STATISTICAL ANALYIS PLAN

September 15, 2023

For the clinical trial entitled:

IDCRP-120 A Pragmatic Assessment of Influenza Vaccine Effectiveness in the DoD (PAIVED)

STUDY SITES:
Carl R. Darnell Army Medical Center (CRDAMC)
Lackland Air Force Base – Wilford Hall (WHASC)
Madigan Army Medical Center (MAMC)
San Antonio Military Medical Center (BAMC)
Naval Medical Center Portsmouth (NMCP)
Walter Reed National Military Medical Center (WRNMMC)
Naval Medical Center San Diego (NMCSD)
United States Naval Academy (USNA)
Uniformed Services University of the Health Sciences (USUHS)
Womack Army Medical Center (WAMC)

STUDY SPONSORS:

INVESTIGATORS:

T.H Burgess, R. Colombo, C.L. Coles, L.C. Collins, P.C. Graf, T. Lalani, R.C. Maves, G. Utz, T. Warkentien, L.M. White

PREPARED BY:
Study Team

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1.0 Study Background

Globally, seasonal influenza epidemics cause three to five million severe cases and 300,000 to 500,000 deaths annually according to the World Health Organization. A century after the 1918 pandemic, influenza remains a leading cause of morbidity and a major threat to operational readiness in the United States Armed Forces. More than 90% of active duty personnel in the United States receive influenza vaccinations annually. Despite high coverage, ILI frequently leads to clinical visits, missed duty days and hospitalizations.

Seasonal strain-specific vaccination remains the foundation of influenza prevention and control. The effectiveness of seasonal influenza vaccines varies considerably by season and has generally been higher against influenza A (H1N1) pdm09 and B viruses than against A (H3N2) viruses, even when well-matched with circulating strains. The overall vaccine efficacy (VE) is estimated to be 19% in armed forces personnel, despite 90% vaccination coverage, while the overall VE is 51% in military beneficiaries where vaccine coverage is approximately 50%. In contrast, mid-season estimates of overall adjusted VE against influenza associated with medically attended acute respiratory infections in the general United States population is 36%. The reasons for the disparities in VE are not yet known.

Multiple factors may contribute to suboptimal influenza vaccine effectiveness, including host factors such as prior influenza exposure, vaccination history, age, and coexisting conditions. Vaccination timing and vaccine failure may also be related to transmission or virulence of influenza in this population. Another factor that may alter the effectiveness of influenza vaccines is the substrate used to produce them. In the United States, most influenza-vaccine viruses are propagated in eggs, although some are produced either in cell culture or by a recombinant protein expression system. Residue changes in the vaccine virus hemagglutinin protein, the antigen responsible for virus attachment and a target of neutralizing antibodies that arise during passage in eggs have been suggested to confer antigenic difference that may result in decreased vaccine effectiveness in specific circumstances. Additional studies are needed to assess whether VE against circulating influenza viruses varies by vaccine formulation, including comparisons between egg-based and non-egg-based vaccines.

This is a trial to compare relative effectiveness of non-egg-based influenza vaccines (cell-based and recombinant-based) to egg-based influenza vaccines in the prevention of laboratory confirmed influenza.

2.0 Trial Objectives

2.1. Primary Objective

Compare the relative effectiveness (prevention of laboratory-confirmed influenza illness) of cell-culture-based (FlucelvaxR) and recombinant HA (FluBlokR) influenza vaccines to that of egg-based influenza vaccines.

2.2. Secondary Objective (Immunogenicity Sub-Study)

Compare the immunogenicity and antigenicity of the cell-culture-based (FlucelvaxR) and recombinant HA (FluBlokR) influenza vaccines to that of egg-based influenza vaccines for:

1. A/H1N1
2. A/H3N2
3. B/Victoria
4. B/Yamagata

2.3 Exploratory Objectives

Compare the disease burden associated with receiving cell-culture-based (FlucelvaxR) and recombinant HA (FluBlokR) influenza vaccines to egg-based influenza vaccines.

Relative risk of laboratory-confirmed influenza hospitalization
Relative risk of ILI Mean workdays lost due to laboratory-confirmed influenza
Mean workdays lost due to ILI

3.0 Methods

3.1 Main trial

This study will be a multi-center, pragmatic, prospective, non-blinded randomized controlled trial to assess the relative effectiveness of the licensed egg-based inactivated influenza vaccine to two other licensed vaccines, the cell-culture-based inactivated influenza vaccine and the recombinant influenza vaccine, in the prevention of laboratory-confirmed influenza infection in active duty members, military retirees and beneficiaries over four consecutive influenza seasons. Subject recruitment will take place at military treatment facilities.

Subjects will be active duty service members or other adults at least 18 years of age who are DEERS-eligible for care in the military treatment facility.

A total of 18,000 eligible subjects will be randomly allocated in a 1:1:1 ratio by study investigative team staff to one of the following groups:

- Group 1: Cell-culture-based (FlucelvaxR) influenza vaccine (~6,000 subjects)
- Group 2: Recombinant HA (FluBlokR) influenza vaccine (~6,000 subjects)
- Group 3: Egg-based influenza vaccines (~6,000 subjects)

Vaccine type will be known to the study investigative team and the participant.

Study Period

All participants who are not Marine Corps recruits will undergo surveillance for ILI during the influenza season (from October 2018-May 2019, September 2019-May 2020, September 2020-May 2021, and September 2021-May 2022).

Process

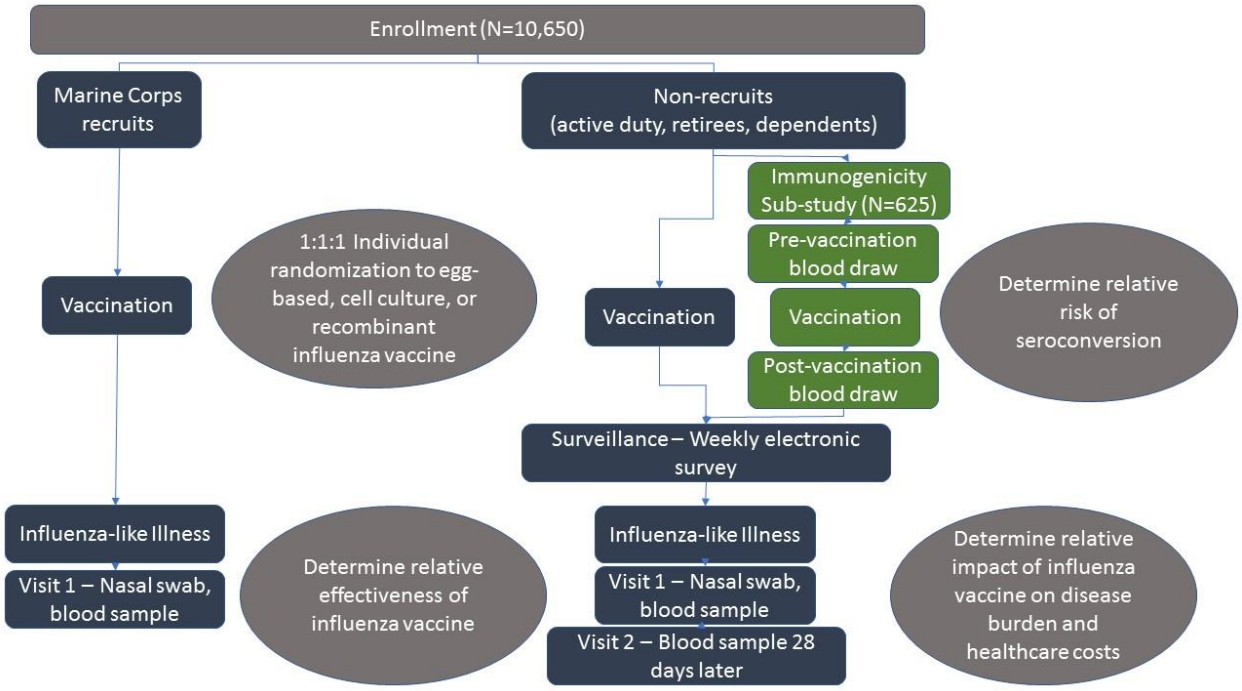
During the influenza season, automated weekly email or text messages will be sent to subjects asking them if they have experienced ILI symptoms in the past 7 days. Subjects who meet the ILI case definition will be asked to complete an online 7-day symptom severity questionnaire and to schedule an acute ILI visit with a study coordinator. The questionnaire takes approximately 10 minutes to complete. At the acute ILI visit (Visit 1), study coordinators will record subjects' vital signs, ILI history, information on prescribed medications, hospitalization status, and work absences. They will also collect a nasal swab for diagnostic purposes and a blood sample for serologic studies and immune function. Subjects will contribute another blood specimen at a convalescent ILI visit approximately 28 days after the acute ILI visit (Visit 2). Information on ILI duration, hospitalization status, concomitant medications and work absences will be updated at this visit. Subjects will be monitored through as many ILI episodes as they experience, though subjects will only be compensated for the first 3 episodes. ILI episodes must be separated by at least 30 days. Information on ILI health care utilization and cost will be abstracted from the Military Health System Data Repository database by IDCRP study staff at the end of each influenza season.

Due to their lack of access to technology, participants who are Marine Corps recruits will not participate in the weekly surveillance; they will present when ill at the health clinic and nasopharyngeal and blood samples will be collected during Visit 1. Recruits are not required to schedule a Visit 2.

3.2 Immunogenicity Sub-Study

Nested within this trial is an immunogenicity sub-study, in which 1,000 subjects will have their blood drawn prior to influenza vaccination and again at 21-35 days post-vaccination to assess changes in immune responses to the vaccines. Collection of a buccal swab for host genomic studies will be optional at enrollment or the 21-35 days post-vaccination visit.

	Recruits	Non-recruits	Non-recruits; Sub-Study
Consent and sociodemographic data collection	X	X	X
Sub-Study blood draw (pre-vaccination)			X
Vaccination with one of 3 vaccines	X	X	X
Weekly surveillance begins for non-recruits 14 days after vaccination		X	X
Sub-Study blood draw (post-vaccination)			X
ILIs - Visit 1 (if ILI is experienced) - nasopharyngeal swab, blood sample	X	X	X
ILI severity survey (if ILI is experienced)		X	X
ILIs - Visit 2 (if ILI is experienced) - blood sample ~28d after Visit 1		X	X



4.0 Analysis Definitions

4.1. Endpoints

4.1.1 Laboratory confirmed influenza:

Laboratory confirmed influenza as determined by RT-PCR from nasopharyngeal swab specimens collected upon presentation with ILI.

4.1.2 Immune Response

4.1.2.1 Hemagglutination inhibition (HI) titers for A/H1N1, B/Yamagata, and B/Victoria

4.1.2.2 Microneutralization (MN) titers for influenza A/H3N2

4.1.2.3 Seroconversion Rate (SCR): Proportion of study participants with either a pre-vaccination titer <10 and a post-vaccination titer $\geq$40, or a pre-vaccination titer $\geq$10 and a $\geq$ 4-fold increase in post-vaccination titer

4.1.2.4 Geometric mean titers (GMT)

4.1.2.5 Mean-fold rise (MFR): (post-sample / pre-sample) vaccination titers

4.1.3 Other

4.1.3.1 Laboratory-confirmed influenza hospitalization

Laboratory-confirmed influenza as determined by RT-PCR from nasopharyngeal swab specimens collected upon presentation with ILI and hospitalized within ***14*** days of laboratory confirmed influenza.

4.1.3.2 ILI

ILI is defined as:

1. Fever OR feeling feverish OR chills/night sweats
   AND
2. Cough OR sore throat
   AND
3. Muscle/body aches OR fatigue (tiredness)

4.2 Analysis terms

Subgroup analysis may be performed using:

- Risk groups (e.g., pregnant women)
- Healthcare workers
- Chronic conditions
- Sex
- Time since vaccination
- Previous influenza vaccinations
- Age
- Race/Ethnicity
- Smoking status

5.0 Statistical Methods

5.1 Primary Endpoint (full study)

The primary analysis is the relative effectiveness analysis for the laboratory confirmed influenza endpoint (definition 3.1.1)

5.1.1 Evaluating balance between the three treatment arms

Randomization is used to control for, or reduce, selection bias among study participants. Randomization should control for both measured and unmeasured confounders/covariates as well as both known and unknown confounders/covariates and background characteristics.

Comparison between groups will occur at the interim analysis at the end of the first influenza season and at the end of the study.

We will compare the three vaccination groups for basic background characteristics and known measured confounders (See Appendix *X*).

5.1.2 Relative Vaccine Effectiveness Estimate

Relative vaccine effectiveness (RVE) of cell-culture-based (FlucelvaxR) influenza vaccine as compared to egg-based influenza vaccines will be calculated using the following formula:

$$RVE_{cell} = \left(\frac{R_{egg} - R_{cell}}{R_{egg}}\right) X100\% = \left(1 - \frac{R_{cell}}{R_{egg}}\right) X100\%$$

Where:
R_{cell}=incidence rate among the cell-culture-based influenza vaccination group
R_{egg}=incidence rate among the egg-based influenza vaccination group

and

Relative vaccine effectiveness of recombinant HA (FluBlokR) influenza vaccines as compared to egg-based influenza vaccine will be calculated using the following formula:

$$RVE_{recombinant} = \left(\frac{R_{egg} - R_{recombinant}}{R_{egg}}\right) X100\% = \left(1 - \frac{R_{recombinant}}{R_{egg}}\right) X100\%$$

Where:
$R_{recombinant}$=incidence rate among the recombinant HA influenza vaccination group
R_{egg}=incidence rate among the egg-based influenza vaccination group

The incidence rate equals the number of laboratory-confirmed influenza (definition 3.1.1) divided by the number of vaccinated individuals in each vaccination group.

The formula for the incidence rate will be:

$$Incidence\ Rate = \frac{Number\ of cases}{Number\ of\ vaccinated}$$

Confidence intervals (95%) will be calculated using the standard formulas for relative risk. The upper and lower confidence intervals for relative vaccine effectiveness will be one minus the upper and lower calculated confidence intervals for the relative risk. The efficacy will be statistically significant if the calculated relative risk has a p-value of less than or equal to 0.025 (one-sided test). The interim analysis for relative effectiveness after the first influenza season uses a stricter p-value (<0.0001). Because of this, the final analysis will not need to adjust the p-value out of concerns about multiple looks.

5.1.3 Relative Vaccine Effectiveness Estimate by Vaccine type

We have an *a priori* interest in evaluating relative vaccine effectiveness by vaccine type regardless of whether the overall relative vaccine effectiveness differs significantly between either cell-culture based influenza vaccine and egg-based influenza vaccine OR recombinant HA influenza vaccine and egg-based influenza vaccine. Vaccine types include:

1. A/H1N1
2. A/H3N2
3. B/Victoria
4. B/Yamagata

Relative vaccine effectiveness comparing cell-culture based influenza vaccine and egg-based influenza vaccine and recombinant HA influenza vaccine and egg-based influenza vaccine will be estimated for each vaccine type.

5.1.4 Power of the study for the primary endpoint

The sample size estimates for this study were based on a power of 0.80 a one-sided alpha of 0.25, and an assumed incidence of five per hundred among the egg-based vaccination group to detect a relative vaccine effectiveness of at least 30%.

5.1.5 Plan for handling missing data and extreme values

Data will come from multiple sources including an enrollment document and from medical records. Care will be taken to minimize missing data, but sometimes this will still occur. For each variable, the data managers will review the data and calculate the percent missing. In addition, extreme values will be

identified. Participants with missing vaccination or outcome data will be excluded. A sensitivity analysis will be performed using only those non-recruit participants with at least 50%/75% of the weekly survey data in order to ensure that findings are consistent.

5.2 Immunogenicity Endpoint (Sub-Study)

5.2.1 Evaluating balance between the three treatment arms

Randomization is used to control for, or reduce, selection bias among study participants. Randomization should control for both measured and unmeasured confounders/covariates as well as both known and unknown confounders/covariates and background characteristics.

Comparison between groups will occur at the interim analysis at the end of the first influenza season and at the end of the study.

We will compare among the subset the three vaccination groups for basic background characteristics and known measured confounders (See Appendix *X*).

5.2.2 Endpoints

5.2.2.1 Relative risk for seroconversion

Relative risk of seroconversion among participants who received cell-culture-based influenza vaccine (FlucelvaxR) as compared to those receiving egg-based influenza vaccine will be calculated using the following formula:

$$RR_{cell} = \frac{p_{cell}}{p_{egg}}$$

Where:
p_{cell} = proportion seroconvert among the cell-culture-based influenza vaccination group
p_{egg} = proportion seroconvert among the egg-based influenza vaccination group

and

Relative risk of seroconversion among participants who received recombinant HA (FluBlokR) influenza vaccine as compared to those receiving egg-based influenza vaccine will be calculated using the following formula:

$$RR_{recombinant} = \frac{p_{recombinant}}{p_{egg}}$$

Where:
$p_{recombinant}$ = proportion seroconvert among the recombinant HA influenza vaccination group
p_{egg} = proportion seroconvert among the egg-based influenza vaccination group

The proportion that seroconvert equals the number seroconvert (definition 3.1.2.3) divided by the number of vaccinated individuals that were enrolled in the Sub-Study and had the pre- and post-blood draws.

Confidence intervals (95%) will be calculated using the standard formulas for relative risk. The relative risk will be statistically significant if the p-value is less than or equal to 0.025 (one-sided test). The interim analysis for relative risk after the first influenza season uses a stricter p-value (<0.0001). Because of this, the final analysis will not need to adjust the p-value out of concerns about multiple looks.

Relative risk for seroconversion as defined in 5.2.2.1 will be evaluated for each vaccine-type (A/H1N1, B/Yamagata, B/Victoria, and A/H3N2);

5.2.2.2 Geometric Mean Titers (GMTs) – GMTs will be calculated pre- and post-vaccination. Non-inferiority will be based on a 2-sided test comparing post-vaccination GMTs between vaccine types.

5.2.2.3 Mean-Fold Rise – Ratios of participant post-vaccination titers divided by the pre-vaccination titers.

5.3 Other

5.3.1 Influenza-confirmed hospitalization – Relative risk of laboratory confirmed influenza hospitalization in cell-culture-based vaccine recipients compared with egg-based vaccine recipients (and comparing recombinant vaccine with egg-based vaccine)5.3.2 Influenza-like illness - Relative risk of laboratory confirmed influenza hospitalization in cell-culture-based vaccine recipients compared with egg-based vaccine recipients (and comparing recombinant vaccine with egg-based vaccine)

5.3.3 Work days lost to influenza and ILI – Compare mean work days lost to influenza and ILI between vaccines using t-tests and/or multivariate linear models.

5.3.4 Healthcare costs/utilization for influenza and ILI - TBD.

6. Interim Analysis after first influenza season (2018/19)

6.1 Objective

The first year of the study includes a relatively small number of participants and it is highly unlikely that the relative vaccine effectiveness of at least 30% between either the cell-culture based compared to the egg-based or the recombinant HA compared to the egg-based would be achieved. The primary goal of the interim analysis is to ensure that all study systems are working appropriately and to identify any potential problem areas. As such, it is anticipated that no statistical comparisons will be conducted, although point estimates and 95% confidence intervals will be generated.

6.2 Methods

6.2.1 Analysis database

After the conclusion of the 2018/2019 influenza season, all participants will be included in the interim analysis. The database will include vaccination information, ILI information, and laboratory-confirmed influenza diagnoses. For participants included in the immunogenicity sub-study, all laboratory results will be included.

6.2.2 Relative Vaccine Effectiveness Estimation

The steps outlined in 5.1 will be completed. For this interim analysis, it is anticipated that all participants will be included, and no subgroup analyses are planned.

6.2.3 Relative Vaccine Effectiveness Stopping Criterion

A p-value of less than 0.0001 will be used as the cut-off value for evaluating statistical significance of the relative vaccine effectiveness at the interim analysis. This would be applied to both pairwise comparison. A statistically significant p-value for one or both vaccines being evaluated would not necessarily be used for stopping the study. If a positive point estimate were observed, the decision to stop the trial for overwhelming relative effectiveness for one or both vaccines might be considered.

6.2.3 Immunogenicity Estimation

The steps outlined in 5.2 will be completed. For this interim analysis, it is anticipated that all participants will be included, and no subgroup analyses are planned.

Appendix X. Demographic and Baseline Characteristics Among Trial Participants by Study Arm

	Vaccine Type			
	Egg-based n (%)	Cell-culture n (%)	Recombinant HA n (%)	p-value
Characteristic				
Age (median, IQR)				
Age group (years) 17-24 25-29 30-39 40+				
Male				
Race White, Only Black, Only Other Unknown				
Hispanic				
Education Level Less than High School High School/GED Associate degree Bachelor Degree Graduate Degree				
Duty Status Active Duty Military Recruit Trainee Retire Military Dependent				
DOD Affiliation Army Navy Coast Guard Marines Air Force				
Smoking Status Current smoker Former smoker Non-smoker				
Enrollment Site (?) Site A Site B Etc.				
Previous Influenza Vaccination None 1-2 doses 3 or more doses				
Underlying disease? Diabetes Cardiovascular Disease COPD				