Official Title: Immunologic response to influenza vaccination

ClinicalTrials.gov ID (NCT number): NCT03614975

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Scientific Background:

The Advisory Committee on Immunization Practice (ACIP) sets civilian immunization policy for the United States. In adults, cell-based recombinant vaccine has been shown to work better immunologically against the H3 strain due to lack of egg-based adaptations; however, it is not licensed for use in children. All of the published efficacy trial data on Flucelvax, the cell-based influenza vaccine approved by the FDA in 2012, were conducted when it was based on the egg-adapted strain; it first used the cell-based strain in the Fall of 2017 so effectiveness data have not been published on this update. Egg-based vaccine has only worked reasonably well against the H3 in preliminary effectiveness data for younger children; it did not work well against H3 in adult recipients due to egg-based adaptions. This study would be among the first head-to-head comparison of the current versions of these two different licensed influenza vaccines to compare the differences in antibody response among children/teens/young adults. Given recent changes in the formulation of Flucelvax and the lack of published data on this new formulation, reasonable equipoise can be assumed among these vaccines.

Influenza is a major public health burden, each year causing millions of illnesses and outpatient visits and tens of thousands of hospitalizations and deaths in the U.S. The primary influenza prevention method is vaccination; however, influenza vaccine effectiveness (VE) is variable. Host factors (e.g., age and antibody landscape), environment (e.g., tobacco exposure), virus and vaccine characteristics, such as the match between the vaccine and circulating strains, contribute to VE. This project will quantify pre- and post influenza immune response to different influenza vaccine types licensed for use (e.g., eggbased, egg-free).

Study Objectives:

Using a RCT for children 4-20 years of age, two influenza vaccines will be assessed for their immunogenic outcomes: Flucelvax (egg-free inactivated flu shot) and Fluzone (egg-based inactivated flu shot).

The study objectives are to determine the serologic, proteomic and/or peripheral blood mononuclear cells (PBMCs)/RNA responses to:
1) cell-based quadrivalent influenza vaccine (Flucelvax) and 2) egg-based quadrivalent influenza vaccine (Fluzone) in a randomized controlled trial (RCT) among racially diverse children 4-20 years of age.

Study Design and Methods:

We will conduct a non-blinded, randomized controlled trial of influenza vaccine immunogenicity in children aged 4-20 years. Influenza vaccination will occur in primary care settings with standard medical care use according to CDC guidelines with FDA approved and licensed vaccines. Bloodwork will be conducted at baseline and post-vaccination for research purposes during the vaccination season (e.g., August thru December).

Parents of child/teen patients will be asked to complete the appropriate screening questionnaire which addresses inclusion and exclusion criteria in lay language where possible. If they do not meet all inclusion criteria, have an exclusion criterion, or are unwilling to complete study activities then they will not be eligible for enrollment and will not be consented by the clinician. The screening questionnaire may be done in person, by phone, by mailed form or by electronically sent form. The questionnaire takes <10 minutes to complete.

Research coordinators will meet with parents and children/teens to review study requirements and provide the written informed consent form for them to read through. Licensed doctoral level medical providers (e.g., MDs) or licensed pharmacists (e.g., PharmD) will be present either in person or by electronic device (e.g., face time) to address any parent's/child/teen's questions regarding the study and to obtain written consent (parents) and assent (children/teens), including written permission from parents to access their child's electronic medical record (EMR) in order to obtain data related to medical history, medication use, and vaccination history.

Randomization to licensed flu vaccine arms: Non-blinded randomization will be generated by computers and done as a 1:1 allocation to each of the licensed flu vaccine arms. Influenza vaccine will be given by clinical staff as part of the child/teen's routine immunization care. Influenza vaccine information will be collected via EMR review and/or documented at baseline using a research form noting the type of influenza vaccine received (e.g., vaccine manufacturer, valence, brand name).

Blood draws will be done at UPMC or Children's clinical recruitment practices. All participants will have a blood draw at baseline (may be during summer before vaccination) and at two subsequent follow-up visits. Total blood volume drawn across the study visits is ~90 mL (~30 mL drawn at each study visit). If not enough blood is obtained during study visit we will ask participants to return for an additional blood draw(s) in order to obtain the volume needed for analysis in the same study visit window; total volume for re-draws dependent upon tube type needed to fill (e.g, CPT tube for PBMCs, paxgene tube for RNA, serum tube for HI). Addendum consents for blood re-draws will be signed prior to drawing blood.

All blood samples will be collected by trained personnel with experience drawing blood from children. The blood samples will be collected using Vacutainer tubes, with uniformly colored tops according to type of processing and appropriately labeled with a uniform scheme (including study ID and collection time point). To support better understanding of the immune response following influenza vaccination, specimens will be banked for future studies that might involve proteomics, immunology, transcriptomics, or genomics. Collection of survey data done at UPMC clinical recruitment practices. Survey data will be collected from all enrolled participants at the baseline visit. If participant time constraints limits collection of the survey at the baseline visit, this information will be collected at a subsequent study visit.

Cells will be prepared and frozen according to current protocols. Plasma and cells will be stored at -80C until all specimens are collected. They will be inventoried and maintained in a database in the laboratory. Upon completion of sample collection (pre-vacc, and postvacc) samples will be sent to the funder and other contracted laboratories for further analysis. Peptoid and other laboratory testing will be conducted per current protocols.

Specific to East Liberty Family Health Center (ELFHC) clinical recruitment site: ELFHC clinical staff's (e.g., MA/RN) activities will be limited to 1) helping to identify potential subjects, drawing blood on and providing influenza vaccination to consented and enrolled participants. Pitt research staff along with the specified MD will conduct the informed consent process with parents and children/teens and/or adult (18-20 years) participants and Pitt research staff will administer the survey at this site. Prior to staffing ELFHC for study recruitment, ELFHC helping with this research project will complete the Community Partner Research Education and Training (CPRET) prior to engagement in research activities. This training will be conducted by the Principal Investigator.

All participants will have a blood draw at baseline and at two subsequent follow-up visits. Total blood volume drawn across the study visits is ~90 mL (~30 mL drawn at each study visit: baseline, 2nd study visit, 3rd study visit). If another blood draw visit needs to be scheduled due to lack of volume collected at the prior visit (e.g., unable to collect total per visit mLs due to participant dehydration) or due to poor recovery of PBMCs, then additional blood will be collected dependent upon the type of tube needed. In some circumstances (e.g., inadequate vacuum draw/clotting issue/observed hemolysis), up to an extra tube (e.g., up through 10mL) may be drawn at any particular visit. Research staff Phlebotomists are fully trained in phlebotomy with appropriate clinical phlebotomy experience (e.g., experience with drawing blood on children). Clinical site/hospital lab Phlebotomists are fully trained and employed by UPMC. ELFHC clinical staff are fully trained in phlebotomy and vaccination.

Specific endpoints that will result in withdrawal from the study include non-compliance with allotted windows for visits in study protocol. Baseline blood draw and 2 return blood-draw visits: day 7 (range 6-9 days) and day 21 (range 21-35 days). Acceptability of samples with minor deviations from these windows will be determined by the research team on a case-by-case basis. Persons who do not meet these criteria will be dropped from the study. Onset of a new severe bleeding disorder that precludes further phlebotomy. If a participant misses day 7 but can still make the day 21 visit, they may be retained.

The PI and co-investigators will monitor the progress of the research study, including assessments of data quality and timeliness and participant recruitment, accrual and retention, subject’s confidentiality, any adverse event data, unanticipated problems and external factors or relevant information that might have an impact on the safety or ethics of the study. The PI and co-investigators will meet on a regular basis to discuss these

issues. Minutes will be recorded for each of the meetings which will occur on a bimonthly to quarterly basis, depending on the stage of the study. At the time of the IRB renewal the PI will submit in writing to the IRB a summary report of the data and safety monitoring activities from the past year. The CDC and University of Pittsburgh will hold phone calls at least quarterly to address the progress of the research study, including assessments of data quality and timeliness and participant recruitment, accrual and retention, subject's confidentiality, any adverse event data, unanticipated problems and external factors or relevant information that might have an impact on the safety or ethics of the study. Minutes will be recorded for each of the meetings. Adverse events will be reported to the HRPO per its Policies and Procedures Manual.

Eligibility Criteria:
Inclusion Criteria:
1. Males and females aged 4-20 years whose prior vaccination history is available (which can be determined based on the medical record or state registry for the prior year or participation in our previous studies of childhood influenza vaccine (previous participants are a high priority for enrollment);
2. Planning to receive seasonal influenza vaccination at one of our recruiting sites
3. >= 37 pounds

Exclusion Criteria:
1. Unable or unwilling to complete all required study activities, including informed consent and bloodwork
2. Already received the influenza vaccine for the current year
3. Have a known immunocompromising condition or on an immunosuppressing medication (e.g., high dose steroids)
4. Known to be pregnant
5. Have a history of severe allergy to eggs or to influenza vaccine or any of its components

Statistical Considerations:

As an initial step in the data analysis, descriptive statistics will be generated for each of the variables of interest, including their distributional properties. Through analyses of these distributional property assessments and relevant statistical tests, the necessary assumptions for the planned statistical tests will be investigated. If the assumptions are not met for any planned statistical test, then either the data will be transformed or a nonparametric alternative will be used.

For the analysis of the survey data, methods appropriate for the analysis of counts and proportions, depending on the specific question will be used. Due to the distributional shape in geometric mean titers (GMT), either nonparametric tests or transformation of the data will be needed; log transformation will be used for the primary endpoint. The analysis will be conducted with transformed data. The natural log of the

GMT will be the dependent variable to normalize the distribution. The mean and standard deviation of log titers will be calculated. Exact tests will be used where needed for small cell sizes. Regression models will also be used for predictors of immunogenic outcomes.

Power calculations show the need to enroll 40 per vaccine arm with 74 per vaccine arm preferred for publication; we have noted 90 enrolled per arm to account for possible attrition. A one-sided, two-sample t-test with group sample sizes of 40 and 40 achieves 98% power to detect a ratio of 2.0 when the ratio under the null hypothesis is 1.0. The coefficient of variation on the original scale is 1.0. The significance level (alpha) is 0.050. A one-sided, two-sample t-test with group sample sizes of 74 and 74 achieves 80% power to detect a ratio of 2.0 when the ratio under the null hypothesis is 1.0. The coefficient of variation on the original scale is 4.0. The significance level (alpha) is 0.050.