Examining vaccine effectiveness (VE) of Flublok® Quadrivalent (Influenza Vaccine) relative to standard dose inactivated influenza vaccine among Kaiser Permanente Northern California members aged 18-64 years

Phase IV, multi-center, modified-cluster randomized study to assess the effectiveness of Flublok Quadrivalent vaccine compared to standard dose inactivated influenza vaccine in adults

Statistical Analysis Plan (SAP) - Core Body Part

Study Code:	VAP00003
Development Phase:	Phase IV
Sponsor:	Kaiser Permanente Northern California Kaiser Permanente Vaccine Study Center
Product(s):	Flublok Quadrivalent vaccine *Standard-Dose Inactivated Influenza Vaccine (SD-IIV)**
	* to be determined prior to influenza season
Form / Route:	Liquid/Intramuscular injection
Indication For This Study:	Comparative vaccine effectiveness of 2018-2019 and 2019-2020 formulations of Flublok Quadrivalent vaccine and SD-IIV in adults 18 through 64 years of age
Manufacturer:	Sanofi Pasteur Inc. Discovery Drive, Swiftwater, PA 18370-0187, USA
Principal Investigator:	Nicola Klein, MD, PhD
Contributing Investigators:	Bruce Fireman, MA Ned Lewis, MPH Laurie Aukes, RN John Hansen, MPH Amber Hyman, MPH
Project Manager and Study Leader	Amber Hyman, MPH
Version and Date of the Protocol:	Version 1.0 dated 16 June 2020

Study Code:	VAP00003
Development Phase:	Phase IV
Sponsor:	Kaiser Permanente Northern California Kaiser Permanente Vaccine Study Center 1 Kaiser Plaza, 16th Floor Oakland, CA 94612
Product(s):	Flublok Quadrivalent vaccine *Standard-Dose Inactivated Influenza Vaccine (SD-IIV)** * to be determined prior to influenza season
Form / Route:	Liquid/Intramuscular
Indication For This Study:	Comparative vaccine effectiveness of 2018-2019 and 2019-2020 formulations of Flublok Quadrivalent vaccine and SD-IIV in adults 18 through 64 years of age
Version and Date of the SAP core body part:	Version 1.0 16-JUN-2020
ClinicalTrials.gov:	NCT03694392

Table of Contents

List of Abbreviations

CAP	Community-Acquired Pneumonia
COPD	Chronic Obstructive Pulmonary Disease
DxCG	Diagnostic Cost Groups
EMR	Electronic Medical Record
HA	Hemagglutinin
HAI	Hemagglutination Inhibition
ICD-10	International Classification of Diseases, 10th Revision
IRB	Institutional Review Board
KPNC	Kaiser Permanente Northern California
KPVSC	Kaiser Permanente Vaccine Study Center
LRTI	Lower Respiratory Tract Infection
PCR	Polymerase Chain Reaction
RSV	Respiratory Syncytial Virus
rHR	Relative Hazard Ratio
rVE	Relative Vaccine Effectiveness
SAP	Statistical Analysis Plan
SD-IIV	Standard-Dose Inactivated Influenza Vaccine
SD-IIV4	Standard-Dose Quadrivalent Inactivated Influenza Vaccine
VE	Vaccine Effectiveness

1 Introduction

Influenza is transmitted through inhalation of virus-containing droplets from infected individuals. The incubation period is usually 1 to 2 days. The virus multiplies in the ciliated columnar epithelium of the upper- and lower-respiratory tract, causing cellular necrosis and sloughing. Virus shedding typically begins just before illness onset (within 24 hours), rapidly peaks, and remains elevated for 1 to 2 days before rapidly declining to low levels. Usually, virus shedding lasts a total of 5 to 10 days.

There is considerable variation in the severity of illness in different individuals, partly due to age, general health, and immune status relative to previous influenza infections and vaccination. The classic symptoms include rapid onset (12 hours or less) of malaise, fever, myalgia, headache, and a non-productive cough or sore throat. Most symptoms last several days, but malaise and cough may last for a week or more. Complications of influenza include primary viral pneumonia, secondary bacterial pneumonia, and exacerbation of underlying medical conditions such as chronic obstructive pulmonary disease and congestive heart failure.

While influenza affects all age groups, seniors, children younger than 5 years of age, and persons with underlying health problems are at increased risk for complications. Members of high-risk groups who become ill with influenza are more likely than the general population to require hospitalization for complications of the infection (e.g., pneumonia and cardiovascular events). Groups considered at higher risk for influenza-related complications include persons aged ≥65 years due to immunosenescence and higher likelihood of chronic medical conditions that put them at increased risk for severe influenza illness, and persons 50–64 years of age and older (also more likely to have chronic medical conditions that put them at increased risk).

Vaccination with influenza vaccine is the primary method for preventing influenza and its severe complications. It has been shown to be effective in reducing influenza-associated morbidity and mortality in groups at increased risk for influenza-related complications such as infants and young children and persons 50 years of age and older. Of note, immune responses to the vaccine are lower in seniors than those in young healthy adults. Strategies to improve immune responses to the vaccine in the elderly population could provide significant additional reductions in influenza-associated morbidity and mortality. One approach is to increase the dose of hemagglutinin (HA) in vaccines. Previous studies evaluating the immune responses in terms of hemagglutination inhibition (HAI) antibodies with higher doses of HA per strain in different influenza vaccines support a dose-response effect.

Flublok Quadrivalent vaccine is a recombinant HA influenza vaccine indicated for active immunization against disease caused by influenza A subtype viruses and type B viruses contained in the vaccine for adults 18 years of age and older. Use of recombinant DNA techniques to produce vaccine antigen expressed in cell culture is a method that avoids growing the influenza viruses in embryonated hens' eggs. Recombinant technology also allows control over the sequence of the full-length HA produced. The recombinant proteins are highly purified and the vaccine contains no egg protein, preservatives, or antibiotics, any of which may produce hypersensitivity reaction in some individuals.

Flublok Quadrivalent vaccine contains 45 μg HA per virus strain per dose, 3 times the amount of HA compared to standard-dose inactivated quadrivalent influenza vaccine (SD-IIV4). The high purity of a recombinant antigen enables administration of a higher concentration of HA antigen without an increase in adverse effects in humans. This higher dose of antigen may be a particular advantage to older adults whose immune responses to influenza vaccines can be suboptimal.

During this study, Flublok Quadrivalent or SD-IIV will be administered according to the guidelines in the Prescribing Information materials and only to persons for whom it is indicated. The 2018–2019, 2019–2020, and 2020–2021 formulations of recombinant influenza vaccine (Flublok Quadrivalent vaccine) and SD-IIV will be evaluated as described below.

The overall objective of this study is to describe the effectiveness of Flublok Quadrivalent vaccine compared to SD-IIV in adults 18 through 64 years of age.

2 Study Objectives

2.1 Primary Objective

Among all adults 50–64 years who receive either Flublok Quadrivalent or SD-IIV during the 2018–2019, 2019–2020, and/or 2020–2021 influenza seasons, we will estimate the relative vaccine effectiveness (rVE) of Flublok Quadrivalent vaccine versus SD-IIV against the following outcome:

- All PCR-confirmed influenza.

2.2 Secondary Objectives

Among all adults 50–64 years who receive either Flublok Quadrivalent or SD-IIV during the 2018–2019, 2019–2020, and/or 2020–2021 influenza seasons, we will estimate the rVE of Flublok Quadrivalent vaccine versus SD-IIV against the following outcomes:

- PCR-confirmed hospitalized influenza
- Hospitalized community-acquired pneumonia (CAP) (we will only include primary diagnoses for this outcome)
- Hospitalized cardio-respiratory events combined (e.g., CAP, other lower respiratory tract infections [LRTI], acute myocardial infarction, congestive heart failure, stroke, atrial fibrillation) (we will only include primary diagnoses for this outcome)
- All PCR-confirmed influenza A
- All PCR-confirmed influenza B

For these 5 secondary objectives, adjustment for multiplicity will be applied.

Sub-analyses of *primary* outcome in sub-groups defined by pre-existing conditions (listed below) will also be conducted among adults 50–64 years. Additionally, sub-analyses of *secondary*

outcomes of sub-groups defined by pre-existing conditions will be conducted as feasible among adults 50–64 years.

"Pre-existing" will be defined as history of a condition in the 3 years prior to vaccination date.

i. **Cardiovascular preexisting conditions composite, which may include:** myocardial infarction, cerebrovascular accident, atrial fibrillation, arrhythmias, coronary artery disease, valvular heart disease/valvuloplasty, chronic/congestive heart failure and/or congenital heart defects, CABG, angioplasty/stents, cardioversion, pacemaker, implantable cardioverter defibrillator, LVAD, heart transplant, hypertensive disease
ii. **Respiratory preexisting conditions composite, which may include:** COPD and/or pulmonary HTN
iii. **Cardiorespiratory preexisting conditions composite:** combined pre-existing conditions (a) and (b) above
iv. **Obesity:** based on available height/weight data for a calculated BMI ≥30 or an obesity diagnosis (e.g., ICD-10 E66.9)
v. **Diabetes:** based on a diagnosis of diabetes

2.3 Exploratory Objectives

- To estimate the rVE of Flublok Quadrivalent vaccine versus SD-IIV in vaccinees aged 50–64 years against the following exploratory outcomes if time allows, potentially including:
    a. Diagnosed with influenza (includes [1] adults with a PCR-positive influenza and [2] adults clinically-diagnosed with influenza in any setting but not tested); this outcome will exclude those with a clinically-diagnosed influenza but are tested and PCR-negative)
    b. All-cause hospitalizations (excluding pregnancy-related hospitalizations)
    c. All-cause mortality

- To estimate the rVE of Flublok Quadrivalent vaccine versus SD-IIV in other age ranges of vaccinees—aged 18–64 years and aged 18–49 years—against the following outcomes, if time allows:
    a. All PCR-confirmed influenza
    b. All PCR-confirmed influenza A and influenza B (separate rVE estimates)
    c. PCR-confirmed hospitalized influenza
    d. Hospitalized CAP
    e. Hospitalized cardio-respiratory events combined
    f. All-cause hospitalization (excluding pregnancy-related hospitalizations)
    g. All-cause mortality

- Sub-analyses of the primary and secondary outcomes of sub-groups defined by pre-existing conditions (as described above in Section 2.2) will be conducted among adults 18–49 years as feasible

- To estimate the rVE of Flublok Quadrivalent vaccine versus SD-IIV among adults aged 18–64 years for PCR-confirmed influenza A subtypes (A(H1N1)pdm09 and A(H3)) and lineage for type B influenza (B/Victoria, B/Yamagata) as feasible

- Other exploratory objectives may be developed, such as vaccination waning or changes in subtypes circulating during an influenza season

3 Description of the Overall Study Design and Plan

3.1 Study Design

To estimate the rVE of Flublok Quadrivalent vaccine versus SD-IIV against all PCR-confirmed influenza in vaccinees aged 18–64 years, this study will compare the incidence of PCR-confirmed influenza among Flublok Quadrivalent vaccinees versus SD-IIV vaccinees. Our primary comparison will be focused on adults aged 50–64 years at KPNC during the 2018–2019, 2019–2020, and 2020–2021 influenza seasons. We will also assess all adults aged 18–64 years for both influenza seasons.

3.2 Trial Plan

This study will employ a design whereby KPVSC will ensure that all clinics in KPNC will receive alternating shipments of Flublok Quadrivalent vaccine or SD-IIV. All KPNC facilities will follow a schedule that alternates which vaccine is supplied to each influenza clinic throughout the 3-year study period.

We will block facilities and randomize within blocks (see protocol **Section 5.1.4**), ensuring that roughly half the facilities within each block initially administer Flublok Quadrivalent vaccine and the other half administer a SD-IIV. After the initial supply, the half that started with Flublok Quadrivalent vaccine will be supplied with a SD-IIV, and the half that started with a SD-IIV will be supplied with Flublok Quadrivalent vaccine (see **Figure 1**). The supply of each clinic will then alternate between the two vaccines; that is, Flublok Quadrivalent vaccine alternating with a SD-IIV with each delivery of vaccine to the clinics.

Figure 1: Visual of block randomization influenza vaccine administration schedule

	Week 1	Week 2	Week 3
Block A			
Facility 1 Facility 3 Facility 5 Facility 7 Facility 9	Flublok	Comparator IIV	Flublok
Block B			
Facility 2 Facility 4 Facility 6 Facility 8 Facility 10	Comparator IIV	Flublok	Comparator IIV

Data Extraction

Pertinent data will be extracted from the KPNC databases to create study datasets in the required file format for analysis (e.g., Excel spreadsheets, SAS datasets). Databases from which data will be extracted include various encounters (inpatient, outpatient, hospitalizations, emergency department, etc.), laboratory tests, documentation of immunizations (e.g., influenza vaccination history), and clinical outcomes and diagnoses, as well as demographic and membership data.

The data analyst will perform validation of the study data throughout the data management process by utilizing the following types of checks as applicable:

- Internal consistency checks (e.g., data undergo range checks, including consistency of dates of birth with vaccination dates, checks for completeness and missing values, etc.)
- External data checks (e.g., data from one database may be compared with data from other databases)
- Error checks written for specific databases.

4 Endpoints and Assessment Methods

4.1 Primary Endpoints and Assessment Methods

Among all adults 50–64 years who receive either Flublok Quadrivalent or SD-IIV during the 2018–2019, 2019–2020, and/or 2020–2021 influenza seasons, we will estimate the relative vaccine effectiveness (rVE) of Flublok Quadrivalent vaccine versus SD-IIV against all PCR-confirmed influenza.

All microbiological testing is conducted at KPNC laboratories that have used real-time PCR tests for influenza since 2006. PCR results are categorized as positive for influenza A; positive for influenza B; or negative for both (PCR results are also categorized as

positive or negative for respiratory syncytial virus [RSV]). In the KPNC system, influenza PCR tests are used for clinical purposes, thus the "trigger" for an influenza PCR test is based on clinical presentation. The decision to test is up to each physician. KPNC monitors the influenza season which can affect the total amount of influenza testing in adults. Once the KPNC laboratories complete the PCR testing, the test result is updated in the patient's EMR.

Among adults aged 50–64 years who are vaccinated with Flublok or an SD-IIV, we plan to assess the number of adults who have a PCR-positive test ≥14 days after vaccination up through to the end of each influenza season (as determined by KPNC, which is generally when the proportion of influenza-positive PCR tests drops below 10%) or the first week of May, whichever is later, compared with those who do not have a PCR-positive test by vaccine received. To be included in the cohort for analysis, the PCR test must occur during the influenza season. The influenza season is defined as the first day that KPNC begins use of the Cepheid GeneXpert PCR assay for the influenza season (generally the first Tuesday of October); the end of the season is generally determined when the proportion of influenza-positive PCR tests drops below 10%, or the first week of May, whichever is later.

We will only count the first PCR positive test per patient in any single season.

4.2 Secondary Endpoints and Assessment Methods

For each of the secondary outcomes listed below, we will count the first event of the outcome per influenza season and censor follow-up after the first event. The influenza season is defined as the first day that KPNC begins use of the Cepheid GeneXpert PCR assay for the influenza season (generally the first Tuesday of October); the end of the season is generally determined when the proportion of influenza-positive PCR tests drops below 10%, or the first week of May, whichever is later.

For all secondary objectives excluding the sub-analyses by pre-existing conditions, adjustment for multiplicity will be applied following the Holm adjustment method[1]:

1. We will rank order the 5 p-values obtained from tests of the 5 null hypotheses about rVE with respect to each of the 5 secondary outcomes listed above.
2. The 5 p-values will be ranked in order from the smallest p-value to the largest: $p_{(o1)} \leq p_{(o2)} \leq p_{(o3)} \leq p_{(o4)} \leq p1_{(o5)}$
3. The corresponding alpha critical values to determine significance are to be used based on rank:
   i. $\alpha_{(C\_o1)} = \alpha/5 = 0.0100$
   ii. $\alpha_{(C\_o2)} = \alpha/4 = 0.0125$
   iii. $\alpha_{(C\_o3)} = \alpha/3 = 0.0167$
   iv. $\alpha_{(C\_o4)} = \alpha/2 = 0.0250$
   v. $\alpha_{(C\_o5)} = \alpha/1 = 0.0500$

The 5 hypothesis tests will be done sequentially in the order given above, until one of the p-values is found to be higher than the corresponding critical value. If the lowest p-value is below 0.01, we

will reject the corresponding null hypothesis, and proceed to the next-lowest p-value. If, and only if, both the 1st and 2nd p-values are below their corresponding critical values, we will proceed to the 3rd lowest p-value. We will test the 5 secondary hypotheses sequentially in this way, comparing the next p-value to its critical value only if all of the lower p-values were below their corresponding critical values.

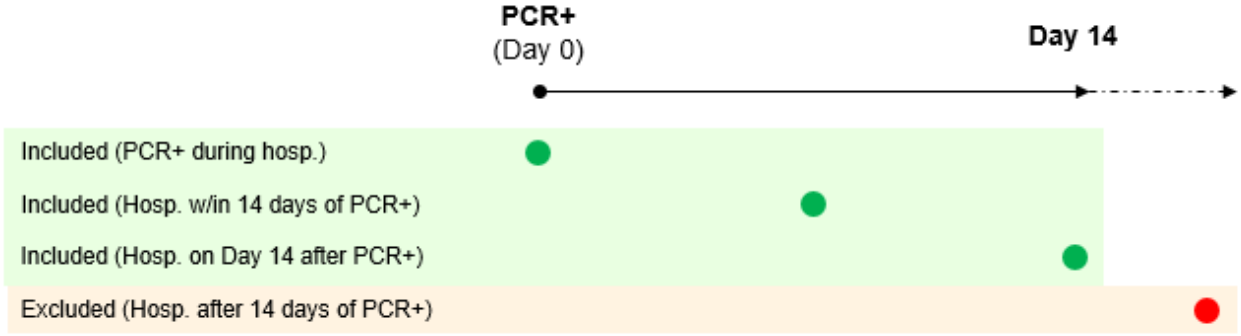
Among all adults 50–64 years who receive either Flublok Quadrivalent or SD-IIV during the 2018–2019, 2019–2020, and/or 2020–2021 influenza seasons, we will estimate the rVE of Flublok Quadrivalent vaccine versus SD-IIV against the following outcomes:

- *PCR-confirmed hospitalized influenza*

Among adults aged 50–64 years who are vaccinated with Flublok or an SD-IIV, we plan to assess the number of adults who have a PCR-positive test during the influenza season ≥14 days after vaccination and are hospitalized.

The PCR-positive test must occur either: (1) during the hospital stay, or (2) a hospitalization that begins ≤14 days after the PCR-positive test. Visually (where the ● represents a hospitalization and the PCR-positive result occurs on Day 0):

Figure 1: Timing of PCR-positive test relative to hospitalization outcome



- *Hospitalized community-acquired pneumonia (CAP)*

Among adults aged 50–64 years who are vaccinated with Flublok or an SD-IIV, we plan to assess the number of adults who were hospitalized with a diagnosis of community-acquired pneumonia (CAP) regardless of whether a PCR test was done.

The hospitalization for CAP must occur during the influenza season and no later than 14 days after the last day of the influenza season, as determined by KPNC.

To identify diagnoses of CAP, we will start with preliminary list of ICD-10 codes that has been previously used by KPNC (**Table 1**). Case ascertainment of CAP may be refined using an iterative process during the data analysis stage.

- *Hospitalized cardio-respiratory events combined (e.g., CAP, other lower respiratory tract infections [LRTI], acute myocardial infarction, congestive heart failure, stroke)*

Among adults aged 50–64 years who are vaccinated with Flublok or an SD-IIV, we plan to assess the number of adults who were hospitalized with a diagnosis of a cardio-respiratory event regardless of whether a PCR test was done.

The hospitalization for a cardio-respiratory event must occur during the influenza season and no later than 14 days after the last day of the influenza season, as determined by KPNC. To identify diagnoses of a cardio-respiratory event, we will start with preliminary list of ICD-10 codes that has been previously used by KPNC (**Table 1**).

- *All PCR-confirmed influenza A*

Among adults aged 50–64 years who are vaccinated with Flublok or an SD-IIV, we plan to assess the number of adults who have influenza A as identified by a PCR-positive test ≥14 days after vaccination up through to the end of each influenza season, compared with those who do not have a PCR-positive test by vaccine received. To be included in the cohort for analysis, the PCR test must occur during the influenza season.

- *All PCR-confirmed influenza B*

Among adults aged 50–64 years who are vaccinated with Flublok or an SD-IIV, we plan to assess the number of adults who have influenza B as identified by a PCR-positive test ≥14 days after vaccination up through to the end of each influenza season, compared with those who do not have a PCR-positive test by vaccine received. To be included in the cohort for analysis, the PCR test must occur during the influenza season.

- *Sub-analyses by pre-existing conditions*

"Pre-existing" will be defined as history of a condition as identified using ICD-10 codes in the 3 years prior to vaccination date.

i. **Cardiovascular preexisting conditions composite, which may include:** myocardial infarction, cerebrovascular accident, atrial fibrillation, arrhythmias, coronary artery disease, valvular heart disease/valvuloplasty, chronic/congestive heart failure and/or congenital heart defects, CABG, angioplasty/stents, cardioversion, pacemaker, implantable cardioverter defibrillator, LVAD, heart transplant, hypertensive disease
ii. **Respiratory preexisting conditions composite, which may include:** COPD and/or pulmonary HTN
iii. **Cardiorespiratory preexisting conditions composite:** combined pre-existing conditions (a) and (b) above
iv. **Obesity:** based on available height/weight data for a calculated BMI ≥30 or an obesity diagnosis (e.g., ICD-10 E66.9)

v. **Diabetes:** based on a diagnosis of diabetes

4.3 Exploratory Endpoints and Assessment Methods

As with the primary and secondary objectives, for each of the exploratory outcomes below, we will only count the first event of the outcome per season and censor after the first event.

For any of the exploratory endpoints below, interpretation of any findings will be challenging. If a significant rVE is found, we will not be able to rule out various sources of confounding as alternative explanations (as opposed to the interpretation that this is the true rVE against any outcome). Any exploratory findings should be interpreted with caution.

- To estimate the rVE of Flublok Quadrivalent vaccine versus SD-IIV in vaccinees aged 50–64 years against the following exploratory outcomes if time allows:

    a. Diagnosed with influenza
    We will consider for inclusion in the analysis adults aged 50–64 years who were (1) found to be PCR-positive for influenza and (2) clinically-diagnosed with influenza using ICD-10 codes, such as J09*, J10*, or J11* in any setting, and were untested. Additional ICD-10 codes may be considered upon review of relevant diagnoses. This outcome will exclude those with a clinically-diagnosed influenza but are tested and PCR-negative.

    The diagnosis must occur during the influenza season and no later than 14 days after the last day of the influenza season, as determined by KPNC.

    b. All-cause hospitalizations

    We will consider for inclusion in the analysis adults aged 50–64 years who were hospitalized. Hospitalizations following a scheduled elective surgery or similar may be excluded. The hospitalization must occur during the influenza season and no later than 14 days after the last day of the influenza season, as determined by KPNC.

    c. All-cause mortality

    We will consider for inclusion in the analysis adults aged 50–64 years who died for any reason. The death must occur during the influenza season and no later than 14 days after the last day of the influenza season, as determined by KPNC.

- To estimate the rVE of Flublok Quadrivalent vaccine versus SD-IIV in other age ranges of vaccinees — aged 18–64 years and aged 18–49 years — against the following outcomes if time allows:
    a. All PCR-confirmed influenza

b. All PCR-confirmed influenza A and influenza B (separately)
c. PCR-confirmed hospitalized influenza
d. Hospitalized CAP
e. Hospitalized cardio-respiratory events combined
f. All-cause hospitalization (excluding pregnancy-related hospitalizations)
g. All-cause mortality

- Sub-analyses of the primary and secondary outcomes of sub-groups defined by pre-existing conditions (as described in Sections 2.2 and 4.2) will be conducted among adults 18–49 years as feasible

- To estimate the rVE of Flublok Quadrivalent vaccine versus SD-IIV among adults aged 18–64 years for PCR-confirmed influenza A subtypes (A(H1N1)pdm09 and A(H3)) and lineage for type B influenza (B/Victoria, B/Yamagata) as feasible

  For at least the first influenza season (2018–2019) and potentially another season, we will subtype a proportion of PCR positive outpatient specimens from adults aged 18–64 years.

  We will prioritize subtyping of adults aged 18–64 years who were vaccinated with Flublok or SD-IIV. After subtyping those specimens, we will then prioritize subtyping a fraction of unvaccinated cases by time interval to ensure we have subtyping data throughout the season. We will create time intervals from which to randomly sample unvaccinated adults (e.g., weeks with higher incidence of influenza at the peak of the season may be broken down into 1-week intervals, while lower incidence weeks may be 2-week intervals). We will prioritize sampling unvaccinated adults aged 50–64 years (primary analysis age group), followed by aged 18–49 years, resources permitting.

  The data on subtypes from vaccinated and unvaccinated PCR-positive adults will be used to determine what strains were circulating at any point during the influenza season. This subtype data will then be used to impute what the subtype likely would have been for those adults who were found to be PCR-positive for influenza, but for which no subtype data are available. For example, if at the peak of our influenza season, we find that 90% of influenza A cases are H1N1, we would assume that this proportion of PCR positive influenza A cases (for which we do not have subtype data) from that time period were also H1N1.

  Because we will have subtype data by calendar date, if there is a change in the predominant circulating strain at some point in the influenza season, we may be able to determine whether rVE also changes with the shift in subtype.

  We may describe the proportion of influenza subtypes in unvaccinated versus vaccinated adults. The unvaccinated adults will serve as a comparison group for descriptive purposes.

For the above outcomes for the additional age groups aged 18–64 years and 18–49 years, we will use the same assessment methods as described in Sections 4.1, 4.2, and 4.3.

5 Statistical Methods and Determination of Sample Size

The statistical analyses will be performed under the responsibility of KPVSC using SAS® Version 9.4 software or later. The results of the statistical analysis will be available in the final study report.

5.1 Statistical Methods

5.1.1 Hypotheses and Statistical Methods for Primary, Secondary, and Exploratory Objectives

5.1.1.1 Hypotheses

For the primary objective, we plan to test the null hypothesis that there is no difference in effectiveness between Flublok and SD-IIV. We will use a 2-sided test ($p<0.05$) with an associated 95% confidence interval to assess superiority. The same null hypothesis of no difference in effectiveness will be tested for all secondary objectives. Analyses in subgroups defined by pre-existing conditions will not adjust for multiplicity; all other secondary analyses will adjust for multiplicity by the Holm method specified in Section 4.2.

5.1.1.2 Statistical Methods

The primary, secondary, and exploratory analyses will use Cox regression on a calendar timeline to obtain an adjusted estimate of the relative hazard ratio (rHR) for each outcome, adjusted for age, sex, race/ethnicity, and facility or service area. The null hypothesis (that the log rHR = 0) will be tested using the Wald test (based on the ratio of the adjusted log rHR estimate to its corresponding standard error estimate). The following covariates have been selected *a priori* to include in our model (via the propensity score, described further below):

- Age at time of vaccination (continuous)
- Sex
- Race (some small categories may be combined, if necessary):
    - White
    - Black
    - Asian
    - Pacific Islander
    - Native American
    - Multiracial
    - Unknown/Other
- Ethnicity:

  - Hispanic
  - Non-Hispanic/Unknown
- KPNC Facility or Service Area

In addition to estimating the rHR, conditional on the covariates above, we will consider whether the rVE of Flublok varies across subgroups or over time. For example, we may examine whether the rHR varies with:

- Time-since-vaccination: number of days, counting from Day 15 after vaccination (where day 0 is the day of vaccination)
- Time-since-beginning-of-the-season
- Season: Year 1, Year 2, or Year 3
- Comorbidities: Diagnostic cost groups (DxCG) risk scores from the ICD-based proprietary risk adjustment and predictive modeling system may be used. DxCG calculates projected healthcare costs for each member for the upcoming year based on demographics, diagnoses, costs, and utilization in the previous year. It may also be included as a proxy for overall health status of the individual.
- Prior healthcare utilization (e.g., number of emergency department visits or hospitalizations in prior year, outpatient visits in prior year)
- Influenza vaccine type received (or none) during the previous year

Adults who are included in our analysis must receive either Flublok or an SD-IIV. The majority of influenza vaccinations are administered in the KPNC region by the end of calendar year. It is possible that Flublok will not last until the end of the influenza season, which is typically in the spring of the following year, as determined by KPNC. Based on our alternating weekly schedule—one week of Flublok followed by one week of an SD-IIV—weeks are "paired" for balance.

The following analyses will be conducted. The single-season analyses are for the purposes of assessing whether any season-specific considerations (or otherwise) need to be made for the overall analysis.

At the end of the third influenza season, we will assess:

- Single-season rVE against PCR-confirmed influenza (primary objective) based on data from the first influenza season (2018–2019)
- Single-season rVE against PCR-confirmed influenza (primary objective) based on data from the second influenza season (2019–2020)
- Single-season rVE against PCR-confirmed influenza (primary objective) based on data from the third influenza season (2020–2021)
- Potential single-season rVE against select secondary outcomes as feasible based on data from the first influenza season (2018–2019)

- Potential single-season rVE against select secondary outcomes as feasible based on data from the second influenza season (2019–2020)
- Potential single-season rVE against select secondary outcomes as feasible based on data from the third influenza season (2020–2021)

We expect that the featured analysis of rVE against each of the primary, secondary, and exploratory outcomes will be the overall analysis including all subgroups over both influenza seasons. If we find variation in rVE across subgroups and over time that is substantial, it will also be reported.

Risk sets will be conditioned (i.e., stratified) on calendar day, using a calendar timeline for the Cox regression. We expect many people to be vaccinated both years, and some might get "breakthrough" influenza both years. Getting Flublok in one year should be entirely unrelated to getting Flublok in another year, so robust standard errors are not needed. We will check our assumption that the vaccine received in one year is not related to vaccine received the next year. For each type of outcome, follow-up would end at the first outcome event in a season but could restart the next year when the individual is vaccinated again.

We do not know whether prior vaccination (i.e., booster or priming effect) affects VE of either Flublok Quadrivalent vaccine or SD-IIV, but we hypothesize that prior vaccination might be an effect modifier but not a potential confounder because what patients are vaccinated with in prior years should not be associated with what type of vaccine they receive in either of the 2 study seasons. We will test to ascertain if, as expected, there is (1) no correlation between prior vaccination (before the study period) and type of vaccination during the 3 study seasons, and (2) no correlation between the type of vaccine received in the one season and the type of vaccine received in the another study season. If we find that there is a relationship in any of these scenarios, we can treat prior vaccination ("prior" meaning before the study commenced, or "prior" as in vaccination in the first study season relative to the second study season) as a potential confounder. We would add variables that adjust for whether a patient was vaccinated during the past year(s), as well as which vaccine type they received. Further, if prior vaccination does modify VE, we may have adequate power to assess whether there is a boosting effect given our large sample size.

There is little possibility of confounding that may occur between patients tested versus those who are not tested. While not everyone with suspected influenza will be tested, the randomization process in vaccine supply (Flublok Quadrivalent vaccine versus SD-IIV) should result in an equal distribution of those who are and are not tested between the two vaccine groups throughout the season. We will attempt to mitigate possible bias and confounding as much as possible upfront by implementing our plans for supplying clinics with alternating shipments of vaccines. Because factors related to testing should be balanced, there is no reason to believe that the likelihood of being tested (conditional on influenza infection and severity) will be related to whether a person received Flublok Quadrivalent vaccine or SD-IIV. (It may be helpful to compare the RSV results of Flublok vs. SD-IIV vaccinees to assess our assumption of no residual confounding.)

We will also be monitoring the balance of potential confounders that may be related to influenza and to PCR testing in order to confirm that we are achieving the balance intended by our quasi-randomized shipment of vaccines to clinics. Once vaccinations begin, we will monitor on a weekly basis the balance of Flublok vaccinees versus SD-IIV vaccinees with respect to facility,

sex, and age group (18–49 years and 50–64 years). Additional demographic variables such as race and ethnicity may also be monitored. We will also monitor balance with respect to comorbidities such as chronic obstructive pulmonary disease (COPD), asthma, and diabetes. If we find that any facility is using Flublok or SD-IIV more (or less) than needed to achieve balance, we will work with the influenza program manager to adjust what is being administered.

By design, inpatient facilities did not participate in the quasi-randomization of influenza vaccine administration. We will therefore exclude patients who received Flublok or SD-IIV during an inpatient stay as these may be higher risk. Facilities that used only Flublok or only SD-IIV will also be excluded from the analysis.

In preparing our dataset for analysis, we will assess the balance in several ways to determine if the imbalance is substantially more than what we would expect to see. Despite our efforts, we anticipate that there will still be imbalances. We will create a facility-specific propensity score for each vaccinee, scoring their probability of receiving Flublok versus SD-IIV as a function of covariates, including but not limited to age, sex, race, ethnicity, comorbidity scores, influenza vaccination, and prior healthcare utilization history. Our Cox regression will be adjusted for the propensity score.

For PCR-confirmed outcomes, we plan to assess the outcomes that occur ≥14 days after vaccination up through to the end of each influenza season (as determined by KPNC, which is generally when the proportion of influenza-positive PCR tests drops below 10%) or the first week of May, whichever is later. For outcomes that are not tied to PCR-confirmation, the outcome or diagnosis must occur on or after the first day that KPNC begins use of the Cepheid GeneXpert PCR assay for the influenza season (generally the first Tuesday of October), but must begin no later than 14 days after the last day of the influenza season, as determined by KPNC.

It is possible that patients enter, exit, and re-enter the KPNC population during the study period. Only patients who are members at the time of vaccination will be included in the analysis in order to most accurately ascertain the vaccine received. If additional exclusions need to be made based on membership, we will make the most appropriate determination for how to handle such cases at the analysis stage, but we anticipate that this would be a very small number of patients. Missing data will not be imputed.

For outcomes not based on PCR-confirmed influenza (e.g., hospitalized pneumonia and cardio-respiratory events), we will include in the analysis all such outcomes occurring within 14 days after the last day of the influenza season (as determined by KPNC) to account for influenza that may still be circulating after the “official” end of the season.

5.2 Interim / Preliminary Analysis

An interim analysis will not be performed. All recommendations will be based on the final analyses that includes all seasons.

No data will be shared beyond the immediate VSC study team as specified and approved by the KPNC IRB, namely:

- Nicola P. Klein, MD, PhD (Principal Investigator)
- Bruce Fireman (Biostatistician)
- Ned Lewis (Data Manager)

- John Hansen (Project Manager)
- Amber Hyman (Project Manager)
- Laurie Aukes (Project Manager)
- Arnold Yee (Senior Data Consultant)
- Karen Nunley (Senior Data Consultant)
- Sharareh Modaressi (Senior Data Consultant)

5.3 Determination of Sample Size and Power Calculation

We calculated the least rVE that is detectable with 80% power in a 3-year study for several aims. We plan to test the null hypothesis that Flublok is not any more or less effective than the comparator, using a 2-sided test, $p<0.05$. Our allocation ratio of 1:1 implies that each year, KPNC would give Flublok Quadrivalent vaccine to 400,000 members aged 18–64 years, and SD-IIV to another 400,000 similar KPNC members.

Because the comparison group will not be unvaccinated—they are getting SD-IIV—we will be estimating and testing rVE: i.e., the percent reduction in the incidence of "breakthrough cases" (e.g., influenza episodes in vaccinees) that results from the use of Flublok Quadrivalent vaccine rather than the SD-IIV vaccines.

We may potentially conduct analyses of rVE within subgroups and periods of time defined by:

- Time-since-vaccination: number of days, counting from Day 15 after vaccination (where day 0 is the day of vaccination)
- Time-since-beginning-of-the-season
- Season: Year 1 or Year 2
- Comorbidities: Diagnostic cost groups (DxCG) risk scores from the ICD-based proprietary risk adjustment and predictive modeling system may be used. DxCG calculates projected healthcare costs for each member for the upcoming year based on demographics, diagnoses, costs, and utilization in the previous year. It may also be included as a proxy for overall health status of the individual.
- Prior healthcare utilization (e.g., number of emergency department visits or hospitalizations in prior year, outpatient visits in prior year)
- Influenza vaccine type received (or none) during the previous year

5.4 Data Review for Statistical Purposes

Since vaccinations began on September 16, 2018, we have been monitoring on a weekly basis the balance of Flublok vaccinees versus SD-IIV vaccinees with respect to service area, facility, pregnancy status, and age group. Additional demographic variables will be monitored as appropriate.

5.5 Changes in the Conduct of the Study or Planned Analyses

No significant change occurred during the conduct of the study that is not documented in a protocol amendment.

6 References List

1. US Food and Drug Administration, “Multiple Endpoints in Clinical Trials Guidance for Industry,” January 2017. Available at https://www.fda.gov/regulatory-information/search-fda-guidance-documents/multiple-endpoints-clinical-trials-guidance-industry. Accessed 29 Jan 2020.

List of Tables

Table 1. Preliminary ICD-10 codes for identifying cardio-respiratory outcomes

Outcome	ICD-10 Code	Description
Community-acquired pneumonia (CAP)	J10.*	Influenza due to other identified influenza virus
	J11.*	Influenza due to unidentified influenza virus
	J12.*	Viral pneumonia, not elsewhere classified
	J13	Pneumonia due to Streptococcus pneumoniae
	J14	Pneumonia due to Hemophilus influenzae
	J15.*	Bacterial pneumonia, not elsewhere classified
	J16.*	Pneumonia due to other infectious organisms, not elsewhere classified
	J17	Pneumonia in diseases classified elsewhere
	J18.* (except for J18.2: Hypostatic pneumonia, unspecified organism)	Pneumonia, unspecified organism
Acute myocardial infarction	I21.x	Acute myocardial infarction
Heart failure	I50.x	Heart failure
Lower respiratory tract infections (LRTI)	*(All codes from CAP above)*	
	J20.x	Acute bronchitis
	J40	Bronchitis, not specified as acute or chronic
	J44.0	Chronic obstructive pulmonary disease with acute lower respiratory infection
Stroke	I60.x	Nontraumatic subarachnoid hemorrhage
	I61.x	Nontraumatic intracerebral hemorrhage
	I63.x	Cerebral infarction
Atrial fibrillation	I48.1	Persistent atrial fibrillation

Note: ".x" indicates that all subcodes listed under the main code may be used.

Table 2. Proposed ICD-10 codes for identifying pre-existing conditions

Pre-existing Condition	Sub-category	ICD-10 Code	Description
Cardiovascular preexisting conditions composite	Myocardial infarction, coronary heart disease	I20.*	Angina pectoris
		I21.*	Acute myocardial infarction
		I22.*	Subsequent myocardial infarction
		I23.*	Certain current complications following acute myocardial infarction
		I24*	Other acute ischaemic heart diseases
		I25*	Chronic ischaemic heart disease
	Cerebrovascular accident	I60.*	Subarachnoid haemorrhage
		I61.*	Intracerebral haemorrhage
		I62.*	Other nontraumatic intracranial haemorrhage
		I63.*	Cerebral infarction
		I64	Stroke, not specified as haemorrhage or infarction
		I65.*	Occlusion and stenosis of precerebral arteries, not resulting in cerebral infarction
		I66.*	Occlusion and stenosis of cerebral arteries, not resulting in cerebral infarction
		I67.*	Other cerebrovascular diseases
		I68.*	Cerebrovascular disorders in diseases classified elsewhere
		I69.*	Sequelae of cerebrovascular disease
	Atrial fibrillation	I48.*	Atrial fibrillation and flutter
	Arrhythmias	I49.*	Other cardiac arrhythmias
		I47.*	Paroxysmal tachycardia
		I44.*	Atrioventricular and left bundle-branch block
		I45.*	Other conduction disorders

	Valvular heart disease	I05.*	Rheumatic mitral valve diseases
		I06.*	Rheumatic aortic valve diseases
		I07.*	Rheumatic tricuspid valve diseases
		I08.*	Multiple valve diseases
		I09.1	Rheumatic diseases of endocardium, valve unspecified
		I09.8	Rheumatic disease of pulmonary valve
		I34.*	Nonrheumatic mitral valve disorders
		I35.*	Nonrheumatic aortic valve disorders
		I36.*	Nonrheumatic tricuspid valve disorders
		I37.*	Pulmonary valve disorders
		I38	Endocarditis, valve unspecified
		I39.*	Endocarditis and heart valve disorders in diseases classified elsewhere
	Chronic/congestive heart failure	I50.*	Heart failure
		I09.9	Rheumatic heart failure
		I11.0	Hypertensive heart disease with (congestive) heart failure
		I13.0	Hypertensive heart and renal disease with (congestive) heart failure
		I13.02	Hypertensive heart and renal disease with both (congestive) heart failure and renal failure
	CABG/valvuloplasty/angioplasty/stents/pacemaker/cardioverter	Z95.*	Presence of cardiac and vascular implants and grafts
		T82.*	Complications of cardiac and vascular prosthetic devices, implants and grafts
	Congenital heart defects	Q20.*	Congenital malformations of cardiac chambers and connections
		Q21.*	Congenital malformations of cardiac septa
		Q22.*	Congenital malformations of pulmonary and tricuspid valves
		Q23.*	Congenital malformations of aortic and mitral valves
		Q24.*	Other congenital malformations of heart
		Q25.*	Congenital malformations of great arteries

		Q26.*	Congenital malformations of great veins
		Q27.*	Other congenital malformations of peripheral vascular system
		Q28.*	Other congenital malformations of circulatory system
	Hypertensive disease	I10.*	Essential (primary) hypertension
		I11.*	Hypertensive heart disease
		I12.*	Hypertensive chronic kidney disease
		I13.*	Hypertensive heart and chronic kidney disease
		I15.*	Secondary hypertension
		I16.*	Hypertensive crisis
Respiratory preexisting conditions composite	Chronic obstructive pulmonary disease	J40.*	Bronchitis, not specified as acute or chronic
		J41.*	Simple and mucopurulent chronic bronchitis
		J42.*	Unspecified chronic bronchitis
		J43.*	Emphysema
		J44.*	Other chronic obstructive pulmonary disease
	Pulmonary hypertension	I27.*	Other pulmonary heart diseases
		I28.*	Other diseases of pulmonary vessels
		I26.*	Pulmonary embolism
Obesity	*Based on calculated height/weight BMI≥30 and/or presence of ICD-10 codes*	E66.0	Obesity due to excess calories
		E66.1	Drug-induced obesity
		E66.2	Extreme obesity with alveolar hypoventilation
		E66.8	Other obesity
		E66.9	Obesity, unspecified
Diabetes	*N/A*	E10.*	Type 1 diabetes mellitus
		E11.*	Type 2 diabetes mellitus
		E12.*	Malnutrition-related diabetes mellitus
		E13.*	Other specified diabetes mellitus
		E14.*	Unspecified diabetes mellitus

Note: ".x" indicates that all subcodes listed under the main code may be used.