Study Protocol

Revealing Protective Immunity to Influenza Using Systems Immunology (PRISM)

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PROTOCOL TITLE: Revealing protective immunity to influenza using systems immunology (PRISM)

EXTERNAL (NON-EMORY) COLLABORATORS

Adriana Tomic, PhD

Depts. of Biomedical Engineering and Virology, Immunology & Microbiology

Boston University

PRINCIPAL INVESTIGATOR:

Daniel Graciaa, MD, MPH, MSc

Hope Clinic of the Emory Vaccine Center

Division of Infectious Diseases, Department of Medicine

Office Phone [redacted]

Cell Phone [redacted]

Email: [redacted]

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REVISION HISTORY

Revision #	Version Date	Summary of Changes

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1. Study Summary

Project Title	Revealing protective immunity to influenza using systems immunology (PRISM)
Project Design	Open-label
Primary Objective	To evaluate the immunogenicity of the live attenuated influenza vaccine (LAIV)
Secondary Objectives	To analyse the effect of the LAIV on eliciting influenza-specific immune responses To assess the safety profile of LAIV
Exploratory Objectives	[redacted]
Research Intervention(s)/Interactions	Single dose live attenuated influenza vaccine (LAIV)
Study Population	Healthy and immunocompetent adults aged 18 to 49 years
Sample Size	50 participants
Study Duration for individual participants	1 month
Study Specific Abbreviations/ Definitions	AR Adverse Reaction BU Boston University CI Chief Investigator

	CEIRR Centers of Excellence for Influenza Research and Response (CEIRR) GCP Good Clinical Practice GMC Geometric Mean Concentration(s) GMT Geometric Mean Titer(s) HA Hemagglutinin HAI Hemagglutination Inhibition titer ICF Informed Consent Form ICH International Conference on Harmonization IMP Investigational Medicinal Product LAIV Live Attenuated Influenza Vaccine LAR Legally Appointed Representative NEIDL National Emerging Infectious Diseases Laboratories PBMC Peripheral blood mononuclear cells PI Principal Investigator PIL Participant/ Patient Information Leaflet REC Research Ethics Committee SAR Serious Adverse Reaction SOP Standard Operating Procedure TCR T cell receptor TIV Trivalent inactivated influenza vaccine
Funding Source (if any)	Boston University funds

2. Objectives

Primary Objective:
Evaluate the immunogenicity of the live attenuated influenza vaccine (LAIV)

Secondary Objectives:
1. Analyze the effect of LAIV on eliciting influenza-specific immune responses.
2. Assess the safety profile of LAIV

Exploratory Objectives:

[redacted]

[redacted]

3. Background

The influenza virus is notorious for its continuous mutation and potential to cause pandemics. This essentially creates a moving target for vaccine development, posing a significant challenge for global health. Current vaccines offer a certain degree of protection but are not fully effective due to the virus's ever-changing nature. In response to this, a live attenuated influenza vaccine (LAIV) was developed with the objective of providing a higher degree of protection. This type of vaccine has demonstrated remarkable effectiveness in children, with protection rates as high as 85%, significantly surpassing the protection provided by inactivated flu vaccines. This made LAIV a preferred choice for administration to young children.

However, when it comes to adults (individuals over 18 years of age), the scenario is markedly different. Recent studies have indicated a significant decline in LAIV's effectiveness in adults compared to children. Various clinical trials have reported that LAIV exhibits approximately 85% efficacy in children under the age of 18, which dramatically decreases to around 40%-60% in adults (18-49 years). This suggests that adults do not respond as well to LAIV as children do, despite the vaccine being highly potent in younger populations. In adults, the LAIV-induced immunity appears to be short-lived, often lasting for only one influenza season. This is startling, especially considering that in children, LAIV has shown to provide long-lasting protection even against mismatched strains. Understanding the factors behind this discrepancy is a critical need if we aspire to improve the protective efficacy of influenza vaccines across all age brackets. Unraveling this mystery could open the doors to the development of a superior flu vaccine, one that offers robust protection across all age groups.

To find the solution to this enigma, it's essential to study why adults don't respond as efficiently to LAIV as children do. This forms the foundation of our research proposal: to understand the potential factors influencing the varied response to LAIV in adults. [redacted]

To attain a comprehensive understanding, we will utilize a systems immunology approach and incorporate 'omics' technologies along with artificial intelligence (AI). This blend of cutting-edge

technologies will facilitate exploring a broad spectrum of aspects within the immune response and build a more complete picture of the complex interactions within the immune system.

The comprehensive insights from this study will enhance our understanding of how LAIV induces immunity. This will significantly contribute to the development of an improved, universally effective flu vaccine that could provide robust protection across all age groups, ultimately reducing the global burden of influenza.

4. Study Endpoints

Primary Endpoint:

• Quantification of antibody titers using hemagglutination inhibition (HAI) assays at day 30

Secondary Endpoints:

- Mean geometric fold rise in HAI titers at Day 30
- Frequency of serious adverse events until Day 30 after vaccination

Exploratory Endpoints:

5. Study Intervention/Investigational Agent

5.1 Description

Live attenuated influenza vaccine
The FDA-approved live attenuated seasonal influenza vaccine (LAIV) (FluMist®, AstraZeneca) is licensed in the US for people 2-49 years of age. The 2024-25 seasonal influenza vaccine will be a trivalent vaccine which will contain three distinct strains[3]:

- A/Victoria/4897/2022 (H1N1)pdm09-like virus
- A/Thailand/8/2022 (H3N2)-like virus
- B/Austria/1359417/2021 (B/Victoria lineage)-like virus.

The approved seasonal LAIV at the time of study enrollment will be obtained from the manufacturer.

5.2 Drug Handling

LAIV will be stored at the Emory Investigational Drug Service (IDS) per the manufacturer's instructions. It will be prepared by an IDS pharmacist on site at the Hope Clinic satellite IDS. Logs of receipt, temperature, maintenance, and disposal will be maintained in the study file. LAIV will be administered by a study nurse or provider at the Hope Clinic as a single 0.2mL dose given as 0.1mL spray in each nostril.

5.3 Accountability Procedures for Study Products

The IDS pharmacist will maintain accurate drug accountability logs which will be kept at the IDS pharmacy until the completion of the study. Upon completion of the study, a final drug accountability will be done. Following the completion of the drug accountability, the original copies of the logs will be maintained. When the vaccine is administered to a participant, the date, time, and location of administration (intranasal) will be recorded in the participant's study source documentation records.

6. Procedures Involved

For a schedule of procedures, see Appendix A.

6.1 Screening, Consenting, Vaccination Visit (D0)

6.1.1 Potential participants interested in the study will respond to study ads. Recruiters from the Hope Clinic will then contact them for a telephone screening, during which the research study will be explained in lay terms and eligibility criteria will be reviewed. If all eligibility criteria are met and the participant remains interested, an in-person screening appointment will be scheduled. Prior participants in other studies conducted at the Hope Clinic, and who had indicated their willingness to participate in other studies may also be contacted for a telephone screening visit.

6.1.2 At the in-person appointment, study staff will review the informed consent form with the participant and will answer all questions related to the study. Once the participant signs the informed consent, they will be assigned a unique study participation number. The following procedures will be performed:

- Recording of demographic information and review of medical history including recent influenza infection, current medication use, and vaccination history (previous receipt of influenza vaccines).
- Vital signs (all participants) and targeted physical exam as indicated based on review of participant health status.
- A urine pregnancy test will be performed if the participant is of child-bearing potential.
- Blood sample collection for baseline assays (60-70 mL)
- Nasal samples (nasopharyngeal swabs, nasosorption from nares) collection for baseline assays

The following actions will be performed for vaccination:

- Review of inclusion and exclusion criteria to confirm eligibility.
- Participants will receive the CDC influenza vaccine information sheet and review it prior to vaccination.
- Participants will then receive LAIV via the intranasal route, administered by a clinical research nurse or provider.
- Participants will be observed for a minimum of 15 minutes for any immediate hypersensitivity reactions.

The visit will last approximately 60 minutes.

6.2 Follow-up Visits (D2, D28)

All participants will return for follow-up visits following vaccination. During these visits, study personnel will:

- Review the participant's current health status.
- Note any changes in health history since the screening visit, including current medication use and vaccination history.
- Collect blood samples for immunologic assays (60-70 mL)
- Collect nasal samples (nasopharyngeal swabs, nasosorption from nares)

The visit will last approximately 30 minutes.

Total blood volume for all visits will be approximately 210 mL.

7. Statistical Analysis Plan

This is an exploratory, non-placebo controlled analysis of immune responses obtained from blood and nasal samples. This will provide us with descriptive data to analyze immune responses.

7.1 Statistical methods

The statistics will be descriptive, reported in the form of percentages, frequencies, geometric mean titres and geometric mean fold rises with 95% confidence intervals. For geometric means and fold rises, data will be logged, and confidence intervals calculated on this log scale, prior to transforming back onto the original scale for reporting and interpretation. Confidence intervals for proportions will be calculated using the binomial exact method.

7.2 Sample size

A sample size of 50 participants will be recruited. We have estimated, using published data[4], that a sample size of 40 individuals will be enough to be adequately powered to find differences in functionality of T cells after vaccination at the 98% power to detect a mean of paired differences of 0.1 (estimated standard deviation of paired differences of 0.1, significance level of 0.05 using a two-sided paired t-test). Therefore, we will analyze 50 individuals to allow for up to 20% drop-out rate, and to adjust for interindividual variability and any skewness in the data distribution. The following analyses will be performed on all participants in the per-protocol (PP) cohorts.

7.3 Analysis of outcome measures

Samples will be processed and stored at the Hope Clinic Processing Laboratory prior to being shipped to the laboratory of Prof. Adriana Tomic (Boston University, MA, USA) for analysis.

7.3.1 Analysis of HAI titers and analytes in serum

Frozen plasma will be transferred to Boston University for HAI titer determination and for O-link/proteomics technology. The HAI assay will be performed on sera from day 0 and day 28 after vaccination. Briefly, serum samples will be diluted 2-fold with PBS in duplicate 96-well V-bottom plates and virus will be added for control and serially diluted from concentration corresponding to 8 HA units to 0.03 HA units. Plates will be incubated for 15min at RT before 50uL of 0.5% chicken red blood cell solution will be added and for additional 1h before reading HAI activity. The HAI titre of a given sample is defined as the highest dilution of serum that prevents hemagglutination. A titre of five will be assigned to all samples in which the first dilution (1:10) was negative.

Cytokines in serum samples before and after vaccination will be measured by a O-Link technology (Target 96 and/or Explore384 panels, O-Link, Boston, MA, US) according to manufacturer's recommendations. Each sample will be measured in duplicate. Plates will be read using a Luminex LabMap200 instrument with a lower bound of 100 or 50 beads per sample per cytokine. For each well, we will consider the median fluorescence intensity (MFI) of all beads measured for a given cytokine and averaged the MFI of the two replicates. Values will be normalized to a control sample ran in each of the plates.

7.3.2 Analysis of adaptive immune responses

Isolated and frozen peripheral blood mononuclear cells (PBMCs) will be transferred to Boston University for analysis of immune cells subsets using spectral and/or mass cytometry. PBMCs from samples obtained on day 0 and days 2-3 and 28 after vaccination, will be thawed and rested overnight. Next day 106 PBMCs/well will be stimulated overnight (12-16 hours) with the influenza peptide pool in 96-well V-bottom plates. Influenza peptide pool contains 483 peptides (20mers with 11aa overlap, Sigma Aldrich) spanning the entire influenza proteome and 24 peptides with HLA-A*0201-specificity (9-10mers, Sigma Aldrich) generated against the influenza strain A/California/07/2009. Samples will then be stained with antibodies for analysis on the Cytek Aurora spectral cytometer. After acquisition, data will be normalised, and further data analysis will be performed using FlowJo v10 software.

8. Data and/or Specimen Banking

Particiants will be asked for permission for the principal investigator to keep any remaining (residual) specimens derived from venous blood collection for possible use in future research studies, such as examining additional immunological assessments or testing for antibodies against other viruses or bacteria. Future use samples/specimens will not be sold or used directly for production of any commercial product. These samples/specimens are protocol-required; thus, participants must agree to future use of these samples/specimens as a condition of their study participation.

All samples/specimens will be processed at the Hope Clinic and shipped regulary to Prof. Tomic's lab at Boston University. The de-identified (all personal identifying information of the

participant will be removed) samples/specimens will be labeled and stored in freezers at -80°C or in liquid nitrogen tanks.

In keeping with NIH policy on scientific data sharing, immunologic assay results will be made publicly available through public database deposition and peer-reviewed publication. Participants will be fully de-identified (i.e., disseminated data will not contain direct identifiable information). Demographic data (e.g., age, race and/or ethnicity, sex/gender) associated with participants will be kept strictly separate from all experimental results. This practice has become standard to protect participant privacy.

9. Sharing of Results with Participants

Immunologic assay results will not be reported to participants as these are intended for research purposes only and therefore cannot be reported to patients per Clinical Laboratory Improvement Amendment (CLIA) regulations.

Incidental findings
Incidental findings such as pregnancy test results or physical exam findings identified during study conduct will be disclosed and explained to the participant by the study investigator, who will provide a referral to the appropriate clinical specialist and information on how to obtain health insurance to secure treatment if needed. Participants will be directed to follow up with primary care providers.

10. Study Timelines

Individuals will participate in this study for a total of 1 month; the maximum time between V1 and V3 will be 35 days. Recruitment of all participants will require 8 months. The primary analysis should be concluded within 48 months of start date. The study will be active for up to 6 years to complete secondary and exploratory analyses.

11. Inclusion and Exclusion Criteria

Inclusion Criteria

1. Able to understand and give informed consent
2. Age 18-49 years
3. Participants of child bearing potential must agree to use effective birth control for the duration of the study. A negative urine pregnancy test must be documented prior to vaccination.

Exclusion Criteria

1. History of allergy or serious adverse reaction, including Guillain-Barré syndrome, to a vaccine or vaccine products

2. History of a medical condition resulting in impaired immunity such as active solid tumors, leukemia, lymphoma, chemotherapy or radiation therapy, autoimmune conditions, or splenic dysfunction. Persons with previous skin cancers or cured non-lymphatic tumors are not excluded from the study.
3. History of asthma, cochlear implant, or active cerebrospinal fluid leak
4. Use of immune modifying drugs including: systemic steroids for more than 1 week (such as prednisone > 20mg/day), chronic administration (more than 14 days total) of immunosuppressive or immunomodulatory drugs in the prior 3 months
5. History of HIV, Hepatitis B or Hepatitis C infection
6. Chronic clinically significant medical problems that could be considered active or unstable (i.e diagnosed within the past 3 months or requiring a change in medication within the past 3 months). This is including (but not limited to):
    a. Insulin dependent diabetes
    b. Severe heart disease (including arrhythmias)
    c. Severe lung disease
    d. Severe liver disease
    e. Severe kidney disease
    f. Severe hypertension: defined as life-threatening consequences (e.g., malignant hypertension, transient or permanent neurologic deficit).
    g. Congenital genetic syndromes (e.g., trisomy 21)
7. BMI > 35
8. Pregnancy or breast feeding, or plans to become pregnant in the next month
9. History of influenza infection or vaccination within the current or previous influenza season
10. Receipt of blood products or immune globulin product within the prior 3 months
11. History of excessive alcohol consumption, drug use, psychiatric conditions, social conditions or occupational conditions that in the opinion of the investigator would preclude compliance with the trial
12. Receipt of any live vaccines 30 days before, or plans to receive any live vaccines 30 days after vaccination
13. Receipt of any inactivated vaccines 14 days before, or plans to receive any inactivated vaccines 14 days after vaccination
14. Receipt of any non-registered or other investigational product in 30 days before, or plans to receive any other investigational product 30 days after vaccination

Temporary Exclusion Criteria

1. Fever (temperature ≥38.0°C) or coryzal symptoms within 72 hours prior to vaccination
2. Receipt of antipyretics within 6 hours prior to vaccination

Participants meeting time-limited exclusion criteria may be re-screened at investigator discretion.

12. Population

No vulnerable populations will be enrolled. Specifically, pregnant persons, children, prisoners, and cognitively impaired persons will not be enrolled. Non-English speakers will not be excluded.

13. Local Number of Participants

We anticipate needing to screen up to 70 participants locally to identify 50 participants to complete the research procedures.

14. Recruitment Methods

14.1 Patients will be recruited following IRB approval.
14.2 Participants will be recruited from the general population of metro Atlanta with the methods detailed below.
14.3 Participants may be identified and recruited from past subjects who agreed to be contacted for future studies or by self-referral from IRB approved advertisements from

- postings of IRB-approved flyers in and around the Emory University campuses as well as on electronic notification boards ; posting approved flyers on Emory and partner institution shuttles (e.g., Georgia Tech);
- use of various social media platforms such as Facebook, Twitter, Instagram, and other mobile apps in compliance with Emory policy; Study staff will use the official Emory University Facebook account and Instagram Emory Get Involved account, where IRB approved social media ads will be posted. The potential volunteers fill in their contact information in the “lead document form” provided by Facebook by default. The form collects the potential participant’s basic contact information such as name, best contact phone number and email address etc. Facebook collects the leads and provides it to the recruitment staff, who save the data in a password protected database on the Emory Hope Clinic’s shared drive. See social media plan for additional detail.
- listservs (such as CDC, Emory University, Emory Vaccine Center, and Vaccine Dinner Club), and clinical trial recruitment websites such as Research Match.org and ClinicalConnection; listing of clinical trials on the Hope Clinic website (hopeclinic.emory.edu) and Emory clinical trials database (clinicaltrials.emory.edu); contacting past participants from the HIPAA-compliant clinical trials database at the Hope Clinic who have agreed to be contacted for future studies; presentations by Hope Clinic faculty at various University and community venues; and volunteer word-of-mouth (direct referrals).
- Potential subjects may also be recruited from a list of potentially eligible volunteers identified through a general screening online survey (created with the Emory-approved Qualtrics platform) hosted on the Hope Clinic website. The survey captures contact, demographic, and basic health-related questions (i.e. flu vaccine status). Participants who meet preliminary study eligibility criteria for this study will be contacted via their indicated preferred method of contact, provided more information about this study, and administered a study-specific phone screen by a Hope Clinic recruiter.

Study recruiters/coordinators will contact potential volunteers via email or phone call once identified and tell them about the study and see if they are interested. If the potential subject is interested, the recruiter will obtain an oral consent and prescreen them for the study using an IRB-approved screening checklist. Qualified subjects will be scheduled to come into the clinic and be fully consented and proceed with screening/enrollment.

15. Withdrawal of Participants

Participants may voluntarily withdraw their consent from all future study activities including follow up at any time without penalty or loss of benefits to which they are otherwise entitled. Participants may be terminated from the study prior to study completion for reasons that might include, but are not limited to, those listed below. The investigator will inform the participant that all data acquired prior to termination will be included in the study analysis unless participant withdraws consent.

15.1 Participant no longer meets eligibility criteria.
15.2 The participant is considered by the PI to be “lost to follow-up” (i.e., no further followup is possible because attempts to reestablish contact with the subject have failed).
15.3 The participant chooses not to allow future use of samples for research purposes.
15.4 The participant dies.
15.5 As deemed necessary by the PI or designee for noncompliance of any nature.
15.6 The participant becomes pregnant.
15.7 If the study is prematurely terminated by the sponsor or the investigator for any reason, the investigator will promptly inform the study participantsand assure appropriate follow-up, as necessary. The investigator will provide a detailed written explanation of the termination to the IRB.

Participants with early termination status (before Day 28) are replaced as needed to preserve adequate sample size for the primary endpoint analysis.

16. Risk to Participants

16.1 Risks of Investigational Product

Seasonal influenza vaccine
The potential harms of receiving the approved seasonal influenza vaccine include, but are not limited to: nasal congestion, runny nose, wheezing, headache, cough, fever, or muscle aches. These reactions occur in 10-20% of adults who receive influenza vaccine. Serious adverse reactions including anaphylaxis and Guillain-Barré syndrome are possible, but rare. Potential participants with a history of severe allergic reaction to any component of the vaccine or Guillain-Barré syndrome after previous influenza vaccination will not be enrolled. For further details, see the attached package insert.

16.2 Risks of Study Procedures

Blood draws
The risks of blood sample collection include a transient feeling of discomfort and may result in a vasvogal reaction. This risk is controlled by having the participant lie down prior to collection, if needed. A bruise might form at the site of the blood draw, and this can be avoided by maintaining pressure to this site following the blood draw. The sites of blood draw are potential sites of infection, but this risk is made very unlikely by the use of sterile technique.

Nasal sampling
The risks of nasal sample collection are minimal including discomfort, eye watering, or mild nosebleed. The amount of discomfort and risk of nose bleeds will be minimized by using experienced staff or ensuring that new staff are properly trained and observed. If a nosebleed occurs, clinical staff will assist the participant with applying pressure or other treatments as needed.

16.3 Risk of Concomitant Medications, Prophylactic Medications and Rescue Medications
While we do not expect to use any additional medication, in case of anaphylactic or hypersensitivity reactions, we have readily available epinephrine (1:1000) and diphenhydramine injections. The use of epinephrine injection may cause side effects such as high blood pressure, arrhythmia, lightheadedness, nervousness, restlessness, tremor, shortness of breath, and diaphoresis; however, the frequency of these side effects is not determined. Diphenhydramine injection may also be necessary to treat potential allergic reactions, and its use may cause low blood pressure, arrhythmia, confusion, dizziness, sedation, restlessness, diarrhea, nausea, and urinary retention, but the frequency of these side effects is also unknown.

When facing a medical emergency, the clinic staff will follow the institutional SOP by calling 911 first (Hope Clinic). If needed, the participant will be transferred to Emory University Hospital or Emory Decatur Hospital Emergency Department for further care.

Participants are permitted to use acetaminophen or other symptomatic treatment if they experience a moderate to severe local or systemic side effect after vaccine administration.

17. Potential Benefits to Participants

Participants will receive the FDA-approved seasonal influenza vaccine, which may protect against influenza viruses. The United States Advisory Committee on Immunization Practices (ACIP) recommends the influenza vaccine for individuals aged 6 months and above, as it is known to be effective in preventing influenza virus infection.

18. Compensation to Participants

As compensation for expenses/travel and time, participants will receive $125 for the screening/vaccination visit and $75 for each follow-up visit that involves a blood draw. Compensation is provided in the form of a gift debit/credit card (ClinCard). If a participant

completes all visits, total compensation will be $275. In the event gift debit/credit cards (ClinCard) are unavailable, gift card reimbursement may be used instead.

19. Data Management and Confidentiality

19.1 Steps taken to secure data

All faculty and staff at the Hope Clinic receive HIPAA, human participants, and EHSO training as part of their onboarding and continuing training. Each participant will be assigned a unique study participant identification number (PTID) and these numbers rather than names will be used to collect and store participant information, including omics data.

Investigators and study personnel will keep accurate records to ensure that the conduct of the study is fully documented. Clinical data from this study, including participant birthdate and demographics, will be associated with the PTID, maintained on a HIPAA compliant clinical management database (Clinical Conductor) and a HIPAA compliant RedCap database and/or password protected Excel file, respectively at Hope Clinic accessible to investigators and study personnel only. Hard copy clinical data forms, for example source or protocol-specific Hope Clinic CRFs, associated with the PTID will be stored at Hope Clinic in a locked filed cabinet and accessible to investigators and clinical staff only. A file linking the participant PII (i.e., their name and contact information) to their PTID will be maintained at the Hope Clinic in a separate locked file cabinet and HIPAA compliant clinical management database accessible to investigators and clinical staff only. Study personnel will only send documents containing personal PII via fax or encrypted email in accordance with HIPAA regulations (e.g., to send/receive medical records request to/from primary care provider).

19.2 Quality Control

The Principal Investigator (or designee) will keep accurate records to ensure that the conduct of the study is fully documented. The investigator will ensure that all Hope Clinic CRFs and participant study files are legible and complete for every participant. The Principal Investigator (or designee), through the use of an internal Quality Management Plan, appropriate site quality control, and quality assurance monitoring staff, will be responsible for the regular review of the conduct of the study for verifying adherence to the protocol, and for confirming the completeness, consistency, and accuracy of all documented data and accuracy of source documentation verification. All charts will be 100% Quality Controlled. The first five volunteers enrolled will receive a 100% Quality Assurance review. The reports of the internal site monitor will be submitted to the Principal Investigator (or designee). The RedCap data entry system will include checks for data that are outside feasible limits (e.g., dates that are in the future) or skipped answers.

19.3 Data and specimen handling

Blood tubes will be labeled at the Hope Clinic with a unique identifier and will be transported to the lab at the Hope Clinic in EHSO-approved transport containers. All de-identified samples will be stored at the Hope Clinic by laboratory staff using only the study number, PTID, date of visit,

and visit number. Data from immunological assays will be linked to PTID, maintained by Dr. Tomic's laboratory at Boston University, and accessible to investigators and laboratory staff. Laboratory data and demographic data may be linked by PTID for statistical analysis by investigators or collaborators (e.g., to control for age); however, PTID will not be published in order to protect participant privacy.

Specimens and demographic data linked by PTID will be stored indefinitely, but all personal identifiable information (PII) and PII/PTID links will be destroyed once the laboratory analysis associated with this protocol is complete and the last manuscript associated with this protocol has been published.

Study investigators and personnel will maintain the highest degree of confidentiality for the clinical and research information obtained from the participants. Medical and research records will be maintained in the strictest confidence. However, as a part of the quality assurance and legal responsibilities of a clinical study, investigators will permit authorized representatives of regulatory authorities to examine (and when required by applicable law, to copy) clinical records for the purpose of quality assurance reviews and evaluations of study safety and progress. Unless required by laws that permit copying of records, only the PTID associated with documents or with other participant data may be copied (and all personally identifying information will be removed). Authorized representatives described above are bound to maintain the strict confidentiality of medical and research information that is linked to identify individuals.

20. Plans to Monitor the Data to Ensure Safety of Participants and Data Integrity

☐ **No more than minimal risk**

Participants in this study will not receive investigational vaccine. Minimal risk is involved.

Select one of the following (do not delete this table; review the guidance document for definitions):	
☐ Medium Complexity	
☐ High Complexity Category A	
☒ High Complexity Category B *If choosing this category for a study under an IND or IDE because you believe the study intervention does not significantly impact morbidity or mortality, please provide your rationale:*	

DSMP Requirement	How this Requirement is Met	Frequency	Responsible Party(ies)
Real-time review of participant data during initial data collection.	100% QC review by CRC/CRN and peer review within 3 business days. QM will review first 5 participants in real time.	*Expectation is that this happens every time you obtain information.*	CRC/CRNs QM Manager/Team
Site Monitoring at pre-determined intervals: The Principal Investigator has a responsibility to ensure that the study is following all aspects of the protocol.	*There should be a standard operating procedure to review data (whether a sample or 100%) at pre-determined intervals to ensure that there is adequate documentation of critical elements such as eligibility criteria. Monitoring is required at the following timepoints (but may be done more frequently):* • *study initiation* • *at least every six months while participants are receiving intervention and* • *annually while participants are in follow-up*	*At a minimum, a review is required annually when no one has been enrolled or the study is in long term follow up. Additional risk-based interim monitoring may be required at least once every 12-24 weeks based on the site activity, to include the possibility of remote monitoring. A longer frequency could be acceptable with justification about risk to participants.*	*Delegate a responsible party for each requirement below.* Self-assessment is acceptable*. *Self-assessment*: a process for self-assessment of protocol compliance and data integrity which can be part of an overall DSMP. See CTAC's self-assessment tool on their webpage.
100% review of regulatory files	Site QM will conduct 100% review of reg files at the beginning and at close-out visit at a minimum.	*Reviewed at a minimum of first and close-out visits*	QM Manager/Team Regulatory Team
100% review of consent forms	CRC/CRNs will perform peer review in real time for 100% of ICFs.	*Real time*	CRC/CRNs QM Manager/Team

	QM will conduct 100% review of ICFs.		
Review of credentials, training records, the delegation of responsibility logs (if applicable)	QM and Regulatory Team will conduct 100% review of credentials/training records/DoA files quarterly at a minimum.	*Quarterly*	QM Manager/Team Regulatory Team
Comparison of case report forms (CRF) to source documentation for accuracy and completion	QM will conduct 100% review for first 5 participants in real time and on a monthly basis throughout the study.	*Monthly*	QM Manager/Team
Review of documentation of all adverse events	SAEs will be reviewed by PI and designee and peer reviewed by CRC/CRNs and verified by QM team and evaluated by the ISM and Emory IRB.	*As events occur*	CRC/CRNs QM Manager/Team
Monitoring of critical data points (eligibility, study endpoints, etc.)	QM will conduct 100% review for first 5 participants in real time and on a monthly basis throughout the study.	*Monthly*	QM Manager/Team
Laboratory review of processing and storage of specimens	Peer review of sample log in done in real time and answering queries regularly	*Reviewed at first and close-out visits and at least biannually*	Lab Manager or designee
Assessment of laboratory specimens stored locally	Peer review of sample log in done in real time and answering queries regularly-shipment done within 2 weeks	*Real time*	Lab Manager or designee
Test article accountability review	Day to day and monthly per IDS pharmacist	*Reviewed at first and close-out visits and at least biannually*	Research Pharmacist
Accountability logs, dispensing records,	Monthly per IDS pharmacist	*At least biannually*	Research Pharmacist

and other participant records			
For FDA regulated studies, the following requirements apply:		Timing, frequency, and intensity of monitoring	
Monitoring methods (may include centralized, on-site, and self-assessment)	Monitoring visits will include, but are not limited to, review of regulatory files, accountability records, electronic case report forms (eCRFs), ICFs, medical and laboratory reports, site study intervention storage records, training records, and protocol and GCP compliance. Site monitors will have access to each participating site, study staff and all study documentation according to the Hope Clinic CQMP. Study monitors will meet with all participating site PIs to discuss any problems and outstanding issues and will document site visit findings and discussion.	Monitoring visits are expected to occur monthly or bimonthly. Details of clinical site monitoring are documented in a clinical monitoring plan (CMP). The CMP describes in detail who will conduct the monitoring, at what frequency monitoring will be done, at what level of detail monitoring will be performed, and the distribution of monitoring reports.	Monitoring for this study will be performed by the QM team.
*For international studies, you are required to engage a CRO that is working in the site country and/or to consult with Emory's legal counsel regarding compliance with the country's clinical research regulations.			

20.1 Study Oversight

The Principal Investigator and the research team (co-Investigators, research nurses, study coordinators, and data managers) are responsible for identifying adverse events. Adverse events will be reviewed regularly by the research team.

20.2 Adverse Events

This section defines the types of adverse events that may occur, and outlines the procedures for appropriate adverse event collecting, grading, recording, and reporting.

Information in this section complies with 21CFR 312; ICH Guideline E2A: Clinical Safety Data Management: Definitions and Standards for Expedited Reporting and Good Clinical Practice;

and applies the standards set forth in the National Cancer Institute (NCI), Common Terminology Criteria for Adverse Events Version 5.0 [Published: November 27, 2017; http://evs.nci.nih.gov/ftp1/CTCAE/About.html]. These criteria have been reviewed by the study investigators and have been determined to be appropriate for this study population.

20.2.1 Adverse Events Definition

An adverse event (also referred to as an adverse experience) can be any unfavorable and unintended sign, symptom, or disease temporally associated with the use of the procedure, without any judgment about causality.

After vaccination, the following adverse events in the indicated intervals may be reported to Vaccine Adverse Event Reporting System (VAERS).

- Anaphylaxis or anaphylactic shock (7 days)
- Vasovagal syncope (7 days)
- Any acute complication or sequelae (including death) of above events (interval - not applicable)
- Events described in manufacturer's package insert as contraindications to additional doses of vaccine: hypersensitivity, including severe allergic reactions after previous dose of influenza vaccine

20.2.3 Suspected Adverse Reaction (SAR)

Suspected adverse reaction means any adverse event for which there is a reasonable possibility that the drug or procedure caused 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.

SARs after vaccine administration may include:

- Local reactions: congestion, runny nose, wheezing
- Systemic reactions: headache, fever, nausea, dizziness.

SARs after phlebotomy may include:

- Local reactions: pain at the site of venipuncture, bruising at the site of venipuncture, infection at the site of venipuncture
- Systemic reactions: lightheadedness or fainting

20.2.4 Serious Adverse Event (SAE) or Serious Suspected Adverse Reaction

An adverse event or suspected adverse reaction is considered ''serious'' if, in the view of the investigator, it results in any of the following outcomes:

- Death
- A life-threatening adverse event
- Inpatient hospitalization or prolongation of existing hospitalization
- A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
- A congenital anomaly/birth defect

Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the participant and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Serious adverse events will be reported and recorded for the entire duration of the study.

An adverse event or suspected adverse reaction is considered ‘‘life-threatening’’ if, in the view of the investigator, its occurrence places the patient or participant at immediate risk of death. It does not include an adverse event or suspected adverse reaction that, had it occurred in a more severe form, might have caused death.

Any serious adverse event (for duration of study) will be recorded and reported within 24 hours to ISM.

20.2.5 Unexpected Adverse Event or Unexpected Suspected Adverse Reaction
An adverse event or suspected adverse reaction is considered ‘‘unexpected’’ if it is not listed in the Summary of Product Characteristics or is not listed at the specificity or severity that has been observed; or, if the Summary of Product Characteristics is not required or available, is not consistent with the risk information described in the general investigational plan or elsewhere in the current application, as amended.

20.2.6 Independent Safety Monitoring
The ISM is a physician with relevant expertise in clinical studies whose primary responsibility will be to provide independent safety monitoring in a timely fashion and to provide recommendations regarding the safe continuation of this study.

The ISM, Dr. David Rimland, will evaluate safety data generated from study participants against the known safety profile of the study product or study procedure to assess for possible changes to the overall risk of the study. In addition to the reporting timelines noted in section 20.3.3, the ISM will review safety data annually and communicate with the Principal Investigator as needed. The study has provisions for a back-up ISM to ensure that independent safety monitoring happens at all times during the study.

20.2.7 Collecting and Recording Adverse Events and Pregnancy
Adverse events may be identified during this study through any of these methods:

- Examination of the participant during study visits.
- Questioning the participant during study visits.
- Receiving a safety contact from the participant at any time during the study

Note: participants will be asked to call the site if they develop any of the following:

- Any adverse event that limits self-care activities of daily living (e.g. bathing, dressing and undressing, feeding self, using the toilet, taking medications, and not bed ridden) even if he/she decides not to seek medical care

Serious adverse events will be recorded if they occur at any time during the study.
A complete recording of safety events in the CRF will include event term; date(s) of onset and resolution/stabilization; assessment of severity; relationship to procedures/intervention(s); expectedness; determination of whether the AE qualifies as serious or non-serious; treatment required; action taken with study anticipation; and outcome. AEs qualifying as serious also require a narrative of the event. Updates in safety events will be recorded as additional information becomes available.

Information on pregnancies will be collected from the time a participant signs the consent until the participant completes study participation. If a participant becomes pregnant after study entry, the investigator will discuss with the participant and/or the treating provider the known possible risks to the fetus. Participants becoming pregnant after study entry will be withdrawn from the study and followed until the end of the pregnancy for safety. A pregnancy resulting in congenital anomaly/birth defect will be considered a SAE. Any premature termination of the pregnancy will also be reported and assessed as an SAE as needed.

20.3 Grading and Attribution of Adverse Events

20.3.1 Grading Criteria

Adverse events will be graded according to the criteria set forth in the National Cancer Institute's Common Terminology Criteria for Adverse Events Version 5.0 [November 27, 2017; http://evs.nci.nih.gov/ftp1/CTCAE/About.html] This document (referred to herein as the NCI-CTCAE manual) provides a common language to describe levels of severity, to analyze and interpret data, and to articulate the clinical significance of all adverse events. All adverse events whether or not listed in the NCI-CTCAE will be graded on a scale from 1 to 5 according to the following standards in the NCI-CTCAE manual (A semicolon indicates 'or' within the description of the grade):

- Grade 1 Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated.
- Grade 2 Moderate; minimal, local or noninvasive intervention indicated; limiting age-appropriate instrumental ADL*.
- Grade 3 Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care ADL**.
- Grade 4 Life-threatening consequences; urgent intervention indicated.
- Grade 5 Death related to AE.

*Instrumental Activities of Daily Living (ADL) refer to preparing meals, shopping for groceries or clothes, using the telephone, managing money, etc.
**Self-care Activities of Daily Living (ADL) refer to bathing, dressing and undressing, feeding self, using the toilet, taking medications, and not bedridden.

Not all Grades are appropriate for all AEs; therefore, some AEs are listed with fewer than five options for Grade selection.

Anaphylaxis is a disorder characterized by an acute inflammatory reaction resulting from the release of histamine and histamine-like substances from mast cells, causing a hypersensitivity immune response. Clinically, it presents with breathing difficulty, dizziness, hypotension, cyanosis and loss of consciousness and may lead to death. Severity grading of anaphylaxis as per the NCI-CTCAE manual is as follows:

- Grade 1= not applicable
- Grade 2= not applicable
- Grade 3= Symptomatic bronchospasm, with or without urticaria; parenteral intervention indicated; allergy-related edema/angioedema; hypotension
- Grade 4= Life-threatening consequences; urgent intervention indicated
- Grade 5= Death

20.3.2 Definition of Attribution

The site investigator will initially determine the relationship of an adverse event. The relationship of an AE to study participation will be determined as RELATED or UNRELATED.

20.3.3 Reporting Timelines

Reporting Adverse Events to the Independent Safety Monitor

Any unexpected adverse event grade 3 or higher related to phlebotomy up to 7 days after the procedure will be reported within 7 days to ISM.

Any SAE will be reported to the ISM by email within 24 hours of becoming aware of the event. The ISM may request further information if necessary and possibly request changes to the protocol or consent form as a consequence of the adverse event.

Notifying Institutional Review Board

The Principal Investigator will ensure the timely dissemination of SAE information, including SAEs requiring expedited review by the ISM and to the IRB in accordance with IRB regulations and guidelines. Serious adverse events that are unanticipated, related to study participation, and involving risk to participant or others will be reported to the IRB within 10 business days of event occurrence or of the PI becoming aware of the event per IRB guidelines.

21. Provisions to Protect the Privacy Interest of Participants

A participant's privacy and confidentiality will be respected throughout the study. Each participant will be assigned a unique patient identification number (PTID) and these numbers rather than names will be used to collect and store participant information and samples. A file linking the participant identity, i.e., name, to their unique study participant ID (PTID) is maintained at the Hope Clinic in a separate locked file cabinet and on a HIPAA compliant clinical management database. Screening, consenting, and study visits will take place in private clinic rooms.

Any publications from this study will not use information that will identify participants by name or PTID. A description of this trial will be available on http://www.ClinicalTrials.gov, as required by US Law. This web site will not include information that can identify participants . At most, this web site will include a summary of the results. Site personnel will only transmit documents containing personal identifiable information (PII) using encrypted email or fax in accordance with HIPAA regulations (e.g., to send/receive safety lab data and medical records to/from primary care provider).

22. Economic Burden to Participants

There is no cost to participants for the research tests, procedures, or study product while taking part in this study. Procedures and treatment for clinical care may be billed to the participant, participant's insurance or third party. Participants may be compensated for their participation in this study. Compensation will be in accordance with the local IRB policies and procedures, and participant to IRB approval.

If it is determined by the principal investigator that an injury occurred to a participant as a direct result of the tests, procedures or treatments that are done for this study, then referrals to appropriate health care facilities will be provided to the participant. Study personnel will try to reduce, control, and treat any complications from this study. Immediate medical treatment may be provided by the participating site. No financial compensation will be provided to the participant for any injury suffered due to participation in this study.

23. Informed Consent

23.1 Statement of Compliance

This study was designed to ensure the protection of participants according to the ethical principles of the Declaration of Helsinki and amendments concerning medical research in human participants. This clinical study will be conducted using current good clinical practice and according to the criteria specified in this study protocol. Before study initiation, the protocol and the informed consent documents will be reviewed and approved by the IRB, as well as any other appropriate health authorities. Any amendments to the protocol or to the consent materials will also be approved by the appropriate bodies listed above prior to implementation.

23.2 Informed Consent Process

The informed consent form will provide information about the study to a prospective participant to allow for an informed decision about participation in the study. Prospective participants must be given ample opportunity to review the informed consent and inquire about the results of the study. The consent form will be provided electronically to the prospective participant during the first screening phone call for their review. A copy of the informed consent form will again be provided to a participant for review prior to any study procedure during the in person screening visit. The Principal Investigator or an approved designee will discuss the consent with the prospective participant and answer questions. Study staff will read through the consent form with potential participants and answer any questions. Participants will be allowed sufficient time

to consider participation in the study, after having the nature and risks of the trial explained to them and have the opportunity to discuss the trial with their family, friends. The prospective participant will be told that being in the study is voluntary and that he or she may withdraw from the study at any time, for any reason. The consenting process will take place in private exam rooms at the Hope Clinic. We anticipate that the consent process will take about 30-45 minutes.

All participants must read, sign, and date a consent form before undergoing any study procedures. Consent materials will be provided in the English language, however, shortforms and a qualified interpreter are available and IRB approval will be requested for use in consenting non-English speaking participants. A copy of the signed consent form will be given to the participant. The informed consent form will be revised and receive IRB approval whenever important new safety information is available, whenever the protocol is amended, and/or whenever any new information becomes available that may affect participation in the study.

24. Setting

Potential participants will be recruited and screened from the general population of metro Atlanta. Participants will be enrolled at the Hope Clinic, Decatur, Georgia.

25. Resources Available

The Hope Clinic has a database of more than 5000 previous volunteers who can be considered for screening for the study. The Hope Clinic also has a website and the ability to reach out to potential participants through advertising around the Emory University campus.

25.1 Facilities

The Hope Clinic is a community-based vaccine research clinic and is the clinical arm of the Emory Vaccine Center.

25.2 Participant Support

Referrals to appropriate medical or psychological health care facilities will be provided by the investigators to the participant as needed due to the anticipated consequences of human research. Study personnel will try to reduce, control, and treat any complications from this study. Immediate medical treatment may be provided by the relevant site (Hope Clinic).

25.3 Study Personnel Training

All faculty and staff at the Hope Clinic receive HIPAA, human participants , and EHSO (e.g., bloodborne pathogens) training as part of their onboarding and continuing training. In addition, all study personnel will complete ongoing approved protocol review and provide documentation of this training to study quality management personnel.

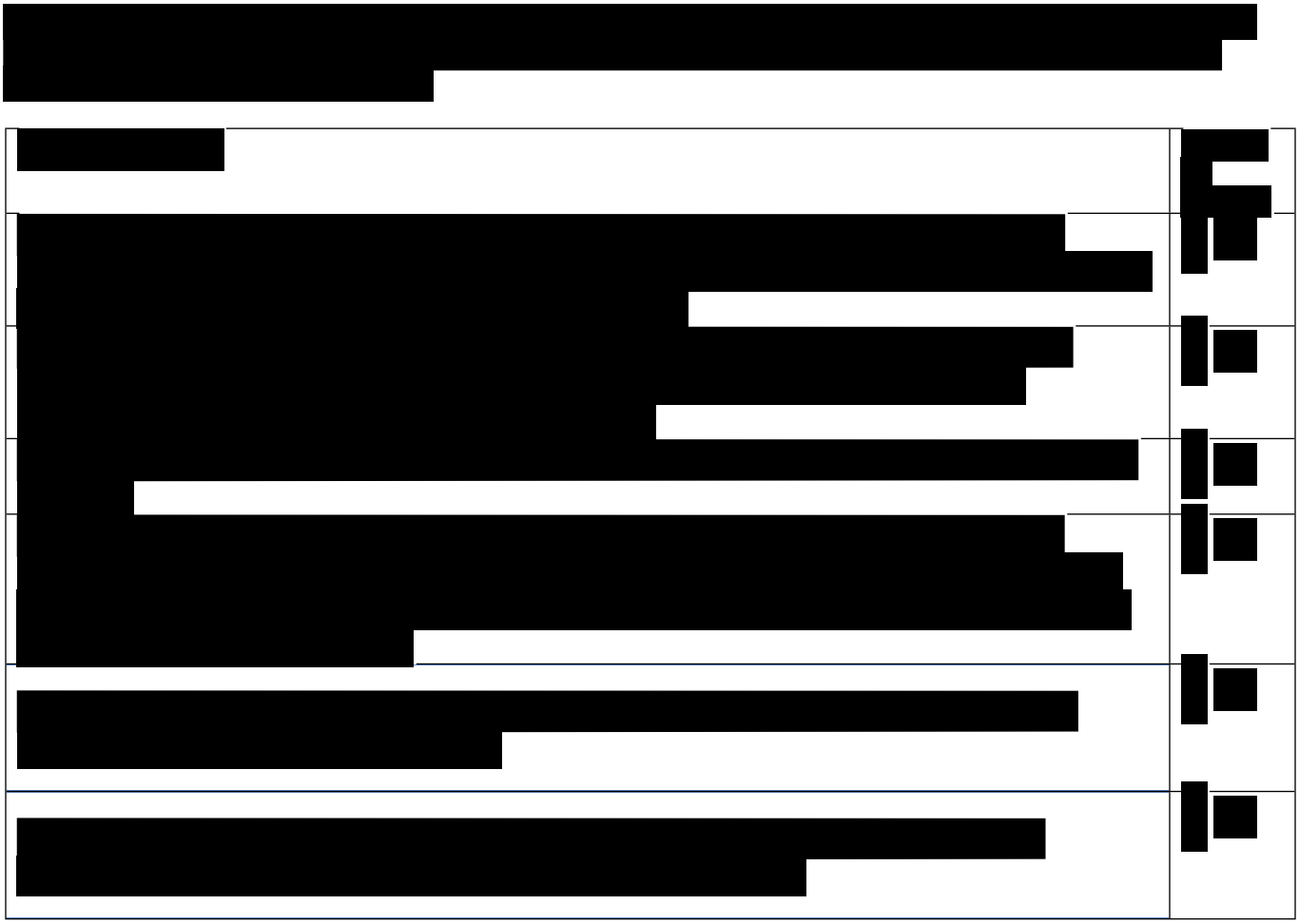
26. Multi-Site or Collaborative Research

All human subjects research activity for this protocol will occur at the Hope Clinic. Deidentified samples will be transported to Dr. Tomic's lab as described in section 19.

27. References

1. Tomic A, Tomic I, Rosenberg-Hasson Y, Dekker CL, Maecker HT, Davis MM. SIMON, an Automated Machine Learning System, Reveals Immune Signatures of Influenza Vaccine Responses. *J Immunol.* 2019;203(3):749-759.
2. Santos A, Colaco AR, Nielsen AB, et al. A knowledge graph to interpret clinical proteomics data. *Nat Biotechnol.* 2022;40(5):692-702.
3. U.S Food and Drug Administration. Use of Trivalent Influenza Vaccines for the 2024-2025 U.S. Influenza Season. https://www.fda.gov/vaccines-blood-biologics/lot-release/use-trivalent-influenza-vaccines-2024-2025-us-influenza-season. Accessed 7 March 2024.
4. Sridhar S, Begom S, Bermingham A, et al. Cellular immune correlates of protection against symptomatic pandemic influenza. *Nat Med.* 2013;19(10):1305-1312.

28. Protocol Checklist



Appendix A. Schedule of Procedures

	D0	D2 (2-3)	D28 (26-32)
	V1	V2	V3
Informed consent	x		
Demographics, medical history	x		
Interim medical history		x	x
Medications	x	x	x
Targeted physical exam	x	(x)	(x)
Vital signs	x	(x)	(x)
Pregnancy test (if applicable)	x		
Vaccination	x		
Blood draw	x	x	x
Nasal sampling	x	x	x

Conducting a visit outside window is allowed at investigator discretion
Parentheses indicate a given procedure will be conducted if relevant for the visit