablast and stimulated B memory cell assays

Immediately after collection, the sodium citrate tubes for stimulated plasmablasts and stimulated B memory cells testing will be inverted 6-10 times and stood upright at room temperature. Tubes will then be shipped overnight to the research laboratory of Dr. Patrick Wilson at the University of Chicago for further processing and testing.

7.1.2.2.5 Processing of whole blood for T cell assays

Immediately after collection, the sodium citrate tubes for stimulated T cells will be inverted 6-10 times and stood upright at room temperature. Tubes will then be processed by the site research laboratory within 2 hours of collection per the tube manufacturer instructions. Recovered PBMCs will be immediately resuspended in a freezing solution, placed in cryovials and the cryovials placed into a freezing container filled with isopropyl alcohol before freezing to -65°C or lower. PBMCs will be shipped frozen to the laboratory of Dr. Florian Krammer at ISMMS in New York, New York for further processing and testing. During the processing of the blood collection tubes, cellular components will be separated from plasma. The leftover plasma (which would otherwise be discarded) will be collected, aliquoted and frozen to -65°C or lower for future use. No

serum/plasma is otherwise collected for this time point and these samples could be used to integrate findings in the cellular assays with humoral responses.

7.1.2.2.6 Processing of whole blood for transcriptomic assays

Immediately after collection, the PAXgene Blood RNA Tube for transcriptomic assays will be inverted 8-10 times and stood upright at room temperature before being processed as per instructions provided in the Laboratory Manual prior to long-term storage at -65˚C or lower. Tubes will be shipped frozen to the laboratory of Dr. Adolfo Garcia-Sastre at ISMMS in New York, New York for further processing and testing.

7.1.3 Saliva specimens

Saliva will be collected via an oral swab for quantification of anti-stalk salivary and secretory IgA. The subject will be asked to chew on the cotton swab until the subject cannot keep themselves from swallowing the collected saliva, before the swab is removed and placed back into the test tube and the cap inserted. The sample will be sent to the laboratory for processing. Processing procedures will be further defined in specimen handling SOPs.

7.1.4 Urine specimens

Urine (or serum, as required by site) will be collected for pregnancy testing during screening and prior to administration of any dose of study vaccine (or placebo). Urine will also be collected at initial screening for opiate use (e.g., 6-MAM, codeine, fentanyl, hydrocodone, hydromorphone, meperidine, morphine, naloxone, naltrexone, norfentanyl, normeperidine, oxycodone, oxymorphone, sufentanil, tramadol).

No urine specimens will be stored after testing.

7.2 Clinical laboratory tests

Protocol mandated clinical screening and safety laboratory tests will be conducted in real time by laboratories that are properly accredited and subscribe to a proficiency testing program.

7.2.1 Hematology and biochemistry

Hematology and biochemistry assays for safety assessment will be performed using standardized procedures in a certified clinical laboratory at the site (Table 8).

7.2.2 Pregnancy testing

Pregnancy testing will be performed using standardized procedures in a certified clinical laboratory at the site (Table 8).

7.2.3 HIV testing

HIV testing for screening will be performed using standardized procedures in a certified clinical laboratory at the site (Table 8).

Table 8 Hematology/biochemistry/virology

System	Discipline	Component	Method	Scale**	Laboratory
Whole blood	Hematology	Leukocytes (White Blood Cells)	As per site clinical laboratory procedure	Quantitative	Site
		Neutrophils*			
		Lymphocytes*			
		Basophils*			
		Monocytes*			
		Eosinophils*			
		Hemoglobin			
		Platelets			
		Erythrocytes (Red Blood Cells)			
Serum	Biochemistry	ALT	As per site clinical laboratory procedure	Quantitative	
		AST			
		Creatinine***			
		Urea nitrogen***			
Urine	Biochemistry	Human chorionic gonadotropin (hCG)	As per site clinical laboratory procedure	Qualitative	
Serum	Virology	HIV-1/2 antigen/antibody and/or nucleic acid	As per site clinical laboratory procedure	Qualitative	

*For White Blood Cell differential count.

**Grading of laboratory parameters assessed as AEs will be based on the FDA Guidance for Industry "Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials" (see Appendix 2)

***BUN-to-creatinine ratio is to be calculated.

Laboratory results will be reviewed promptly by the site PI or designee. Participants will be notified of any clinically significant abnormalities. If clinically significant abnormalities are identified during screening, subjects will be referred to their primary health provider or appropriate medical center. If identified during the study, subjects may be asked to return to the study site for further evaluation, including clinical evaluation and repeat laboratory testing as warranted.

Table 9 specifies hematologic, biochemical, and virologic read-outs by sampling time point and study group.

Table 9 Read-outs for hematology/biochemistry/virology

Sampling time point		Number of subjects	Component
Visit time point	Sampling time point		
Screening	SCR	All screened subjects	Hematology: leukocytes, neutrophils, lymphocytes, basophils, monocytes, eosinophils, hemoglobin, platelets, erythrocytes
			Biochemistry: ALT, AST, creatinine*, urea nitrogen*
			Virology: HIV antibody/antigen
Visit 03** Visit 09 Visit 10 Visit 11 Visit 12 Visit 13 Visit 14 Visit 15	PRE PId7 PId28 PId84 PIId7 PIId28 PIIm6 PIIm12	All Subjects	Hematology: leukocytes, neutrophils, lymphocytes, basophils, monocytes, eosinophils, hemoglobin, platelets, erythrocytes
			Biochemistry: ALT, AST, creatinine*, urea nitrogen*

SCR = screening

*The BUN-to-creatinine ratio is to be calculated.

**Will occur at Visit 02 (one day prior to Visit 03) for subjects in LAIV-IIV treatment arms (Groups 1, 2, & 3)

7.3 Immunological laboratory assays

7.3.1 Humoral and mucosal immune responses

Multiple assays will be used to probe humoral immunologic responses to the study LAIV and IIVs with chimeric HAs. Serological assays for quantification of 1) anti-stalk IgG and IgA and anti-neuraminidase antibodies detected by ELISA, 2) neutralizing antibodies detected by MN assay, 3) antibodies with anti-H1 stalk activity demonstrated after passive transfer in an influenza challenge model in mice, 4) antibodies with Fc-receptor ADCC activity, and 5) antibodies with Fc-receptor mediated ADCP activity will be performed at ISMMS (laboratory of Dr. Florian Krammer) or in a laboratory designated by ISMMS (e.g., Neomed) using standardized procedures. Serologic assays for quantification of antibodies with HI activity will be performed by a designated research laboratory using standardized procedures. Additional assays for quantification of anti-stalk salivary and secretory IgA will be performed at ISMMS (laboratory of Dr. Florian Krammer) or in a laboratory designated by ISMMS (e.g., Neomed) using standardized procedures. All planned assays for characterization of humoral and mucosal immune responses are listed in Table 10. Additionally, Table 10 specifies humoral and mucosal immunologic read-outs by sampling time point and study group. In case of insufficient blood or saliva sample volume to perform assays for all antibodies, the samples will be analyzed according to priority ranking provided in Table 11.

Below are brief descriptions of each planned assay.

7.3.1.1 cH6/1 stalk IgG by ELISA

This ELISA will measure IgG antibodies against the H1 stalk domain by using a chimeric protein that contains an exotic HA head domain (that the vaccinees have not previously been exposed to) and the H1 stalk domain as substrate. Most subjects are expected to have been previously exposed to H1N1 seasonal influenza viruses and have baseline titers to H1 stalk; thus, this assay is more aimed at detecting increases from baseline. **This assay is considered the primary immunologic read-out of the trial and one of the five key immunologic read-outs of the trial.**

7.3.1.2 cH6/1 stalk IgA ELISA (serum)

Likewise, this ELISA will measure serum IgA antibodies against the H1 stalk domain by using a chimeric protein that contains an exotic hemagglutinin head domain (that the vaccinees have not previously been exposed to) and the H1 stalk domain as substrate. Again, most subjects are expected to have been previously exposed to H1N1 seasonal influenza viruses and have baseline titers to H1 stalk; thus, this assay is more aimed at detecting increases from baseline.

7.3.1.3 cH6/1 stalk secretory IgA ELISA (saliva)

Likewise, this ELISA will specifically measure secretory IgA antibodies against the H1 stalk domain in saliva by using a chimeric protein that contains an exotic HA head domain (that the vaccinees have not previously been exposed to) and the H1 stalk domain as substrate. Secretory IgA is actively secreted into the mucosa. Again, most subjects are expected to have been previously exposed to H1N1 seasonal influenza viruses and may have baseline titers to H1 stalk; thus, this assay is more aimed at detecting increases from baseline. **This assay is considered one of the five key immunologic read-outs of the trial.**

7.3.1.4 cH6/1 stalk total IgA ELISA (saliva)

Likewise, this ELISA will measure total IgA antibodies against the H1 stalk domain in saliva by using a chimeric protein that contains an exotic HA head domain (that the vaccinees have not previously been exposed to) and the H1 stalk domain as substrate. This includes IgA present in the mucosa through both active and passive transfer processes. Again, most subjects are expected to have been previously exposed to H1N1 seasonal influenza viruses and may have baseline titers to H1 stalk; thus, this assay is more aimed at detecting increases from baseline.

7.3.1.5 cH6/1 stalk total IgG ELISA (saliva)

Likewise, this ELISA will measure IgG antibodies against the H1 stalk domain in saliva by using a chimeric protein that contains an exotic HA head domain (that the vaccinees have not previously been exposed to) and the H1 stalk domain as substrate. Again, most subjects are expected to have been previously exposed to H1N1 seasonal influenza viruses and have baseline titers to H1 stalk; thus, this assay is more aimed at detecting increases from baseline.

7.3.1.6 H2 IgG ELISA

This ELISA will measure the induction of cross-reactive IgG antibodies to a Group 1 influenza A virus with a heterosubtypic HA that is moderately distantly related to the currently circulating influenza A/H1 viruses. Influenza viruses expressing H2 HAs have previously circulated in the human population. See Figure 1. H2N2 viruses circulated for only 11 years, from 1957 to 1968. Subjects born after 1968 typically do not possess neutralizing titers against H2N2 viruses because of lack of exposure, and hence it is not expected that subjects in the current trial would have any immunologic history with this virus. For this study, an avian H2 HA antigen will be used for detection of cross-reactive antibodies to H2.

7.3.1.7 H9 IgG ELISA

This ELISA will measure the induction of cross-reactive antibodies to a Group 1 influenza A virus with heterosubtypic HA that is moderately distantly related to the currently circulating influenza A/H1 viruses. See Figure 1. H9N2 viruses circulate in multiple avian species and are the source of some genes for H9N7 viruses that have infected humans over the past several years. For this study, an avian H9 HA antigen will be used for detection of cross-reactive antibodies to H9.

7.3.1.8 H18 IgG ELISA

This ELISA will measure the induction of cross-reactive antibodies to a Group 1 influenza A virus with heterosubtypic HA that is distantly related to the currently circulating influenza A/H1 viruses. See Figure 1. H18 viruses have only been detected in bat species. For this study, a chiropteran H18 antigen will be used for detection of cross-reactive antibodies to H18.

7.3.1.9 H3 IgG ELISA

This ELISA will measure the induction of cross-reactive antibodies to a Group 2 influenza A virus with heterosubtypic HA. See Figure 1. Antibodies that cross-react between Group 1 and Group 2 influenza A viruses are rare. H3N2 viruses have widely circulated in humans since 1968. Thus, most subjects are expected to have baseline antibody levels of antibodies to H3. However, increases from baseline, in the absence of detected laboratory-confirmed H3N2 ILI in a subject or substantial H3N2 circulation during the trial period, would suggest cross-reactive responses to this Group 2 influenza A virus.

7.3.1.10 N1 IgG ELISA

This ELISA will measure the induction of antibodies against the NA of H1N1. All vaccine components contain N1 and antibodies against NA could confer additional protection. Most subjects are expected to have been previously exposed to H1N1 seasonal influenza viruses and have baseline titers to N1; thus, this assay is more aimed at detecting increases from baseline.

7.3.1.11 cH6/1N5 MN assay

This MN assay will measure neutralizing antibodies against the H1 stalk domain by using a virus that expresses a cHA that contains an exotic HA head domain (that the vaccinees have not previously been exposed to) and the H1 stalk domain. This virus furthermore contains an exotic neuraminidase for which humans are generally naïve. **This assay is considered one of the five key immunologic read-outs of the trial.**

7.3.1.12 pH1N1 MN assay

This MN assay will measure the neutralizing potential of antibodies against the currently circulating human H1N1 isolate which is similar to the stalk used in study vaccines.

7.3.1.13 asH1N1 MN assay

This MN assay will measure the neutralizing potential of cross-reactive antibodies to a Group 1 influenza against an H1N1 virus isolate that currently circulates in animals and is antigenically different from current human isolates. This avian-swine H1N1 virus, while isolated in swine, is believed to be of avian origin.

7.3.1.14 H5N8 MN assay

This MN assay will measure the neutralizing potential of cross-reactive antibodies to a Group 1 influenza virus with a heterosubtypic HA from a recent avian influenza virus.

7.3.1.15 Serum ADCC activity

This assay will measure the potential of the vaccination regimens to induce antibodies that mediate ADCC activity via the Fc-receptor. **This assay is considered one of the five key immunologic read-outs of the trial.**

7.3.1.16 Phagocytic serum activity

This assay will measure the potential of the vaccination regimens to induce antibodies that mediate antibody-dependent cellular phagocytosis (ADCP) via the Fc-receptor.

7.3.1.17 cH6/1N5 challenge after passive transfer

This passive serum transfer assay will measure in vivo in an influenza challenge model in mice protective antibodies against the H1 stalk domain by using a virus that expresses a cHA that contains an exotic HA head domain (that the vaccinees have not previously been exposed to) and the H1 stalk domain. This virus furthermore contains an exotic neuraminidase for which humans are generally naïve. Sera from subjects in each study group will be pooled, and sets of mice will each receive a peritoneal injection of either the pre- or post-vaccination serum pool. Mice will then be challenged with the cH6/1N5 virus, with survival, weight loss, and viral lung titers subsequently measured 14 days post-challenge.

7.3.1.18 H1N1 challenge after passive transfer

This passive serum transfer assay will measure in vivo in an influenza challenge model in mice protective antibodies against the currently circulating human H1N1 isolate. Sera from subjects in each study group will be pooled, and sets of mice will each receive a peritoneal inoculum from either the pre- or post-vaccination serum pool. Mice will then be challenged with the H1N1 virus, with survival, weight loss, and viral lung titers subsequently measured 14 days post-challenge.

7.3.1.19 cH8/1N1 HI

This HI assay will measure the induction of antibodies reactive against the head domain of the cH8/1 in study LAIV. Subjects receiving study cH8/1 LAIV are expected to have measurable HI responses post-vaccination.

7.3.1.20 cH5/1N1 HI

This HI assay will measure the induction of antibodies reactive against the head domain of the cH5/1 in study IIV. Subjects receiving study cH5/1 IIV are expected to have measurable HI responses post-vaccination.

7.3.1.21 cH6/1N5 HI

This HI assay will measure the induction of antibodies reactive against the head domain of a cH6/1. As neither study vaccine contains an H6 head, induction of antibodies against this domain is not expected. If cH6/1 HI antibodies are measured, it would imply that the study vaccines induced broadly cross-reactive HA head antibodies.

7.3.1.22 H1N1 HI

This HI assay will measure the induction of antibodies reactive against the head domain of the H1 of the currently circulating human H1N1 virus. As neither study vaccine contains an H1 head, induction of antibodies against this domain is not expected. If H1 HI antibodies are measured, it would imply that the study vaccines induced broadly cross-reactive HA head antibodies.

7.3.1.23 Avian-swine H1N1 HI

This HI assay will measure the induction of antibodies reactive against the head domain of an H1N1 virus isolate that currently circulates in animals and is antigenically different from H1N1 current circulating in humans. As neither study vaccine contains an asH1 head, induction of antibodies against this domain is not expected. If asH1 HI antibodies are measured, it would imply that the study vaccines induced broadly cross-reactive HA head antibodies. This H1N1 virus, while isolated in swine, is believed to be of avian origin.

7.3.1.24 H5N8 HI

This HI assay will measure the induction of antibodies reactive against the head domain of a group 1 influenza virus with a heterosubtypic HA from a recent avian influenza virus. As the cH8/1N1 LAIV and IIV do not contain an H5 head, lack of measurable antibodies in this assay post cH8/1N1 administration would suggest most of the responses measured in the H5N8 MN are stalk associated.

Table 10 Humoral and mucosal immunity assays

System	Component (Strain or Antigen Description)	Method	Kit/Manufacturer	Unit	Cut-off*	Laboratory
ELISAs						
Serum	**cH6/1 HA** = Recombinant antigen based on A/mallard/Sweden/81/2002 (H6N1) head domain with stalk domain from HA of H1N1 virus A/California/04/09	Anti-H1 HA-stalk IgG ELISA	In-house assay (Developed by GSK)	ELISA units per mL (EU/mL)	65.3	Neomed
	H2 HA full length = Recombinant antigen based on A/mallard/Netherlands/5/1999 (H2N9) HA	Anti- H2 HA-full length IgG ELISA			22.0	
	H18 HA full length = Recombinant antigen based on A/flat-faced bat/Peru/033/2010 (H18N11) HA	Anti- H18 HA-full length IgG ELISA			42.3	
	H9 HA full length = Recombinant antigen based on A/chicken/Hong Kong/G9/1997 (H9N2) HA	Anti-H9 HA-full length IgG ELISA			31	
	N1 NA = Recombinant antigen based on A/California/04/2009 (H1N1) NA	Anti-N1 NA ELISA	In-house assay	Endpoint titer	100	ISMMS
	H3 HA full length = Recombinant antigen based on A/Hong Kong/4801/2014 (H3N2) HA	Anti- H3 HA-full length IgG ELISA	In-house assay	Endpoint titer	100	
	cH6/1 HA = Recombinant antigen based on A/mallard/Sweden/81/2002 (H6N1) head domain with stalk domain from HA of H1N1 virus A/California/04/09	Anti-H1 HA-stalk IgA ELISA	In-house assay	Endpoint titer	100	
Saliva	**cH6/1 HA** = Recombinant antigen based on A/mallard/Sweden/81/2002 (H6N1) head domain with stalk domain from HA of H1N1 virus A/California/04/09	Anti-H1 HA-stalk secretory IgA ELISA	In-house assay	Endpoint titer	4	
	cH6/1 HA = Recombinant antigen based on A/mallard/Sweden/81/2002 (H6N1) head domain with stalk domain from HA of H1N1 virus A/California/04/09	Anti-H1 HA-stalk total IgA ELISA	In-house assay	Endpoint titer	10	
	cH6/1 HA = Recombinant antigen based on A/mallard/Sweden/81/2002 (H6N1) head domain with stalk domain from HA of H1N1 virus A/California/04/09	Anti-H1 HA-stalk IgG ELISA	In-house assay	Endpoint titer	10	

Table 10 Humoral and mucosal immunity assays (continued)

System	Component (Strain or Antigen Description)	Method	Kit/Manufacturer	Unit	Cut-off*	Laboratory
Microneutralization (MN) assays						
Serum	**cH6/1N5 virus (on PR8 backbone [A/Puerto Rico/8/34]):** HA head: A/mallard/Sweden/81/2002 (H6N1) stalk: A/California/04/2009 (H1N1pandemic) N5: A/mallard/Sweden/86/2003 (H12N5)	Anti-H1 HA-stalk MN Assay	In-house assay	1/DIL Endpoint titer	10	ISMMS
	H5N8 virus: RG reassortant virus based on PR8 for 6 genes with 2 surface proteins: HA and NA from A/Gyrfalcon/Washington/41088-6/2014 (H5N8)	Anti-heterosubtypic HA Group 1 virus MN Assay			10	
	H1N1 avian-swine influenza virus: A/Swine/Jiangsu/40/2011 (asH1N1)	Anti-heterologous HA Group 1 virus MN Assay			10	
	H1N1 strains**	Anti-heterologous HA Group 1 virus MN Assay			10	
Hemagglutination Inhibition (HI) Assay						
Serum	Chimeric vaccine strain cH8/1N1; cH5/1N1; (cH6/1N5; H5N8; asH1N1; H1N1)***	HI assays	In-house assay	1/DIL	10	CCHMC
Other Assays						
Serum	cH6/1N5 challenge	Passive Transfer to mice	In-house assay	NA	NA	ISMMS
Serum	H1N1** challenge	Passive Transfer to mice	In-house assay	NA	NA	ISMMS
Serum	cH6	ADCC	In-house assay	AUC	1	ISMMS
Serum	cH6	ADCP	In-house assay	AUC	1	ISMMS

TBD = to be determined; DIL = dilution; NA = not applicable

*Cut-off value may change following set-up/qualification data

**The H1N1 strain will depend on the World Health Organization recommendation for the 2017/2018 season.

***These additional HI assays will also use the same four antigens as used for MNs

Table 11 Humoral and mucosal immunologic read-outs

Blood sampling time point		Component	Components priority rank
Visit time point	Sampling time point		
ELISAs			
Visit 03* Visit 10 Visit 11 Visit 13 Visit 14** Visit 15	PRE PId28 PId84 PIId28 PIIm6 PIIm12	Anti-H1 HA-stalk IgG ELISA (serum)	1
		Anti-H1 HA-stalk IgG ELISA (saliva)	(3)***
		Anti-H1 HA-stalk total IgA ELISA (serum)	4
		Anti-H1 HA-stalk total IgA ELISA (saliva)	(2)***
		Anti-H1 HA-stalk secretory IgA ELISA (saliva)	(1)***
		Anti-H2 HA-full length IgG ELISA	6
		Anti-H9 HA-full length IgG ELISA	7
		Anti-H18 HA-full length IgG ELISA	8
		Anti-H3 HA-full length IgG ELISA	14
		Anti-N1 NA ELISA	15
Microneutralization (MN) Assays			
Visit 03* Visit 10 Visit 11 Visit 13 Visit 15	PRE PId28 PId84 PIId28 PIIm12	Anti-H1 HA-stalk MN assay	2
		Anti-heterosubtypic HA Group 1 virus MN assay (H5N8)	9
		Anti-heterosubtypic HA Group 1 virus MN assay (avian-swine H1N1)	10
		Anti-heterosubtypic HA Group 1 virus MN assay (H1N1)	11
Hemagglutination Inhibition (HI) Assays			
Visit 03* Visit 10 Visit 11 Visit 13 Visit 15	PRE PId28 PId84 PIId28 PIIm12	HI with cH5/1N1	16
		HI with cH8/1N1 virus	17
		HI with cH6/1N5	18
		HI with H5N8	19
		HI with asH1N1	20
		H1 with H1N1	21
Other Assays			
Visit 03* Visit 10 Visit 11 Visit 13 Visit 15	PRE PId28 PId84 PIId28 PIIm12	ADCC Activity	3
		ADCP Activity	5
Visit 03* Visit 13 Visit 15	PRE PIId28 PIIm12	Passive transfer to mice with cH6/1N5 challenge	12
		Passive transfer to mice with H1N1 challenge	13

*Will occur at V02 (one day prior to V03) for subjects in LAIV-IIV treatment arms (Groups 1, 2, & 3)

**At Visit 14, only anti-H1 HA-stalk IgG detection by ELISA in serum will be done.

***Parentheses indicate components priority rank for testing of saliva. All other rankings are for testing of serum.

7.3.2 Cell-mediated immunity

Multiple assays will be used to probe cell-mediated immunologic responses to the chimeric HA LAIV and IIVs. T-cell responses will be evaluated by ISMMS (laboratory of Dr. Florian Krammer) using standardized procedures (Table 12). B-memory cell and plasmablast responses will be evaluated by the University of Chicago (laboratory of Dr. Patrick Wilson) using standardized procedures (Table 12).

Table 13 specifies cell-mediated immunologic read-outs by sampling time point and study group. In case of insufficient blood sample volume to perform assays for all antibodies, the samples will be analyzed according to priority ranking provided in Table 13

7.3.2.1 Plasmablast response (ELISPOT for cH6/1)

Plasmablasts represent predominantly the reactivation of past memory B cells with little further adaptation to the current immunogen. Plasmablasts are expected to be present at peak levels 7 days post-vaccination with study vaccine. One third to one quarter of each sample will be used for Enzyme-Linked ImmunoSpot (ELISPOT) assays to evaluate total IgG and IgA+ antibody-secreting cells (ASC) and the proportions of these cells that are reactive to the cH6/1 recombinant HA (rHA) protein and to control rHA protein. The units this assay generates is ASCs/10^6 PBMCs. The frequency of ASCs to rHA proteins will be calculated as (ASCs to rHA per million PBMCs)/(ASCs to IgG per million PBMCs).

To reduce cell deaths plasmablasts will not be frozen before processing. The laboratory will also sort single plasmablast cells for use in generating monoclonal antibodies (mABs) and evaluating the immunoglobulin gene repertoires. The antibodies will be used to evaluate the frequency and functionality of the antibody response as well as the epitopes that are targeted.

7.3.2.2 Memory B-cell response (ELISPOT for cH6/1)

Memory B-cells generated at baseline, 4 weeks post-vaccination and maintained out to 12 months post-Dose 2 will be studied. Day 28 memory B cells represent B cells that may have undergone further adaptation to the immunogen. Memory B cells can be detected by ELISPOT after in vitro stimulation to induce differentiation into IgG and IgA secreting cells. The lab will test the PBMCs for antigen specific B cells in ELISPOT assays. This will determine the proportions of these cells that are reactive to the cH6/1 rHA protein and to control rHA protein. Again, the units this assay generates is ASCs/10^6 PBMCs. The frequency of memory cells to rHA proteins will be calculated as (memory cells to rHA per million PBMCs)/(memory cells to IgG per million PBMCs). **This assay is considered one of the five key immunologic read-outs of the trial.**

In addition, the laboratory will isolate antigen-specific memory B cells by flow cytometry with memory B cell-specific cell surface markers. The antibodies will be used to evaluate the frequency and functionality and maturation of the antibody response in response to vaccination.

7.3.2.3 T-cell response (ELISPOT)

This assay will allow us to measure the potential of the vaccination regimens to induce T cell responses. The protective role of antibodies in the context of influenza virus vaccination has been well established. However, less is known about T cell responses to influenza virus vaccination. It has been previously shown that T cell responses can be elicited with live virus vaccination. In this study, a live-attenuated virus prime, followed by an inactivated intramuscular vaccination will be compared to two intramuscular vaccinations with inactivated vaccine. To measure differences in T cell activation, as well as long term T cell responses specific to influenza viruses, the lab will collect PBMCs and quantify the number of cytokine producing cells in response to influenza specific antigens (including whole virus stimulation and peptide pools) and compare them to the response against an irrelevant peptide as a negative control.

To perform an interferon gamma ELISPOT assay, for example, a standardized amount of PBMCs are pipetted into each well, stimulated with the antigen of choice and incubated overnight. Cells that become activated will release interferon gamma that gets bound to the bottom of the well. The plates are then developed and the individual spots (representing a single interferon gamma releasing cell) are counted.

Table 12 Cell-mediated immunologic assays

System	Component	Challenge	Method	Unit	Laboratory
PBMCs	B-cells reactive to "challenge" antigens	cH6/1	B-memory cells and ELISPOT (monoclonal antibodies will also be cloned from select subjects)	Frequencies of antigen-specific memory B-cells/millions memory B-cells	UChicago
PBMCs	Plasmablasts detected using HA-SA biotinylated probe	cH6/1	Plasmablast detection to HA by flow cytometry	Frequencies of antigen-specific plasmablasts/millions plasmablasts	UChicago
PBMCs	T-cells	cH6/1 and other antigens, to be determined	ELISPOT	Frequencies of IFN-γ positive spots	ISMMS

PBMC = Peripheral blood mononuclear cells; IFN-γ = interferon-gamma

Table 13 Cell-mediated immunologic read-outs

Cell-mediated immunity			
Visit time point	**Sampling time point**	**Component**	**Components priority rank**
Visit 03* Visit 10 Visit 11** Visit 13 Visit 15**	PRE PId28 PId84 PIId28 PIIm12	B memory cells (including ELISPOT for cH6/1)	1
Visit 09 Visit 12	PId7 PIId7	Plasmablast detection to HA by flow cytometry	2
Visit 03* Visit 09 Visit 11 Visit 12 Visit 15	PRE PId7 PId84 PIId7 PIIm12	T-cell response (ELISPOT)	3

PRE = pre-vaccination; PI = post-Dose 1; PII = post-Dose 2; D = day; M = month
*Will occur at V02 (one day prior to V03) for subjects in LAIV-IIV treatment arms (Groups 1, 2, & 3)
**ELISPOT only

For "universal" influenza vaccines based on the approach of using vaccines with chimeric HA constructs, no generally accepted immunological correlate of protection has been demonstrated so far.

Additional exploratory testing related to the vaccine and/or to the disease under study may be performed within the framework of the study if deemed necessary for accurate interpretation of the data, for test improvement or development of analytical methods, or should such assay(s) become available.

7.4 Transcriptomic laboratory assays

Analysis of global changes in blood associated with vaccination might allow the identification of biomarkers associated with protection. Changes to the blood transcriptome and cell composition will be analyzed using RNA sequencing at ISMMS (laboratory of Dr. Adolfo Garcia-Sastre) using standardized procedures (see Table 14). Comparison of the differences between the different vaccination regimens, induced immune responses, blood transcriptomes and cell type compositions will tell us the specific blood parameters associated with immunogenicity, and in the long run, potentially with protection.

Table 14 Transcriptomic read-outs

Transcriptomics			
Visit time point	**Sampling time point**	**Component**	**Components priority rank**
Visit 03*	PRE	RNA sequencing	1
Visit 05	PId2		
Visit 09	PId7		
Visit 10	PId28		
Visit 11*	PId84		
Visit 12	PIId7		
Visit 13	PIId28		
Visit 14	PIIm6		
Visit 15*	PIIm12		

*Will occur at V02 (one day prior to V03) for subjects in LAIV-IIV treatment arms (Groups 1, 2, & 3)

7.5 Influenza virologic assays

At each site, nasal and OP swab specimens collected from subjects in Groups 1, 2, & 3 while they are in the inpatient clinical isolation unit will be tested daily (within 24 hours of collection) for the presence of influenza A virus using standardized procedures. Primer-probe sets appropriate for the detection of seasonal influenza A viral RNA by real time RT-PCR will be used. Swab specimens may also be tested by RT-PCR using primer-probe sets designed to detect specifically genes of A/Leningrad/134/17/1957, the master donor virus used to create study LAIV and/or to detect the cH8/1 HA gene. Specimens testing positive will be shipped to a single designated

laboratory for detection of viable vaccine virus in MDCK cell culture using standardized procedures (Table 15).

To maintain the observer-blind study status insofar as is possible, the results of the daily influenza A virus test performed on subjects in the inpatient clinical isolation unit will only be provided to a designated unblinded member of the site team. The unblinded member will only communicate positive results to the blinded Investigator and study team members on a need to know basis. For example, positive results from the Day 4 and later specimens need to be communicated so that the study team can ensure that the subject has met all the required discharge criteria prior to discharge from the inpatient clinical unit.

For subjects in any study arm (Groups 1-5) reporting ILI during the course of the study follow-up, nasal and OP swab specimens will be tested for evidence of seasonal influenza infection by RT-PCR (A/B typing followed by A/H1 or A/H3 subtyping).

Table 15 Influenza virology

System	Discipline	Component	Method	Scale**	Laboratory
Nasal and OP swabs	Virology	Influenza A RNA detection	As per research laboratory procedure	Qualitative	Site
		H3N2 HA RNA detection			
		H1N1 HA RNA detection			
		Influenza B RNA detection			
		cH8/1 HA RNA detection		Qualitative	CCHMC
		Cytopathic effect in MDCK cell culture		Qualitative	CCHMC

Table 16 specifies influenza virologic read-outs by sampling time point and study group.

Table 16 Read-outs for influenza virology

Sampling time point		Number of subjects	Component
Type of contact and time point	Sampling time point		
Visit 02 (Day -1) Visits 04-08 (Days 2-6)	PRE PId1-6	Subjects in Groups 1-3	Influenza A RNA detection (may also include second detection of master donor virus specific or cH8/1 specific RNA segments)
Visit 02 (Day -1) Visits 04-08 (Days 2-6)	PRE PId1-6	Subjects in Groups 1-3	Cytopathic effect in MDCK cell culture and staining with cH8/1 monoclonal antibody*
ILI Visit (unscheduled visit)	Any	All Subjects	Influenza A RNA detection • H3N2 HA RNA detection • H1N1 HA RNA detection Influenza B RNA detection

*Only for those specimens influenza A positive by RT-PCR in post-vaccination specimens.

7.6 Assays qualification, standardization, validation

Immunologic and transcriptomic assays used in this first-in-humans trial are for research purposes only and have adequate reference standards and controls, where available. No immunologic or transcriptomic assay is qualified or validated at this stage.

7.7 Future use of stored samples

At the completion of all the testing at the laboratories, the samples will be either destroyed or stored at an appropriate place in a designated freezer at the laboratories or at a PATH-designated facility. PATH will be responsible for the oversight of sample storage and destruction. As part of the consent process, subjects will be asked whether they consent to any remaining samples being used for other, ethically approved research which could be used in new or different laboratory tests, to provide information for the development of new vaccines, or for the studies of influenza or other infections. The samples might be shared with researchers at other study centers. All samples will be used only for research purposes. Human genetic (DNA) tests will not be performed on these samples. Samples obtained in this study may result in the development of a product that could be patented or licensed. There are no plans to provide financial compensation to the subject should this occur.

7.8 Biohazard containment

As exposure to blood-borne pathogens can occur through contact with contaminated needles, blood, and blood products, appropriate blood and secretion precautions will be employed by all personnel in the drawing of blood and shipping and handling of all specimens for this study as recommended by the US Centers for Disease Control and Prevention (CDC). All biological specimens will be transported using packaging mandated by CFR 42 Part 72. All dangerous goods materials, including diagnostic specimens and infectious substances, must be transported according to instructions detailed in the International Air Transport Association Dangerous Goods Regulations. Biohazardous waste will be contained according to institutional, transportation/carrier, and all other applicable regulations.

8 SAFETY ASSESSMENT AND REPORTING

8.1 Definitions

8.1.1 Adverse event (AE)

An AE is defined as any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product, which does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, finding during physician examination, or disease

temporally associated with the use of the investigational medical product(s), whether or not considered related to the investigational medical product(s).

The occurrence of an AE might come to the attention of study personnel during study visits or during interviews of a study subject who presents separately for medical care. Information to be collected on AEs includes event description, time of onset, assessment of severity, relationship to study product (assessed only by those with the training and authority to make a diagnosis), and time of resolution/stabilization of the event.

This definition includes exacerbations of pre-existing conditions. Stable pre-existing conditions which do not change in nature or severity during the study are not considered AEs; however, these should be reported as part of the medical history. However, if this condition deteriorates (e.g., increases in frequency or severity grade) during the study, it should be recorded as an AE.

8.1.1.1 Solicited local and general reactions

Solicited AEs are pre-specified local and general (systemic) adverse events that are common or known to be associated with vaccination that are actively monitored as indicators of vaccine reactogenicity. Investigators are not required to assess causality of solicited adverse events if the onset is during the solicitation periods. Solicited adverse events with onset after the solicitation period should be captured as unsolicited AEs.

For this trial, solicited AEs will be assessed by study staff 60 (+10) minutes after each vaccination and then by study subjects daily for 7 days (day of vaccination and subsequent 6 days). Participants will be provided a diary card for recording the presence or absence of solicited (and unsolicited) AEs, for scoring for severity of the solicited (and unsolicited) AEs and for documenting use of concomitant medication and any pain relief and/or antipyretics taken by the subject to correct the AE(s). Subjects will be instructed to measure and record the oral body temperature in the evening regardless of the occurrence of any symptoms. Should additional temperature measurements be performed at other times of day, subjects will be instructed to record the highest temperature in the diary card. Solicited local AEs will be tailored to the site of administration of each product. However, because influenza vaccines containing chimeric HAs have not been previously administered to humans (outside of GSK's parallel Ph1/2 trial), solicited general AEs will be common across both study LAIV and IIV products.

The following local site (nasal) AEs will be solicited post-administration of study LAIV (or placebo) for subjects in Groups 1, 2, and 3:

Table 17 Solicited local adverse events - post-LAIV dose

Nasal congestion
Rhinorrhea

The following local site (injection) AEs will be solicited post-administration of any study IIV (or placebo). This includes post-Dose 1 for subjects in Groups 4 and 5 and post-Dose 2 for subjects in all groups:

Table 18 Solicited local adverse events - post-IIV dose

Pain at injection site
Redness at injection site
Swelling at injection site

The following general AEs will be solicited post-administration of study LAIV (or placebo) and study IIV (or placebo) for subjects in all Groups:

Table 19 Solicited general adverse events - post-LAIV or post-IIV dose

Abdominal pain
Arthralgia
Cough
Diarrhea
Fatigue
Fever
Headache
Myalgia
Nausea
Shivering
Sore throat
Vomiting
Wheezing

Investigators will review diary cards with the subject to ensure the solicited AEs are appropriately documented. The investigator will then transcribe all solicited AEs to the appropriate section of the eCRF.

8.1.1.2 Unsolicited adverse events

Unsolicited AEs are any AEs reported spontaneously by the subject, observed by the study personnel during study visits or those identified during review of medical records or source documents, such as diary cards.

Subjects will be instructed to record any unsolicited symptoms or other illness description in their diary card during the 28 days after each vaccination (day of vaccination and subsequent 27 days) and then until the end of the study. Investigators will review diary cards with the subject to ensure the unsolicited AE is appropriately described. The investigator will then transcribe unsolicited AEs to the appropriate section of the eCRF.

8.1.2 Adverse drug reaction / suspected adverse reaction

An adverse drug reaction is any AE in which the casual relationship to the investigational vaccine is at least a reasonable possibility, i.e., the relationship cannot be ruled out. Having a reasonable suspected causal relationship to the investigational vaccine qualify as an adverse drug reaction. The concept of “reasonable causal relationship” is meant to convey in general that there are facts (evidence) or arguments to suggest a causal relationship.

Suspected adverse reaction is any adverse event for which there is a reasonable possibility that the drug (vaccine) caused the adverse event. For the purposes of IND safety reporting, "reasonable possibility" means there is evidence to suggest a causal relationship between the drug (vaccine) and the adverse event. Suspected adverse reaction implies a lesser degree of certainty about causality than adverse reaction, which means any adverse event caused by a drug (vaccine).

Adverse reaction is any adverse event caused by the drug (vaccine). Adverse reaction is a subset of suspected adverse reactions where there is reason to conclude that the drug (vaccine) caused the event.

Unexpected suspected adverse reaction is considered "unexpected" if it is not listed in the investigator brochure or is not listed at the specificity or severity that has been observed.

8.1.3 Serious adverse event (SAE)

A serious adverse event is an AE that meets one of the following conditions:

- Results in death.

- Is life threatening (i.e., puts the subject at immediate risk of death). (The term “life-threatening” in the definition of “serious” refers to an event in which the subject is/was, in the opinion of the investigator or Sponsor, at risk of death at the time of the event; it does not refer to an event that hypothetically might have caused death if it were more severe.)

- Requires inpatient hospitalization or prolongation of existing hospitalization. (Continuation of stay in the isolation unit beyond 7 days post-administration of either dose of study vaccine or placebo because of late detected viral shedding in a subject shall not be considered a “prolongation of existing hospitalization” for SAE recording or reporting.)

- Results in persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions

- Results in congenital anomaly/birth defect. (Only in the case of a woman becoming pregnant during the study period after administration of at least one dose of study vaccine. All pregnancies must be followed to term and outcome reported to the Sponsor and regulatory agencies.)

- Is an important medical event that may not meet one of the above conditions, but may jeopardize the well-being of the subject or require medical or surgical intervention to prevent one of the outcomes listed above. (Appropriate medical judgment should be exercised in deciding whether reporting these events is appropriate.)

Suspected unexpected serious adverse reaction is any suspected adverse reaction that is both unexpected and serious.

8.1.4 Clinical laboratory parameters and other abnormal assessments qualifying as adverse events or serious adverse events

In absence of diagnosis, abnormal clinical laboratory findings (i.e., clinical biochemistry and hematology) or other abnormal assessments (i.e., physical examination findings) that are judged by the investigator to be clinically significant will be recorded as an AE or SAE if they meet the definition of an AE or SAE. To the extent possible, all normal ranges for clinical laboratory test results will be pre-specified in site reference range documents, but the investigator will exercise his/her medical and scientific judgment in deciding whether an abnormal laboratory finding or other abnormal assessment is clinically significant. Clinically significant abnormal laboratory findings or other abnormal assessments that are present at baseline and significantly worsen following the start of the study will also be reported as AEs or SAEs.

Any finding not normalizing by the end of the study should be followed by the investigator, as would be done under normal clinical care circumstances. After termination of the trial, the investigator should assure that the subject is referred for medical follow-up, as appropriate.

8.1.5 Medically attended event (MAE)

For each solicited and unsolicited AE the subject experiences, the subject will be asked if he/she received medical attention defined as hospitalization, or an otherwise unscheduled visit to or from medical personnel for any reason, including emergency room visits. This information will be recorded in the eCRF.

8.1.6 Influenza-like illness (ILI)

Beginning from Visit 09 (Day 8), all subjects will be monitored for ILI in order to assess the possibility of increased susceptibility to influenza due to vaccine inducing HA stalk antibodies that are not sufficiently neutralizing and that assist wild-type influenza virus in fusion with the human cell membrane. Laboratory confirmed ILI (LC-ILI) will be considered the safety outcome of interest although all ILI, regardless of laboratory confirmation, will also be analyzed. **For the purpose of this study, ILI is defined as at least 1 systemic symptom (fever [oral temperature, ≥ 37.8°C or ≥ 100°F] or myalgia) AND at least 1 respiratory symptom (cough or sore throat).**

Moderate-to-severe ILI will be defined as ILI accompanied by any of the following:

- maximum recorded oral body temperature of >39°C or 102.2°F
- physician-diagnosed pneumonia, lower respiratory tract infection, bronchiolitis, bronchitis, or laryngotracheitis; or
- physician-diagnosed serious extrapulmonary complications of influenza

For analysis and reporting purposes, only ILIs laboratory-confirmed (Nasal and OP swab positive by RT-PCR) as caused by seasonal influenza virus will be included.

8.1.7 Potential immune-mediated disease (pIMD)

Adverse events of specific interest for safety monitoring include pIMDs, a subset of AEs that include autoimmune diseases and other inflammatory and/or neurologic disorders of interest which may or may not have an autoimmune etiology. AEs that need to be recorded and reported as pIMDs include those listed in Table 20.

However, the investigator will exercise his/her medical and scientific judgment in deciding whether other diseases have an autoimmune origin (i.e., pathophysiology involving systemic or organ-specific pathogenic autoantibodies) and should also be recorded as a pIMD.

Once a pIMD is diagnosed (serious or non-serious) in a study subject, the investigator (or designate) must complete, date and sign an electronic Expedited Adverse Events Report.

When there is enough evidence to make any of the diagnoses listed in Table 20, the AE must be reported as a pIMD. Symptoms, signs or conditions which might (or might not) represent the below diagnoses, should be recorded and reported as AEs but not as pIMDs until the final or definitive diagnosis has been determined, and alternative diagnoses have been eliminated or shown to be less likely.

Table 20 List of potential immune-mediated diseases

Neuroinflammatory disorders	Musculoskeletal disorders	Skin disorders
• Cranial nerve disorders, including paralyses/paresis (e.g., Bell's palsy) • Optic neuritis • Multiple sclerosis • Transverse myelitis • Guillain-Barré syndrome, including Miller Fisher syndrome and other variants • Acute disseminated encephalomyelitis, including site specific variants: e.g., non-infectious encephalitis, encephalomyelitis, myelitis, myeloradiculoneuritis • Myasthenia gravis, including Lambert-Eaton myasthenic syndrome • Immune-mediated peripheral neuropathies and plexopathies, (including chronic inflammatory demyelinating polyneuropathy, multifocal motor neuropathy and polyneuropathies associated with monoclonal gammopathy). • Narcolepsy	• Systemic lupus erythematosus and associated conditions • Systemic scleroderma (Systemic sclerosis), including diffuse systemic form and CREST syndrome • Idiopathic inflammatory myopathies, including dermatomyositis • Polymyositis • Antisynthetase syndrome • Rheumatoid arthritis, and associated conditions including juvenile chronic arthritis and Still's disease • Polymyalgia rheumatica • Spondyloarthritis, including ankylosing spondylitis, reactive arthritis (Reiter's Syndrome) and undifferentiated spondyloarthritis • Psoriatic arthropathy • Relapsing polychondritis • Mixed connective tissue disorder	• Psoriasis • Vitiligo • Erythema nodosum • Autoimmune bullous skin diseases (including pemphigus, pemphigoid and dermatitis herpetiformis) • Alopecia areata • Lichen planus • Sweet's syndrome • Localized Scleroderma (Morphoea) • Cutaneous lupus erythematosus

Vasculitides	Blood disorders	Others
• Large vessels vasculitis including: giant cell arteritis such as Takayasu's arteritis and temporal arteritis. • Medium sized and/or small vessels vasculitis including: polyarteritis nodosa, Kawasaki's disease, microscopic polyangiitis, Wegener's granulomatosis, Churg–Strauss syndrome (allergic granulomatous angiitis), Buerger's disease (thromboangiitis obliterans), necrotizing vasculitis and anti-neutrophil cytoplasmic antibody (ANCA) positive vasculitis (type unspecified), Henoch-Schonlein purpura, Behcet's syndrome, leukocytoclastic vasculitis.	• Autoimmune hemolytic anemia • Autoimmune thrombocytopenia • Antiphospholipid syndrome • Pernicious anemia • Autoimmune aplastic anemia • Autoimmune neutropenia • Autoimmune pancytopenia	• Autoimmune glomerulonephritis (including IgA nephropathy, glomerulonephritis rapidly progressive, membranous glomerulonephritis, membranoproliferative glomerulonephritis, and mesangioproliferative glomerulonephritis) • Ocular autoimmune diseases (including autoimmune uveitis and autoimmune retinopathy) • Autoimmune myocarditis/cardiomyopathy • Sarcoidosis • Stevens-Johnson syndrome • Sjögren's syndrome • Idiopathic pulmonary fibrosis • Goodpasture syndrome • Raynaud's phenomenon

Liver disorders	Gastrointestinal disorders	Endocrine disorders
• Autoimmune hepatitis • Primary biliary cirrhosis • Primary sclerosing cholangitis • Autoimmune cholangitis	• Inflammatory Bowel disease, including Crohn's disease, ulcerative colitis, microscopic colitis, ulcerative proctitis • Celiac disease • Autoimmune pancreatitis	• Autoimmune thyroiditis (including Hashimoto thyroiditis) • Grave's or Basedow's disease • Diabetes mellitus type I • Addison's disease • Polyglandular autoimmune syndrome • Autoimmune hypophysitis

8.1.8 Pregnancy

Any confirmed pregnancy among female subjects after receipt of Dose 1 of study vaccine (or placebo) will be recorded but will not be considered an AE. Only if the pregnancy outcome is stillbirth or any congenital anomaly, then this outcome will be reported as an SAE.

The following should always be considered as SAE and will be reported as described in Section 8.6:

- Spontaneous pregnancy loss, including:
  - Spontaneous abortion, (spontaneous pregnancy loss before/at 22 weeks of gestation).
  - Ectopic and molar pregnancy.
  - Stillbirth (intrauterine death of fetus after 22 weeks of gestation).
- Any early neonatal death (i.e., death of a live born infant occurring within the first 7 days of life).
- Any congenital anomaly or birth defect (as per [CDC MACDP] guidelines) identified in the offspring of a study subject (either during pregnancy, at birth or later) regardless of whether the fetus is delivered dead or alive. This includes anomalies identified by prenatal ultrasound, amniocentesis or examination of the products of conception after elective or spontaneous abortion.

Furthermore, any SAE occurring as a result of a post-study pregnancy AND considered by the investigator to be reasonably related to the study vaccines/products will be reported to GSK Biologicals as described in Section 8.6.2. While the investigator is not obligated to actively seek this information from former study participants, he/she may learn of a pregnancy through spontaneous reporting.

8.2 Monitoring periods for safety events

Adverse events will be collected during the study during the following periods:

- Solicited local and general AEs commonly associated with intranasal or intramuscular influenza vaccination occurring through 7 days following any dose (day of vaccination and subsequent 6 days);
- Unsolicited AEs occurring through 28 days following any dose (day of vaccination and subsequent 27 days);
- Hematological and biochemical clinical laboratory abnormalities during the entire study period post-Dose 1;

- AEs (including SAEs) leading to withdrawal from the study during the entire study period post-Dose 1;
- SAEs during the entire study period post-Dose 1;
- SAEs related to study participation during the entire study period beginning from enrollment in screening;
- MAEs during the entire study period post-Dose 1;
- ILIs during the entire study period post-Dose 1;
- pIMDs during the entire study period post-Dose 1; and
- Pregnancies during the entire study period post-Dose 1.

Any solicited AE that starts during the 7 days post-vaccination and is still ongoing after 7 days post-vaccination should still be reported as a solicited AE and not an unsolicited AE. Any AE, SAE, or other safety event should be followed to resolution or stabilization, if possible, even if resolution or stabilization is after its required reporting period or the end of the trial.

AEs/SAEs that are assessed as being “related” to study participation or study procedures during the study period from enrollment in screening until administration of a study vaccine, even if they occur prior to administration of a pharmaceutical product are also to be collected and reported.

8.3 Severity of adverse events

All AEs, including clinical laboratory test results, will be assessed by a study clinician and the study subject (as applicable) to quantify severity using a protocol-defined grading system stated below. For certain study clinical laboratory parameters, the US Food and Drug Administration “Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials” will be used (see Appendix 2).

8.3.1 Severity of solicited AEs

Solicited symptoms will be graded by subjects as described (see Table 21).

Table 21 Severity grading scales for solicited symptoms in adults

Adverse Event	Intensity Grade	Parameter
Runny nose/nasal congestion	0	None
	1	Mild: Runny nose and/or nasal congestion not interfering with daily activity
	2	Moderate: Runny nose and/or nasal congestion causing some interference with daily activity
	3	Severe: Runny nose and/or nasal congestion preventing daily activity
Pain at injection site	0	None
	1	Mild: Any pain neither interfering with nor preventing normal every day activities.
	2	Moderate: Painful when limb is moved and interferes with every day activities.
	3	Severe: Significant pain at rest. Prevents normal every day activities.
Cough	0	None
	1	Mild: Cough that is easily tolerated
	2	Moderate: Cough that interferes with normal activity
	3	Severe: Cough that prevents normal activity
Fever* (record temperature in °F)	0	< 38.0°C or 100.4°F
	1	≥ 38.0°C or 100.4°F - ≤ 38.5°C or 101.3°F
	2	> 38.5°C or 101.3°F - ≤ 39.0°C or 102.2°F
	3	> 39.0°C or 102.2°F
Headache	0	None
	1	Mild: Headache that is easily tolerated
	2	Moderate: Headache that interferes with normal activity
	3	Severe: Headache that prevents normal activity
Fatigue	0	None
	1	Mild: Fatigue that is easily tolerated
	2	Moderate: Fatigue that interferes with normal activity
	3	Severe: Fatigue that prevents normal activity
Gastrointestinal symptoms (nausea, vomiting, diarrhea and/or abdominal pain)	0	None
	1	Mild: Gastrointestinal symptoms that are easily tolerated
	2	Moderate: Gastrointestinal symptoms that interfere with normal activity
	3	Severe: Gastrointestinal symptoms that prevent normal activity
Arthralgia	0	None
	1	Easily tolerated
	2	Interferes with normal activity
	3	That prevents normal activity
Myalgia	0	None
	1	Easily tolerated
	2	Interferes with normal activity
	3	That prevents normal activity
Redness (record greatest surface diameter in mm)	0	≤ 20 mm
	1	> 20 mm - ≤ 50 mm
	2	> 50 mm - ≤ 100 mm
	3	> 100 mm

Adverse Event	Intensity Grade	Parameter
Shivering	0	None
	1	Easily tolerated
	2	Interferes with normal activity
	3	That prevents normal activity
Sore throat	0	None
	1	Mild: Sore throat that is easily tolerated
	2	Moderate: Sore throat that interferes with normal activity
	3	Severe: Sore throat that prevents normal activity
Swelling (record greatest surface diameter in mm)	0	≤ 20 mm
	1	> 20 mm - ≤ 50 mm
	2	> 50 mm - ≤ 100 mm
	3	> 100 mm
Wheeze	0	None
	1	Mild: Wheeze that is easily tolerated
	2	Moderate: Wheeze that interferes with normal activity
	3	Severe: Wheeze that prevents normal activity

*Fever is defined as temperature ≥ 38.0°C or 100.4°F. The preferred location for measuring temperature in this study will be the oral cavity.

8.3.2 Severity of unsolicited AEs, including SAEs, MAEs, pIMDs, and ILIs

The subject will record his/her assessment of unsolicited AEs on diary cards through the end of the study. The investigator will review all such recorded unsolicited AEs with the subject and ensure that the maximum intensity that occurred over the duration of the event is recorded. For other unsolicited AEs not captured on the diary card (including SAEs, MAEs, pIMDs, and ILIs), the investigator will make his/her assessment of maximum severity based on the investigator's clinical judgment after reviewing signs and symptoms of the event and how the event affected the subject.

The intensity should be assigned to one of the following categories:

Grade 1 (Mild) An AE that is easily tolerated by the subject, causing minimal discomfort and not interfering with everyday activities.

Grade 2 (Moderate) An AE that is sufficiently discomforting to interfere with normal everyday activities.

Grade 3 (Severe) An AE that prevents normal, everyday activities. In adults, such an AE would, for example, prevent attendance at work/school and would necessitate the administration of corrective therapy.

An AE that is assessed as Grade 3 (severe) should not be confused with an SAE. Grade 3 is a category used for rating the intensity of an event; and both AEs and SAEs can be assessed as Grade 3. Any AE that places the subject, in the view of the investigator, at moderately high risk of death from the reaction *as it occurred* should be considered as life-threatening and reported as

an SAE (The investigator should *not* grade a reaction as potentially life-threatening that, had it occurred in a more severe form, might have caused death).

8.4 Causality of adverse event

The investigator is obligated to assess the relationship between study vaccines and the occurrence of each AE/SAE using clinical judgment. In case of concomitant administration of multiple vaccines/products (e.g., study IIV + $AS03_A$), if possible, the investigator should specify if the AE could be causally related to a specific vaccine/product administered (i.e., investigational, control/placebo, or co-administered vaccine). When causal relationship to a specific vaccine(s)/product(s) cannot be determined, the investigator should indicate the AE to be related to all products.

Alternative plausible causes, such as natural history of any underlying diseases, concomitant therapy, other risk factors, and the temporal relationship of the event to the study vaccines will be considered and investigated. The investigator will also consult the Investigator Brochure(s) and/or Summary of Product Characteristics to determine his/her assessment.

There may be situations when an SAE has occurred and the investigator has minimal information to include in the initial report to the Sponsor. However, it is very important that the investigator always makes an assessment of causality for every event prior to submission of an expedited report form to the Sponsor. The investigator may change his/her opinion of causality in light of follow-up information and update the SAE information accordingly. The causality assessment is one of the criteria used when determining regulatory reporting requirements.

During the 7-day follow-up period post each dose, all solicited local reactions will be considered causally related to vaccination. Also, relatedness will also not be assessed for any solicited general (systemic) reactions occurring during this period. Causality of unsolicited AEs occurring at any time should be assessed by the investigator using the following question: “Is there a reasonable possibility that the AE may have been caused by the study vaccine/product?”

YES: There is a reasonable possibility that the study vaccine(s)/product(s) contributed to the AE. "Reasonable possibility” means that there is evidence to suggest a causal relationship between the study product and the AE. **(RELATED)**

NO: There is not a reasonable possibility that the AE is causally related to the administration of the study vaccine(s)/product(s). There are other, more likely causes and administration of the study vaccine(s)/product(s) is not suspected to have contributed to the AE. **(NOT RELATED)**

If an event meets the criteria to be determined as ‘serious’ (i.e., SAE), additional examinations/tests should be performed by the investigator in order to determine ALL possible contributing factors for the SAE. Possible contributing factors include:

- Medical history.
- Other medication.

- Protocol required procedure.
- Other procedure not required by the protocol.
- Lack of efficacy of the vaccine, if applicable.
- Erroneous administration.
- Other cause (specify).

8.5 Outcome assessment

The investigator will assess the outcome of all unsolicited AEs (including SAEs) recorded during the study as:

- Recovered/resolved without sequelae.
- Recovered/resolved with sequelae.
- Recovering/resolving.
- Not recovered/Not resolved.
- Fatal (SAEs only).

8.6 Follow-up of adverse events, serious adverse events, and pregnancies

8.6.1 Follow-up of adverse events and serious adverse events

8.6.1.1 Follow-up during the study

After the initial AE/SAE report, the investigator is required to proactively follow each subject and provide additional relevant information on the subject's condition to the Sponsor (within 24 hours for SAEs).

All MAEs, SAEs, and pIMDs (serious or non-serious) documented at a previous visit/contact and designated as not recovered/not resolved or recovering/resolving will be reviewed at subsequent visits/contacts until the last visit of the subject.

All AEs documented at a previous visit/contact and designated as not recovered/not resolved or recovering/resolving will be reviewed at subsequent visits/contacts until 28 days after the last vaccination.

8.6.1.2 Follow-up after the subject is discharged from the study

The investigator will follow subjects:

- With MAEs, SAEs, pIMDs (serious or non-serious), or subjects withdrawn from the study as a result of an AE, until the event has resolved, subsided, stabilized, disappeared, or until the event is otherwise explained, or the subject is lost to follow- up.

- With other non-serious AEs, until the event is otherwise explained or they are lost to follow-up.

If the investigator receives additional relevant information on a previously reported SAE, he/she will provide this information to the Sponsor using a paper/electronic SAE and/or pregnancy report, as applicable.

The Sponsor may request that the investigator performs or arranges the conduct of additional clinical examinations/tests and/or evaluations to elucidate as fully as possible the nature and/or causality of the AE or SAE. The investigator is obliged to assist. If a subject dies during participation in the study or during a recognized follow-up period, the Sponsor will be provided with any available post-mortem findings, including histopathology.

8.6.2 Follow-up of pregnancies

Pregnant subjects will be followed to determine the outcome of the pregnancy. At the end of the pregnancy, whether full-term or premature, information on the status of the mother and child will be forwarded to the Sponsor using the electronic pregnancy report and the SAE Report, if applicable. Generally, the follow-up period does not need to be longer than six to eight weeks after the estimated date of delivery. Regardless of the reporting period for SAEs for this study, if the pregnancy outcome is an SAE, it should always be reported as an SAE.

After consent is obtained from the pregnant female partner of a male subject, the pregnancy must be reported and outcome followed-up in the same manner as for female subjects.

8.7 General guidance on recording adverse event

To improve the quality and precision of acquired AE data, the investigator should observe the following guidelines:

- Whenever possible, use recognized medical terms when recording AEs on the AE CRF. Do not use colloquialisms and/or abbreviations.

- If known, record the diagnosis (i.e., disease or syndrome) rather than component signs, symptoms and laboratory values (e.g., record congestive heart failure rather than dyspnea, rales, and cyanosis). However, signs and symptoms that are considered unrelated to an encountered syndrome or disease should be recorded as individual AEs (e.g., if congestive heart failure and severe headache are observed at the same time, each event should be recorded as an individual AE).

- AEs occurring secondary to other events (e.g., sequelae) should be identified by the primary cause. A “primary” AE, if clearly identifiable, generally represents the most accurate clinical term to record. If a primary SAE is recorded, events occurring secondary to the primary event should be described in the narrative description of the case. (For example: Orthostatic hypotension→Fainting and fall to floor→Head trauma→Neck pain. The primary AE is orthostatic hypotension.)

- Death is an outcome of an event. The event that resulted in the death should be recorded and reported on the SAE CRF.

- For hospitalizations for surgical or diagnostic procedures, the illness leading to the surgical or diagnostic procedure should be recorded as the SAE, not the procedure itself. The procedure should be captured in the case narrative as part of the action taken in response to the illness.

- Pregnancies that occur in study subjects are not considered AEs and will be recorded on a separate Pregnancy CRF. Pregnancy outcomes that include stillbirth and any congenital anomalies must be reported as SAEs.

8.8 Reporting of SAE

8.8.1 Investigator reporting to sponsor

PATH has designated Emmes Corporation as the Study Coordinating Center with authority to coordinate SAE/pIMD and pregnancy reporting activities. **An appropriate expedited reporting form must be completed and submitted within 24 hours of an investigator’s awareness of an SAE or pIMD, as defined in the protocol.** The site investigator is required to notify the PATH Medical Officer and the Emmes Medical Monitor within 24 hours of knowledge of a pregnancy. **A pregnancy report form must be submitted within 2 weeks of an investigator's awareness of a pregnancy.** Details of the process will be specified in a study-specific Safety Monitoring Plan document.

The investigator must not wait to collect additional information to fully document the event before submitting the appropriate expedited reporting form (e.g., SAE Form). When additional information becomes available, follow-up submission(s) must be completed. The initial expedited reporting form should be completed with all information known at the time and should include as much as possible the following:

- Name and contact of the investigator submitting the expedited reporting form
- Subject ID number
- Date subject received study vaccine/s, including inpatient/outpatient cohort group
- Description of the SAE or pIMD and date of event onset
- Investigator’s assessment of severity, causality, and expectedness

- Action taken and current status
- If available, any diagnostic test reports or hospital records that may help the sponsor to evaluate the SAE or pIMD

The investigator will be responsible for notifying his/her institution's IRB. The Sponsor will notify the other site investigator, who will be responsible for notifying his/her IRB, as applicable. The Sponsor will also be responsible for notifying regulatory authorities, as required, and for notifying GSK Biologicals if the event occurs post-administration of any GSK-provided study vaccines.

8.8.2 Notification and review of SAEs

The Sponsor is responsible for evaluating SAEs/pIMDs submitted by the investigator within 24 hours to convene a safety review if the investigator reported the SAE as fatal or life-threatening and suspected to be related to study vaccine. Medical Officers from PATH serving as technical consultants will provide technical guidance regarding SAE management including classification and reporting.

The Study Coordinating Center is responsible for notifying the Sponsor and also convening a safety review within 24 hours by the IDMC if the investigator reported the SAE as fatal or life-threatening and suspected to be related to study vaccine. The designated medical monitor will review all unanticipated events involving risk to subjects or others, SAEs and all subject deaths associated with the protocol and will provide a written report. At a minimum, the designated medical monitor must comment on the outcomes of the event or problem and, in case of an SAE or death, comment on the relationship to participation in the study. The designated medical monitor must also indicate whether he/she concurs with the details of the report provided by the site PI.

8.8.3 Sponsor reporting to regulatory agency

The Sponsor has authorized the Study Coordinating Center to execute its responsibility for safety reporting to the FDA within the following time periods:

- Suspected unexpected serious adverse reaction within 15 days of Sponsor’s awareness.
- Fatal or life-threatening suspected adverse reaction within 7 days of Sponsor’s awareness.

8.9 Other events requiring expedited reporting

All unanticipated problems will be reported in the continuing review report submitted to the IRBs and regulatory authorities per reporting requirements of each regulatory body. All serious unanticipated problems involving risk to subjects or others will be promptly reported (within 48 hours) by telephone, by email, or by facsimile to the Coordinating Center. Follow-up reports will be submitted as soon as additional information becomes available.

9 SAFETY MONITORING

The site PIs and/or designated site clinical staff will be responsible for continuous safety monitoring of all study subjects and for alerting the Sponsor if unexpected concerns arise or study pause/hold criteria are met.

9.1 Routine reviews by protocol safety review team

The PSRT, comprised of the site Principal Investigators, the PATH Medical Officer, and the Emmes medical monitor, will routinely monitor safety throughout the duration of the trial. The PSRT will be chaired by the PATH Medical Officer and may seek additional independent expert medical opinion as dictated by needs. An Emmes statistician with assistance of Emmes Data Management staff will prepare blinded safety reports for review by the PSRT. These reports will provide at a minimum the following information:

- Accrual data and subject status data with regard to completion of/discontinuation from the study, sorted by site.
- Summaries of solicited adverse events, classified by severity.
- Unsolicited AEs (including SAEs/pIMDs) sorted by MedDRA term, severity and relatedness to study vaccine.
- Safety laboratory test results outside of normal institution reference ranges, classified by severity grading scale (irrespective of whether assessed as AEs).
- Any new or updated AEs that have occurred in the interval from the previous report.

All SAEs/pIMDs will be provided to the PSRT, with history and subsequent follow-up information as pertains to the SAE/pIMD, within the first 24 hours following site awareness and notification of the SAE/pIMD.

The PSRT safety review will be conducted by teleconference approximately one month post completion of dosing with each dose and at approximately 6 and 12 months after completion of dosing with the second dose. In addition to safety review, the PSRT may elect to discuss trial conduct issues that impact study integrity and subject safety. These may include but not limited to data quality, critical monitoring findings, study product, research specimens, etc. The Study Coordinating Center will also notify the PSRT of ad hoc safety reviews whenever it is aware of a suspected unexpected serious adverse reaction or adverse events that meet pre-specified study pause criteria as per section 9.3

9.2 Independent data monitoring committee

An Independent Data Monitoring Committee will be established by PATH for the purpose of monitoring the study and to provide independent, non-binding advice on safety and ethics. The IDMC will be comprised of independent medical experts in vaccinology and infectious diseases

who will periodically review the conduct and safety of study. The IDMC will be supported by an unblinded secretary and an unblinded biostatistician, both from Emmes. The responsibilities and procedures of the IDMC are defined in the IDMC Charter. During the whole study period, there will be IDMC reviews at pre-defined time points (The frequency of these reviews may be adapted upon IDMC recommendation if deemed necessary). At least three pre-defined meeting time points will be as follows:

- The IDMC will review safety data through Visit 10 (28 days post-Dose 1) for all subjects prior to any subject receiving Dose 2 of study vaccine.
- The IDMC will review safety data through Visit 13 (28 days post-Dose 2) for all subjects.
- The IDMC will review safety data through Visit 15 (12 months post-Dose 2) for all subjects.
- The IDMC may also be convened to review events meeting pause rules if deemed necessary by the PSRT. In such cases, the IDMC and PSRT may convene by teleconference to jointly review the data.

The IDMC reviews will be summarized with recommendations to the study Sponsor as to whether there are safety concerns and whether the study should continue without change, be modified, or be terminated. If at any time, a decision is made to permanently discontinue administration of study vaccinations in all subjects, the Sponsor will notify the FDA and the site PIs will notify the responsible IRBs expeditiously.

9.3 Study pause rules

Study pause rules based on safety data are defined in Table 22. If a pause rule 1a through 1d is observed by the investigator, it will be reported promptly to the Study Coordinating Center within 24 hours. In order to assess pause rules 2a through 2c, these pause rules have been written under the assumption that the safety data of all subjects will be available. Therefore, the investigator will be requested to record safety data in the eCRF within 72 hours after reception of the data to ensure that all necessary information is available for timely PSRT and IDMC reviews. If the data from all subjects are not available, the pause rules will be assessed on a pro-rata basis.

The pause rules will automatically pause or halt further vaccinations. However, subjects already enrolled will continue to be followed for safety during the hold. These pause rules pertain to suspected adverse reactions and will be triggered automatically if any of the events are met during the conduct of the study at the parameters specified.

Table 22 Study pause rules

Pause rules	Event	Number of subjects
1a	Death or any life-threatening **SAE**	≥ 1
1b	Any **SAE** that cannot reasonably be attributed to a cause other than vaccination	≥ 1
1c	Any **withdrawal** from the study or **withdrawal** from study vaccine(s)/product(s) (by investigator or subject request) following a grade 3 AE that cannot reasonably be attributed to a cause other than vaccination	≥ 1
1d	Any local or general solicited AE leading to **hospitalization**, or **fever > 40°C or 104°F** (oral route) that cannot reasonably be attributed to a cause other than vaccination, or **necrosis** at the injection site, within the 7-day (Days 1-7) post-vaccination period	≥ 1
2a	Any **grade 3 solicited local** AE lasting 48h or more in an investigational group, within the 7-day (Days 1-7) post-vaccination period	≥ 15% (and ≥ 2 subjects in one group)
2b	Any **grade 3 solicited general** AE lasting 48h or more in an investigational group, that cannot reasonably be attributed to a cause other than vaccination, within the 7-day (Days 1-7) post-vaccination period	≥ 20% (and ≥ 2 subjects in one group)
2c	Any **grade 3 unsolicited** AE in an investigational group, that cannot reasonably be attributed to a cause other than vaccination, within the 7-day (Days 1-7) post-vaccination period OR Any grade 3 abnormality in the same pre-specified hematological or biochemical **laboratory parameters*** in an investigational group, that cannot reasonably be attributed to a cause other than vaccination, within the 7 days post-vaccination	≥ 20% (and ≥ 2 subjects in one group)

*Grading of laboratory parameters will be based on the FDA Guidance for Industry "Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials" (refer to Appendix 2).

9.4 Study hold procedure

Once a pause rule criterion has been met, vaccinations at both sites must be put on hold as a consequence. The Study Coordinating Center will ensure immediate notification to both sites and the PSRT and IDMC, which will convene ad hoc reviews. The investigators are not permitted to continue enrollment or administering any dose of study vaccines to enrolled subjects until receipt of approval to continue dosing is received by the PSRT and IDMC.

If a study pause is initiated, randomized subjects will continue with their scheduled follow-up visits, but not vaccination visits. In that case, the visit will be on hold during the pause; when the study is resumed, the subject will still be considered to be within their vaccination window, and all future visits adjusted based on the date of resumed vaccination. Such a pause in the study would not constitute a protocol deviation in regards to subject visit windows, and the pause would be taken into consideration for restarting the assigned vaccination visit schedule.

The PSRT and IDMC will expeditiously (within 48 hours) convene to review all available, relevant information. The PSRT and IDMC reviews will be summarized with consensus recommendations to the study Sponsor as to whether there are safety concerns and whether the study should

continue without change, be modified, or be stopped. To be clear, the IDMC must approve lifting the hold in order for enrollment and study vaccination to continue.

If at any time, a decision is made to discontinue administration of study product in all subjects, expeditious notification will be provided by the Sponsor to the FDA and by the site PIs to their IRBs within 48 hours.

10 DATA HANDLING AND RECORDKEEPING

The Principal Investigator is responsible for assuring that the data collected is complete, legible, attributable, accurate, and recorded in a timely manner. Data collection is the responsibility of the clinical trial staff at the site under the supervision of the site PI. All source documents and laboratory reports must be reviewed by the clinical team and data entry staff, who will ensure that they are accurate and complete

Emmes has been designated by PATH for data management activities, including quality review, analysis, and reporting. The study will utilize Advantage eClinical, an EDC system uniquely suited for clinical research. Site staff will use the EDC tool to securely enter data into eCRFs from source documents, or from medical or clinical records, directly into the database.

10.1 Source documents and access to source data/documents

Prior to the start of the trial it will be determined which documents completed by the investigative team will be considered source documents. Only authorized study staff and representatives of the Sponsor and authorized regulatory agencies may have direct access to source documents containing study data. Data for this study will include biographical, medical history, clinical (signs, symptoms, prescription and non-prescription medical treatments, and non-study vaccinations), safety data, and immunologic laboratory data. All information in original records and certified copies of original records, clinical findings, or observations will be considered source data for this study.

Documents and data pertaining to the study will be kept in a locked cabinet under the responsibility of the investigator. PATH and/or Emmes will conduct periodic monitoring visits to ensure that the data is safe and stored in this secure place and that only those authorized study staff have access to the data.

10.2 Data capture methods (case report form development and completion)

The clinical data in CRFs or other source documents (such as clinical laboratory results) will be entered directly into a 21 CFR Part 11-compliant electronic data capture system (Advantage eClinical) provided by The Emmes Corporation by trained and qualified study staff. The eCRF for the EDC system will be developed by Emmes with input from study staff and approval of PATH. The data system includes password protection and internal quality checks, such as automatic

range checks, to identify data that appear inconsistent, incomplete, or inaccurate. Clinical data for each subject will be entered directly into the eCRF from the CRFs or source documents.

It is the site PIs' responsibility to ensure the accuracy, completeness, and timeliness of the data reported in the subject's eCRF and any supporting documentation. All CRF source documents should be completed in a neat, legible manner to ensure accurate interpretation of data. All source documentation supporting the eCRF data should document the dates and details of study procedures, AEs, and subject status. The site PIs/institutions will maintain the information collected from CRFs and in the eCRFs and all source documents that support the data collected from each subject in a secure area and treated as confidential material.

10.3 Data management

Emmes will develop the data management plan describing the tools and processes for the generation of the clinical database and data management activities from project set-up through data lock and transfer. The project set-up period will include a planning phase for the design of the system environment and the development of a detailed structure for logistical operations. Instructions for database management tasks will include data processing, data coding, query generation and tracking, and any other tasks that are unique to project requirements. The plan for managing electronic laboratory generated data will include instructions for data import, and upload directly into the Advantage eClinical system. The system and programmatic requirements for the transfer of data sets and associated data components will be identified in the plan. Emmes' data management SOPs will be referenced to ensure that the data management plan for the study addresses the risk management aspects for all processes and accommodates for shifts in workload activity.

Emmes will generate reports through Advantage eClinical for missing forms and missing values, and quality control checks between the clinical and laboratory results. Anomalies will be communicated to the designated site personnel and documented at the site using procedures compliant with International Conference on Harmonisation Good Clinical Practices (ICH-GCP), and the site will update the data system if any of these anomalies require modifications to the records.

10.4 Retention of study record

Clinical trial data will be stored securely for a period of 2 years following the date that a marketing application is approved or not approved or until 2 years after the study is closed and the national regulatory authority is notified. PATH will be responsible for providing the site with date of approval or IND/regulatory withdrawal.

No records will be destroyed without the written consent of PATH. Storage of all trial-related documents will be such that confidentiality will be strictly maintained to the extent provided by local law.

11 STATISTICAL CONSIDERATIONS

11.1 Overview and general design

This is a prospective, multi-center, randomized, controlled, observer-blind, Phase 1 trial in healthy male and female adults 18 through 39 years of age. Up to 65 eligible subjects will participate and will be randomized to one of five groups to receive a first dose of study cH8/1N1 LAIV (or placebo) or study cH8/1N1 IIV + $AS03_A$ adjuvant (or placebo) followed three months later by study cH5/1N1 IIV +/- $AS03_A$ adjuvant (or placebo). Eligible enrolled subjects will be randomized to any of the treatment arms (LAIV-IIV, Groups 1, 2, and 3 or IIV-IIV, Groups 4 and 5) under one allocation sequence to allow comparability between study groups, particularly LAIV-IIV vs IIV-IIV regimens (Groups 1 vs 4). While subjects will be blinded to their exact treatment group and whether they received active study vaccine versus placebo, subjects will certainly know if they received LAIV (or placebo) vs IIV (or placebo) at Dose 1, given the different presentations and routes of administration of these products. The primary objective is to assess the reactogenicity and safety after each dose of study vaccine, a key secondary objective is to assess the potential superiority of the immune responses elicited by LAIV-IIV prime-boost compared to those by IIV-IIV.

11.2 Randomization procedures

The randomization plan will be developed in coordination with Emmes. Randomization will be stratified by site and include a blocking factor. The original allocation ratio for LAIV-LAIV Groups 1, 2 and 3 was 3:3:1, and the original allocation ratio for IIV-IIV Groups 4-5 was 3:2. To preserve comparability of subjects across treatment groups, subjects will be randomized to all groups under one allocation sequence. Among LAIV-LAIV subjects, Group 1 is our primary interest, especially for comparison to Group 4. To guard against loss of power from possible drop-out between randomization and admission into the inpatient clinical isolation unit, Group 1 will be over-randomized by five subjects, for a final randomization scheme of 4:3:1:3:2. A secure IWRS will be utilized for eligibility verification, subject registration, randomization, and treatment assignment. IWRS allows authorized staff from study centers to perform subject enrollment 24 hours a day, 7 days a week. The system guides users through the process of specifying the subject identifier, completing demographic information, and finally, completing the protocol-specific inclusion-exclusion checklist, which confirms a subject's eligibility, which is generally predetermined at the site from screening information.

11.3 Primary endpoints

11.3.1 Reactogenicity and safety

- Occurrence of solicited local and general AEs post-vaccination:
  - Occurrence of solicited local AEs during a 7-day follow-up period (i.e., on the day of vaccination and 6 subsequent days) after the first and the second dose in each study group.
  - Occurrence of solicited general AEs during a 7-day follow-up period (i.e., on the day of vaccination and 6 subsequent days) after the first and the second dose in each study group.
- Occurrence of unsolicited AEs post-vaccination:
  - Occurrence of unsolicited AEs during a 28-day follow-up period (i.e., on the day of vaccination and 27 subsequent days) after the first and the second dose in each study group.
- Occurrence of hematological and biochemical laboratory abnormalities post-vaccination:
  - Any hematological (red blood cell, white blood cell, and differential count, platelets count and hemoglobin level) or biochemical (ALT, AST, creatinine, BUN and BUN-to-creatinine ratio) laboratory abnormality on Visits 9, 10, 11, 12, and 13 in each study group.
- Occurrence of MAEs, LC-ILIs, pIMDs, and SAEs up to Visit 13:
  - Occurrence of MAEs, LC-ILIs, pIMDs, and SAEs up to Visit 13 in each study group.

(Because Groups 1 and 2 will receive the same study LAIV at Dose 1, data for the period post-Dose 1 will be pooled for these groups.)

11.4 Secondary endpoints

11.4.1 Reactogenicity and safety

- Occurrence of hematological and biochemical laboratory abnormalities:
  - Any hematological (red blood cell, white blood cell, and differential count, platelets count and hemoglobin level) or biochemical (ALT, AST, creatinine, BUN and BUN-to-creatinine ratio) laboratory abnormality at Visits 14 and 15 in each study group.
- Occurrence of MAEs, LC-ILIs, pIMDs and SAEs up to study end at Visit 16:
  - Occurrence of MAEs, LC-ILIs, pIMDs and SAEs up to Visit 16 in each study group.

- Shedding of vaccine virus through 5 days post-vaccination (Groups 1, 2 & 3 only):
  - Percentages of subjects with vaccine virus detectable by RT-PCR each day and overall (at any time).
  - Percentages of subjects with recovery of viable vaccine virus in MDCK culture each day and overall (at any time).

11.4.2 Immunogenicity - descriptive, post-Dose 2

- Humoral and mucosal immunity in terms of anti-H1 stalk immune response measured by ELISA, neutralizing antibodies by MN assay, and activity by ADCC assay at Visit 13 (28 days post-boost):
  - Levels of anti-H1 stalk antibody titers by ELISA, by MN, and by ADCC.
- The following aggregate variables will be calculated for the above parameters with 95% confidence interval (CI):
  - Seropositivity rates and GMTs at Day 1 and Visit 13.
  - Percentage of subjects with a $\geq$ 4-fold increase from Day 1 to Visit 13.
  - Percentage of subjects with a $\geq$ 10-fold increase from Day 1 to Visit 13.
  - MGI from Day 1 to Visit 13.
  - (Post-dose one descriptive immunogenicity will be similarly analyzed at Visit 10 . Because Groups 1 and 2 will receive the same study LAIV at Dose 1, data for the period post-Dose 1 will be pooled for these groups.)

11.4.3 Immunogenicity - descriptive, post-Dose 2 - breadth

- Breadth of the humoral immune response as measured by levels of anti-H2, anti-H9 and anti-H18 antibody titers by ELISA. The following aggregate variables will be calculated for the above parameters with 95% CI:
  - Anti-H2, anti-H9, and anti-H18 seropositivity rates and GMTs at Day 1 and Visit 13.
  - Percentage of subjects with a $\geq$ 4-fold increase in anti-H2, anti-H9 and anti-H18 antibody titers from Day 1 to Visit 13.
  - Percentage of subjects with a $\geq$ 10-fold increase in anti-H2, anti-H9 and anti-H18 antibody titers from Day 1 to Visit 13.
  - MGI in anti-H2, anti-H9 and anti-H18 antibody titers from Day 1 to Visit 13.

- Levels of antibody titers by MN for H5N8; H1N1 avian-swine influenza, and current H1N1pdm09-like vaccine strains. The following aggregate variables will be calculated for the above parameters with 95% CI:

  - Seropositivity rates and GMTs for H5N8, H1N1 avian-swine, and H1pdm09-like neutralizing antibody at Day 1 and Visit 13.

  - Percentage of subjects with a ≥ 4-fold increase in H5N8, H1N1 avian-swine, and H1pdm09-like neutralizing antibody titers from Day 1 to Visit 13.

  - Percentage of subjects with a ≥ 10-fold increase in H5N8, H1N1 avian-swine, and H1pdm09-like neutralizing antibody titers from Day 1 to Visit 13.

  - MGI in H5N8, H1N1 avian-swine, and H1pdm09-like neutralizing antibody titers from Day 1 to Visit 13.

  - (Post-Dose 1 descriptive immunogenicity for breadth will be similarly analyzed at Visit 10 and additional post-Dose 2 descriptive immunogenicity for breadth may be analyzed at Visit 15.)

11.4.4 Immunogenicity - descriptive, post-Dose 2 - persistence

- Persistence of the both humoral and mucosal immune responses as measured by levels of anti-H1 stalk antibody titers by ELISA, by MN, and by ADCC. The following aggregate variables will be calculated for the above parameters with 95% CI:

  - Seropositivity rates and GMTs at Visits 14* and 15.

  - Percentage of subjects with a ≥ 4-fold increase from Day 1 to Visits 14* and 15.

  - Percentage of subjects with a ≥ 10-fold increase from Day 1 to Visits 14* and 15.

  - MGI from Day 1 to Visits 14* and 15.
    **Only for anti-H1 HA-stalk IgG detection by ELISA in serum*

11.4.5 Immunogenicity - comparative, post-Dose 2

- Humoral and mucosal immunity in terms of anti-H1 stalk immune response as measured by ELISA, neutralizing antibodies by MN assay, and activity by ADCC assay 28 days post-boost with LAIV-IIV prime-boost compared to two doses of IIV:

  - Adjusted GMT ratio of the LAIV / IIV + $AS03_A$ group (Group 1) versus the IIV + $AS03_A$ / IIV + $AS03_A$ group (Group 4), 28 days post-vaccination (i.e., at Visit 13 to evaluate the LAIV-IIV prime-boost effect).

- Seroresponse (≥4-fold) rate difference of the LAIV / IIV + $AS03_A$ group (Group 1) versus the IIV + $AS03_A$ / IIV + $AS03_A$ group (Group 4), 28 days post-vaccination (i.e., at Visit 13 to evaluate the LAIV-IIV prime-boost effect).

- Adjusted GMT ratio of the LAIV / IIV group (Group 2) versus the IIV + $AS03_A$ / IIV + $AS03_A$ group (Group 4), 28 days post-vaccination (i.e., at Visit 13 to evaluate the LAIV-IIV prime-boost effect).

- Seroresponse (≥4-fold) rate difference of the LAIV / IIV group (Group 2) versus the IIV + $AS03_A$ / IIV + $AS03_A$ group (Group 4), 28 days post-vaccination (i.e., at Visit 13 to evaluate the LAIV-IIV prime-boost effect).

- LAIV-IIV groups might be pooled in an exploratory analysis if the adjuvant effect appears minimal. Adjuvant effect on the anti-stalk immune response in term of:

  - GMT ratio of the LAIV- IIV + $AS03_A$ group (Group 1) versus the LAIV-IIV unadjuvanted group (Group 2), 28 days post-boost (i.e., at Visit 13 to evaluate the adjuvant effect post-boost).

11.5 Tertiary endpoints

- Evaluation of CMI parameters in terms of frequencies of:

  - Antigen-specific IFN-γ secreting cells upon *in vitro* stimulation at Day 1 and Visits 9, 11, 12, and 15.

  - B-memory cells reactive with the H1 stalk domain and a wild-type H1N1pdm09 virus at Day 1 and Visits 10, 11, 13, and 15.

  - Plasmablasts reactive with the H1 stalk domain and a wild-type H1N1pdm09 virus at Visits 9 and 12.

- Levels of HI antibody to chimeric vaccine strains. The following aggregate variable will be calculated with 95% CI:

  - Seropositivity rates and GMTs at Day 1 and Visits 10, 11, 13, and 15.

  - Seroprotection rate at each time point listed above.

  - Seroconversion rate at Day 1 and Visits 10, 11, 13, and 15.

  - MGI from Day 1 to each subsequent time point listed above.

- Evaluation of the anti-H3 stalk response by ELISA pre-and post-vaccination. Levels of anti-N1 NA antibody by ELISA at Day 1 and Visits 10, 11, 13, and 15.

- Assessment of the *in vivo* protective effect of the anti-stalk antibodies when transferring Day 1, Visit 13 and Visit 15 pooled serum from a subset of subjects of each vaccine groups to mice subsequently challenged with cH6/1N5, or with current H1N1pdm09-like virus recommended by the World Health Organization, using the following endpoints:

  - Survival over 14 days post-challenge (day of death/euthanasia for weight loss > 25% baseline body weight) in groups of mice/serum pool/per vaccine group/time point.

  - Mean weight loss (change from baseline over 14 days post-challenge) in groups of mice/serum pool/vaccine group/time point.

  - Lung virus titer in plaque-forming units (pfu)/g (log_{10} fold change [Day 1 minus Visit 13]), within challenge group.

11.6 Sample size

In this study in which subjects must consent to participate as either inpatient subjects or outpatient subjects for Dose 1 prior to randomization and finding out to which regimen they will be assigned (LAIV-IIV vs IIV-IIV), we assume drop-out is possible between randomization and receipt of Dose 1, especially for subjects randomized to the major commitment of staying in the inpatient clinic. While such drop-out could occur equally in any of the three LAIV-IIV (or placebo) treatment arms, since Group 1 is our primary interest and for cost control, we over-randomized that group by 5 subjects. Admittedly, loss from Group 2 could lower the power in that group as well. We hope that drop-outs will be minimal in both groups. We also assume drop-out is possible between Dose 1 and primary immunogenicity sampling at Visit 13. The following Table 22 summarizes our assumptions, which are applied in the subsequent sample size and power tables.

Table 23 Assumed losses of evaluable subjects from randomization until immunogenicity analyses at Visit 13

Study Arm	Planned sample size per Group	Number of subjects assumed lost between randomization and receipt of Dose 1	Evaluable subjects for safety analyses post-Dose 1	Number of subjects assumed lost between Dose 1 and primary immunogenicity readouts at Visit 13	Evaluable subjects for primary immunogenicity analyses at Visit 13
Group 1	20	3	17	1	16
Group 2	15	2	13	1	12
Group 3	5	1	4	1	3
Group 4	15	0	15	1	14
Group 5	10	0	10	1	9

11.6.1 Descriptive objectives

11.6.1.1 Safety

The primary objectives of the study are to assess the safety of the investigational LAIV and IIVs 28 days after each dose. Because Groups 1 and 2 will receive the same study LAIV at Dose 1, data for the period post-Dose 1 will be pooled for these groups.

Table 24 shows the true proportions associated with a 90% probability to observe an event in 30 LAIV recipients (e.g., SAE, pIMD) post-Dose 1.

Table 24 True proportions associated with a 90% probability to observe a certain number of adverse events pooled LAIV groups (30 subjects)

True proportion	Number of adverse events observed with > 90% probability
0.074	> 0
0.124	> 1
0.168	> 2
0.209	> 3

Table 25 shows the true proportions associated with a 90% probability to observe an event in 15 IIV recipients (e.g., SAE, pIMD) post-Dose 1.

Table 25 True proportions associated with a 90% probability to observe a certain number of adverse events within an IIV group (15 subjects)

True proportion	Number of adverse events observed with > 90% probability
0.142	> 0
0.236	> 1
0.317	> 2
0.393	> 3

Table 26 illustrates the 95% CI for different possible numbers of AEs among all LAIV recipients post-Dose 1.

Table 26 2-sided 95% exact confidence intervals for the true adverse event rate at different possible observed adverse event rates (30 subjects)

Observed number of adverse events	Observed adverse event proportion	95% Exact Confidence Interval	
		Lower Limit	Upper Limit
0	0.000	0.000	0.116
1	0.033	0.001	0.172
2	0.067	0.008	0.221
3	0.100	0.021	0.265
4	0.133	0.038	0.307
5	0.167	0.056	0.347
10	0.333	0.173	0.528
15	0.500	0.313	0.687
20	0.667	0.472	0.827

Table 27 illustrates the 95% CI for different possible numbers of AEs post-receipt of study IIV post-Dose 1.

Table 27 2-sided 95% exact confidence intervals for the true adverse event rate at different possible observed adverse event rates (15 subjects)

Observed number of adverse events	Observed adverse event proportion	95% Exact Confidence Interval	
		Lower Limit	Upper Limit
0	0.000	0.000	0.218
1	0.067	0.002	0.320
2	0.133	0.017	0.405
3	0.200	0.043	0.481
4	0.267	0.078	0.551
5	0.333	0.118	0.616
10	0.667	0.384	0.882

11.6.1.2 Immunogenicity

Table 28 presents the 95% CI for different possible rates of immunological response all LAIV subjects post-Dose 2.

Table 28 2-sided 95% exact confidence intervals for the true immunological response rate at different possible observed response rates (28 evaluable subjects)

Observed number of responses	Observed response proportion	95% Exact Confidence Interval	
		Lower Limit	Upper Limit
10	0.357	0.186	0.559
15	0.536	0.339	0.725
20	0.714	0.513	0.868
25	0.893	0.718	0.977

Table 29 presents the 95% CI for different possible rates of immunological response among Group 1 LAIV-IIV subjects post-Dose 2.

Table 29 2-sided 95% exact confidence intervals for the true immunological response rate at different possible observed response rates (16 evaluable subjects)

Observed number of responses	Observed response proportion	95% Exact Confidence Interval	
		Lower Limit	Upper Limit
5	0.313	0.110	0.587
7	0.438	0.198	0.701
10	0.625	0.354	0.848
13	0.813	0.544	0.960

Table 30 presents the 95% CI for different possible rates of immunological response among IIV-IIV subjects post-Dose 2.

Table 30 2-sided 95% exact confidence intervals for the true immunological response rate at different possible observed response rates (14 evaluable subjects)

Observed number of responses	Observed response proportion	95% Exact Confidence Interval	
		Lower Limit	Upper Limit
5	0.357	0.128	0.649
7	0.500	0.230	0.770
10	0.714	0.419	0.916
13	0.929	0.661	0.998

11.7 Comparative objectives

Secondary objectives include comparisons of the immunogenicity post-Dose two between LAIV-IIV and IIV-IIV groups (Group 1 vs Group 4). These comparisons are purely descriptive with the aim to characterize the differences in immunogenicity between groups. For example, the ratio of GMTs in the LAIV-IIV and IIV-IIV groups will be compared. Table 31 illustrates the power to detect various fold increase in GMT ratios between treatment groups.

Table 31 Power to detect fold increases in GMT ratios for different values of alpha (type I error) assuming a standard deviation of 0.4* in the log_{10} titer values in both comparison groups (14 evaluable subjects per group)**

One Sided Alpha	Fold Increase				
	2.0	2.5	3.0	3.5	4.0
.025	48%	72%	86%	93%	97%
.05	62%	82%	92%	97%	99%
.10	75%	90%	97%	99%	100%

*The assumed standard deviation is unpublished but is based on data from the study: Nachbagauer R, et al. J Virol; 2014 [25]

**For simplicity, we assume equivalent numbers of subjects (n=14) per group, as the power table is purely illustrative.

11.8 Analysis sets

11.9 Exposed set

The Exposed Set (ES) will include all subjects with at least one vaccine administration documented:

- A safety analysis based on the ES will include all vaccinated subjects.
- An immunogenicity analysis based on the ES will include all vaccinated subjects for whom immunogenicity results are available.

The total vaccinated cohort (TVC) analyses will be performed per treatment actually administered.

11.10 Per-protocol set

The Per-Protocol set will be adapted by time point to include all eligible subjects' data up to the time of important protocol deviation, namely:

- Dose of study vaccine not according to protocol procedures and to their random assignment.
- Randomization code broken.
- Non-compliance with the procedures and intervals defined in the protocol (refer to Appendix 1).
- Intake of concomitant medication/product/vaccination leading to elimination from the Per-Protocol analysis.
- Occurrence of medical condition leading to elimination from the Per-Protocol analysis (refer to Section 5.9)

11.11 Statistical analyses

All analyses will be based on data pooled from both sites. However, because of the multi-center nature of this study with randomization stratified by site, sub-analyses by site will also be conducted.

11.11.1 Analysis of demographics

Demographic characteristics (age at study vaccination in years, gender, ethnicity, history of influenza vaccination since the 2014/2015 season) and withdrawal status will be summarized by group in the TVC, using descriptive statistics:

- Frequency tables will be generated for categorical variables such as gender.
- Mean, median, standard deviation will be provided for continuous data such as age.

11.11.2 Analysis of safety

The analysis will be performed on the ES.

All analyses will be descriptive. Data will be presented by dose, overall/dose and overall/subject. Outputs will be presented by study group. Analyses will be repeated pooling LAIV groups (for post-Dose 1 events collected prior to Visit 11).

The percentage of subjects with at least one local AE (solicited and unsolicited), with at least one general AE (solicited and unsolicited) and with any AE during the solicited follow-up period will be tabulated with exact 95% CI. The same calculations will be performed for AEs rated as grade 3.

The percentage of subjects reporting each individual solicited local and general AE during the solicited follow-up period will be tabulated with exact 95% CI. The same tabulation will be performed for grade 3 AEs and for AEs with relationship to vaccination.

The verbatim reports of unsolicited AEs will be reviewed by a physician and the signs and AEs will be coded according to MedDRA. The percentage of subjects with at least one report of unsolicited AE classified by MedDRA and reported up to 28 days after vaccination will be tabulated with exact 95% CI. The same tabulation will be performed for grade 3 unsolicited AEs and for unsolicited AEs with a relationship to vaccination.

At each hematology/biochemistry sampling time point, by study group, individual hematological and biochemical values will be presented as number of subjects out of range (above and below normal range) and tabulated by toxicity grading (refer to Appendix 2). In addition, changes from baseline (median/interquartile range) will be presented.

SAEs, LC-ILIs, MAEs, and pIMDs will be described in detail. Withdrawals due to AEs/SAEs will also be summarized.

11.11.3 Analysis of immunogenicity

The analysis of immunogenicity will be performed primarily on the Per-Protocol set. If 10% or more of the vaccinated subjects are eliminated from the Per-Protocol set, a second analysis will be performed on the TVC.

11.11.3.1 Within group assessment

11.11.3.1.1 Humoral and mucosal immunogenicity assessment

For each study group, at each time point at which the tests are done and results are available, for each humoral immunity parameter, the following analyses will be performed:

- Seropositivity rates and GMTs, with exact 95% CI.
- MGI from Day 1, with 95% CI.

- Percentage of subjects with at least 4-fold increase from Day 1, with exact 95% CI.
- Percentage of subjects with at least 10-fold increase from Day 1, with exact 95% CI.
- Distribution of antibody titers using reverse cumulative distribution curves (RCCs).

The correlation between anti-H1 stalk ELISA and anti-H1 stalk MN assay results will be explored.

11.11.3.1.2 CMI assessment

For each study group, at each time point where a blood sample result is available, the frequency of antigen specific IFN-γ secreting cells, B-memory cells, and plasmablasts will be summarized using descriptive statistics.

11.11.3.2 Between group assessment

GMT ratios and their 2-sided 95% CI will be computed after fitting an ANCOVA model on the $\log_{10}$ transformation of ELISA/MN titers, including vaccine group as fixed effect and the pre-vaccination titer as covariate.

Differences in percentage of subjects with a four-fold increase from baseline and their 95% CIs will be calculated.

The assessment time points will be described in the Statistical Analysis Plan, but generally speaking, the results 4 weeks post-booster (or second IIV dose) will be compared.

The following group ratios/differences will be provided:

- Evaluation of the proof of principle:
  - Dose 1: LAIV and Dose 2: IIV + $AS03_A$ versus Dose 1: IIV + $AS03_A$ and Dose 2: IIV + $AS03_A$.
  - Dose 1: LAIV and Dose 2: IIV versus Dose 1: IIV + $AS03_A$ and Dose 2: IIV + $AS03_A$.
  - Pooled Dose 1: LAIV and Dose 2: IIV groups versus Dose 1: IIV + $AS03_A$ and Dose 2: IIV + $AS03_A$ (only if results suggest $AS03_A$ did increase immune responses post-boost in the LAIV-IIV groups).
- Assessment of the adjuvant system:
  - Dose 1: LAIV and Dose 2: IIV + $AS03_A$ versus Dose 1: LAIV and Dose 2: IIV.

Additional ratios/differences, such as versus placebo groups, will be considered at the time of analysis.

11.12 Interpretation of analyses

For humoral and mucosal immune responses, it is expected that for some assays 100% of subjects will already be seropositive at baseline. Thus, while seropositive rates will be calculated for each assay at each time point, GMTs, MGIs, and percentages of subjects with fold increases from baseline will be most meaningful.

Generally, emphasis will be placed on the following assays for interpretation of immunologic responses in the descending order:

- Anti-H1 HA-stalk IgG by ELISA (using cH6/1 antigen)
- Serum neutralizing antibodies by microneutralization (using cH6/1N5 virus)
- ADCC assay
- Anti-H1 HA-stalk secretory IgA by ELISA (using cH6/1 antigen)
- Proportion of stalk-specific memory B cells by ELISPOT (stimulated by cH6/1 antigen)

Comparative analyses will be descriptive with the aim to characterize the difference in reactogenicity/immunogenicity between groups.

11.13 Conduct of analysis

11.13.1 Sequence of analyses

Staged analyses will be done. Excluding any IDMC analyses, the analyses will be performed in a stepwise manner:

- Interim analyses will be performed when safety, reactogenicity, and immunogenicity (including at least H1 anti-stalk ELISA) data from all subjects are available up to Visit 13.

- A final analysis of all data will be performed when data up to study conclusion are available. This analysis will be reported in a Study Report.

11.13.2 Statistical considerations for interim analyses

The decision to pursue development will be based on the final assessment of Visit 13 data performed at the first analysis.

No statistical adjustment will be made for the interim analyses, which are intended to provide final outputs related to the different endpoints and time points in a phased manner.

At the time of the interim analysis, PATH will neither have access to the individual treatment assignments, nor to the unblinded safety report, but will be provided with aggregated immunogenicity results per group in order to allow strategic decisions for the future of the project.

12 QUALITY ASSURANCE AND QUALITY CONTROL

The study will be conducted in accordance with procedures identified in the protocol and staff will be guided by procedural activities detailed in SOPs.

Site monitoring will be conducted to ensure that human subject protection, study procedures, laboratory procedures, study intervention administration, and data collection processes are of high quality and meet ICH-GCP, sponsor standards, and regulatory guidelines, and that the study is conducted in accordance with the protocol and sponsor SOPs.

12.1 General considerations

The study will be conducted in full compliance with the protocol and ICH-GCP to provide public assurance that the rights, safety, and well-being of trial subjects are protected, and that the clinical trial data are credible. To ensure quality and standardization, the site will develop SOPs for key protocol procedures and conduct the study guided by the study Manual of Procedures or other written guidelines. The site will also develop routine operational checks to verify that critical protocol requirements and procedures are executed correctly and completely at the time the work is being performed. Prior to the initiation of the study, the Sponsor and Emmes will conduct training on the protocol and SOPs for the study staff, including mock runs of study visits/walk-throughs with study staff.

12.2 External monitoring

To ensure that the study is conducted in accordance with ICH-GCP and regulatory requirements, monitoring responsibilities will be provided by Emmes. A site initiation visit will be conducted prior to beginning the study, and monitoring will be conducted at initiation, during, and at closeout of the study. During the course of the study, monitors will visit the clinical site at intervals to verify compliance to the protocol; completeness, accuracy, and consistency of the data and study product accountability; and adherence to ICH-GCP and applicable regulations. As needed and when appropriate, the monitors will also provide clarifications and additional training to help the site resolve issues identified during the monitoring visit. As appropriate and informed by risk assessment, remote centralized monitoring activities may be considered in place of or to supplement onsite monitoring. These may include analysis of data quality (e.g., missing or inconsistent data), identification of data trends not easily detected by onsite monitoring, and performance metrics (e.g., screening or withdrawal rates, eligibility violations, timeliness, and accuracy of data submission).

The extent and frequencies of the monitoring visits will be described in a separate monitoring plan developed prior to study initiation. The investigator will be notified in advance of scheduled monitoring visits. The monitors should have access to all trial related sites, subject medical records, study product accountability, and other study-related records needed to conduct monitoring activities. Emmes will share the findings of the monitoring visit, including any corrective actions, with the site investigator and PATH. The site PI and the monitors must cooperate to

ensure that any problems detected in the course of these monitoring visits are resolved in a predefined timeframe.

To ensure the quality of clinical data for all subjects, a clinical data management review will be performed on subject data received by Emmes. During this review, subject data will be checked for consistency, omissions, and any apparent discrepancies. In addition, the data will be reviewed for adherence to the protocol and ICH-GCP. To resolve any questions arising from the clinical data management review process, data queries and/or site notifications will be sent to the site for resolution as soon as possible and within the time frame described in the monitoring plan; all queries must be resolved prior to database lock.

Essential documents must be filed in the site study file on an ongoing basis and be available for review by Emmes.

12.3 Independent auditing

Sponsor representatives may audit the study to ensure that study procedures and data collected comply with the protocol and applicable SOPs at the clinical site and that data are correct and complete. The site PIs will permit auditors (employees of the Sponsor or employee of a company designated by PATH) to verify source data validation of the regularly monitored clinical study. The auditors will compare the entries in the eCRFs with the source data and evaluate the study site for its adherence to the clinical study protocol and ICH-GCP guidelines and applicable regulatory requirements.

12.4 Regulatory agency auditing

The site PIs must be aware that regulatory authorities may wish to inspect the site records to verify the validity and integrity of the study data and protection of human research subjects. The site PIs will notify PATH within 24 hours following contact by a regulatory authority. The site PIs must make the relevant records available for inspection and will be available to respond to reasonable requests and audit queries made by authorized representatives of regulatory agencies. The site PIs will provide PATH with copies of all correspondence that may affect the review of the current study or his qualification as an investigator in clinical studies conducted by PATH. PATH will provide any needed assistance in responding to regulatory audits or correspondence.

13 ETHICAL CONSIDERATIONS (AND INFORMED CONSENT)

13.1 Ethical standards

This study will be conducted in accordance with the ethical principles set forth in The Belmont Report: Ethical Principles and Guidelines for the Protection of Human Subjects of Research, as drafted by the US National Commission for the Protection of Human Subjects of Biomedical and Behavioral Research and in conformity with ICH-GCP, 45 CFR Part 46 and 21 CFR 50.

13.2 Ethical review

This study will be conducted under the auspices of the following ethics authorities at these institutions: CCHMC IRB, ISMMS IRB, Duke University Health System IRB, and PATH. The intention will be to identify one site IRB to serve as the central IRB of record.

The investigators and Sponsor are responsible for obtaining approval from the IRB or record prior to enrollment of the first subject. The committee will review and approve the protocol, informed consent form, and any recruitment materials (advertising or informational material); including any modifications to these documents prior to, or during the study. All changes to the protocol or informed consent form must be reviewed and approved prior to implementation, except where necessary to eliminate apparent immediate hazard to study subjects. The investigators and Sponsor are also responsible for obtaining continuing review throughout the duration of the study in accordance with existing regulations.

13.3 Recruitment

Participants will be recruited from the local community. The target population will reflect the community at large at each of the participating study sites. Estimated time to complete enrollment in this trial is approximately 2 months. Information regarding this trial may be provided to potential subjects who have previously participated in vaccine trials conducted at the participating study sites. Other forms and/or mechanisms of recruitment may also be used. The local IRB will approve all materials prior to use. Careful recruitment and communication about all aspects of the study will be critical to ensuring eligible subjects who eventually enroll and participate in the trial are committed to participate for the full length of the study.

13.4 Informed consent process

Prior to any study related screening, informed consent will be obtained from each subject. The site PI or designee will fully inform the subject about the aims, procedures, potential risks, and potential benefits of the study. The subject will be given the written, IRB approved ICF, allowed ample time to read the consent form, encouraged to ask questions about the study, have the questions answered and then be given time to decide if s/he would like to participate in the study. It will be emphasized that participation is voluntary, and that the subject has the right to decline to participate or subsequently withdraw from the study at any time without prejudice.

The site PIs or designees must obtain the subject's voluntary, signed and dated ICF (or, if the subject is unable to sign, independently witnessed and documented) before any study-related procedures are performed. Study staff must document the informed consent process. The original, signed ICF must be kept in the site study file. A copy of the informed consent document will be given to the subjects for their records. Prior to any study vaccination activities, subjects must take a written exam about the study and pass with 70% of answers correct.

For the study extension, the site PI or designee will fully inform the subject about the additional visit. The site PIs or designees must obtain the subject's voluntary, signed and dated addendum to the ICF (or, if the subject is unable to sign, independently witnessed and documented) before any study-related procedures are performed

13.5 Participant confidentiality

Every effort will be made to protect subject privacy and confidentiality. Personal identifiers will not be included in any study reports. All study records will be kept confidential to the extent provided by national laws. Medical records containing identifying information will be made available for review when the study is monitored by PATH or an authorized regulatory agency. Direct access may include examining, analyzing, verifying, and reproducing any records and reports that are important in the evaluation of the study.

All study-related information will be stored securely at the study site. All subject information will be stored in locked file cabinets in areas with access limited to study staff. Data collection, process, and administrative forms, and other reports will be identified only by a coded number to maintain subject confidentiality. All local databases will be secured with password-protected access systems. Forms, lists, logbooks, appointment books, and any other listings that link subject ID numbers to other identifying information will be stored in a separate, locked file in an area with limited access. Subjects' study information will not be released without their written permission, except as necessary for monitoring, or as required/permitted by law/regulatory authorities.

13.6 Reimbursement

Pending IRB approval, subjects will be compensated for their time and effort in this study, and be reimbursed for travel to study visits. The study ICF and addendum will state the plan for reimbursement. Participants will not be charged for study injections, research clinic visits, research-related examinations, or research-related laboratory tests. Subject payments will be suitable to the requirements of the protocol and site. Payments will also be designed to be ethically acceptable and ensure retention of subjects in this relatively complicated and demanding trial.

13.7 Compensation for research related Injury

Subjects will be instructed that if they experience any illness they should contact study staff as soon as possible. Subjects will be encouraged to be evaluated at the study clinic, if possible. If subjects seek medical care outside the study clinic, they should inform the health care provider(s) of their participation in this study, provide the health care provider(s) with contact information for the investigator, and separately contact the investigator to inform the investigator of the medically attended illness. There are limited funds available to the study for care of study-related injury. Although the investigators and Sponsor will make every effort to cover the costs of any study-related injury, full coverage cannot be guaranteed, and uncovered costs may fall on subjects and/or their insurers. Subjects will be reimbursed to the extent possible for medical care

necessary for any research related injury. Subjects will be informed that there are limited funds to cover SRI care costs and that there is the possibility that they may ultimately be responsible for at least some of the cost of care (See Financing and Insurance, Section 14).

14 FINANCING AND INSURANCE

The trial is supported by a grant from the Bill & Melinda Gates Foundation to the Icahn School of Medicine at Mt. Sinai, New York. PATH, the study sponsor, is a sub-recipient under this grant. PATH maintains clinical trial and liability insurance with a limited rider for medical coverage for research-related injury. Institutional authorities will make the decision to invoke that additional coverage on an individual case basis.

15 PUBLICATION POLICY

A Clinical Study Report comprised of text and results tables reflecting all safety and immunogenicity data will be generated. The clinical study report (CSR) will be reviewed, approved and signed by the Protocol Chair. The CSR will be compliant with ICH-GCP guidelines.

All data, documents, any recordings and information transferred by PATH to any contractor or obtained or prepared by any contractor, his consultants or persons associated by contractual relationships with any contractor during the trials, belong to PATH.

All confidential information communicated to the Principal Investigator by PATH, ISMMS, University of Chicago, Neomed, or GSK Biologicals shall be kept strictly confidential by him/her or any other person connected with the study and shall not be disclosed, either orally or in written form, by him/her or such person to any third party without prior written consent of the organization of which the information is the exclusive property.

Following completion of the CSR, the investigators, working with PATH, ISMMS, University of Chicago, and GSK representatives, are expected to publish the results of this research in peer-reviewed scientific journal(s) and make the data publicly available. In no way may PATH prohibit the public dissemination of the results of this trial; details of the publication plan are specified in the Agreement between PATH and the investigator’s institution(s). Within any presentation or publication, confidentiality of individual subjects will be maintained, with identification by subject code number and initials, if applicable.

The International Committee of Medical Journal Editors (ICMJE) member journals have adopted a trials-registration policy as a condition for publication. This policy requires that all clinical trials be registered in a public trials registry. It will be the responsibility of PATH representatives to register this trial in an acceptable registry. ICMJE authorship criteria will be strictly followed for publication of any manuscript(s) arising from this trial.

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APPENDIX 1 SUMMARY OF STUDY PROCEDURES FOR LAIV-IIV AND IIV-IIV TREATMENT ARMS

Table 32 and Table 33 detail procedures at each visit for subjects receiving the LAIV-IIV regimen (Groups 1, 2 and 3) and the IIV-IIV regimen (Groups 4 and 5), respectively.

Table 32 List of study procedures for LAIV-IIV subjects (Groups 1, 2, & 3)

Epoch	Screening [a]		Primary													Long-term		
Visit	V00	V01	V02	V03	V04	V05	V06	V07	V08	V##	V09	V10	V11	V12	V13	V14	V15	V16
Time point	56 to 2 days prior to D1		-1 day	D1	D2	D3	D4	D5	D6	D7	D8	D29	D85	D92	D113	M9 (D252)	M15 (D420)	M21 (D588)
Sampling Time point				Pre	PId1	PId2	PId3	PId4	PId5	PId6	PId7	PId28	PId84	PIId7	PIId28	PIIm6	PIIm12	PIIm18
Obtain written informed consent	X																	
Collect demographic data	X																	
Collect/review medical history	X												X					
Collect influenza vaccination history for previous 3 seasons (2014/2015, 2015/2016, 2016/2017)	X																	
Collect serum for biochemistry (ALT, AST, Creatinine, BUN)	X		X								X	X	X	X	X	X	X	
Collect whole blood for hematology (CBC with differential)	X		X								X	X	X	X	X	X	X	
Collect serum for HIV testing	X																	
Collect urine for pregnancy test (Beta-HCG) [b]	X		X										X					
Collect urine for drug testing	X																	
Review interim medical history and record any intercurrent medical conditions		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Measure vital signs (height & weight at screening only)	X			X									X					
Perform complete physical examination		X																
Perform targeted physical examination depending on subject's signs and symptoms	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Check/confirm inclusion/exclusion criteria		X		X														
Randomize to study Group		X																
Admit to inpatient unit			X															
Collect serum for immunology			X									X	X		X	X	X	X
Collect whole blood for CMI			X								X	X	X	X	X		X	
Collect whole blood for transcriptomics			X			X					X	X	X	X	X	X	X	
Collect saliva for immunology			X									X	X		X		X	X
Collect nasal and OP swab for InfA testing by RT-PCR			X [c]		X	X	X	X	X	X [d]								
Test swab specimens using respiratory panel in multiplex PCR in case of any ARI			X	X	X	X	X	X	X	X								
Measure body temperature (orally) pre-vaccination				X									X					
Check contraindications, warnings and precautions				X									X					

Epoch	Screening [a]		Primary													Long-term		
Visit	V00	V01	V02	V03	V04	V05	V06	V07	V08	V##	V09	V10	V11	V12	V13	V14	V15	V16
Time point	56 to 2 days prior to D1		-1 day	D1	D2	D3	D4	D5	D6	D7	D8	D29	D85	D92	D113	M9 (D252)	M15 (D420)	M21 (D588)
Sampling Time point				Pre	PId1	PId2	PId3	PId4	PId5	PId6	PId7	PId28	PId84	PIId7	PIId28	PIIm6	PIIm12	PIIm18
Administer one dose of study vaccine or placebo				LAIV									IIV					
Observe for immediate reactions for at least 60 minutes				X									X					
Instruct subject on use of diary card				X							X	X	X	X	X	X		
Subject records solicited AEs within 7 days post-dose				X	X	X	X	X	X	X			X					
Subject records unsolicited AEs				X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Review and transcribe diary card					X	X	X	X	X	X	X	X	X	X	X	X	X	X
Discharge subject from inpatient unit [e]									X									
Contingency for subject to remain in inpatient unit [f]									*X*	*X*								
Option to begin antiviral treatment [g]									*X*									
Record any concomitant medications/vaccinations	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Record and report AEs and SAEs leading to withdrawal from the study				X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Record and report SAEs, ILIs, MAEs, pIMDs, and pregnancies				X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Record and report SAEs related to study participation		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Subject completion of study																		X

Note: the thick line following Visit 13 indicates two immunogenicity analyses will be performed on all data obtained up to Visit 13 and then up to Visit 15, respectively.
D = day; M = month (= 28 days); SCR = screening; PRE = pre-vaccination; PI = post-Dose 1; PII = post-Dose 2 CMI = cell-mediated immunology, CBC = complete blood count, AE = adverse event, SAE = serious adverse event; MAE = medically attended event; pIMD = potential immune-mediated disease

[a] Screening evaluations may be completed 2 to 56 days before Day 1. Site staff should allow sufficient time between the Screening and Visit 1 to receive and review screening laboratory test results. If a delay occurs such that the interval between Screening and the Visit 1 vaccination exceeds 56 days, a re-screening visit should be scheduled before Visit 1 during which blood sample collection for screening laboratory assessments must be repeated; an interim medical history and physical examination must be obtained and inclusion / exclusion criteria must be re-reviewed. Only data from the re-screening visit, if it occurs, will be recorded in the eCRF and taken into consideration.

[b] Only for female subjects of childbearing potential. A serum pregnancy test instead of a urine pregnancy test should only be considered if required by local or ethics committee regulations.

[c] 18-36 hours prior to Dose 1.

[d] Nasal and oropharyngeal swab collection will continue for each day subject remains in isolation after planned discharge day (D6).

[e] If 3 consecutive swab specimens collected over 48 hours (3 separate days, i.e., D4, D5, and D6) are all InfA negative by RT-PCR.

[f] If any D4, D5 or D6 swab specimen is InfA positive by RT-PCR, and until 3 consecutive swab specimens collected over 48 hours (3 separate days) are all influenza negative by RT-PCR or 24 hours after subject initiates anti-neuraminidase treatment and last swab specimen prior to discharge is InfA negative by RT-PCR

[g] If D6 swab specimen tests InfA positive by RT-PCR.

Table 33 List of study procedures for IIV-IIV subjects (Groups 4 & 5)

Epoch	Screening [a]		Primary							Long-term		
Visit	V00	V01	V03	V05	V09	V10	V11	V12	V13	V14	V15	V16
Time point	56 to 2 days prior to D1		D1	D3	D8	D29	D85	D92	D113	M9 (D252)	M15 (D420)	M21 (D588)
Sampling Time point			Pre	PId2	PId7	PId28	PId84	PIId7	PIId28	PIIm6	PIIm12	PIIm18
Obtain written informed consent	X											
Collect demographic data	X											
Collect/review medical history	X						X					
History of influenza vaccination within previous 3 seasons (2014/2015, 2015/2016, 2016/2017)	X											
Collect serum for biochemistry (ALT, AST, Creatinine, BUN)	X		X		X	X	X	X	X	X	X	
Collect whole blood for hematology (CBC with differential)	X		X		X	X	X	X	X	X	X	
Collect serum for HIV testing	X											
Collect urine for pregnancy test (Beta-HCG) [b]	X		X				X					
Collect urine for drug testing	X											
Review interim medical history and record any intercurrent medical conditions		X	X	X	X	X	X	X	X	X	X	X
Measure vital signs (height and weight at screening only)	X		X				X					
Perform complete physical examination		X										
Perform targeted physical examination depending on subject signs and symptoms	X		X	X	X	X	X	X	X	X	X	X
Check/confirm inclusion/exclusion criteria		X	X									
Randomize to study Group		X										
Collect serum for immunology			X			X	X		X	X	X	X
Collect whole blood for CMI			X		X	X	X	X	X		X	
Collect whole blood for transcriptomics			X	X	X	X	X	X	X	X	X	
Collect saliva for immunology			X			X	X		X		X	X
Measure body temperature (orally) pre-vaccination			X				X					
Check contraindications, warnings and precautions			X				X					
Administer one dose of study vaccine or placebo			**IIV**				**IIV**					
Observe for immediate reactions for at least 60 minutes			X				X					
Instruct subject on use of diary card			X		X	X	X	X	X	X		
Subject records solicited AEs within 7 days post-dose			X	X			X					
Subject records unsolicited AEs			X	X	X	X	X	X	X	X	X	X
Review and transcribe diary card				X	X	X	X	X	X	X	X	X
Record any concomitant medications/vaccinations	X	X	X	X	X	X	X	X	X	X	X	X
Record and report AEs and SAEs leading to withdrawal from the study			X	X	X	X	X	X	X	X	X	X
Record and report SAEs, ILIs, MAEs, pIMDs, and pregnancies			X	X	X	X	X	X	X	X	X	X
Record and report SAEs related to study participation		X	X	X	X	X	X	X	X	X	X	X
Subject completion of study												X

Note: the thick line following Visit 13 indicates two immunogenicity analyses will be performed on all data obtained up to Visit 13 and then up to Visit 15, respectively.

D = day; M = month (= 28 days); PRE = pre-vaccination; PI = post-Dose 1; PII = post-Dose 2, CMI = cell-mediated immunology, AE = adverse event, SAE = serious adverse event; MAE = medically attended event; pIMD = potential immune-mediated disease

[a] Screening evaluations may be completed 2 to 56 days before Day 1. Site staff should allow sufficient time between the Screening and Visit 1 to receive and review screening laboratory test results. If a delay occurs such that the interval between Screening and the Visit 1 vaccination exceeds 56 days, a re-screening visit should be scheduled before Visit 1 during which blood sample collection for screening laboratory assessments must be repeated; an interim medical history and physical examination must be obtained and inclusion / exclusion criteria must be re-reviewed. Only data from the re-screening visit, if it occurs, will be recorded in the eCRF and taken into consideration.

[b] Only for female subjects of childbearing potential. A serum pregnancy test instead of a urine pregnancy test should only be considered if required by local or ethics committee regulations.

APPENDIX 2 FDA SEVERITY GRADING TABLES FOR HEMATOLOGIC AND BIOCHEMICAL PARAMETERS

Only those parameters that will be assessed as part of the study have been maintained in the tables below:

Serum *	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially Life Threatening (Grade 4)**
Creatinine – mg/dL	1.5 – 1.7	1.8 – 2.0	2.1 – 2.5	> 2.5 or requires dialysis
ALT, AST increase by factor	1.1 – 2.5 x ULN	2.6 – 5.0 x ULN	5.1 – 10 x ULN	> 10 x ULN

ULN = upper limit of the normal range.
*The laboratory values provided in the tables serve as guidelines and are dependent upon institutional normal parameters. Institutional normal reference ranges should be provided to demonstrate that they are appropriate.
**The clinical signs or symptoms associated with laboratory abnormalities might result in characterization of the laboratory abnormalities as Potentially Life Threatening (Grade 4). For example, a low sodium value that falls within a Grade 3 parameter (125-129 mE/L) should be recorded as a Grade 4 hyponatremia event if the subject had a new seizure associated with the low sodium value.

Hematology*	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially Life Threatening (Grade 4)
Hemoglobin (Female) - gm/dL	11.0 – 12.0	9.5 – 10.9	8.0 – 9.4	< 8.0
Hemoglobin (Female) change from baseline value - gm/dL	Any decrease – 1.5	1.6 – 2.0	2.1 – 5.0	> 5.0
Hemoglobin (Male) - gm/dL	12.5 – 13.5	10.5 – 12.4	8.5 – 10.4	< 8.5
Hemoglobin (Male) change from baseline value - gm/dL	Any decrease – 1.5	1.6 – 2.0	2.1 – 5.0	> 5.0
WBC Increase - cell/mm^3	10 800 – 15 000	15 001 – 20 000	20 001 – 25 000	> 25 000
WBC Decrease - cell/mm^3	2 500 – 3 500	1 500 – 2 499	1 000 – 1 499	< 1 000
Lymphocytes Decrease - cell/mm^3	750 – 1 000	500 – 749	250 – 499	< 250
Neutrophils Decrease - cell/mm^3	1 500 – 2 000	1 000 – 1 499	500 – 999	< 500
Eosinophils - cell/mm^3	650 – 1 500	1 501 – 5 000	> 5 000	Hyper-eosinophilic
Platelets Decreased - cell/mm^3	125 000 – 140 000	100 000 – 124 000	25 000 – 99 000	< 25 000

*The laboratory values provided in the tables serve as guidelines and are dependent upon institutional normal parameters. Institutional normal reference ranges should be provided to demonstrate that they are appropriate.

REFERENCE for Appendix 2:

FDA Guidance for Industry: Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials (September 2007)