Official Title:

Immunological Response to Influenza Vaccination in Children, Adolescents, and Young Adults: A RCT of FluMist vs. Flucelvax

ClinicalTrials.gov ID (NCT number): NCT03982069

Review Date: September 3, 2020

Research protocol – Updated 8-31-2020

Immunological Response to Influenza Vaccination in Children and Teens: A RCT of FluMist vs. Flucelvax - 2nd year

Short Title Acronym: Mist-C Study

University of Pittsburgh Investigators and Key Personnel:
PI: Richard K. Zimmerman, MD, MPH

Co-Is:
Judith Martin, MD
Mary Patricia Nowalk, PhD, RD
John Alcorn, PhD

IRB Co-Is:
Melissa Andrasko, RN
Sonika Bhatnagar, MD, MPH
Mary Pat Friedlander, MD
Alejandro Hoberman, MD
Amanda Jaber, PharmD
John Maier, MD, PhD
Gysella Muniz Pujalt, MD
Jennifer Nagg, RN, MS
Lindsay Nakaishi, MD, MPH
Marcia Pope, RN
Anne-Marie Rick, MD
Sandy Sauereisen, MD, MPH
Nader Shaikh, MD
Timothy Shope, MD, MPH
Mary Ann Sieber, RN
Lori Stiefel, MD

Katherine Williams MD, MPH,
Study Coordinator

Key Personnel:
Karen Clarke
Mark Collins
Dominic Kramer
Matthew Lee
Chongchiou Lin, Statistician
Leah McKown
Jennifer Opal, RN
Neelima Pathri
Lalicia Roman, RN
Sean Saul
Michael Susick
Bo Zhai

Funding Agency
U.S. Centers for Disease Control and Prevention (CDC)

CDC Project Officer:
Brendan Flannery, PhD

CDC Laboratory Teams:
Min Levine, PhD
Suryaprakash Sambhara, DVM PhD
Shivaprakash Gangappa, DVM PhD

Abstract

Background: Whether the several classes of influenza vaccines are equivalent or not in children is unclear. Live attenuated influenza vaccine (LAIV, FluMist), offers a nasal administration route which may result in higher vaccination rates [1]. Data from 2015-16 showed LAIV had lower effectiveness in children aged 2-17 years [2, 3]. Starting in 2018-19, LAIV was reformulated, with attention to the H1N1 construct, and re-offered as an Advisory Committee on Immunization Practice- (ACIP) approved vaccine choice. Cell-culture inactivated influenza vaccine (ccIIV4, Flucelvax) is another approved option for children aged 4 years and older that, for the first time in 2019-2020, was entirely derived from cell seed strains. Given the concern about lower vaccine effectiveness due to mutations in general egg-grown formulations, having an egg-free comparator to LAIV is advantageous. The LAIV reformulation and interest in avoiding egg-based H3N2 mutations, indicates that a randomized trial between LAIV and ccIIV in children and teens is warranted to compare immune responses between egg-grown LAIV and cell-culture derived vaccine. This study was started in 2019-20, yielding 204 paired serum sets plus a modest number of paired PBMC sets. This study will be extended into 2020-21 in order to:
Compare the serologic responses to cell culture-based quadrivalent influenza vaccine (ccIIV [Flucelvax]) versus live attenuated quadrivalent influenza vaccine (LAIV [FluMist]) in a randomized controlled trial (RCT) among racially diverse children/young adults 4-21 years of age. The primary endpoint is change in antibody titer. The secondary Aims are to: 1. Compare the immune responses between ccIIV and LAIV; 2. The nasal microbiome may both effect and be affected by vaccination (2019 only);

Methods: An unblinded, randomized controlled trial will be conducted by Pediatric and Family Medicine teams at the UPMC and the University of Pittsburgh. Randomization to either LAIV or ccIIV on a 1:1 ratio will be done in blocks of 4. Depending on funding, up to 240-260 will be randomized; assuming a 16% drop-out rate (21% in children under 9 and 11% in ages 9 and up) this would result in approximately 200 participants. Efforts will be made to recruit/include children 4-8 years and persons not vaccinated in 2019-20 for comparison of immune response to those previously vaccinated in 2019-20 . Young persons aged 4-21 years without a contraindication to LAIV who plan to receive seasonal influenza vaccination at one of our recruiting sites are eligible to participate. The primary endpoints are change in antibody titers. Given that LAIV is known to produce limited IgG [4], other potential beneficial responses to nasal administration of intranasal LAIV compared to ccIIV will be examined as secondary endpoints, including changes in serum IgA. Titer levels will be log transformed and reported as seropositivity, mean fold rise (MFR) and seroconversion. Specimens will be collected on a subset of participants for cell-mediated immune responses to be studied at CDC and Pitt.

Introduction

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 and the type of vaccine, contribute to VE. The Advisory Committee on Immunization Practice (ACIP) sets civilian immunization policy for the United States and has no preference among influenza vaccine products [5]. This project will quantify pre- and post influenza immune response to different influenza vaccine types.

LAIV has a storied past. When the formulation was still a trivalent one, a metanalyses showed LAIV to have superior VE and, for one season, was the ACIP preferred vaccine for children 2-8 years of age [6]. LAIV was previously shown to be more effective than IIV against antigenically different or drifted strains—thus, egg-based LAIV may provide broader protection than IIV. However, after introduction of the quadrivalent formulation, data from 2015-16 showed that LAIV's effectiveness was low in those aged 2-17 years [7], resulting in ACIP recommending against its use [8]. The manufacturer has since reformulated LAIV, specifically the H1N1 component, and it was included as a vaccination option in ACIP's recommendations, starting in 2018-19 [5]. However, its late reintroduction and initial disagreement by AAP and AAFP with the return to routine use, led to limited uptake in the US.

In a community trial of trivalent products in adults aged 18-49 years in 2007-8, IIV was efficacious whereas LAIV was not [9]. In a military population in 2006-7, the duration in years of being vaccine-naïve was correlated with trivalent LAIV effectiveness but not with trivalent IIV effectiveness[10] and the effect of vaccination was statistically significant for LAIV (P=0.04) but not for TIV (P=0.63).

Cell-culture inactivated influenza vaccine (Flucelvax) was approved by the FDA in 2012, and includes cell seed strains for all four components starting in 2019-2020. CcIIV most closely approximates the antigenicity of the influenza viruses circulating at the time of vaccine strain selection [11]. Among elderly persons in 2017-18, vaccine effectiveness was 10% greater for cell-cultured influenza vaccines relative to egg-based quadrivalent vaccines [12], however limited data are available for children and teens.

There are several factors that may complicate the serological immune response to LAIV. Compared to adults, children have been shown to have a significant IgA response to LAIV [4] which may afford protection not demonstrable by IgG levels alone. In addition, the presence of different microbial species in the nose, as well LAIV-related

alterations in the microbial species present in the nose, may result in variations in IgA antibody production [13].

Another consideration in vaccine response is the individual's prior vaccine history. Both T cells and B cells are required for effective immunity to influenza infection and vaccination. B cells secrete antibodies, which represent a major likely correlate of vaccine-mediated protection. T cells play two potential roles: first, CD8+ T cells can directly recognize and kill influenza-infected cells, and second, antibody production requires "help" from CD4+ T cells. The relative contributions of CD8+ and CD4+ T cells to influenza immunity in humans is less clearly understood than is the role of B-cell-secreted antibodies, but all these responses are likely to be important in controlling influenza virus replication. Children are likely to have different immune response profiles against influenza from adults, since each person's immune "repertoire" is shaped by his/her lifetime exposure to influenza viruses. Most adults have been infected with and/or vaccinated against influenza multiple times in their lives. Therefore, they enter each influenza season with a pre-existing repertoire of "memory" responses against virus strains to which they have been exposed previously, which may cross-react to varying degrees with antigens in the current season's vaccine. In contrast, children have few or no lifetime influenza infections and have fewer pre-existing memory responses when infected or vaccinated. A better understanding of the humoral and cell-mediated immune response to repeat influenza vaccination and different influenza vaccine types (LAIV and ccIIV) in children and teens with and without a recent history of vaccination is needed.

This study is the first head-to-head comparison of the current versions of LAIV and ccIIV, both of which are FDA-approved options for children aged 4 years and older. We will compare the differences in response among children/teens who were unvaccinated in the previous year in a randomized trial. We will also focus our recruitment efforts on children ages 4-8 years old, regardless of prior vaccination status, as this group was under-represented in 2019-20. The data on responses to repeated doses of ccIIV and of LAIV are limited and this study will enable determination of those responses by reenrolling persons who were enrolled in this study in 2019-20 and randomly assigning them to receive LAIV or ccIIV.

A. Specific Aims:

Primary Aim

Compare the serologic responses to cell culture-based quadrivalent influenza vaccine (ccIIV [Flucelvax]) versus live attenuated quadrivalent influenza vaccine (LAIV [FluMist]) in a randomized controlled trial (RCT) among racially diverse children/young adults 4-21 years of age. The primary endpoint is change in antibody titer.

a. Serology—based on hemagglutination inhibition assay (HAI) titers: Hypotheses: Mean fold rise (MFR) based on HAI titers and seroconversion will differ between LAIV and ccIIV.

Secondary Aims:

1. Compare the immune responses between ccIIV and LAIV.
2. The nasal microbiome may both effect and be affected by vaccination (2019 only)

B. Study Design and Methods

B.1 Study Design and population Years 1 and 2 <u>combined</u>

1. Total number of subjects to be enrolled at this site up to 480 enrolled over two years (of up to 650 screened)
2. Describe and explain the study design:
   This study will be an unblinded randomized controlled trial of 200-220 (2019) and up to 260 (2020) children, adolescents and young adults to examine their immune response following influenza vaccination. For 2020, all participants will have a baseline draw and a Day 28 (range 21-35 days) blood draw. A subset of up to 120 participants ages 9-21 **only** will have a blood draw on Day 7 (range 6-9 days)
3. Describe the primary and secondary study endpoints:
   Primary:
   -Seroconversion based on hemagglutinin inhibition (HAI) titers
   Secondary:
   - Day 0 and Day 28 Seroprotection based on HAI titers
   - Day 0 and Day 28 geometric mean comparisons of HAI titers

   The overall purpose is to understand immune responses related to influenza vaccination and their determinants. We will also look at serum IgG and IgA, cell mediated immunity indices, transcriptomics, genomic, protonomic, cell-mediated immunity, nasal microbiome (2019 only) and/or past vaccine experience effects on the immune response to influenza vaccination.

B.2 Study Design and Population Year 2 2020-2021 only

This study will be a randomized controlled trial of up to 260 children, adolescents and young adults to examine their immune response following influenza vaccination. All participants will have a blood draw at baseline and at Day 28 (range 21-35 days). A subset (up to 120) of participants ages 9 -21 will have a blood draw at Day 7 (range 6-9 days).

Actual recruitment numbers depend on when study can start and pandemic restrictions in place at the time of the study because a) the Day 28 blood draw should be completed before the influenza disease season so that influenza infections do not impact laboratory results; and b) the main student body is leaving the University in mid November per 2020 University policy. Therefore, we anticipate a possible enrollment decrease of 10% per week, given that the enrollment period has been shortened); and c)extra protections in place for participant and worker safety; this limits throughput due to the extra cleaning and social distancing requirements. (The University has granted official permission for this study in the Fall of 2020 after special application).

Recruitment and enrollment of the study population will take place at family medicine and pediatric clinics as well as at other sites, such as the University of Pittsburgh.

Screening for eligibility will be conducted by a Research Assistant either via phone, online, or in person with data entered directly into REDCap, a secure online database management system. (For screening eligibility surveys that are conducted via paper, information collected on this form will be entered into REDCap post-hoc.) Research assistants will meet with parents of potentially eligible children, or young adults themselves, to review study requirements and the consent form, giving parents/patients time to read through it thoroughly and answer questions. Then research assistant will obtain parental or young adult consent and child/teen assent. For research assistants without medical training, a physician or doctoral level pharmacist will be available either in person or via electronic device to review the study protocol and vaccine choices and answer any questions the parents and/or child/teen or young adult may have.

Remotely, written informed consent may also be collected after performing screening procedures, but prior to performing any of the research interventions/interactions. A REDCap PDF of the consent form will be emailed to the potential participant and reviewed with them. The participant receives the email and clicks on a link to see the consent. If they agree, they initiate the electronic consent process within REDCap, sign and submit the form. The signed consent document is stored securely in REDCap. The consenting party will document that they consented the participant by going back into the participant's consent to complete the fields on the electronic consent form or by a note to file in the participant record.

B.2.1 Inclusion and Exclusion Criteria

A. Inclusion criteria:

For 2019 Enrollment

1. Persons aged 4-21 years who have not received 2019-2020 vaccination and meet one of the following criteria:

a. Denies vaccination in 2018-19.

b. Participated in our 2018-19 study of childhood influenza vaccine.

c. Contingency if enrollment low: Persons with record-confirmed type of influenza vaccination in 2018-19.
2. Willing to receive seasonal influenza vaccine by randomization to LAIV or ccIIV;

For 2020 Enrollment
1. Persons aged 4-21 years who have not received 2020-21 season influenza vaccination;
2. Willing to receive seasonal influenza vaccine by randomization to LAIV or ccIIV;

B. Exclusion criteria:
1. Unable or unwilling to complete all required study activities, including informed consent and bloodwork;
2. Have a known immunocompromising condition or taking high doses of an immunosuppressant medication (e.g., high dose steroids defined as >/= 20 mg prednisone equivalent per day for >2 weeks);
3. Known to be pregnant;
4. Have a history of severe allergy to eggs or to influenza vaccine or any of its components.
5. Chronic use of aspirin or aspirin containing medication
6. In the last twelve months, have had asthma, wheezing, treatment for heart disease, treatment for seizures, or treatment for diabetes mellitus.
7. Participants who would place at risk (due to FluMist as a live attenuated vaccine) severely immunocompromised persons as a household or close contact.

B.2.2 Enrollment

Targeted enrollment numbers

Targeted enrollment is up to 130 individuals in each vaccine arm for 2020. Approximately ½ of the subjects would be recruited from our returning 2019-2020 vaccinated cohort of patients with three prior visits. Among non-cohort recruits, we will prioritize persons ages 4-8 years regardless of 2019-20 vaccination status and persons 9-21 years who were not vaccinated with seasonal influenza vaccine in the 2019-20 season. Enrollment in this study will begin after IRB and CDC approval is obtained and will continue until the target sample size for each group is reached with the goal of completing all baseline study visits by mid November of the current year. We will recruit from primary care and pediatric locations such as UPMC Shadyside Family Health Center; Children's Community Pediatrics (CCP) Primary Care Oakland; and, perhaps, East Liberty Family Health Center. We will also recruit past participants who were vaccinated at Falk Pharmacy. Because the Pitt+Me database was highly successful in 2019-20, we will use it to recruit new participants. Inclusion of older teenagers and young adults aged 18-21 years allows for college students using Falk pharmacy, as well as returning cohort members.

B.2.4 Enrollment procedures: Data and Blood Sample Collection

B.2.4.1a Enrollment visit

Eligible patients who agree to enroll will be consented prior to conducting further baseline visit enrollment procedures. Written informed consent includes permission to access medical records and/or state registry records to obtain data related to medical history, medication use, vitals, high-risk codes, and vaccination history.

A survey of demographics and health status will be collected at baseline, or if insufficient time, at a follow-up study visit. Data will be entered directly into REDCap (or if collected via paper will be entered into REDCap post-hoc.)

All participants will have blood drawn at baseline enrollment visit (may be done during the summer prior to vaccination). Refer to Table for blood volumes and tube types to be collected. The total blood volume for a particular participant is based on several factors including size of the participant's veins, limitations on number of PBMC specimens that the lab can process in a particular day, past PBMC yield from the participant (if known), enrollment site, past vaccination status, and courier transportation availability, given the need to process PBMCs quickly.

Upon consent, participants will be randomized to one of two vaccine types (LAIV or ccIIV) using a 1:1 computer generated simple randomization scheme. Randomization block size will be conducted in sets of four (2 LAIV and 2 ccIIV).

Table. Specimens to be collected for 2020

Age Group	Blood tube type	Day 0 visit	Day 7 post vax visit	Day 28 post vax visit	Total
4-8 years	SST Serology	5-10 mL	No visit	5-10 mL	10-20 mL
	CPT-PBMCs	4 mL	No visit	4 mL	8 mL
9-21 years	SST Serology	10 mL	--	10 mL	20 mL
	CPT-PBMCs on subset	24 mL	24 mL	24 mL	72 mL

B.2.4.1b Vaccination visit

All participants will receive influenza vaccine after the pre-vaccination blood draw according to their randomized influenza vaccine allocation. Both vaccines are FDA approved, licensed and recommended for these age groups. Vaccinations will be recorded in the EMR and Vaccine Information Sheets will be provided following standard clinical protocol. This visit may occur at the time of the enrollment visit provided that the vaccine to which the participant is randomized is available.

B.2--4.1c Blood draw procedures

All blood will be collected by trained personnel with experience drawing blood from children. The blood samples will be collected using Vacutainer tubes, appropriate for type of processing, and appropriately labeled with a uniform scheme (including study ID and collection time point).

Serum specimens will be aliquoted and frozen. Individual aliquots will be either shipped to CDC for analysis, analyzed by labs affiliated with the University of Pittsburgh or UPMC, or banked for future studies that might involve proteomics, immunology, transcriptomics, or genomics. .

B.2.4.2 Follow-up study visits

All participants will have a blood draw at Day 28 (range 21-35 days). A subset (up to 120) of participants ages 9 -21 will have a blood draw at Day 7 (range 6-9 days).

The amount of blood to be drawn at each timepoint is noted in the Table.

B.2.4.3 Specimen Processing and Sharing with Collaborators

Dr. John Alcorn's lab at the Rangos Research Building will process all blood specimens for storage and handling following standard protocols by trained study staff. Specimens will be shipped to CDC and CDC's chosen laboratory contractors as well as to other contracted laboratories for processing and analysis.

Flow cytometry results from CDC will be shared with Pitt so that further bioinformatics analyses can be conducted.

B. 3 Laboratory Methods

The laboratory tests consisting of assessing CMI responses including multiparametric flowcytometry of T and B cells, ILC, DC, antigen-specific T cells by ICCS, B-cell seq and Ig-seq to determine antibody clonotypes and their specificities, serological analyses including ADCC, HI, microneutralization, avidity and reactivities against historical strains will be conducted by CDC or CDC's chosen contractors.

B. 3.1 Hemagglutination Inhibition Antibody and Microneturalization Testing

Serum specimens will be tested by hemagglutination inhibition assays (HAI) to estimate antibody titer response against the vaccine strains. HAI testing will be conducted at CDC or by a CDC-designated laboratory. Miconeutralization titers may be conducted by CDC for selected antigen(s). Serum antibody levels may be measured by enzyme immunoassays at CDC.

B. 3.2 Peripheral Blood Mononuclear Cells (PBMCs)

PBMC will be analyzed at the CDC laboratory for cell mediated immunity. Briefly, innate immune subsets (NK cells, innate lymphoid cells, gamma/delta T cells), antigen-specific B cell plasmablasts, HA-specific memory B cells, phenotypic (subsets) and functional (cytokine production) characterization of CD4 and CD8 T cells will be done using multi-parametric flow cytometry. For the proposed flowcytometric analysis $20x10^6$ PBMC with greater than 85-90% viability are required. In addition, analysis of the changes in the B cell repertoire along with Ig-seq with LC/MS in response to prior vaccination status and/or vaccine types will be considered for a subset of vaccinees. For LC/MS, 1.5 ml-2 ml of sera is needed.

B. 3.3 Additional laboratory testing to be done if funding permits.

Pitt may utilize spectral flow cytometry to generate single cell protein expression data. We have recently adapted our flow cytometry panels to take advantage of the Cytek Aurora platform capabilities here at the University of Pittsburgh. This approach utilizes deconvolution of fluorescence data to allow for resolution of overlapping emission spectra. Our current panel for human PBMC is the following 26 antigens: CCR7, CD19, CD16, TCRγδ, CD14, CD8, PD1, CD56, CD4, CD28, CD11c, CD45Ra, CD3, CD25,

IgD, CD95, CD11b, CD38, CD57, CD27, CD123, CD127, HLADR, KLRG1, CD45R0, CD154. With this panel we can identify granulocyte, monocyte, and lymphocyte subsets with high resolution. For example, this panel allows for identification of CD8+ T cells, NKT cells, CD4+ T cells, B cells. NK cells, and lineage negative ILC, further segregated by activation and memory markers. All of this information is derived from a single flow cytometry run on $5x10^6$ cells.

Data are analyzed by multidimensional algorithms such as t-stoichastic neighborhood embedding (tSNE) or sequential pattern discovery using equivalence classes (SPADE) to cluster cell types based on the full panel of antigens stained rather than typical two dimensional staining analyses. tSNE works by first creating a probability distribution that dictates the relationships between various neighboring data points in high-dimensional space. It then tries to recreate a low dimensional space (two dimensional plot) that follows that probability distribution as best as possible to segregate cells based on similarity of marker expression. SPADE uses a similar approach to determine relationships between individual cells, but relays this information as a two dimensional branching tree plot that indicates the degree of similarity between cells. These algorithms allow for identification in three dimensions of overlapping cell subsets in two dimensional space and consideration of multiple stains at the same time, with the potential to identify new cell subsets.

C. Statistical Analysis

Pre- and post- vaccination geometric mean antibody titers (GMT) will be $\log_2$ transformed and compared among participants who received LAIV or ccIIV. The mean fold rise, seroconversion and seropositivity at Day 28 will be calculated.

Seroconversion will be defined as either a pre-vaccination HAI titer <1:10 and a post-vaccination HAI titer ≥1:40 or a pre-vaccination HAI titer ≥1:10 and a minimum four-fold rise in post-vaccination HAI antibody titer.

Fold-rise in HAI titers is the ratio of Day 28 log titers to Day 0 log titers and will be conducted with baseline titer as a covariate.

C.1 Power Analyses

Using a power calculation for a Chi square test with alpha set at 0.05 reveals the following:

Effect size	Final sample size	Power	Comments
0.3	90	.81	Fairly large effect size; unlikely to occur
0.3	81	.77	Fairly large effect size; assumes 10% failure to complete
0.1	81	.15	No power for small effect size
0.2	200	.81	Good power for moderate effect size
0.1	200	.29	No power for small effect size

https://www.anzmtg.org/stats/PowerCalculator/PowerChiSquare

If funding is available for the full sample, analyses based on prior 2019-20 vaccine history (none, egg-based, cell-based) will provide additional insight into vaccine immunogenicity.

D. Human Subjects

D.1. Consent

Participants in this study will provide written consent (parents and young adults) and assent (children/teens) as applicable for specimen collection, influenza vaccination, and medical record review.

Further details regarding data privacy and protection of human subjects are provided in the IRB application document.

D.2. Participant Compensation

Participants will be compensated for each visit:

- First visit = $35 plus $5 travel for a total of $40
- Second visit on subset = $25 plus $5 travel for a total of $30
- Final visit = $45 plus $5 travel for a total of $50

The maximum possible payment will be $90 (two visits)-$120 (three visits). If a separate vaccine visit is required, participants will be paid $30 for the blood draw and enrollment visit and $10 for a separate vaccine visit.

D.3. Threats to study and contingencies

In the event of a late start, some aspects of recruitment may be impacted: (1) PBMCs are time-consuming to process, leading to a limited throughput; thus, the sample size for PBMCs is constrained by starting later. (2) vaccination in the community is likely to start early, due to vaccination by community pharmacies and to mass purchase by UPMC; later recruitment is more difficult because some will have already been vaccinated.

Contingency plans: In the event of a late start, several contingencies will be used. First, a greater emphasis will be given to recruiting young adults, who are less likely to have been vaccinated early. Second, if one vaccine is delayed, then persons may be recruited per the randomization scheme and those randomized to the missing vaccine may have their baseline blood drawn but be asked to return for the rest of the study once the vaccine is available. Third, given that quality processing of the PBMCs is time consuming with limited throughput, overall target sample sizes may be reduced.

D.4 Timeline

	Aug	Sept- Nov	Dec	Jan	Feb-May	June-July
Prep, Supplies, Train						
Enroll, vaccinate, draw blood, process specimens						
Organize and ship to CDC (or Battelle) lab						
Conduct initial HAI						
Advanced Lab assays						
HAI statistical analyses						
Papers						
ClinicalTrials.gov Final						

REFERENCES

1. Fogel, B. and S. Hicks, *Influenza vaccination rates in children decline when the live attenuated influenza vaccine is not recommended.* Vaccine, 2017. **35**(39): p. 5278-5282.
2. Jackson, M.L., et al., *Influenza Vaccine Effectiveness in the United States during the 2015-2016 Season.* N Engl J Med, 2017. **377**(6): p. 534-543.
3. Zimmerman, R.K., et al., *2014–2015 influenza vaccine effectiveness in the United States by vaccine type.* Clinical Infectious Diseases, 2016. **63**(12): p. 1564-1573.
4. Manenti, A., et al., *Comparative analysis of influenza A(H3N2) virus hemagglutinin specific IgG subclass and IgA responses in children and adults after influenza vaccination.* Vaccine, 2017. **35**(1): p. 191-198.
5. Grohskopf, L.A., et al., *Prevention and Control of Seasonal Influenza with Vaccines: Recommendations of the Advisory Committee on Immunization Practices-United States, 2018-19 Influenza Season.* MMWR Recomm Rep, 2018. **67**(3): p. 1-20.
6. Osterholm, M.T., et al., *Efficacy and effectiveness of influenza vaccines: a systematic review and meta-analysis.* Lancet Infect Dis, 2012. **12**(1): p. 36-44.
7. Jackson ML, et al., *Influenza Vaccine Effectiveness in the United States — 2015/16 Season.* New England Journal of Medicine, 2017. **377**(6): p. 534-543.
8. Grohskopf, L.A., et al., *Prevention and Control of Influenza with Vaccines: Recommendations of the Advisory Committee on Immunization Practices, United States, 2015-16 Influenza Season.* MMWR Morb Mortal Wkly Rep, 2015. **64**(30): p. 818-25.
9. Monto, A.S., et al., *Comparative efficacy of inactivated and live attenuated influenza vaccines.* N Engl J Med, 2009. **361**(13): p. 1260-7.
10. Wang, Z., et al., *Live attenuated or inactivated influenza vaccines and medical encounters for respiratory illnesses among US military personnel.* JAMA, 2009. **301**(9): p. 945-53.
11. Ambrose, C.S., et al., *The efficacy of intranasal live attenuated influenza vaccine in children 2 through 17 years of age: A meta-analysis of 8 randomized controlled studies.* Vaccine, 2012. **30**(5): p. 886-892.
12. Izurieta, H.S., et al., *Relative effectiveness of cell-cultured and egg-based influenza vaccines among the U.S. elderly, 2017-18.* J Infect Dis, 2018. **220**(8): p. 1255-1264.
13. Salk, H.M., et al., *Taxa of the Nasal Microbiome Are Associated with Influenza-Specific IgA Response to Live Attenuated Influenza Vaccine.* PLoS One, 2016. **11**(9): p. e0162803.

CONSENT TO ACT AS A PARTICIPANT IN A RESEARCH STUDY

Title of Study: Immunological Response to Influenza Vaccination in Children, Adolescents, and Young Adults: A RCT of FluMist vs. Flucelvax

Principal Investigator: Richard K. Zimmerman MD MPH

Sponsor: Centers for Disease Control and Prevention

SUMMARY OF THE STUDY

This is a **voluntary research study** for persons **ages 4-21 years** to understand how well the influenza (flu) vaccine protects people from the flu.

What will you and/or your child do?

- Sign a consent form
- Answer some survey questions
- Make 1 visit before and 1 or 2 research visits after getting the flu vaccine this year
- A separate visit to receive vaccine may be needed based on vaccine availability

What will you as a 4-21 year-old study participant do?

- Allow us to draw blood at each visit to test for flu virus
- Allow us to review and collect information from your medical record

What will you or your child receive?

- $40 for the first visit for enrollment and vaccine; if two visits are required due to vaccine not available, you will receive $30 for enrollment visit and $10 for the vaccine visit for a total payment of $40
- $30 for the optional second visit 1 week after the vaccine visit
- $50 for the third visit at 4 weeks after the vaccine visit
- The potential total payment is $120 for three visits ($90 for two visits)
- Vaccine is provided at no charge, but your insurance may be billed for the clinical staff to administer it

How long will it take?

- Approximately 30 minutes for the first visit and 15 minutes for each additional visit

Are there any risks?

This is a low risk study, but there is a potential for

- Common risks of the blood draw include mild discomfort and bruising. Infrequent risks include excessive bleeding, scarring, infection, lightheadedness, nausea, or fainting.
- A breach of confidentiality or discomfort discussing personal matters in the survey

FULL CONSENT FORM
CONSENT TO ACT AS A PARTICIPANT IN A RESEARCH STUDY

Title of Study Immunological Response to Influenza Vaccination in Children, Adolescents, and Young Adults: A RCT of FluMist vs. Flucelvax

Principal Investigator
Richard K. Zimmerman MD MPH
Department of Family Medicine, University of Pittsburgh
4420 Bayard St. Suite 520
Pittsburgh PA 15260
(412) 383-2355

Co-Investigators

Children's Primary Care Oakland and University Center
Phone: (412) 692-7382
Dr. Judy Martin
Dr. Alejandro Hoberman
Dr. Nader Shaikh
Dr. Timothy Shope
Dr. Sonika Bhatnagar
Dr. Gysella Muniz Pujalt
Dr. Anne-Marie Rick
Jennifer Nagg, RN
Marcia Pope, RN
Melissa Andrasko, RN
Mary Ann Sieber, RN

Falk Pharmacy
Phone: (412) 623-6222
Dr. Amanda Jaber

Shadyside Family Health Center
Phone: (412) 692-2287
Dr. Lindsay Nakaishi
Dr. Lori Stiefel

East Liberty Family Health Center
Phone: (412) 661-2802
Dr. Richard Zimmerman

Univ. of Pittsburgh, Family Med.
Phone: (412) 383-2355
Dr. Mary Patricia Nowalk
Dr. John Maier
Dr. Katherine Williams

Lawrenceville Family Health Center
Phone: (412) 622-7343
Dr. Sandy Sauereisen
Dr. Mary Pat Friedlander

Sponsor Centers for Disease Control and Prevention (CDC)

We are conducting a research study with people of age 4 to 21 years who are receiving a flu vaccine from their doctor to help us understand how people respond to flu vaccine, also called influenza vaccine, and to better understand the body's protective response against flu. We will ask up to 260 people ages 4 through 21 years to take part in this study.

If you agree to participate on behalf of yourself or your child, we will ask you to complete a survey, allow us to review and collect information for your medical records, and draw blood before and after the flu vaccine this year.

The requested information will be collected by a research staff member and the blood draw performed by a trained person. The research assistants work for Dr. Zimmerman or the other listed Co-Investigators.

Flu vaccine is standard medical care and is highly recommended by physicians because it is recommended by the CDC for anyone 6 months of age and older. The flu vaccine will be one of two available licensed vaccines selected randomly, which is like flipping a coin to decide which vaccine will be given. The two vaccines randomly assigned are:

1. **FluMist** quadrivalent flu vaccine given as a nasal spray. FluMist flu vaccine is licensed for use in people ages 2-49 years old. FluMist quadrivalent vaccine was approved by the Food and Drug Administration (FDA) in 2010.

2. **Flucelvax** quadrivalent flu vaccine given as a shot. Flucelvax flu vaccine is licensed for use in patients 4 years of age and older. Because some patients are allergic to chicken eggs that are used in the manufacturing process of flu vaccine, Flucelvax, is an alternative flu vaccine that uses cell culture technology instead of eggs to grow the flu vaccine. Flucelvax was approved by the FDA in 2012 and available to patients in the United States starting in 2016.

The flu vaccine itself will be provided free of charge to every study participant but the cost of administrating it may be billed to your insurance provider by the clinic. Children ages 4-8 years who have not previously received flu vaccine will need two doses of either vaccine product.

Participation in this research involves storage of samples. We will collect and store blood at 2 visits as indicated below. For 9-21 year-olds, approximately 2 tablespoons (36 mL) of blood will be drawn at each study visit for a total amount of blood equal to approximately 5 tablespoons (72 mL). The optional 1-week visit involves drawing 2 tablespoons of blood (24 mL) for a total amount of blood equal to approximately 7 tablespoons (108 mL) for three visits. We may schedule an additional blood draw if not enough blood was collected at a previous visit, typically this would result in approximately 2 tablespoons of blood being collected for that visit in order to collect the volume needed for analysis. Children ages 4-8 years old would have only 2 teaspoons (10 mL) drawn at each of two study visits (enrollment and 1 month) for a total amount of blood of 4 teaspoons (20 mL).

All samples will have all personal identifiers removed, a research code applied, and may be tested for genomic and transcriptomics (e.g., messages that the body sends in response to vaccine which instructs the immune system on how to respond), and proteomic interactions (e.g. how the body fights infection) and may be used in other research tests to help us better understand immune response. These blood samples will be stored and banked for an indefinite time under the supervision of Dr. Alcorn, one of the Co-investigators, a CDC designated lab and/or at their contractor laboratories and provided to other contracted laboratories for testing.

The study schedule is outlined in the table below:

Day of study	**What**
Enrollment	Blood work, survey, $30 payment
Vaccine visit	Vaccine will be given at enrollment or at a separate visit, $10 payment
Visit 2 (6-9 days) after flu vaccine visit (Optional for ages 9-21 years only)	Blood work, $30 payment
Visit 3 (21-35 days) after flu vaccine visit	Blood work, $50 payment
Visit 4 (6-16 months) after flu vaccine visit	Additional visit if funded, and if you are interested in continuing

For those children ages 4-8 years who require 2 doses of vaccine, the second vaccine visit will include a payment of $10 and the timing of the blood draws would be as follows: Day 0 prior to first vaccine dose and Day 28 after second vaccine dose.

As part of this research, we will use past, current and future (until August 2021) information from medical records on 4 to 21-year-old participants. As part of this research study, we are requesting your authorization or permission to review your medical records to determine whether you meet the conditions for participation in this study, to compare your earlier test results to the self-reported information from this study, and if possible, to use your previous exam results in place of, or in addition to, some of the exams needed for this study. This authorization is valid for an indefinite period of time. The following information may be collected: height, weight, medical problem lists, medications, vaccination history, and diagnosis codes. This information will be collected by members of the research staff under the direction of Dr. Zimmerman and it will also be given a unique research code before names are removed.

This identifiable medical record information will be made available to members of the research team for an indefinite period of time.

You can always withdraw your authorization to allow the research team to review your medical records by contacting the investigator listed on the first page and making the request in writing. If you do so, you will no longer be permitted to participate in this study. Any information obtained from you up that point will continue to be used by the research team.

Data and samples without patient names will be shared with the multicenter research team including the CDC and their chosen lab contractors. Any information that could identify you or your child will be removed before being sent out.

This information may be used by our research team for the purposes of this project, and/or for any retrospective research studies. We will keep this information for an indefinite period of time, to be used for research studies related to influenza and response to vaccination.

How will the privacy of medical record information be protected?
Only Dr. Zimmerman, the co-investigators, the research team, and clinical staff providing the flu vaccine will be aware of your or your child's participation in this study. We will not link names to any of the information we release. This information will be identified by a code number, and the information linking these numbers with you or your child's identity will be kept separately. You or your child's identity will not be revealed in any description or publications. Although we will do everything in our power to protect your or your child's privacy and the confidentiality of these records, just as with the use of medical information for healthcare purposes, we cannot guarantee the privacy of research records.

De-identified research data and blood will be placed in a database called the Gene Expression Omnibus (GEO) and may be shared with investigators conducting other research; this information will be linked by assigning the same research identification code to both the research data and blood samples.

These research data/samples may contribute to a new discovery or treatment. In some instances, these discoveries or treatments may be of commercial value and may be sold, patented, or licensed by the investigators and the University of Pittsburgh for use in other research or the development of new products. You or your child will not retain any property rights nor will you or your child share in any money that the investigators, the University of Pittsburgh, or their agents may realize.

Authorized representatives from the study sponsor, federal regulatory agencies, and the University of Pittsburgh Office of Research Protections may review your or your child's identifiable information for the purpose of monitoring the conduct of this study. To confirm vaccination status, state and federal immunization records will be checked. In unusual cases, these research

records may be released in response to an order from a court of law.

We will protect your privacy and the confidentiality of your records, as described in this document, but cannot guarantee the confidentiality of your research records, including information obtained from your medical records, once your personal information is disclosed to others outside UPMC or the University.

There is the possibility that you or your child may be eligible for other research studies independent of this one. You may be contacted by members of our research teams to determine you/your child's interest in other studies, but there is never under any obligation to participate.

Potential Risks of the Survey: The risks associated with this study include the potential for a breach of confidentiality. To reduce the risk of that happening, we will protect the confidentiality of this information. You or your child may also feel some discomfort or embarrassment discussing personal matters. You or your child may stop answering questions at any time. We will also inform you if we learn of any new significant study risks associated with this research.

Although every reasonable effort has been taken, confidentiality during Internet communication activities cannot be guaranteed and it is possible that additional information beyond that collected for research purposes may be captured and used by others not associated with this study.

Risks of the Vaccine: Flucelvax may commonly cause pain, bruising, or redness at the site of injection. FluMist may commonly cause runny nose or nasal congestion. Both Flucelvax and FluMist may less commonly cause headache, tiredness or crankiness, muscle aches, loss of appetite or overall feeling unwell. Rarely, Flucelvax and FluMist may case a fever or a severe allergic reaction. Participants who receive FluMist should avoid contact with severely immunocompromised individuals for at least 7 days following vaccination to avoid possibly infecting them with the flu virus.

Risks of the Blood Tests: Bruising, soreness, or rarely, excessive bleeding, infection, or scarring may occur as a result of the needle sticks to obtain blood.

If you believe that the research procedures have resulted in an injury to you or your child, immediately contact the Principal Investigator who is listed on the first page of this form. Emergency medical treatment for injuries solely and directly related to participation in this research study will be provided by the hospitals of UPMC. Your insurance provider may be billed for the costs of this emergency treatment, but none of those costs will be charged directly to you. If your research-related injury requires medical care beyond this emergency treatment, you will be responsible for the costs of this follow-up care. At this time there is no plan for any additional financial compensation. You do not, however, waive any legal rights by signing this form.

Research Use of Genetic Information: The risks associated with gene studies include the potential for a breach of confidentiality which could affect future insurability, employability, or reproduction plans, or have a negative impact on family relationships and/or result in paternity suits or stigmatization.

A federal law, called the Genetic Information Nondiscrimination Act (GINA), generally makes it illegal for health insurance companies, group health plans, and most employers to discriminate against you based on your genetic information. This law will protect you in the following ways:

• Health insurance companies and group health plans may not request your genetic information that we get from this research.

• Health insurance companies and group health plans may not use your genetic information that we get from this research when making decisions regarding your eligibility or premiums.
• Employers with 15 or more employees may not use your genetic information that we get from this research when making a decision to hire, promote, or fire you or when setting the terms of your employment.

Be aware that this new federal law does not protect you against genetic discrimination by companies that sell life insurance, disability insurance, or long-term care insurance, nor does it protect you against genetic discrimination by all employers.

The data, samples, and genetic data generated from samples may be shared with other researchers and with federal repositories, in a de-identified manner (without identifiers).

You or your child will benefit from participating in this research by receiving the flu vaccine at no charge. Your insurance may be billed for the clinical staff to administer the vaccine. The information we obtain may help us better understand how immune system responses to flu vaccines differ in and how well flu vaccines work to prevent flu.

Compensation for this study will go to you or your child as the 4- through 21-year-old participant whose name, address, and social security number will be released to the Accounting Office at the University of Pittsburgh. All compensation is taxable income to the participant regardless of the amount. If a participant receives $600 or more in a calendar year from one organization, that organization is required by law to file a Form 1099 – Miscellaneous with the IRS and provide a copy to the taxpayer. Individuals who do not provide a social security number may still participate in the research, but the IRS requires that 28% of the payment be sent by the institution to the IRS for 'backup withholding'; thus you would only receive 72% of the expected payment.

Participation in this research study is completely voluntary. If you or your child do not agree to participate in this research study, this decision will have no effect on you or your child's current or future relationship with the University of Pittsburgh, UPMC or its affiliated healthcare providers or healthcare insurance providers. You or your child can still receive flu vaccine from your doctor's office, even if you or your child/teen does not agree to participate in this study. You or your child may be withdrawn from the study by the research team unable to return for follow-up blood draw visits, if not enough blood is able to be collected, or if you or your child develops a new health condition that arises that prevents future participation.

If you choose not to participate in this study, you can choose to receive an influenza vaccination from your physician's office or local pharmacy through your medical insurance plan.

If you decide you no longer wish for you or your child to participate after you have signed the consent form, you should **contact Dr. Zimmerman or his research team at (412) 383-1130**. A decision to withdraw from this study will have no effect on you or your child's current or future relationship with the University of Pittsburgh or with UPMC or its affiliate health care and insurance operations. If you or your child withdraw (or are withdrawn) from this study, the data and blood samples collected before the date of withdrawal may still be used and shared with other researchers and CDC.

****

VOLUNTARY CONSENT

The above information has been explained to me and all of my current questions have been answered. I understand that I am encouraged to ask questions about any aspect of this research study during the course of this study, and that such future questions will be answered by a qualified individual or by the investigator(s) listed on the first page of this consent document at the telephone number(s) given. I understand that I may always request that my questions, concerns or complaints be addressed by a listed investigator.

I understand that I may contact the Human Subjects Protection Advocate of the IRB Office, University of Pittsburgh (1-866-212-2668) to discuss problems, concerns, and questions; obtain information; offer input; or discuss situations in the event that the research team is unavailable.

By signing this form, I consent to participate in this research study and provide my authorization to share my medical records with the research team.

PARTICIPANT AGE:_____. PARTICIPANT STUDY ID _____

CONSENT FOR PARTICIPANTS AGE 18 THROUGH 21

_____	_____	_____
Participant's Signature	Printed Name of Participant	Date/Time

****

ASSENT FOR CHILD PARTICIPANT AGE 14-17 YEARS

This research has been explained to me, and I agree to participate (for children 14-17 years of age or younger who may be developmentally able to provide assent).

_____
Participant's (Child's) Name (Print)

_____ _____
Participant's (Child's) Signature Date/Time

If child is developmentally too young to assent, check here: _____

PARENTAL CONSENT FOR MINOR CHILD LESS THAN 18 YEARS

I understand that, as a minor (age less than 18 years), the above-named child/teen is not permitted to participate in this research study without my consent. Therefore, by signing this form, I give my consent for his/her participation in this research study and authorize the use of his/her medical record information for the purposes described above. A copy of this consent form will be given to me.

_____ _____ _____
Parent Signature Relationship to child (i.e. parent) Date/Time

VERIFICATION OF EXPLANATION

I certify that I have explained the nature and purpose of this research study to the above-named individual(s), and I have discussed the potential benefits and possible risks of study participation. Any questions the individual(s) have about this study have been answered, and we will always be available to address future questions, concerns or complaints as they arise. I also certify that I have carefully explained the purpose and nature of this research study to the child subject in age appropriate language. He/she has had an opportunity to discuss it with me in detail. I have answered all his/her questions and he/she has provided affirmative agreement (i.e. assent) to participate in this study.

_____ _____
Signature of Person Obtaining Consent Date/Time

_____ _____
Printed Name of Person obtaining Consent Role in Research Study

Voluntary Consent

The above information has been explained to me and all of my current questions have been answered. I understand that I am encouraged to ask questions about any aspect of this research study during the course of this study, and that such future questions will be answered by a qualified individual or by the investigator(s) listed on the first page of this consent document at the telephone number(s) given. I understand that I may always request that my questions, concerns or complaints be addressed by a listed investigator.

I understand that I may contact the Human Subjects Protection Advocate of the IRB Office, University of Pittsburgh (1-866-212-2668) to discuss problems, concerns, and questions; obtain information; offer input; or discuss situations in the event that the research team is unavailable.

By signing this form, I consent to participate in this research study and provide my authorization to share my medical records with the research team.

Parental Consent for Minor Child (< 18 Years Old)

I understand that, as a minor (age less than 18 years), the below-named child/teen is not permitted to participate in this research study without my consent. Therefore, by signing this form, I give my consent for his/her participation in this research study and authorize the use of his/her medical record information for the purposes described above. A copy of this consent form will be given to me.

Guardian First Name	_____
Guardian Last Name	_____
Please click on the link to the right to add guardian's signature.	_____
Date of Guardian Signature	_____
Participant Aged 14-17 is developmentally able to provide assent?	◯ Yes ◯ No
Participant is developmentally too young to assent	◯ Yes ◯ No (14-17 years of age or younger who may be developmentally able to provide assent)

Participant Information

Participant First Name	_____
Participant Last Name	_____
Participant Date of Birth	_____
Calculated age at enrollment	_____ (Must be < =21 years at Enrollment)

Participant Assent

This research has been explained to me, and I agree to participate

Please click on the link to the right to add participant's signature.

_____

Study Member Certification

VERIFICATION OF EXPLANATION

I certify that I have explained the nature and purpose of this research study to the above-named individual(s), and I have discussed the potential benefits and possible risks of study participation. Any questions the individual(s) have about this study have been answered, and we will always be available to address future questions, concerns or complaints as they arise. I also certify that I have carefully explained the purpose and nature of this research study to the child subject in age appropriate language. He/she has had an opportunity to discuss it with me in detail. I have answered all his/her questions and he/she has provided affirmative agreement (i.e. assent) to participate in this study.

Study Team Member Obtaining Consent

_____

Study Team Member Role in Research Study

◯ MD/RN
◯ RA

Remember to obtain Physician Video Consent Signature through Physician Documentation Survey in Redcap.

Study Team Member Signature

_____

Date of Study Team Member Signature

_____