Randomized Single-Blind Trial to Compare the Immunogenicity of Recombinant Hemagglutinin Influenza Vaccine with Conventional Egg-Based Influenza Vaccines among Healthcare Personnel in Israel

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ABBREVIATIONS

ADCC	Antibody-dependent cell-mediated cytotoxicity
GMT	Geometric mean titers
HCP	Healthcare Personnel
HA	Hemagglutinin
HI	Hemagglutination Inhibition
ILI	Influenza-like illness
IIV	Inactivated Influenza Vaccine
MDCK	Madin-Darby Canine Kidney
GMR	Geometric Mean fold rise
MN	Microneutralization
NA	Neuraminidase
NAI	Neuraminidase antibody mediated inhibition
rHA	Recombinant Hemagglutinins
RIV	Recombinant Influenza Vaccine
SCR	Seroconversion rate
SHIRI	Study of Healthcare Personnel with Influenza and other Respiratory Viruses in Israel

1. PROJECT SUMMARY

Healthcare personnel (HCP) are believed to be at increased risk of influenza virus infection due to a higher rate of exposure compared with the general population.[1] Close contact with patients may further result in transmission to patients.[2] Vaccination is the most effective method of preventing influenza, but there is limited information on the effectiveness of influenza vaccines among HCP.[3] Some studies have demonstrated reduced influenza vaccine immunogenicity and effectiveness among persons with a history of frequent influenza vaccination,[4,5] emphasizing the need to examine influenza vaccine effects among HCP who may be frequently vaccinated or demonstrate a high baseline immune response to influenza viruses.

The primary objective of the study is to compare humoral immune responses to a single dose of recombinant hemagglutinin quadrivalent influenza vaccines (RIV4) or to standard egg-

based unadjuvanted quadrivalent influenza vaccines (IIV4) among HCP ≥ 18 years old who were vaccinated in the 2018-19 season with IIV4. The trial will be conducted at two hospital sites in Israel during the upcoming influenza season (2019-20) among HCP who were enrolled in the Study of Healthcare Personnel with Influenza and other Respiratory Viruses in Israel (SHIRI).

The study design is a randomized, single-blind, trial. During the study, eligible HCP at each site who consent to participate will be randomized 1:1 to receive a single dose of RIV4 (Flublok™ Quadrivalent by Sanofi, Inc., 45µg of HA per strain) versus a single dose of IIV4 (VaxigripTetra™ by Sanofi, Inc., 15µg of HA per strain). Participants will be blinded to the vaccine that they receive; study staff will not be blinded. Eligible HCP who report already having been vaccinated with Vaxigrip at the time they are approached to join the study will be eligible to participate. All randomized participants will have blood collected just prior to vaccination; all participants, including those who were vaccinated with Vaxigrip outside of the study, will have blood collected , approximately 28 days after to evaluate humoral immune responses to vaccination. Relative efficacy of single doses of study vaccines will be assessed by comparing immunologic responses to vaccination among participants receiving RIV4 and those receiving IIV4. In addition, the effect of prior vaccination in potentially modifying antibody immune responses to vaccine will be evaluated.

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3. INTRODUCTION

3.1 Background Information

HCP are believed to be at increased risk of influenza virus infection due to a higher rate of exposure compared with the general population.[1] Close contact with patients may further result in transmission to patients.[2] Vaccination is the most effective method of preventing influenza, and HCP were identified by the World Health Organization as a priority population to target for influenza vaccination.[6] However, there is limited information on the effectiveness of influenza vaccines among HCP.[3] Reports of reduced influenza vaccine effectiveness among persons with a history of frequent influenza vaccination in some studies[4,5] and seasons[7] also make it especially important to examine influenza vaccine effects among HCP. Recent studies of influenza vaccine immunogenicity among HCP have demonstrated that repeated vaccination can blunt the antibody response to hemagglutinin (HA)[8] and neuraminidase (NA).[9]

Influenza vaccine efficacy varies annually depending on the match between vaccine strains and circulating strains, and by influenza virus type and subtype.[10] Reduced influenza vaccine effectiveness has been observed for A/H3N2 viruses over the past several influenza seasons in the United States,[11,12] and in Israel,[13,14] and has been associated with mutations that can occur during the production of inactivated influenza vaccines (IIV). Specifically, circulating influenza viruses are adapted for growth in embryonated chicken eggs, and then grown in large scale to produce the vaccine.[15-17] Both the adaptation process and serial passage of influenza viruses in chicken eggs can result in the development of mutations that have been associated with diminished vaccine effectiveness against both A/H3N2 and A/H1N1 viruses.[17-21]

New influenza vaccines manufactured without the use of eggs have been licensed in the United States, including a recombinant HA influenza vaccine (Flublok® by Sanofi Pasteur). Flublok® is formulated with purified influenza HA protein from cell-culture isolates of the vaccine reference virus. The influenza HA is grown using baculovirus vector technology that efficiently expresses the recombinant HA (rHA) proteins in large volumes in an insect cell line from the fall armyworm. The rHA is then purified from the cells and stored in phosphate-buffered saline (PBS). Since production of Flublok® does not use egg-adapted reference viruses, the rHA proteins may more closely match the vaccine reference virus for specific components in some seasons.[22]

Flublok®, which contains more influenza antigen compared to most other vaccines licensed in the United States (45 µg vs. 15 µg of HA per strain in IIV), has been shown to be efficacious and immunogenic in a number of trials. In a phase III randomized placebo-controlled trial of trivalent Flublok® among healthy adults aged 18-49 years, Flublok® was 45% (95% CI 19-63%) efficacious against influenza infection during the 2007-2008 influenza season when the majority of circulating influenza A/H3N2 and B viruses were drifted from the vaccine strains.[23] Flublok® was also found to be highly immunogenic for all three vaccine strains; ≥96% of participants had an antibody titer of ≥1:40 against each vaccine strain at 28 days post-vaccination.[23] In a phase III randomized trial comparing quadrivalent Flublok® to quadrivalent Fluarix, an egg-based vaccine, among adults aged ≥50 years, Flublok® was 30% (95%CI 10-47%) more efficacious against influenza infection during the 2014-2015 influenza season, when drifted A/H3N2 viruses predominated.[24] Flublok® was found to be well tolerated, with equivalent reactogenicity compared to IIV.[25]

Flublok® was licensed in the United States based on demonstrations of clinical efficacy and non-inferiority using immunogenicity outcomes according to the criteria of the Centers for Biologic Evaluation and Research. Sub-analyses in the clinical trials demonstrated a greater response to Flublok compared with IIV among previously vaccinated individuals. However, this same effect has not been studied among HCP, a population who are repeatedly vaccinated against influenza and frequently exposed to influenza viruses. It also remains unclear whether recombinant HA influenza vaccines that are free from mutations in the viral HA introduced during the egg adaptation and passage process of egg-based vaccine production are more effective against circulating influenza viruses compared to egg-based vaccines. Historically, the immunogenicity of influenza vaccines has been assessed by measuring antibody responses to egg-grown influenza viruses, which may be a suboptimal measure of efficacy if egg-grown viruses differ antigenically from circulating wild-type viruses. Studies evaluating Flublok have measured antibody responses to cell culture-grown viruses, but to date, there are few data directly comparing the immunogenicity of recombinant HA influenza vaccines to egg-based vaccines using the same immunogenicity outcome measures against the same antigenic targets.

This randomized, single-blind trial will assess humoral responses to recombinant HA quadrivalent influenza vaccine (RIV4, 45µg of HA per strain) compared with conventional egg-based quadrivalent standard dose (IIV4, 15µg of HA per strain) among HCP ≥ 18 years old using both cell-grown and egg-grown vaccine reference viruses as antigenic targets.

The primary study hypothesis is that a single dose of RIV4 may be more immunogenic than a single dose of egg-based standard-dose IIV4 to vaccine virus strain components among persons ≥ 18 years old.

3.2 Justification

Influenza vaccines are the most effective method of influenza prevention. However, the efficacy of licensed influenza vaccines varies annually depending on the match between vaccine strains and circulating strains and varies by influenza virus type and subtype. Data from the last several influenza seasons showing lower vaccine effectiveness against influenza A/H3N2 viruses combined with studies documenting challenges with growing recently circulating influenza A/H3N2 viruses in embryonated chicken eggs for vaccine production have raised questions about the impact of egg adaptation and propagation of vaccine viruses on the effectiveness of

egg-based influenza vaccines. Furthermore, studies conducted among HCP repeatedly vaccinated for influenza have demonstrated a reduced antibody response to vaccination with IIVs. Influenza vaccines, including recombinant HA influenza vaccines, made by alternative methods of production that avoid the egg adaptation and/or egg propagation steps are now licensed in the United States. Studies are needed to compare the immunogenicity of conventional egg-based vaccines with vaccines manufactured using alternative methods of production using standard assays and endpoints. Data from such studies will inform whether a preferential recommendation for vaccines based on a specific method of production are warranted, particularly for HCP.

3.3 Expected benefits from the proposed study

Influenza results in substantial morbidity and mortality, and annual vaccination remains the most effective method to prevent influenza and its complications. Through this study, we will gain a better understanding of whether RIV4 (Flublok® Quadrivalent) offers greater protection to HCP than the conventional egg-based IIV4 (Vaxigrip® Quadrivalent) as measured primarily by humoral immune responses to MDCK cell-grown vaccine strains.

In the United States, both vaccines are licensed for use in adults aged ≥18 years based on phase III trials demonstrating efficacy in comparison to placebo[23] or non-inferiority to conventional egg-based influenza vaccines. Therefore, study participants may directly benefit from receipt of study vaccine by receiving protection against circulating influenza viruses during the 2019-20 influenza season. In Israel, only IIV4 is licensed for use in adults aged ≥18.

4. STUDY OBJECTIVES

4.1 Primary Objective

- Compare humoral immune responses to a single dose of RIV4 versus IIV4 at approximately 28 days after vaccination in the 2019-20 season among HCP ≥ 18 years old who were vaccinated in the 2018-19 season with IIV4 (defined as Group One) as measured by
    - hemagglutination inhibition (HI) titers for egg-grown influenza viruses A/H1N1, B/Yamagata, and B/Victoria, and egg-grown and cell-grown A/H3N2
    - microneutralization (MN) titers for egg-grown and cell-grown A/H3N2 viruses

4.2 Secondary Objectives

- Compare humoral immune responses to a single dose of RIV4 versus IIV4 at 28 days post-vaccination in the 2019-20 season among HCP with a history of PCR-confirmed influenza infection during any of the three study years (2016-17 through 2018-19) compared to those without PCR-confirmed influenza infection (defined as Group Two);
- Compare humoral immune responses at 28 days post-vaccination as stated in the primary objective but with secondary indicators of humoral immune response using MN titers for other virus subtypes/lineages, as appropriate;
- Examine whether the number of prior influenza vaccinations during the preceding 10 years, as documented by medical records, modifies humoral immune responses after a single dose of RIV4 or IIV4 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

4.3 Exploratory Objectives

- Examine whether any association between prior vaccination and immune response to 2019-20 vaccination is mediated by antibodies to the prior vaccine strains
- Gather information on any adverse events following vaccination
- Characterize humoral immune responses as measured by HI, MN titers, and other appropriate humoral immunity assays among subsets of enrollees of particular interest due to their age or demographic characteristics, health status, and/or occupational roles and responsibilities.

5. STUDY ENDPOINTS

5.1 Primary Endpoints

The primary endpoints will be assessed by the following laboratory tests. The choice of laboratory assay may depend in part on how the 2019-2020 vaccine components and circulating strains affect which laboratory assay is most effective in assessing specific antigens.

- HI and/or MN responses to *egg*-grown vaccine reference viruses for all vaccine viruses for each study season at approximately 28 days, 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
    - Geometric mean titers (GMT) and the ratio of post-vaccination titers between the two vaccines (post-vaccination GMT ratio);
    - Geometric mean-fold rise (GMR) defined as the ratio of the post-vaccination titer value to the pre-vaccination value
    - Elevated post-vaccination titers at thresholds greater than 1:40, 1:80, 1:160

- MN responses to the *cell*-grown influenza A/H3N2 (using MDCK-SIAT or other appropriate cell line) vaccine reference viruses at approximately 28 days post-vaccination, including the following endpoints:
    - SCR, post-vaccination GMT ratio, GMR, and elevated post-vaccination titers

5.2 Secondary Endpoints

- HI responses to *egg*-grown influenza A/H3N2 vaccine reference viruses at approximately 28 days, including the following endpoints: SCR, post-vaccination GMT ratio, GMR, and elevated post-vaccination titers

5.3 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, post-vaccination GMT ratio, GMR, and elevated post-vaccination titers
    - SCR, post-vaccination GMT ratio, and GMR as measured by MN, as appropriate
- GMT as measured by NAI pre- and post-vaccination.
- Indicators of immune response to vaccination based on other immunologic assays not listed above (as appropriate)

6. METHODS

6.1 Overview of Study Design

This is a randomized, single-blind study design. Starting in July 2019, approximately 550 to 700 HCP from two hospitals (275-350 per hospital site) in Israel will be enrolled. Following completion of a written consent form, participants will complete an enrollment survey and grant permission for review of information collected during the SHIRI study, including vaccination status and immune response during the SHIRI study years. Participants will complete an enrollment survey and grant permission to include and integrate information collected during the SHIRI study into the current study's data, including health history, influenza vaccination history, and immune response to vaccination during the SHIRI study years. HCP will be randomly assigned 1:1 to receive a single dose of IIV4 licensed in Israel (expected to be Vaxigrip® Quadrivalent, 15µg of HA per strain) or RIV4 (Flublok® Quadrivalent by Sanofi Pasteur, 45µg of HA per strain) during August-October of 2019. Participants will be blinded to the vaccine that they vaccine, but study staff will be aware of the vaccine. Adverse events following vaccination will be monitored and documented. Blood specimens will be collected prior to vaccination and approximately 28 days after to evaluate immune responses to vaccination.

Eligible HCP who were already vaccinated with Vaxigrip, the vaccine that is routinely available in Israel, at the time they are approached to join the study, will be invited to join the study as part of the Vaxigrip arm. Like randomized participants, participants who were vaccinated with Vaxigrip outside the study will complete a written consent form, an enrollment survey and grant permission for review of information collected during the SHIRI study, including vaccination status and immune response during the SHIRI study years. As part of the consent form, participants will record the estimated date that they received the vaccine, and the location, and will authorize study staff to verify the date of current-year Vaxigrip vaccination in the Clalit EMR and with the hospital vaccination staff. Participants will complete an enrollment survey and grant permission to include and integrate information collected during the SHIRI study into the current study's data, including health history, influenza vaccination history, and immune response to vaccination during the SHIRI study years. Blood specimens will be collected approximately 28 days after to evaluate immune responses to vaccination.

6.2 Study Setting and Source Population

This study will be conducted among HCP who were enrolled in SHIRI for at least one year. SHIRI was conducted during the 2016-2017, 2017-2018, and 2018-2019 influenza seasons at the following two hospitals:

Beilinson Hospital, Rabin Medical Center, Petah Tikva, Israel

Soroka Medical Center, Beersheva, Israel

6.3 Participant Identification and Recruitment

Consent, screening, enrollment, and all study visits will take place at designated study site locations.

6.3.1 Inclusion and Exclusion Criteria

The following inclusion and exclusion criteria will be applied to determine eligibility for enrollment.

Inclusion criteria:

1. Aged ≥18
2. Are currently a member of Clalit Health Services
3. Consent to randomized receipt of influenza vaccination with either RIV4 or IIV4, or, if already vaccinated in the current year outside of the study, consent to receive a post-vaccination blood draw
4. HCP Group One participation criteria:
    - Participated in the SHIRI study and received influenza vaccination during the 2018-19 influenza season
5. HCP Group Two participation criteria:
    - Participated in any year of the SHIRI study and had PCR-confirmed influenza virus infection during any of the three study years;
6. HCP Group One Supplemental criteria:
    - Participated in any year of the SHIRI study, not vaccinated in 2018-2019, but vaccinated in at least one study year;
    - Participated in the SHIRI study during all three years, regardless of vaccination status

Exclusion criteria

1. ~~Already received an influenza vaccine during the current influenza season~~
2. Not willing or able to get the flu vaccines being used in this study;
3. Previous hypersensitivity reaction to the study vaccines, or any vaccine, as reported by the subject
4. Received any non-influenza vaccine (e.g., Hepatitis B or other vaccine recommended for HCP) in the 4 weeks prior to the first study visit or plans to receive a vaccine in the 4 weeks following the first study visit
5. 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.
6. Any condition or employment status that the potential participant or local study site principle investigator (PI) believes may interfere with successful completion of the study prior to December 2019, including being currently pregnant.

Withdrawal criteria

In addition to exclusion criteria identified prior to enrollment, the following criteria will be used to identify withdrawal from the study:

1. Withdrawal of consent by participant for any reason
2. As deemed necessary by the principal investigator for noncompliance or other reasons
3. Lost to follow up defined as not returning for scheduled study visit 2
4. Receipt of influenza vaccine outside of the study and after enrollment

6.3.2 Pre-screening and recruitment

The study will be open to HCP meeting the eligibility criteria at the two study sites. Prior to enrollment, study staff will identify potentially eligible HCP from SHIRI enrollment records. Pre-screening procedures may vary by enrollment facility. HCP will be recruited using a combination of methods that will be customized at each study site, including face-to-face meetings, phone calls, and printed material. Each site will describe the methods employed to recruit HCP potentially eligible for enrollment. All potential enrollees will be recorded in a recruitment log (**Appendix A. Recruitment Form**) in order to track invitations, acceptance, and refusal. All HCP who are interested in participation will have a screening and consent

appointment with a study physician, as required in Israel, in order to discuss the study purpose and procedures.

From the start of enrollment, sites will make efforts to recruit HCP from the priority Group One. In parallel HCP from the secondary Group Two will be recruited. If Group One recruitment goals cannot be reached, HCP from the Group One Supplemental will be targeted. A minimum target of 550 participants will be enrolled, including 275 at each site (see Section 9. Data Analysis). The target population of HCP will include 440 HCP from Group One, and 110 HCP from Group Two.

6.3.3 Screening, consent and enrollment questionnaire

During the screening and consent appointment, the study physician will describe the study briefly and then conduct a brief interview to determine study eligibility (**Appendix B. Eligibility and Screening Form**).

The study physician will administer consent for eligible HCP interested in participating. The consent form will explain study details, risks, and benefits, and participating HCP will be asked to read, sign, and date the consent form (**Appendix C. Consent Form**). In addition to receiving a copy of the full consent form, all participants will receive a brief summary of the consent form and an outlined schedule of study visits. All potential study participants will be free to decide if they want to participate in the study. The consent will include permission to access medical records and link data from the previous study with data collected during the current study.

After consenting to the study, participants will complete a brief questionnaire about health status (self-perceived health status), hours and types of patient contact encountered as part of work duties (**Appendix D. Enrollment Questionnaire**).

6.3.4 Randomization

Using the list of HCP consented to participate in the study, HCP will be randomized 1:1 to receive one 0.5 mL intramuscular dose of Flublok® Quadrivalent or Vaxigrip Quadrivalent. Randomization lists will be generated by the epidemiologist and data analyst using block randomization to ensure equal sample size in each group.[26]

Randomization will be conducted after enrollment is completed. Randomization will occur separately for participants in Group One and Group Two.

Randomization will not occur for enrolled participants who were vaccinated with Vaxigrip outside of the study.

6.4 Study visit 1

6.4.1 Pre-vaccination Questionnaire

All study participants, except those who were already vaccinated outside of the study, will complete a second brief questionnaire to determine whether they are currently experiencing fever, decreased energy, muscle aches, headache, or nausea, and will be asked to rate the subjective severity of any symptom(s) as mild, moderate, or severe (**Appendix E. Pre-vaccination Questionnaire/Vaccine Administration Form**). Appendix E will also readdress the study exclusion criteria. Participants reporting subjective fever (of any severity) and those reporting no fever but one or more symptom of moderate or severe subjective severity 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. Study number assignment and pre-vaccination blood draw will take place on the day of vaccine administration.

6.4.2 Blood Draw

All study participants will have 20 ml of venous blood drawn for serologic testing at the first study visit via venipuncture in multiple serum separator tubes with clot activator and gel, according to CDC guidelines. The use of a butterfly needle connected to a vacutainer tube is recommended as the best collection method to minimize hemolysis and to reduce the risk of needle stick injury. As an alternative, the use of a safety needle that connects directly to the vacutainer tube is acceptable. Study staff will document the blood collection in **Appendix F1: Blood Specimen Collection/Tracking Form.**

Study participants who were vaccinated with Vaxigrip outside of the study will not receive a blood draw during the initial study visit. For these participants, the end-of-season blood draw from SHIRI, which was taken at the end of the 2018-2019 influenza season, will be used as their "pre-vaccination" or "baseline" blood draw.

6.4.3 Vaccine administration

Participants will receive a single (0.5ml) dose of study vaccine administered in the deltoid muscle of the arm opposite the blood draw. Vaccine administration will be performed by a qualified nurse trained in the delivery of the study vaccines and documented on **Appendix E. Pre-vaccination Questionnaire/Vaccine Administration Form**. Participants will not be made aware of the vaccine they received until after the second blood draw (Study visit 2).

6.5 Study Visit 2

The optimal timing for Study Visit 2 is 21 to 35 days after vaccination, though 21 to 62 days will be considered acceptable. All study participants will have 20 ml of venous blood drawn for serology testing. Study staff will document the blood collection in **Appendix F1: Blood Specimen Collection/Tracking Form.**

6.6 Post-vaccination safety monitoring

After receiving the vaccine, all randomized participants will be contacted by SMS or by telephone 2-3 days post-vaccination and asked about adverse events (Appendix I). Non-solicited reports of adverse events will be documented from the time of vaccination through the second blood draw. Adverse events reported during this time period that are deemed to be serious by a medical doctor will be investigated. Serious adverse event reporting forms will be completed according to Israel Ministry of Health guidelines. In addition, study staff will provide participants with a phone number to call in case they have any questions or concerns about possible adverse events after vaccination.

6.7 Data Collection

Linked SHIRI Data: The participants of the current study are identified from among HCP that previously participated in the SHIRI study during the 2016-2019 influenza seasons. Subject to participant consent, study investigators will link all data from the previous study with data collected during the current study. This reduces participation burden, since previously supplied information does not need to be repeated, and provides information that is essential to study objectives, including immune profiles in previous seasons and HI response to prior vaccinations.

Enrollment Survey: Updated information on participants' health and occupational responsibilities will be collected by self-report though brief surveys at enrollment. Surveys are designed to be self-administered electronically via the internet using the REDCap program. In rare cases when a participant cannot complete a survey on his or her own, study staff will administer the electronic survey to the participant by telephone or in-person. Data collection elements are outlined in the Enrollment Questionnaire (**Appendix D. Enrollment Questionnaire**).

Electronic Medical Record (EMR) Extraction: For all enrolled HCP, additional data will be extracted from the Clalit Health Services (CHS) EMR, which contains extensive demographic and clinical data. Information on socio-demographic characteristics, chronic medical conditions,

medical care utilization, and influenza vaccination history (including date of receipt, vaccine product name, vaccine lot number, and route of administration) for the preceding 10 years will be extracted from CHS medical records and employee records at enrollment.

7. LABORATORY PROCEDURES

7.1 Blood Collection/Processing/Storage

After collection, blood specimens will be transported to the local laboratory for processing using standard procedures. Each tube will be labeled or barcode linked with the participant ID number, study visit number, and date of collection. Collected blood will be stored at 4°C immediately either by placing the sample on ice, in a 4°C refrigerator, or in a cooler with cold packs. Blood may be stored at 4°C for up to 18 hours. Sometime between 1 hour and 18 hours after collection, the blood collection tube will be centrifuged to separate the clotted blood from the serum and the serum removed to a clean tube labeled or barcode linked with the study participant ID number, study visit number, and date of collection. The clotted blood will be discarded. The serum will then be divided into approximately 8-10 aliquots of 1 mL/aliquot into labeled tubes. After aliquoting, the serum samples will be immediately stored at -20°C or colder.

Participant specimens will be stored indefinitely at a CDC-designated facility for future testing to evaluate aspects of respiratory infection and/or immune responses to influenza vaccine or influenza virus infection, as needed. Participants will also be asked if they would like to be contacted about future research studies related to this study.

7.2 Specimen Testing

Assessment of humoral or serologic immune response to antigens that match influenza virus strains that are components of the vaccines and circulating during the study period typically require multiple laboratory assays. We expect that primary methodologies for this effort will be the HI and MN assay. In recent years, MN assays have been required to assess response to cell-grown A/H3N2 viruses, which are a better match to circulating strains and vaccine strains in vaccines that do not involve egg-based production, such as Flublok. Nonetheless, the combination of assays and antigens most relevant to the study objectives will depend on vaccine components, circulating strains, and performance of assays during the study period.

Additional advance serologic assays on a subgroup of specimens may be required. For example, to examine the extent of narrow vs. broad antibody responses and the potential over-focusing of response on specific antibody sites, it may be necessary to quantify inhibition by serum of antigenic site-specific monoclonal antibodies (mAbs) to HA.[17] Response to the two vaccine types may also differ in the extent "back boosting" of antibodies to historical vaccine and circulating viruses as measured by conducting an antibody landscape.[27]

Sera will be tested by HI and MN to measure antibody titers against cell-grown and egg-grown vaccine reference viruses at approximately 28 days post-vaccination compared to baseline samples drawn at day 0 prior to vaccination during each study season. Sera may also be tested by HI and MN against cell-grown wild-type influenza strains, as appropriate. MDCK-SIAT cells or another appropriate cell line will be used to cultivate cell-grown virus strains. A subset of sera from each study arm may also be tested by other immunologic assays as appropriate to measure activity against antigens represented in the study vaccines at day 0 and at approximately 28 days at each site.

8. VACCINE PRODUCT

Two vaccine products will be used in this trial: (1) the standard-dose quadrivalent inactivated influenza vaccine (expected to be Vaxigrip® Quadrivalent), and (2) Flublok® Quadrivalent. CDC or study sites will purchase vaccine. Both vaccines are inactivated influenza vaccines that are approved for use in adults aged ≥18 years in the United States. Vaxigrip Quadrivalent is licensed in Israel. Flublok is not licensed in Israel. Both vaccine products will contain vaccine strains representative of the four strains in the 2019-2020 northern hemisphere formulation.

Vaxigrip® Quadrivalent 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 or a 5mL multi-dose vial; the vaccine is administered intramuscularly. Each dose is formulated to contain 15µg of HA per strain. Each 0.5mL dose of vaccine also contains sodium phosphate-buffered isotonic sodium chloride solution, up to 100µg of formaldehyde, and up to 250µg of octylphenol ethoxylate. Multi-dose vials also contain the preservatives,

thimerosal and mercury. The most common reactions occurring after vaccine administration in adults are pain at the injection site, myalgia, headache, and malaise. The vast majority of these reactions are mild to moderate, and severe reactions are very rare.[28]

Flublok® Quadrivalent is a recombinant vaccine manufactured by Sanofi Pasteur. The vaccine is formulated from purified HA protein from cell-culture isolates of the vaccine reference virus grown using baculovirus vector technology in an insect cell line (*expres*SF+®) from cells of the fall armyworm. Influenza HA is removed from cells using Triton® X-100 and then purified by chromatography. The resulting vaccine suspension is clear and is available for use in adults as a prefilled single-dose 0.5mL syringe for intramuscular administration. Each dose is formulated to contain 45µg of HA per strain. Each 0.5mL dose of vaccine also contains 4.4mg of sodium chloride, 0.195µg of monobasic sodium phosphate, 1.3mg of dibasic sodium phosphate, and 27.5µg of polysorbate 20. The vaccine may also contain up to 19mcg of baculovirus and *Spodoptera frugiperda* cell proteins, up to 10ng of baculovirus DNA and cellular DNA, and up to 100µg of Triton X-100.[29,30] The most common systemic symptoms after vaccine administration are injection site tenderness or pain, headache, fatigue, muscle pain, and joint pain. Severe reactions are very rare. The frequency of solicited symptoms and adverse events were comparable or often less frequent than among IIV recipients.[24,25,31]

Vaccine products will not be modified for this study. The vaccines must be stored at 2° C to 8° C [35.6° F to 46.4° F] until needed. Vaccine products should not be frozen and should be protected from natural light. Documentation of proper vaccine storage will be monitored daily and maintained during the duration of the trial. In the event of accidental deep-freezing or disruption of the cold chain, study vaccine will not be administered.

9. DATA ANALYSIS

9.1 Power and Sample Size Considerations

To guide enrollment goals, we estimated the sample size required to do a two-sided test with a significance level of 0.05 and a power of 80%. The Food and Drug Administration criteria for demonstrating non-inferiority between IIV and a new vaccine requires comparisons of both the GMR and the SCR. We based expected values for the SCRs and GMRs comparing RIV3 and IIV3 on previously published estimates from prior vaccine immunogenicity trials. We assume that the SCR for IIV4 is ≥40% and ≥55% for RIV4, based on a clinical trial among adults <65 years demonstrating the SCR for IIV4 to range from 35% to 60% and RIV4 to range from 54%

to 63% against influenza A subtypes (seroconversion rates reported against influenza B viruses reflected trivalent vaccine formulations and were not included).[23,32] We intend to have the power to show a 30% relative increase in SCR in the RIV arm compared to the IIV arm. Using the same assumptions and sample size would also provide adequate statistical power to detect a difference in post-vaccination GMT of ≥2 fold between study arms if post-vaccination GMT value is ≥20 for IIV4 and ≥40 for RIV4. **Table 2** provides estimated sample sizes required for varying SCR and differences in SCR between study arms.

Based on prior experience enrolling participants at the two study sites, we will assume that 5% of participants will drop out before study visit 2.

Table 2. Sample size calculations to detect specified differences in SCR between RIV4 and IIV4 at approximately 28 days post-influenza vaccination

SCR at 28 days post-vaccination in IIV arm	Relative increase (%) in SCR in RIV arm compared to IIV arm at 28 days post-vaccination	Total enrollment for both study arms
30%	10%	7526
30%	20%	1926
30%	30%	874
40%	10%	4778
40%	20%	1208
40%	30%	540
50%	10%	3130
50%	20%	776
50%	30%	340
60%	10%	2032
60%	20%	488
60%	30%	206

9.2 Statistical Analysis

HI and MN results will be measured by four primary parameters: SCR, post-vaccination GMT ratio, GMR, and post-vaccination titers equal or greater than seropositive thresholds at 1:40, 1:80, and 1:160. SCR will be defined as the proportion of participants with paired samples that achieved ≥ 4 fold rise comparing post vs pre-vaccination titers and post vaccination titers ≥ 1:40. GMR will be defined as the mean of the ratio of post-vaccination HI/MN titer and pre-vaccination HI/MN titer for each subject.

Primary Objective

The primary objective of the study is compare humoral antibody responses to IIV4 and RIV4 among Group One HCP as measured by HI (and MN, as appropriate) at baseline (day 0), and approximately 28 days post-vaccination. The proportion of participants that seroconverted (SCR) is defined as ≥ 4-fold increase comparing post- versus pre-vaccination titers. Exact 95% confidence intervals will be calculated and the SCR will be compared between vaccine groups using χ^2 or Fisher exact tests.

Due to the skewed distribution of the resulting titer values, base-2 log transformed titers will be used in analyses and then the summary statistics will be back-transformed to the original scale for presentation of results.[33] The GMT is calculated for each vaccination group as the anti-$\log_2$(mean[$\log_2$(titer$_i$)]), where *i* represents the titer value for each participant, and 95% confidence intervals assume a normal distribution. To allow for calculation of HI GMTs, a titer of 1:5 will be assigned to those with undetectable HI antibody.

Differences in GMT and GMR will be compared between study groups using separate linear regression models or a generalized linear model that accounted for repeated measures. Odds of achieving elevated post-vaccination titers at seropositive thresholds > 1:40, 1:80, and 1:160 will be compared between study groups by logistic regression.

Secondary Objectives

To compare immune responses among Group Two participants with a history of PCR-confirmed influenza infection a linear regression predicting log transformed titer will be created with independent variables for year of influenza infection, type of influenza virus detected, study arm, and potential confounders. We assume confounding will be eliminated by randomization and that models will not be adjusted unless differences in study arms are found during univariate analysis.

To assess prior vaccination status among Group One participants (number of influenza vaccinations received during the preceding 10 years) as an effect modifier of differences in GMTs, a linear regression predicting log transformed titer will be created with independent variables for vaccine history, study arm, and potential confounders, and an interaction term between study arm and vaccine history.

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

10. ETHICAL CONSIDERATIONS

10.1 Possible Risks

The potential discomforts from study participation include having blood drawn, intramuscular injection of vaccine, and possible reactions to vaccine. Blood samples will be taken from all participants at each study visit. Participants may experience some minimal pain and/or bruising at the site of the blood draw. To prevent or lessen bruising, study staff will apply pressure or ask participants to apply pressure to the draw site for several minutes after the blood draw. There is also a slight risk of infection associated with blood draws. Study staff will swab the site with alcohol and use sterile equipment to make infection at the site where blood is drawn or where vaccination is given extremely unlikely.

Both study vaccines are licensed by the US FDA. Vaxigrip Tetra is licensed in Israel. Flublok is not licensed in Israel. Occasionally, adult recipients of influenza vaccines may develop influenza-like reactions such as fever, body aches, headache, malaise, myalgia, and/or nausea. If present, these symptoms usually occur soon after vaccination and may last up to 1-2 days post vaccination. Some participants may develop reactions at the site of vaccination (redness, swelling, pain, or tenderness). Analgesics such as ibuprofen or acetaminophen and rest will generally relieve or moderate these symptoms. Acute and potentially life-threatening allergic reactions are also possible, though extremely unlikely; severe reactions from influenza vaccine are estimated to occur in < 1 per 4 million persons vaccinated. Signs of a severe allergic reaction may include shortness of breath, wheezing, hives, hoarseness, difficulty swallowing, swollen face/ tongue/ pharynx, tachycardia, and dizziness.

10.2 Remuneration

Participants will receive small gifts or incentives (such as a gift card for a local restaurant) at study milestones:

- At completion of enrollment (complete enrollment survey);
- At the time of pre-vaccination blood draws and vaccination;
- At the time of the second blood draw.

The total value of the gifts will be in the range of 100-150 shekels.

10.3 Provisions for protecting privacy/ confidentiality:

All data collected in this study will be kept confidential. All written information will be stored in locked cabinets with limited access, and electronic information will be stored on secured servers. Blood samples that will be sent to collaborating institutions for processing and analysis will not contain any personal identification information. Collaborating institutions will destroy any stored data after completion of the study and data analysis. During the study, only a few members of the research team will have access to personal identifying information about the study participants. All members of the research team, including the study investigators, research nurses, research assistants, and research coordinators, will have access to de-identified data during the study. Individuals who are not a part of the research team will not have access to the study information or data while the study is taking place. With an official letter and a clearly stated objective, scientists who are not a part of the research team may receive access to de-identified data, if approved by the Steering Committee (described below).

10.4 Consent forms for study volunteers:

Written informed consent will be obtained for all potential participants prior to enrollment. In understandable language, trained project staff, including a medical doctor, will explain study procedures to potential participants and discuss the advantages and disadvantages of participating. Participants will be given a copy of the full consent form. Participants will be asked to read the full consent before agreeing to be in the study and providing their signature. Study coordinators will emphasize the voluntary nature of the study, the possible benefits and outcomes, alternatives to participation, confidentiality of participation, and the participant's right to refuse and/or withdraw from the study at any time. It will be explained to participants that

discontinuation of participation or choosing not to participate will not affect their professional standing.

10.5 Protocol Deviations

Study staff will report protocol deviations using a Protocol Deviation Log (**Appendix G. Protocol Deviation**) for each participant. Sites may also complete additional protocol deviation forms as required by their local IRBs. Sites will report deviations to their local IRBs according to site-specific reporting requirements.

Protocol Completion or Termination: Study staff will complete a protocol completion or termination form for each participant at the time the participant either completes all protocol procedures or at the time of termination if early termination occurs (**Appendix H. Study Status Change Form**).

11. SITE MONITORING PLAN

CDC will conduct site monitoring through teleconference calls and site visits (as needed) to ensure that human subject protection, study procedures, laboratory procedures, vaccine administration, and data collection procedures are high quality and meet guidelines, and that the study is conducted in accordance with the protocol and sponsor standard operating procedures. CDC will summarize requests to address any areas for improvement or modification that are identified during teleconference calls or site visits through written communication to site PIs.

11.1 Study Oversight, Management, and Guidance for Decision Making

A steering committee consisting of representatives from Clalit Research Institute, the US CDC, Beilinson Hospital, Soroka Medical Center and the University of Michigan School of Public Health will each provide high level input into this project. The steering committee will review and approve a data usage agreement and other guidance documents to aid in decision making prior to the start of the study. The steering committee will be consulted on over-arching project issues including final protocol decisions, adjudicating any protocol deviations that might occur, reviewing and confirming analysis plans, and making final decisions on analyses, manuscripts, and authorship as needed. Upon the completion of all study deliverables and after a

suitable moratorium, external parties may request de-identified study data from the steering committee as specified in U.S. Government Data Sharing guidelines.

12. REFERENCES

1. Kuster SP, Shah PS, Coleman BL, et al. Incidence of influenza in healthy adults and healthcare workers: a systematic review and meta-analysis. PLoS One 2011;6:e26239.
2. Ahmed F, Lindley MC, Allred N, Weinbaum CM, Grohskopf L. Effect of influenza vaccination of healthcare personnel on morbidity and mortality among patients: systematic review and grading of evidence. Clin Infect Dis 2014;58:50-7.
3. Wilde JA, McMillan JA, Serwint J, Butta J, O'Riordan MA, Steinhoff MC. Effectiveness of influenza vaccine in health care professionals: a randomized trial. JAMA 1999;281:908-13.
4. McLean HQ, Thompson MG, Sundaram ME, et al. Impact of repeated vaccination on vaccine effectiveness against influenza A(H3N2) and B during 8 seasons. Clin Infect Dis 2014;59:1375-85.
5. 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.
6. Hollmeyer H, Hayden F, Mounts A, Buchholz U. Review: interventions to increase influenza vaccination among healthcare workers in hospitals. Influenza and other respiratory viruses 2013;7:604-21.
7. Skowronski DM, Chambers C, De Serres G, et al. Serial Vaccination and the Antigenic Distance Hypothesis: Effects on Influenza Vaccine Effectiveness During A(H3N2) Epidemics in Canada, 2010-2011 to 2014-2015. J Infect Dis 2017;215:1059-99.
8. Thompson MG, Naleway A, Fry AM, et al. 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.
9. Laguio-Vila MR, Thompson MG, Reynolds S, 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.

10. Belongia EA, Simpson MD, King JP, et al. Variable influenza vaccine effectiveness by subtype: a systematic review and meta-analysis of test-negative design studies. Lancet Infect Dis 2016;16:942-51.
11. Flannery B, Chung JR, Belongia EA, et al. Interim Estimates of 2017-18 Seasonal Influenza Vaccine Effectiveness - United States, February 2018. MMWR Morb Mortal Wkly Rep 2018;67:180-5.
12. Groth N, Montomoli E, Gentile C, Manini I, Bugarini R, Podda A. Safety, tolerability and immunogenicity of a mammalian cell-culture-derived influenza vaccine: a sequential Phase I and Phase II clinical trial. Vaccine 2009;27:786-91.
13. Mandelboim M, Glatman-Freedman A, Drori Y, et al. Ineffectiveness of the 2014-2015 H3N2 influenza vaccine. Oncotarget 2016;7:1185-92.
14. Stein Y, Mandelboim M, Sefty H, et al. Seasonal Influenza Vaccine Effectiveness in Preventing Laboratory-Confirmed Influenza in Primary Care in Israel, 2016-2017 Season: Insights Into Novel Age-Specific Analysis. Clin Infect Dis 2018;66:1383-91.
15. Skowronski DM, Chambers C, Sabaiduc S, et al. Interim estimates of 2014/15 vaccine effectiveness against influenza A(H3N2) from Canada's Sentinel Physician Surveillance Network, January 2015. Euro Surveill 2015;20.
16. Skowronski DM, Janjua NZ, De Serres G, et al. Low 2012-13 influenza vaccine effectiveness associated with mutation in the egg-adapted H3N2 vaccine strain not antigenic drift in circulating viruses. PLoS One 2014;9:e92153.
17. Zost SJ, Parkhouse K, Gumina ME, et al. Contemporary H3N2 influenza viruses have a glycosylation site that alters binding of antibodies elicited by egg-adapted vaccine strains. Proc Natl Acad Sci U S A 2017;114:12578-83.
18. Robertson JS. An overview of host cell selection. Dev Biol Stand 1999;98:7-11; discussion 73-4.
19. Xu X, Kilbourne ED, Hall HE, Cox NJ. Nonimmunoselected intrastrain genetic variation detected in pairs of high-yielding influenza A (H3N2) vaccine and parental viruses. J Infect Dis 1994;170:1432-8.
20. Raymond DD, Stewart SM, Lee J, et al. Influenza immunization elicits antibodies specific for an egg-adapted vaccine strain. Nat Med 2016;22:1465-9.

21. Liu F, Tzeng WP, Horner L, et al. Influence of Immune Priming and Egg Adaptation in the Vaccine on Antibody Responses to Circulating A(H1N1)pdm09 Viruses After Influenza Vaccination in Adults. J Infect Dis 2018;218:1571-81.
22. Dunkle LM, Izikson R. Recombinant hemagglutinin influenza vaccine provides broader spectrum protection. Expert Rev Vaccines 2016;15:957-66.
23. Treanor JJ, El Sahly H, King J, et al. Protective efficacy of a trivalent recombinant hemagglutinin protein vaccine (Flublok(R)) against influenza in healthy adults: a randomized, placebo-controlled trial. Vaccine 2011;29:7733-9.
24. Dunkle LM, Izikson R, Patriarca P, et al. Efficacy of Recombinant Influenza Vaccine in Adults 50 Years of Age or Older. N Engl J Med 2017;376:2427-36.
25. Izikson R, Leffell DJ, Bock SA, et al. Randomized comparison of the safety of Flublok((R)) versus licensed inactivated influenza vaccine in healthy, medically stable adults >/= 50 years of age. Vaccine 2015;33:6622-8.
26. Kang M, Ragan BG, Park JH. Issues in outcomes research: an overview of randomization techniques for clinical trials. J Athl Train 2008;43:215-21.
27. Fonville JM, Wilks SH, James SL, et al. Antibody landscapes after influenza virus infection or vaccination. Science 2014;346:996-1000.
28. Sanofi Pasteur, 450/477 Fluzone® Quadrivalent Southern Hemisphere. (Accessed April 13, 2018, at https://www.fda.gov/downloads/BiologicsBloodVaccines/Vaccines/ApprovedProducts/UCM305089.pdf.)
29. Summary Basis for Regulatory Action - Flublok Quadrivalent. 2016. (Accessed April 24, 2019, at https://www.fda.gov/BiologicsBloodVaccines/Vaccines/ApprovedProducts/ucm524660.htm.)
30. Flublok Quadrivalent Influenza Vaccine. 2019. (Accessed April 13, 2019, at https://www.fda.gov/downloads/BiologicsBloodVaccines/Vaccines/ApprovedProducts/UCM619551.pdf.)
31. Dunkle LM, Izikson R, Patriarca PA, Goldenthal KL, Cox M, Treanor JJ. Safety and Immunogenicity of a Recombinant Influenza Vaccine: A Randomized Trial. Pediatrics 2018;141.

32. Baxter R, Patriarca PA, Ensor K, Izikson R, Goldenthal KL, Cox MM. Evaluation of the safety, reactogenicity and immunogenicity of Flublok(R) trivalent recombinant baculovirus-expressed hemagglutinin influenza vaccine administered intramuscularly to healthy adults 50-64 years of age. Vaccine 2011;29:2272-8.
33. Gaglani M, Spencer S, Ball S, et al. Antibody Response to Influenza A(H1N1)pdm09 Among Healthcare Personnel Receiving Trivalent Inactivated Vaccine: Effect of Prior Monovalent Inactivated Vaccine. J Infect Dis 2014;209:1705-14.

Table 3: List of Appendices by Relevant Study Activity

Appendix	Screening/ Enrollment	Visit 1 (Pre-vaccination blood draw, vaccination)	Visit 2 (2nd blood draw 21 to 35 days after vaccination)	Post-study analysis
Appendix A. Recruitment Form	X			
Appendix B: Eligibility Screening Form	X			
Appendix C: Consent Form	X			
Appendix D: Enrollment Questionnaire	X			
Appendix E: Pre-vaccination Questionnaire/Vaccine Administration Form		X		
Appendix F1: Blood Specimen Collection/Tracking Form		X	X	
Appendix G: Protocol Deviation Log	(X)	(X)	(X)	
Appendix H Study Status Change Form		(X)	(X)	
Appendix I: List of High-Risk Medical Conditions				(X)
Appendix J. Data Use Agreement	(X)	(X)	(X)	
Appendix K. Scientific Advisory Group	(X)	(X)	(X)	
Appendix I. Adverse Event Follow-Up Form		(X)		

Parentheses indicate that forms will be completed as needed.