Randomized Open-Label Trial to Compare the Immunogenicity of Cell Culture-Based and Recombinant Unadjuvanted Quadrivalent Influenza Vaccines to Conventional Egg-Based Unadjuvanted Quadrivalent Influenza Vaccines among Healthcare Personnel Aged 18-64 Years:

Year 2 Protocol Amendment

NCT03722589

Document Date: July 11, 2019

1. Protocol Title

Randomized Open-Label Trial to Compare the Immunogenicity of Cell Culture-Based and Recombinant Unadjuvanted Influenza Vaccines to Egg-Based Influenza Vaccines among Healthcare Personnel Aged 18-65 Years:

Year Two Amendment

Background

The first year of this randomized, open-label trial conducted during the 2018-2019 influenza season assessed humoral and cell-mediated immune responses to cell culture-based and recombinant unadjuvanted quadrivalent influenza vaccines compared to conventional egg-based unadjuvanted quadrivalent SD (SD) (15µg of HA per strain) influenza vaccines among healthcare personnel (HCP) aged 18-64 years at two sites in the United States. Eligible participants were randomized 2:2:1:1 to receive a single dose of cell culture-based vaccine (Flucelvax™ Quadrivalent by Seqirus, Inc., 15µg of HA per strain) versus recombinant vaccine (Flublok® Quadrivalent by Sanofi Pasteur, 45µg of HA per strain) versus one of two egg-based SD vaccines (Fluzone® Quadrivalent by Sanofi Pasteur, 15µg of HA per strain and Fluarix® Quadrivalent by GlaxoSmithKlein, 15µg of HA per strain) during October 2018. The first year of the trial was designed to address the question of whether HCP who receive cell culture-based or recombinant influenza vaccines for the first time (after receipt of egg-based SD vaccines in previous seasons) have higher antibody titers (surrogates for immune protection[1, 2]) against circulating influenza viruses compared to HCP who receive egg-based SD vaccines.

Since the trial began, preliminary data from another randomized trial comparing cell culture-based, recombinant and egg-based SD vaccines have become available and suggest that recombinant vaccine induces higher antibody titers against both egg- and cell-grown vaccine reference viruses compared to egg-based vaccines, whereas cell culture-based vaccine does not (personal communication, Min Levine, CDC). Data from observational vaccine effectiveness analyses comparing cell culture-based vaccine to egg-based vaccines are mixed with some analyses suggesting little to no difference in effectiveness between vaccines.[3, 4] However, it is unknown whether repeated vaccination with cell culture-based or recombinant vaccines provides additional benefit compared to first-time receipt of these vaccines or whether the improved humoral immune response to recombinant vaccine that contains three times the antigen dose of SD influenza vaccines is driven by response to recombinant antigen, higher antigen dose, or both.

Year two of this trial will examine two hypotheses. The first hypothesis is that repeated vaccination with cell culture-based or recombinant vaccines boosts antibody titers against cell-grown viruses more than first-time

vaccination with these vaccines, after receipt of egg-based SD vaccines. To address this hypothesis, participants from year one of the trial who received cell culture-based or recombinant influenza vaccines will be re-randomized to receive one of these two vaccines in year two of the trial and participants who received egg-based SD vaccines will be re-randomized to receive a cell-culture based, recombinant or egg-based SD vaccine (represented by Fluzone® Quadrivalent). The second hypothesis is that higher antigen doses, regardless of whether egg- or cell-grown, will result in higher antibody titers against cell-grown viruses. To address this hypothesis, the trial will enroll additional participants at the Kaiser Permanente Northwest site into a non-randomized vaccine arm in which participants will receive Fluzone® High-Dose influenza vaccine. Fluzone® High-Dose is an egg-based vaccine that contains four times the antigen content (60 μg of HA per strain) compared to SD influenza vaccine and is licensed for use in persons aged ≥65 years. In this trial, Fluzone® High-Dose will be used off-label among persons aged 18-64 years (see Rationale for Off-Label Use of Fluzone® High-Dose Among Healthcare Personnel Aged 18-64 Years).

Year two of this trial may also afford the opportunity to evaluate whether cell culture-based, recombinant, or high-dose egg-based vaccines boost antibody response among HCP with low responses to SD egg-based vaccines, if low-responders are identified among participants who received SD egg-based vaccines in year one. Year two of this trial will also investigate differences in cell-mediated immune responses to repeated versus first-time vaccination with cell-based and recombinant vaccines in a subset of participants (approximately 100) at the Baylor Scott & White Health site. Effects of repeated vaccination with non-egg-based vaccines on cell-mediated immune responses have not been studied.

Rationale for Off-Label Use of Fluzone High-Dose among Healthcare Personnel Aged 18-64 Years

Off-label use of Fluzone® High-Dose has been studied in randomized controlled trials in healthcare personnel aged 18-64 years,[5] adults with HIV infection[6], and adult and pediatric oncology patients[7-9] and has been shown to be well tolerated. In a randomized trial comparing Fluzone® High-Dose to Fluzone® Quadrivalent in HCP aged 18-64 years in Canada, fatigue, weakness and injection site reactions were more frequently reported after Fluzone® High-Dose, but the majority of adverse events were mild and none required medical attention.[5] In the same study, 92% of Fluzone® High-Dose recipients reported willingness to receive the same vaccine in the future based on their experiences with adverse events. Other trials of off-label use of Fluzone® High-Dose have confirmed a similar safety profile with either no difference in adverse events compared to Fluzone® Quadrivalent or more reactogenicity or systemic events that were generally mild and self-limited among Fluzone® High-Dose recipients.[6-9] These findings are consistent with a large pre-licensure trial comparing the safety profile of Fluzone® High-Dose to Fluzone® Quadrivalent among persons aged ≥65 years which found that solicited injection-site reactions and systemic adverse events were more common after Fluzone® High-Dose but most reactions occurred within three days of vaccination and were mild and self-limited.[10] A formal Investigational

New Drug waiver was obtained for this trial from the US Food and Drug Administration Division of Vaccines and Related Products (Appendix M, FDA IND Exemption Letter).

HCP are believed to be at increased risk of influenza virus infection and are one of the most vaccinated target populations in the United States. The frequent influenza vaccination history of HCP may predispose them to a lower antibody response to SD influenza vaccines, as some studies of influenza vaccine immunogenicity among HCP suggest that repeated vaccination can blunt the antibody response to hemagglutinin[11] and neuraminidase.[12] In addition, some studies have found that influenza vaccines are less effective in persons with a history of frequent influenza vaccination,[13, 14] Fluzone® High-Dose has been shown to elicit higher antibody titers to some or all vaccine strains compared to Fluzone® Quadrivalent among persons aged ≥65 years and other populations in which the vaccine has been used off-label.[5-8] Year two of this trial will evaluate whether Fluzone® High-Dose elicits an improved immune response in HCP aged 18-64 years with a history of frequent vaccination compared to cell-culture based, recombinant, and egg-based SD influenza vaccines. The addition of Fluzone® High-Dose vaccine in year two will also provide the opportunity to evaluate the role of higher antigen content versus vaccine type (egg-based vs. recombinant) in eliciting improved immune responses to vaccination if higher post-vaccination titers are observed among Flublok® Quadrivalent recipients.

STUDY OBJECTIVES, YEAR TWO

- **Primary Objectives**

    1) Compare serologic responses after two consecutive years of vaccination with cell culture-based influenza vaccine (Flucelvax™ Quadrivalent) or recombinant influenza vaccine (Flublok® Quadrivalent) versus first time receipt of cell culture-based or recombinant vaccines (following vaccination with egg-based SD influenza vaccines during the prior season) at approximately 28 days after receipt of the 2019-20 vaccines as measured by
        - hemagglutination inhibition (HI) titers for influenza A/H1N1, influenza B/Yamagata, and influenza B/Victoria strains, and
        - microneutralization (MN) titers for influenza A/H3N2 viruses
    2) Compare serologic responses after cell culture-based influenza vaccine (Flucelvax™ Quadrivalent) versus recombinant influenza vaccine (Flublok® Quadrivalent) at approximately 28 days after receipt of the 2019-20 vaccines, regardless of prior vaccination status, as measured by
        - HI titers for influenza A/H1N1, influenza B/Yamagata, and influenza B/Victoria strains, and
        - MN titers for influenza A/H3N2 viruses
    3) Compare serologic responses after two consecutive years of vaccination with cell culture-based influenza vaccine (Flucelvax™ Quadrivalent) or recombinant influenza vaccine (Flublok®

Quadrivalent) versus egg-based SD vaccine (Fluzone Quadrivalent) at approximately 28 days after receipt of the 2019-20 vaccines as measured by

- HI titers for influenza A/H1N1, influenza B/Yamagata, and influenza B/Victoria strains, and
- MN titers for influenza A/H3N2 viruses

4) Compare immune responses after first time vaccination with cell culture-based influenza vaccine (Flucelvax™ Quadrivalent), recombinant influenza vaccine (Flublok® Quadrivalent), or high-dose egg-based vaccine (Fluzone® High-Dose) versus repeated vaccination with egg-based SD vaccine at approximately 28 days after receipt of the 2019-20 vaccines as measured by:

- HI titers for influenza A/H1N1, influenza B/Yamagata, and influenza B/Victoria strains, and
- MN titers for influenza A/H3N2 viruses

Secondary Objectives

1) Compare serologic responses at 28 days post-vaccination as stated in the primary objective but with secondary indicators of immune response, including by:

- HI titers for influenza A/H3N2 viruses, as appropriate
- MN titers for other virus subtypes/lineages, as appropriate

- **Exploratory Objectives**

1) Characterize cell-mediated immune responses of B cells, innate lymphoid cells (ILC), CD4 and CD8 T-cells by multiparametric flowcytometry with antigen tags, B-cell receptor seq and antibody-seq to Flucelvax™ Quadrivalent, Flublok® Quadrivalent, Fluzone® Quadrivalent, and Fluzone® High-Dose at approximately 7 and 28 days after vaccination in a sub-set of vaccinees.
2) Characterize the avidity of antibodies produced in response to Flucelvax™ Quadrivalent, Flublok® Quadrivalent, Fluzone® Quadrivalent and Fluzone® High-Dose at approximately 7 and 28 days after vaccination.
3) Compare immune responses among persons vaccinated with either a single dose of Flucelvax™ Quadrivalent, Flublok® Quadrivalent, or Fluzone® High-Dose versus a single dose of Fluzone® Quadrivalent at approximately 28 days after vaccination measured by

- NAI and antibody-dependent cell-mediated cytotoxicity (ADCC) titers
- Other relevant immunological markers

STUDY ENDPOINTS, YEAR TWO

Primary Endpoints

- MN responses to the *cell*-grown influenza A/H3N2 (using MDCK-SIAT or other appropriate cell line) vaccine reference viruses for each study season at approximately 28 days post-vaccination, including the following endpoints:
    - Seroconversion rate (SCR) defined as the proportion of participants with paired samples that achieved ≥ 4 fold rises comparing post- versus pre-vaccination titers, and post vaccination titers ≥ 40.
    - Geometric mean titers (GMT)
    - MFR mean-fold rise (MFR) defined as the ratio of the post-vaccination titer value to the pre-vaccination value
    - GMT ratio defined as the ratio of GMTs between study arms at 28 days post-vaccination
- HI responses to the *cell*-grown influenza A/H1N1, influenza B/Yamagata, and influenza B/Victoria vaccine reference viruses for each study season at approximately 28 days post-vaccination, including the following endpoints:
    - SCR
    - GMT
    - MFR
    - GMT ratio

Secondary Endpoints

- HI responses to *cell*-grown influenza A/H3N2 vaccine reference viruses for each study season at approximately 28 days, including the following endpoints
    - Post-vaccination titers ≥ seropositive thresholds at 1:40, 1:80, 1:160, SCR, GMT, MFR, and GMT ratio
- HI responses to *cell*-grown influenza A/H1N1, influenza B/Yamagata, and influenza B/Victoria vaccine reference viruses for each study season at approximately 28 days, including the following endpoints
    - Post-vaccination titers ≥ seropositive thresholds at 1:40, 1:80, 1:160

Exploratory Endpoints

- HI and/or MN responses to *cell*-grown *wild-type* influenza viruses at approximately 28 days (if appropriate), including the following endpoints
    - SCR, GMT, MFR, GMT ratio and post-vaccination titers ≥ seropositive thresholds at 1:40, 1:80, 1:160 as measured by HI
    - SCR, GMT, MFR, and GMT ratio as measured by MN, as appropriate
- GMT as measured by NAI and ADCC pre- and post-vaccination.
- Mean percentages of strain-specific activation markers on T, B and ILC populations, frequencies of antibody secreting plasmablasts and memory B cells to hemagglutinin (HA) with HA-tags, interferon-gamma (IFN- gamma), interleukin 2 (IL-2), and Tumor Necrosis Factor-alpha (TNF-alpha) secreting T

cell responses to *wild-type* cell-grown strains, B cell repertoire, antibody-seq, fine-specificities of antibody reactivities and single cell transcriptome analysis (where feasible) at approximately 7 and 28 days post-vaccination.

- Indicators of immune response to vaccination based on other immunologic assays not listed above (as appropriate)

METHODS, YEAR TWO MODIFICATIONS

Unless otherwise specified in the sections below, study methods will remain the same as those described in the original protocol.

Inclusion Criteria

- Healthcare personnel, including dentists and other dental health personnel
- Have been receiving routine medical care in the Baylor Scott & White Health System for at least one month before enrolling in the study

  or

  enrolled in the Kaiser Permanente health network for at least one month before enrolling in the study
- Aged 18-65 years for participants who were originally enrolled in year one of the study
- Aged 18-64 years for participants newly enrolled in year two of the study
- Available and willing to participate in study follow-up through approximately 1 month after study vaccination in the 2019-2020 influenza season
- Received an egg-based SD influenza vaccine during the 2018-2019 influenza season (for participants enrolled for the first-time during year two)

Exclusion Criteria

- Already received an influenza vaccine during the current influenza season
- Previous hypersensitivity reaction to the study vaccines as reported by the subject
- Received any vaccine in the 4 weeks prior to the first study visit or plans to receive a vaccine (other than influenza vaccine provided through the study protocol) in the 4 weeks following the first study visit
- Currently participating in a study that involves an experimental agent (vaccine, drug, biologic, device, blood product, or medication), or has received an experimental agent within 1 month prior to enrollment in this study, or expects to receive an experimental agent during participation in this study
- Any condition that the research staff believes may interfere with successful completion of the study

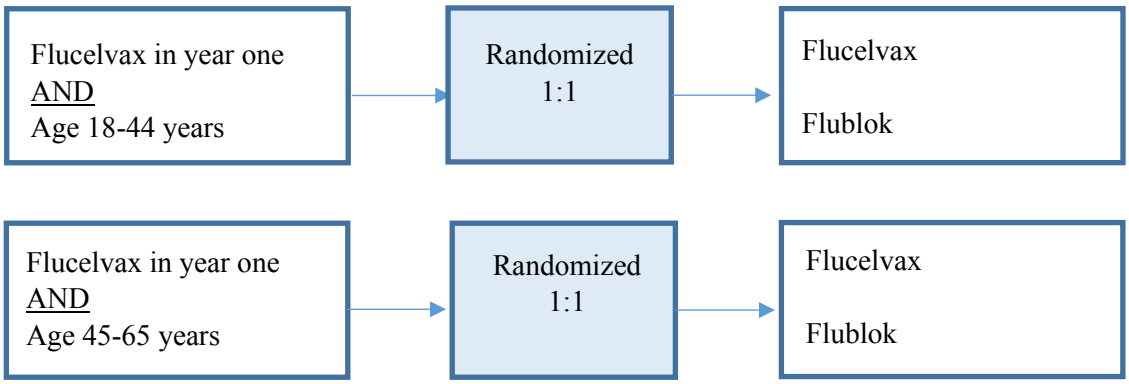
- You are not willing or able to get the licensed flu vaccines being used in this study;
- For enrollment into the non-randomized Fluzone® High-Dose arm at KPNW, the following exclusion criterion will also apply for female HCP aged 18-49 years:
    - Pregnant by participant report or study-administered urine pregnancy test

Study Design: Year two of the trial will be open-label and will include both randomized and non-randomized arms (Figure 3). Since the proposed trial design is a modification of the original protocol, the consent process will be repeated for all participants from year one of the trial. Participants from year one who received Flucelvax™ Quadrivalent or Flublok® Quadrivalent will be randomized 1:1 to receive Flucelvax™ Quadrivalent or Flublok® Quadrivalent in year two (Figure 3). Participants who received an egg-based SD vaccine in year one (Fluzone® Quadrivalent or Fluarix Quadrivalent) will be randomized 1:1:1 to receive Flucelvax™ Quadrivalent , Flublok® Quadrivalent , or Fluzone® Quadrivalent in year two.

Both sites may also aim to enroll additional participants to achieve a total of 150 participants per site (including participants who continue from year one plus additional newly enrolled participants) who received egg-based SD influenza vaccine during the 2018-2019 influenza season and who will be randomized 1:1:1 to receive Flucelvax™ Quadrivalent, Flublok® Quadrivalent, or Fluzone® Quadrivalent in year two. The KPNW site will also enroll up to 80 new participants for a non-randomized study arm that will receive Fluzone® High-Dose.

The study will prioritize enrollment of HCP who participated in a previous study of influenza vaccine effectiveness that was conducted at both study sites during the 2010-2011 influenza season. Stratified enrollment will be used for both randomized and non-randomized arms to ensure an even mix of age groups (18-44 years and 45-65 years) in each study arm.

Figure 3 Stratified randomization and sample size goals for groups with new enrollees <u>per site</u>



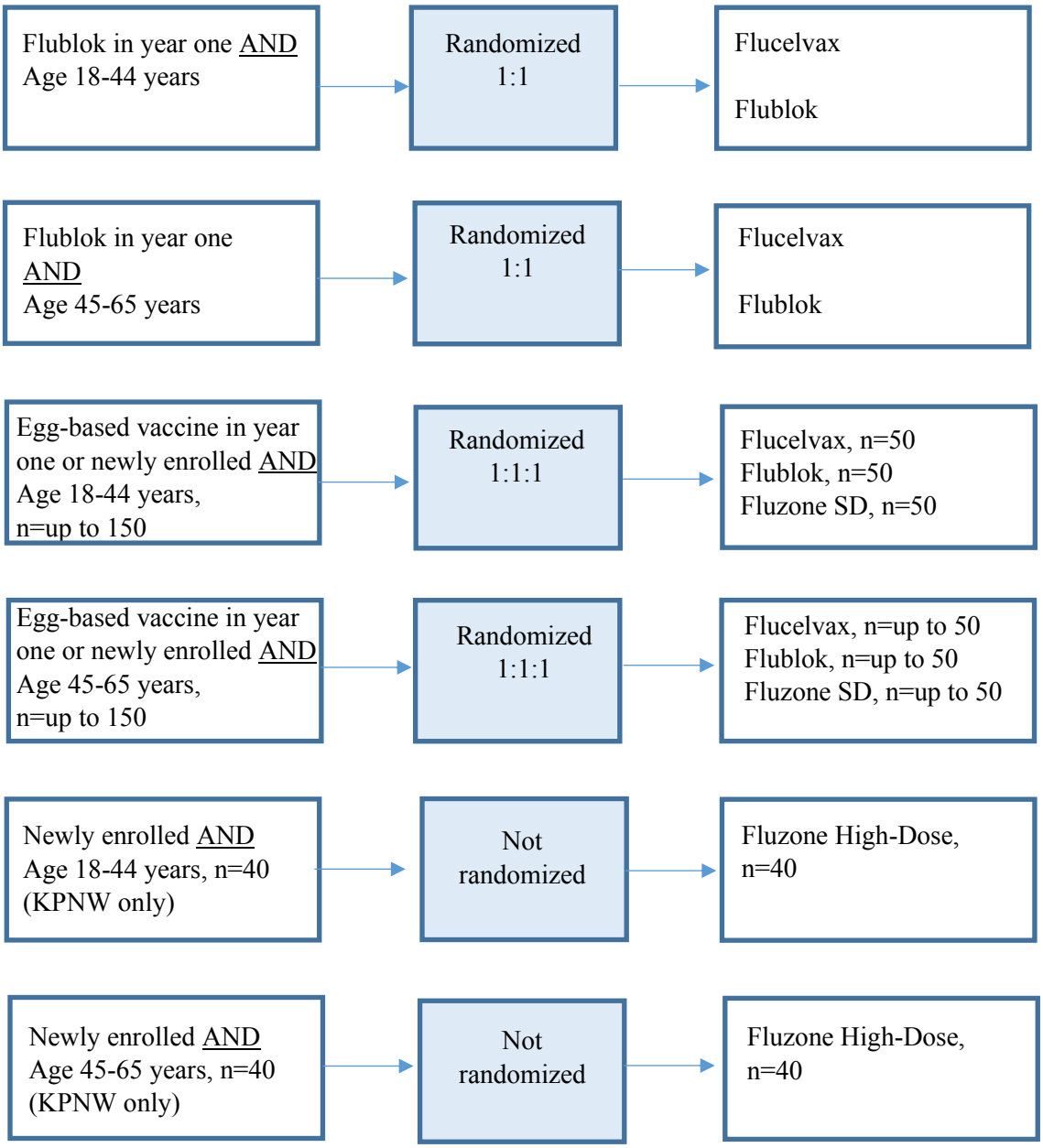
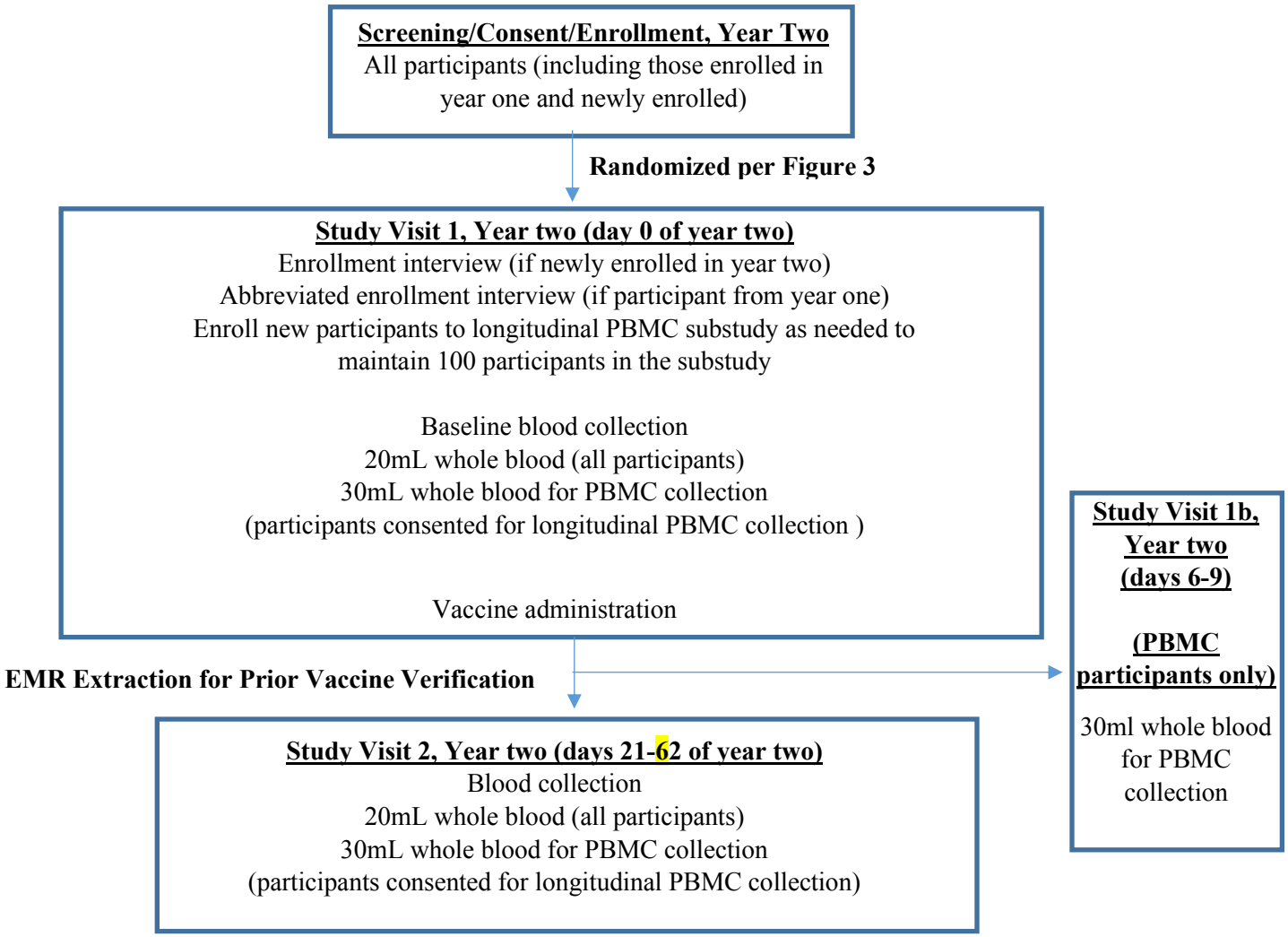

Flublok in year one AND
Age 18-44 years
Randomized
1:1
Flucelvax
Flublok
Flublok in year one
AND
Age 45-65 years
Randomized
1:1
Flucelvax
Flublok
Egg-based vaccine in year one or newly enrolled AND
Age 18-44 years,
n=up to 150
Randomized
1:1:1
Flucelvax, n=50
Flublok, n=50
Fluzone SD, n=50
Egg-based vaccine in year one or newly enrolled AND
Age 45-65 years,
n=up to 150
Randomized
1:1:1
Flucelvax, n=up to 50
Flublok, n=up to 50
Fluzone SD, n=up to 50
Newly enrolled AND
Age 18-44 years, n=40
(KPNW only)
Not
randomized
Fluzone High-Dose,
n=40
Newly enrolled AND
Age 45-65 years, n=40
(KPNW only)
Not
randomized
Fluzone High-Dose,
n=40

Figure 4 Study activity flow diagram, Year two



Consent/Screening/Enrollment:

Participants from year one of the study will be recruited for re-enrollment in year two of the study. In addition, both sites may recruit additional participants for enrollment into the main trial for randomization to Flucelvax™ Quadrivalent, Fluzone® Quadrivalent or Flublok® Quadrivalent (Figure 4). The KPNW site will recruit additional participants for enrollment to a non-randomized sub-study of Fluzone® High-Dose influenza vaccine. All potentially eligible HCP including those who participated in year one of the trial and newly recruited HCP will take a self-administered survey or study staff may administer a survey to determine whether inclusion/exclusion criteria for year two of the trial are met (Appendix N, CDC FluVaxTrial: Eligibility Screening Form, Year 2). Participants will be enrolled in the study if they meet inclusion criteria and provide consent to be in the second year of the study (Appendices O, Consent Form, Year 2; Appendix P, Non-Randomized Fluzone High-Dose Trivalent Study Consent Form).

Additional Eligibility Screening for Fluzone High-Dose Substudy

After providing informed consent to participate in the Fluzone® High-Dose arm of the trial, female HCP aged 18-49 years will be screened for pregnancy using a step-wise process. First, women will be asked if they are pregnant. Women who report that they are not pregnant will be asked to provide the start date of their last menstrual period. A urine pregnancy test will be performed (with consent) for any woman who reports that she is uncertain about whether she is pregnant, does not know the start date of her last menstrual period, or reports a start date of last menstrual period that is >28 days prior to the date of screening. Women who are pregnant based on self-report or a positive urine pregnancy test will be excluded from participation. Notification of urine pregnancy test results will be done according to site-specific requirements.

Study Visit 1 of Year Two [day 0]

Participants from year one of the study will complete an enrollment questionnaire during study visit 1 of year two (Appendix Q, CDC HCP Flu VaxTrial: Enrollment Questionnaire, Year 2). All study participants will also complete a brief questionnaire to determine whether they are currently experiencing fever or other acute illness symptoms (Appendix R, Pre-vaccination Questionnaire/Vaccine Administration Form, Year 2).

Participants who present with moderate to severe illness with fever and/or acute illness during the study visit will not receive study vaccine until their illness has resolved. In this case, a follow-up appointment will be made after resolution of the illness, at which time the participant will receive the vaccine.

Randomization of Participants in Year two of the Study

Study nurses administering vaccine will consult participants' study files to confirm which study vaccine participants from year one received during the first year of the study. For the purposes of randomization, newly enrolled participants into the main trial who did not participate in year one will be treated as part of the group who received Fluzone® Quadrivalent or Fluarix ® Quadrivalent during year one.

Stratified block randomization will be used at each site to ensure even allocation of age groups (18-44 years and either 45-65 years) to each study arm (Figure 3). Within each age stratum:

- participants who received Flucelvax™ Quadrivalent in year one will be randomized 1:1 to receive one 0.5 mL intramuscular dose Flucelvax™ Quadrivalent or Flublok® Quadrivalent;
- participants who received Flublok® Quadrivalent in year one will be randomized 1:1 to receive one 0.5 mL intramuscular dose of Flucelvax™ Quadrivalent or Flublok® Quadrivalent; and
- participants who received Fluzone® Quadrivalent or Fluarix ® Quadrivalent in year one or who were newly enrolled into the main trial will be randomized 1:1:1 to receive one 0.5 mL intramuscular dose of Flucelvax™ Quadrivalent, Flublok® Quadrivalent , or Fluzone® Quadrivalent.

Randomization lists will be generated using a computerized random-number generator to select randomly permuted group blocks of six with either three in each block assigned to Flucelvax™ Quadrivalent and Flublok® Quadrivalent for those who received Flucelvax™ Quadrivalent or Flublok® Quadrivalent in year one or two in each block assigned to Fluzone® Quadrivalent or Flucelvax™ Quadrivalent or Flublok® Quadrivalent for those who received Fluzone® Quadrivalent in year one or are newly enrolled to the main trial in year two. The next available sequential study-number will be assigned to each enrolled participant upon study-entry.

Participants who are newly enrolled into the Fluzone® High-Dose substudy will not be randomized and will all receive Fluzone® High-Dose.

'Opt-In Consent for Longitudinal PBMC Collection:

After participants are randomized, those who were in the Longitudinal PBMC Collection substudy in year one at BSWH will be invited to participate in longitudinal PBMC collection that requires collection of an additional 30mL of blood at study visits 1 and 2 in year two plus an additional study visit 1b as outlined in the original protocol. Additional participants who received either Fluzone® Quadrivalent or Fluarix ® Quadrivalent in year one of the study may be recruited to maintain a total of 100 participants in the Longitudinal PBMC

Collection substudy. Participants are considered fully enrolled and contributing to the sample size goals for longitudinal PBMC collection once they have completed PBMC collection at study visits 1 and 1b in year two. KPNW will not participate in the Longitudinal PBMC Collection substudy.

Blood Draw, Vaccine Administration, Blinding, and Post-Vaccination Safety Monitoring Procedures

Same as described for year one of the trial. Information related to blood collection will be recorded on blood collection forms (Appendix S1, S2, S3, CDC HCP Flu VaxTrial: Blood Specimen Collection/Tracking Form, Year 2, Study Visits 1, 1b, and 2).

Monitoring for Adverse Events

Since the safety and tolerability of the study vaccines, including off-label use of Fluzone® High-Dose, have been established in prior studies and data from this study will not be used to seek a licensure change for Fluzone® High-Dose, data on reactogenicity events and adverse events will not be solicited. However, study staff will provide participants with a phone number to call in case they have any questions after vaccination.

Study Visit 1b of Year Two [days 6-9]

Blood Draw: Participants who had blood collected at day 0 for collection of PBMCs will be instructed to return on day 7 (acceptable range days 6-9) to have 30 ml of venous blood drawn for collection of PBMCs to assess cell-mediated immune responses to vaccination.

Study Visit 2 of Year Two [optimal timing days 21-35 (acceptable timing days 21-62)]

Blood Draw: All study participants will have 20 ml of venous blood drawn for serology testing. Participants who had blood collected at day 0 for collection of PBMCs will also have an additional 30 ml of venous blood drawn for collection of PBMCs.

Active Surveillance for Influenza-Like Illness

Active surveillance for influenza-like illness will not be conducted during year two of this trial.

Electronic Medical Record Extraction/Abstraction

For newly enrolled participants, site investigators will extract (or abstract if necessary) data from participants' electronic medical records and state immunization registries on influenza vaccination history during the preceding ten years to include, date of receipt, vaccine product name, vaccine lot number, and route of administration (Appendix T, EMR/Chart Extraction/Abstraction Form, Year 2). Participants may be asked to sign release of medical information forms to allow access to vaccination records as needed to meet site-specific data access requirements.

For participants who were in year one of the trial, site investigators will limit extraction (or abstraction) to collecting data on influenza vaccination during the 2011-2012 and 2012-2013 influenza season.

Statistical Analysis for Year Two:

Humoral antibody responses as measured by HI (and MN, NAI and ADCC, as appropriate) at baseline (day 0) and approximately 28 days post-vaccination will be compared between participants in each study arm as outlined in Table 5; additional comparisons may be made as appropriate. Ninety-five percent confidence intervals will be calculated for relevant antibody response measures as outlined in the study endpoints at each time point (day 0 and approximately 28 days post-vaccination). Post-vaccination GMTs and MFRs at each time point post-vaccination may be compared between study arms using repeated measures mixed models with time point and vaccine as well as their interaction as independent variables; ANOVA will be used to allow for adjustment for imbalances between vaccine groups. Titers $\geq$ seropositive thresholds at 1:40, 1:80, 1:160 and SCR may be compared with Chi Square tests. Adjustment for multiple comparisons to the meet the primary objectives will be done using a Bonferonni correction approach.

Cell-mediated immune responses at baseline and approximately 7 days and 28 days will be compared between participants in each study arm. ANOVA will be used to carry out comparisons of cell-mediated immunity studies, and Bonferonni correction may be used to adjust for multiple comparisons.

All primary analyses will be conducted as intention-to-treat. All tests will be 2-tailed with a level of significance of .05.

Table 5 Planned statistical comparisons for primary objectives and endpoints in year two

Primary Objective	**Comparison Group 1**	**Comparison Group 2**
Compare immune responses after two consecutive years of vaccination with cell-based influenza vaccine (Flucelvax™ Quadrivalent) or recombinant influenza vaccine (Flublok® Quadrivalent) versus first time receipt of these vaccines following vaccination with SD egg-based influenza vaccines.	Flucelvax™ Quadrivalent year one/year two	SD egg-based vaccine year one/Flucelvax™ Quadrivalent year two
	Flublok® Quadrivalent year one/year two	SD egg-based vaccine year one/Flublok® Quadrivalent year two
Compare immune responses after two consecutive years of vaccination with cell-based influenza vaccine (Flucelvax™ Quadrivalent), recombinant influenza vaccine (Flublok® Quadrivalent), or SD egg-based vaccine (Fluzone SD).	Flucelvax™ Quadrivalent year one/year two	SD egg-based vaccine year one/year two
	Flublok® Quadrivalent year one/year two	SD egg-based vaccine year one/year two

Compare immune responses after cell culture-based influenza vaccine (Flucelvax™ Quadrivalent) versus recombinant influenza vaccine (Flublok® Quadrivalent) at approximately 28 days after receipt of the 2019-2020 vaccines, regardless of prior vaccination status.	Flucelvax™ Quadrivalent year 2 (regardless of year one vaccine)	Flublok® Quadrivalent year 2 (regardless of year one vaccine)
Compare immune responses after first time vaccination with cell-based influenza vaccine (Flucelvax™ Quadrivalent), recombinant influenza vaccine (Flublok® Quadrivalent), or high-dose egg-based vaccine (Fluzone High-Dose) following vaccination with SD egg-based influenza vaccines to repeated vaccination with SD egg-based vaccine.	SD egg-based vaccine year one/Flucelvax™ Quadrivalent year two	SD egg-based vaccine year one/year two
	SD egg-based vaccine year one/Flublok® Quadrivalent year two	
	Fluzone HD year two	

SD: Standard dose; HD: High dose

Power/Sample Size Calculations:

Study sample size was determined based on available resources. An overall sample size of up to 830 with

- 90 participants each in the Flublok® Quadrivalent year one/ Flucelvax™ Quadrivalent year two group and Flublok® Quadrivalent year one/year two group
- 135 participants each in the Flucelvax™ Quadrivalent year one/year two group and Flucelvax™ Quadrivalent year one/ Flublok® Quadrivalent year two group
- 100 participants each in the SD (SD) egg-based vaccine year one/Flucelvax™ Quadrivalent year two, SD egg-based vaccine year one/Flublok® Quadrivalent year two, and SD egg-based vaccine year one/year two groups, and
- 80 participants in the Fluzone HD year two group

would provide adequate statistical power to detect a post-vaccination GMT ratio of 1.2 between comparison groups as outlined in table 5 after adjusting for up to seven comparisons using a Bonferroni correction. Table 6 provides estimated sample sizes per comparison group required for varying post-vaccination GMT ratios after adjustment for up to eight comparisons.

Table 6. Sample size calculations to detect specified post-vaccination GMT ratios between two comparison groups after adjustment for eight primary comparisons using a Bonferroni correction

GMT ratio	N per group
1.5	16

1.4	22
1.3	34
1.2	69
1.1	245

Vaccine Product:

Four vaccine products will be used during the 2019-2020 season of this trial: Fluzone® Quadrivalent, Flucelvax™ Quadrivalent, Flublok® Quadrivalent, and Fluzone Trivalent High Dose. CDC or study sites will purchase vaccine. Fluzone® Quadrivalent, Flucelvax™ Quadrivalent, and Flublok® Quadrivalent are inactivated influenza vaccines that are approved for use in adults aged ≥18 years in the United States. Fluzone Trivalent High Dose is an inactivated vaccine that is licensed for use in adults aged ≥65 years in the United States and will be used off-label in this trial. All four vaccine products will contain vaccine strains representative of the following three strains in the 2019-2020 formulation: an A/Brisbane/02/2018 (H1N1)pdm09-like virus; A/Kansas/14/2017 (H3N2)-like virus; and a B/Colorado/06/2017-like virus (B/Victoria/2/87 lineage). All quadrivalent vaccine products will also include a B/Phuket/3073/2013-like virus (B/Yamagata/16/88 lineage).

Fluzone® High-Dose is a split-virus/subvirion vaccine manufactured by Sanofi Pasteur. It is formulated from influenza virus grown on embryonated chicken eggs. Vaccine virus is harvested, inactivated with formaldehyde, purified by zonal centrifugation using a sucrose gradient, split by Triton® X-100, and then further purified to a split-virus/subvirion form. The resulting vaccine suspension is clear and is available for use in adults as a prefilled single-dose 0.5mL syringe; the vaccine is administered intramuscularly. Each dose is formulated to contain 45 μg of HA per strain. Each 0.5mL dose of vaccine also contains sodium phosphate-buffered isotonic sodium chloride solution, up to 100mcg of formaldehyde, and up to 250mcg of octylphenol ethoxylate. The most common reactions occurring after vaccine administration in adults are pain at the injection site, myalgia, headache, malaise, and erythema at the injection site. The majority of these reactions are mild to moderate.[15]

References

1. Hobson D, Curry RL, Beare AS, Ward-Gardner A. The role of serum haemagglutination-inhibiting antibody in protection against challenge infection with influenza A2 and B viruses. J Hyg (Lond) 1972;70:767-77.
2. Potter CW, Oxford JS. Determinants of immunity to influenza infection in man. Br Med Bull 1979;35:69-75.
3. Izurieta HS, Chillarige Y, Kelman J, Wei Y, Lu Y, Xu W, Lu M, Pratt D, Chu S, Wernecke M, et al. Relative effectiveness of cell-cultured and egg-based influenza vaccines among the U.S. elderly, 2017-18. J Infect Dis 2018.
4. Klein N, Fireman B, Goddard K, Zerbo O, Asher J, Zhou J, King J. Vaccine Effectiveness of Flucelvax Relative to Inactivated Influenza Vaccine During the 2017-18 Influenza Season in Northern California. In: ID Week. San Francisco; 2018.

5. Volling C, Coleman BL, Katz K, Simor AE, Muller M, Powis J, McElhaney J, McGeer A. Immunogenicity and reactogenicity of high- vs. standard-dose trivalent inactivated influenza vaccine in healthcare workers: a pilot randomized controlled trial. Clin Microbiol Infect 2019;25:217-24.
6. McKittrick N, Frank I, Jacobson JM, White CJ, Kim D, Kappes R, DiGiorgio C, Kenney T, Boyer J, Tebas P. Improved immunogenicity with high-dose seasonal influenza vaccine in HIV-infected persons: a single-center, parallel, randomized trial. Ann Intern Med 2013;158:19-26.
7. Halasa NB, Savani BN, Asokan I, Kassim A, Simons R, Summers C, Bourgeois J, Clifton C, Vaughan LA, Lucid C, et al. Randomized Double-Blind Study of the Safety and Immunogenicity of Standard-Dose Trivalent Inactivated Influenza Vaccine versus High-Dose Trivalent Inactivated Influenza Vaccine in Adult Hematopoietic Stem Cell Transplantation Patients. Biol Blood Marrow Transplant 2016;22:528-35.
8. Jamshed S, Walsh EE, Dimitroff LJ, Santelli JS, Falsey AR. Improved immunogenicity of high-dose influenza vaccine compared to standard-dose influenza vaccine in adult oncology patients younger than 65 years receiving chemotherapy: A pilot randomized clinical trial. Vaccine 2016;34:630-5.
9. McManus M, Frangoul H, McCullers JA, Wang L, O'Shea A, Halasa N. Safety of high dose trivalent inactivated influenza vaccine in pediatric patients with acute lymphoblastic leukemia. Pediatr Blood Cancer 2014;61:815-20.
10. Falsey AR, Treanor JJ, Tornieporth N, Capellan J, Gorse GJ. Randomized, double-blind controlled phase 3 trial comparing the immunogenicity of high-dose and standard-dose influenza vaccine in adults 65 years of age and older. J Infect Dis 2009;200:172-80.
11. Thompson MG, Naleway A, Fry AM, Ball S, Spencer SM, Reynolds S, Bozeman S, Levine M, Katz JM, Gaglani M. Effects of Repeated Annual Inactivated Influenza Vaccination among Healthcare Personnel on Serum Hemagglutinin Inhibition Antibody Response to A/Perth/16/2009 (H3N2)-like virus during 2010-11. Vaccine 2016;34:981-8.
12. Laguio-Vila MR, Thompson MG, Reynolds S, Spencer SM, Gaglani M, Naleway A, Ball S, Bozeman S, Baker S, Martinez-Sobrido L, et al. Comparison of serum hemagglutinin and neuraminidase inhibition antibodies after 2010-2011 trivalent inactivated influenza vaccination in healthcare personnel. Open Forum Infect Dis 2015;2:ofu115.
13. McLean HQ, Thompson MG, Sundaram ME, Meece JK, McClure DL, Friedrich TC, Belongia EA. Impact of repeated vaccination on vaccine effectiveness against influenza A(H3N2) and B during 8 seasons. Clin Infect Dis 2014;59:1375-85.
14. Belongia EA, Skowronski DM, McLean HQ, Chambers C, Sundaram ME, De Serres G. Repeated annual influenza vaccination and vaccine effectiveness: review of evidence. Expert Rev Vaccines 2017;16:1-14.
15. Fluzone High-Dose Package Insert. (Accessed June 11, 2019, at https://www.fda.gov/media/119870/download.)

Amendment Appendices

Appendix Letter	Title
M	FDA IND Exemption Letter
N	CDC Flu VaxTrial: Consent Form, Year 2
O	CDC Flu VaxTrial: Non-Randomized Fluzone® High-Dose Trivalent Study Consent Form
P	CDC Flu VaxTrial: Eligibility Screening Form, Year 2
Q	CDC HCP Flu VaxTrial: Enrollment Questionnaire, Year 2
R	CDC HCP Flu VaxTrial: Pre-vaccination Questionnaire/Vaccine Administration Form, Year 2
T1	CDC Flu VaxTrial: Blood Specimen Collection/Tracking Form Year 2 Study Visit 1 [day 0 defined as day of vaccination]
T2	CDC HCP Flu VaxTrial: Blood Specimen Collection/Tracking Form Year 2 Study Visit 1b [days 6-9 with day 7 as target]
T3	CDC Flu VaxTrial: Blood Specimen Collection/Tracking Form Year 2 Study Visit 2 [days 21-42 with days 21-35 as target]
U	EMR/Chart Extraction/Abstraction Form, Year 2