2.1.2 Secondary Objectives

1. To compare the proportions of local and systemic reactions (other than moderate/severe injection site pain) after aIIV3 and IIV3-HD in the full study population and by age-group (65-79 years and ≥80 years)
2. To describe and compare changes in health-related quality of life after aIIV3 and IIV3-HD in the full study population and by age-group
3. To compare serum hemagglutination inhibition (HAI) antibody titers after aIIV3 and IIV3-HD for each of the three influenza vaccine strains contained in the respective vaccine for that season in the full study population and by age (except for seroconversion for the H3N2 strain in the full study population)

2.1.3 Exploratory Objectives:

1. To describe how the reactogenicity events affect health-related quality of life after IIV3 and IIV3-HD in the full study population and by age-group
2. To describe and compare participant perceptions about the vaccination experience
3. To describe participant perceptions of methods of adverse event monitoring in older adults receiving influenza vaccines.
4. To describe injection site pain immediately after vaccination after aIIV3 and IIV3-HD
5. To describe and compare the proportions of immediate adverse events and unsolicited adverse events after aIIV3 and IIV3-HD in the full study population and by age-group (65-79 years and ⩾80 years)
6. To describe factors (e.g., statin use) associated with reactogenicity to aIIV3 and IIV3-HD
7. To describe the safety of aIIV3 and IIV3-HD after repeat administration of the same product over 2 consecutive influenza seasons in a subset of subjects
8. To describe the relationship between reactogenicity and immunogenicity for aIIV3 and IIV3-HD
9. To explore factors (e.g., statin use) associated with immunogenicity for aIIV3 and IIV3-HD
10. To describe the immunogenicity of aIIV3 and IIV3-HD after repeat administration of the same product over 2 consecutive influenza seasons in a subset of subjects
11. To assess changes in serum hemagglutination inhibition at 1 month after aIIV3 and IIV3-HD vaccination, and at 6 months after vaccination in a subset of subjects

2.2 Study Outcome Measures

Where possible, all outcome measures will be evaluated and compared between the two treatment groups for the full population and in both age subsets (65-79 years and ≥80 years)

2.2.1 Primary Outcome Measures:

1. Comparison of the proportion of subjects reporting moderate/severe injection site pain within the first week post-vaccination in both treatment groups.

2. The frequency and descriptions of serious adverse events and adverse events of clinical interest observed in the two treatment groups.

3. H3N2 HAI seroconversion: The proportion of subjects achieving H3N2 seroconversion at day 29 (an HAI titer $\geq$ 1:40 at day 29 if the baseline titer is < 1:10 or a minimum four-fold rise in HAI titer if the baseline titer is $\geq$ 1:10) in the respective season's vaccine.

2.2.2 Secondary Outcome Measures

1. Comparison of local and systemic reactions within the first week post-vaccination in both treatment groups.
2. Change in scores on the Late Life Function & Disability Instrument (Year 1 only), EuroQOL 5 dimensions-5 level (EQ-5D-5L) and EuroQOL visual analogue scale (EQ VAS) pre-vaccination and post-vaccination will be compared between the vaccination groups and age groups.
3. HAI titers by vaccination group and age group (except for seroconversion to H3N2 strain for the full study population):
    a. The proportion of subjects achieving seroconversion at day 29 (an HAI titer $\geq$ 1:40 at day 29 if the baseline titer is < 1:10 or a minimum four-fold rise in HAI titer if the baseline titer is $\geq$ 1:10) for H1N1 and influenza B and H3N2 (by age group only) in the respective season's vaccine
    b. Proportion of subjects with a seroprotective HAI titer (≥ 1:40) pre- and post-immunization at day 29 for each IIV antigen in the respective season's vaccine
    c. The geometric mean HAI titer (GMT) for each IIV antigen in the respective season's vaccine

2.2.3 Exploratory Outcome Measures

1. Associations between moderate/severe local and systemic reactogenicity events and quality of life outcomes in the full study population and by age group
2. Comparison between vaccination groups of proportion of participants with negative and positive perceptions of the vaccination experience based on responses to the Perceptions of Vaccination Experience questionnaire.
3. Proportion of participants with difficulty performing adverse event monitoring based on responses to a questions to the Perceptions of Vaccination Experience questionnaire
4. Mean injection site pain scores immediately after vaccination in both vaccination groups using the Faces Pain Scale
5. Compare the frequency and descriptions of immediate reactogenicity and immediate serious adverse events and adverse events of clinical interest between vaccination groups.
6. Associations between reactogenicity and demographic, co-morbidity and medication factors from subjects' medical histories, including statin use
7. Compare changes in local and systemic reactions in subjects who receive vaccine in both study years
8. Associations between HAI titers and moderate/severe local and systemic reactogenicity events.
9. Associations between HAI titers and demographic, co-morbidity and medication factors in subjects' medical histories including statin use
10. Comparison of seroprotection and seroconversion as defined by HAI titers and geometric mean HAI titers in subjects who receive vaccine in both study years

11. Comparison of seroprotection and seroconversion as defined by HAI titers and geometric mean HAI titers between 1 month and 6 months in the same subjects (Year 1 only)

3 STUDY DESIGN

3.1 Main study design

This study is a prospective, randomized, blinded clinical trial to assess the safety of aIIV3 versus IIV3-HD in ≥440 adults age ≥65 years enrolled at Duke University Medical Center (Lead Contractor), ≥340 adults age ≥65 years enrolled at Boston Medical Center (BMC) (Contributing Contractor), and 100 adults age ≥65 years enrolled at Cincinnati (Contributing Subcontractor). Participants will be enrolled in 2017-18 (Duke and Boston) and 2018-19 (Duke, Boston, and Cincinnati) influenza seasons. Unblinded, licensed staff will perform vaccinations, and all other study personnel and subjects will be blinded throughout the study, with the exception of the Duke Project Manager, Boston Pharmacy, and Cincinnati Pharmacy staff (who have no involvement with study subjects). In addition, the study statistician will be blinded during data analysis. Older adults who have not received IIV during the respective influenza seasons during which they are recruited will be enrolled. Detailed health, demographic and health-related quality of life data will be collected from study participants at baseline prior to influenza vaccine receipt. With Day 1 serving as the day of vaccination, participants will be followed through Day 8 (total 8 days) for symptoms of reactogenicity as described in Section 5.4. Health-related quality of life and vaccination experience data will be collected during this time period. Participants will be followed through Day 43 for serious adverse events and adverse events of clinical interest, including health care utilization, as described in Sections 5.4 and 5.6.

3.2 Laboratory Studies

3.2.1 Influenza Hemagglutination Inhibition Assay

Participants will have blood draws on Day 1 (before vaccination) and Day 29 to be stored for serum hemagglutination inhibition (HAI) antibody titers. During 2017-18 influenza season (Year 1), a subset of approximately 100 patients at Duke will have a blood draw at Day 181 for an additional HAI titer. If funding is available, HAI antibody titers will be compared between groups receiving aIIV3 or IIV3-HD for each of the three influenza vaccine strains contained in the respective vaccines for that season. Additionally, if funding available, 60 year 1 participants will receive repeat immunization with the same vaccine in year 2 at Duke, and HAI titers will be performed on serum from Day 1 and Day 29. Participants will not receive individual HAI antibody titer results; these are not routinely used in clinical practice.

3.2.2 Future studies

Additional blood will be stored for further immune analyses depending on funding availability. Biologic specimens collected as part of this study and used in other studies examining the immune response to influenza vaccine will be linked to information (including identifying information) that participants provided in the current study. Participants are not expected to receive results of any future testing of their specimens.

4 STUDY ENROLLMENT AND WITHDRAWAL

4.1 Subject Inclusion Criteria

Subjects who meet all of the following criteria will be eligible to participate in this interventional study.

1. Persons aged ≥65 years, living in the community
2. Intention of receiving IIV vaccine based on ACIP-CDC guidelines
3. Willing to provide written informed consent prior to initiation of any study procedures
4. Able to speak English
5. Able and willing to complete baseline assessments and questionnaires, and to allow information to be collected from their electronic medical record
6. Able and willing to complete post-vaccine assessments and questionnaires independently or with assistance
7. Able and willing to have blood drawn for the study
8. Able and willing to return in about one month for a follow-up visit including completing questionnaires and having another blood test
9. Access to and ability to use a phone, independently or with assistance
10. Adequate vision and motor skills to complete the symptom diary form independently or with assistance.
11. Not living in a skilled nursing facility/nursing home/long term acute care facility

4.2 Subject Exclusion Criteria

Subjects who meet any of the following criteria will not be eligible to participate in this study:

1. Influenza vaccine receipt during the current influenza season prior to study enrollment
2. Enrolled in this study during the 2017-18 (Year 1) influenza season
   Note: Year 1 study participant will only be enrolled in Year 2 if they are participating in the sub-study on repeat vaccination
3. Has immunosuppression as a result of an underlying illness or treatment, or use of anti-cancer chemotherapy or radiation therapy within the preceding 12 months.
4. Has an active neoplastic disease (excluding non-melanoma skin cancer or prostate cancer that is stable in the absence of therapy) or a history of any hematologic malignancy*
   **Participants with a history of malignancy may be included if, after previous treatment by surgical excision, chemotherapy or radiation therapy, the participant has been observed for a period that in the investigator's estimation provides a reasonable assurance of sustained cure*
5. Thrombocytopenia, bleeding disorder, or anticoagulant use contraindicating intramuscular injection
6. Receipt of blood or blood-derived products in the past three months
7. History of febrile illness ($\geq$ 100.0°F or 37.8°C) within the past 24 hours prior to IIV administration (temporary deferral)
8. Contraindication to IIV receipt including history of severe allergic reaction after a previous dose of any influenza vaccine; or to a vaccine component*, including egg protein; or a latex allergy
   **Formaldehyde, Octylphenol ethoxylate, neomycin, kanamycin, barium, cetyltrimethlyammonium bromide (CTAB)*
9. Any history of Guillain-Barré syndrome

Procedure	Visit 1 Day 1	Visit 2 Day 3 + 2	Unscheduled Visit	Visit 3 Day 9 + 3	Visit 4 Day 29 ± 7	Visit 5 Day 43 + 7	Visit 6 Day 181 + 14[a]
Participant perception of vaccination	X[d]			X[d]	X[d]		
Baseline reactogenicity	X[c]						
Venipuncture	X				X		X
Randomization	X[d]						
Vaccination	X						
Diary & supplies	X						
Assess for injection site pain at least 15 min. after immunization	X						
At ≥ 15 min. assess for any immediate reactogenicity symptoms	X						
Obtain solicited adverse events		X	X[b]	X			
Obtain unsolicited adverse events		X	X	X	X	X	
Obtain SAE information, AEs of clinical interest, and new onset medical conditions		X	X	X	X	X	
Obtain health care utilization data		X	X	X	X	X	

[a]For subset of approximately 100 subjects receiving third blood draw 6 months post-vaccination in 2017-18
[b]For unscheduled visits, solicited AEs will be collected only for days ≤9
[c]Baseline reactogenicity will be performed before venipuncture and vaccination
[d]Does not apply to subjects participating in the Year 2 repeat vaccination during study Year 2
[e]Will collect Day 3 and Day 9 health related quality of life instruments (EQ-5D-5L and Vaccine Reaction Questionnaire) on V3 phone call

Visit 1, Study Day 1 - Screening, Enrollment, and Vaccination (Clinic Visit)

- Obtain written informed consent and release of medical record information
    - Note: In Year 2, subjects participating in the sub-study on repeat vaccination will sign a new consent form
- Determine if subject will participate in long term immunogenicity sub-study or repeat vaccination sub-study (Year 1 only)
- Review and confirm study eligibility
- Perform cognitive assessment with the Mini-Cog tool[22] (and RUDAS if needed)(Appendix B,C)
    - Scores 3-5 on MiniCog will be eligible
    - Scores 0-1 on MiniCog will be ineligible
    - Scores of 2 on MiniCog: person will undergo further screening with RUDAS. If person scores 23 or higher (range = 0-30), they will be eligible.
    - Potential participants may have low scores on the cognitive screening tests and be ineligible for the study. In this case, the study doctor or designee will review the results with the individual and recommend follow-up with the individual's health care provider.

5.4.2 Reporting of Adverse Events

An adverse event (AE) is any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product and which does not necessarily have to have a causal relationship with this treatment.

If indicated, AEs occurring during the study will be reported to the Vaccine Adverse Event Reporting System (VAERS). The National Childhood Vaccine Injury Act requires healthcare providers to report the following AEs to VAERS:

- Any adverse event listed by the vaccine manufacturer as a contraindication to further doses of the vaccine; or
- Any adverse event listed in the VAERS Table of Reportable Events Following Vaccination [PDF - 75KB] that occurs within the specified time period after vaccination.

In addition, CDC encourages reporting of any clinically significant adverse event that occurs in a patient following a vaccination, even if there is uncertainty regarding if a vaccine caused the event.

A serious adverse event (SAE) is defined as an AE that meets one of the following conditions[24]:

- Results in death
- Is life-threatening (defined as immediate risk of death at the time of the event)
- Requires inpatient hospitalization (initial or prolonged)
- Results in a persistent or significant disability/incapacity
- Any other important medical event that may not result in death, be life threatening, or require hospitalization, may be considered an SAE when, based upon appropriate medical judgment, the event may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed above.

SAE and AE reporting will occur consistent with institutional policy. The original verbatim terms used by investigators to identify SAEs and adverse events of clinical interest in the case report form will be mapped to preferred terms using the Medical Dictionary for Regulatory Activities (MedDRA) dictionary (http://www.meddra.org/).

Vaccine-related SAEs will be medically attended per routine care

SAEs will be reported promptly to the overseeing IRBs in accordance with institutional procedures. Any unanticipated problems resulting from study conduct related to participation will be reported promptly to the reviewing IRBs and CDC, in accordance with institutional procedures.

The duration of subject's participation in the study varies based on their involvement in the immunogenicity sub-study. Therefore, the period for monitoring and reporting SAEs varies among subjects as shown below:

- Subjects NOT participating in long-term immunogenicity subset: duration of study participation is 42-days post vaccination
- Subjects participating in long-term immunogenicity subset: duration of study participation is 181 days post-vaccination

The study will report only SAEs occurring during each subject's participation in the study.

More information on potential risks and benefits is located under Human Subjects, section 8.3.

5.4.3 Safety Monitoring Plan

Although FLUAD® and Fluzone® High-Dose are licensed vaccines, there is less US safety experience with these vaccines than with standard influenza vaccines, particularly for FLUAD®, which was recently licensed in the United States (November 24, 2015). Fluzone® High Dose was licensed on December 23, 2009 in the United States. Also, less data are available in persons ≥80 years or for those with mild cognitive impairment than in younger, healthier adults. This is the first study conducted in the CISA Project where at least one death is somewhat likely to occur during the study period in a study participant (due to the older age-group of the participants). Therefore, the goal of the safety monitoring plan is to protect the health of the study population and ensure adequate communication of potential risks, and to provide situational awareness of potential safety signals from this study to CDC Immunization Safety Office (ISO) leadership. This plan is designed to monitor safety while minimizing introduction of bias into the study and minimizing burden to study investigators. The safety monitoring plan is described in Appendix H.

5.5 Health-Related Quality of Life (HRQOL)

5.5.1 Generic Measures of HRQOL

Late-Life Function & Disability Instrument (LLFDI) and Late-Life Function & Disability Instrument – Computer Adaptive Test (LLFDI-CAT) (Year 1 Only)

The Late-Life FDI is an HRQOL instrument for assessing function (ability to perform discrete actions or activities as part of daily routines) and disability (socially-defined tasks) in community-dwelling older adults[25-27]. The function component is a 32-item questionnaire that assesses how much difficulty a person has doing a range of upper extremity, basic lower extremity, and advanced lower extremity functions. The response categories for the function instrument are none, a little, some, quite a lot, cannot do in relation to the question, 'How much difficulty do you have?'. The disability component is a 16-item questionnaire that assesses how frequently a community dwelling older adult performs a particular task and the extent to which they feel limited in doing the task. The response categories for the disability instrument are very often, often, once in a while, almost never, never in response to 'how often do you . . ?' and not at all, a little, somewhat, a lot, completely in response to 'to what extent do you feel limited in?'

Each instrument generates a raw score, scaled score, and standard error for each dimension. The function component displays a total function score as well as separate scores for upper extremity, basic lower extremity, and advanced lower extremity subscales. The disability component displays a frequency total score, with social role and personal role subscale scores, and a limitation total score, with instrumental role and management role subscale scores. The scores range from 0-100 with high scores indicating higher levels of functioning and ability.

For the function section, the minimum clinically important difference ranges from 2.7 – 4.3. The disability section is split into two dimensions: limitations and frequency in doing a task. For limitations, the minimum clinically important difference is 16.7. For frequency, the minimum clinically important difference is 7.8.

The Late-Life Function & Disability Instrument – Computer Adaptive Test (LLFDI-CAT) is a modification of the LLFDI that expands the number of items and incorporates the World Health Organization's International Classification of Functioning, Disability Health domains of activity limitations (function) and participation restriction (disability)[28] (Appendix I). The Computer Adaptive Test method significantly reduces administration time and respondent burden. The LLFDI-CAT uses the same response categories as the LLFDI and generates similar summary and subscale scores

These instruments have several advantages for use in this study. They provide measures of extremity function and multiple activities for community dwelling older adults. They are validated, reliable, and responsive in older populations and various diseases. They are designed for self-completion. They employ a standardized scoring system, and are free of charge.

EQ-5D

The EQ-5D is a standardized, generic measure of health status that provides information on health-related quality of life and activities of daily living relevant to older adults: mobility, self-care, usual activities, pain/discomfort and anxiety/depression (http://www.euroqol.org/)[29]. In addition, the instrument contains the EQ Visual Analogue Scale (EQ-VAS) which measures the respondent's self-rated health.

The EQ-5D-5L is the new version of the EQ-5D that increases the levels of severity from three to five to significantly increase reliability and sensitivity while maintaining feasibility and reducing ceiling effects (Appendix J)[30,31]. The descriptive system comprises 5 dimensions of mobility, self-care, usual activities, pain/discomfort, anxiety/depression. For each of these dimensions, there are 5 response levels: no problems, slight problems, moderate problems, severe problems, and extreme problems. The respondent is asked to indicate his/her health state by ticking in the box against the most appropriate statement in each of the 5 dimensions. This decision results in a 1-digit number expressing the level selected for that dimension. The digits for 5 dimensions can be combined in a 5-digit number describing the respondent's health state from 11111 as best health and 55555 as worst health These numbers are converted to a Utility Index that ranges from -0.109 (worst health) to 1.000 (best health) for US specific values. The minimum clinically important difference ranges from 0.05 to 0.1 depending on health conditions being studied. The EQ VAS records the respondent's self-rated health on a 20 cm vertical, visual analogue scale with endpoints labelled 'the best health you can imagine' (100) and 'the worst health you can imagine' (0). The respondent marks an 'X' on the scale number to indicate how their health is 'today.' The minimum clinically important difference on the VAS is 8.

The EQ-5D-5L, EQ-VAS have several advantages for use in this study. The measure is applicable to a wide range of health conditions and treatments and provide a simple descriptive profile and a single index value for health status. It has been validated in US and international populations and in older adults[29,32,33]. The measure is useful for monitoring the health status of patient groups at different moments in time and assessing the seriousness of conditions at

- All cryovial aliquots will be barcode labelled and contain a unique identifier via REDCap. Numbers should be placed lengthwise on the tube.
- Freeze the cryovials at -80°C in the temperature-monitored research center freezer for future shipment.
- Serum aliquots will be stored in the Duke Human Vaccine Institute Accessioning Lab, the Boston at the Maxwell Finland Laboratory for Infectious Diseases, and at Cincinnati Children's Hospital until planned HAI analyses at which point the samples stored at BMC and Cincinnati will be shipped to Duke where all HAI analyses will be done

6 LABORATORY ANALYSES

6.1 Influenza Hemagglutination Inhibition (HAI) Assay

Influenza Hemagglutination Inhibition (HAI) Assays will be performed on sera collected, contingent on additional funding. Briefly, reference wild-type, reassortant, or vaccine virus strains representative of the specific viral antigens included in the 2017-2018 and 2018-2019 influenza vaccine will be used to evaluate the relative levels of all three influenza strain-specific antibodies in participant serum samples collected pre- and 28 days post-vaccination from all study participants and at 181 days post-vaccination in a subset of 100 participants at Duke. To accomplish these activities all participant samples will be interrogated for influenza antibodies against the strains of interest using the influenza hemagglutination inhibition assay (HI). This assay is considered the "gold-standard" measure by which to evaluate seroconversion/seroprotection in response to seasonal influenza vaccination. This assay will be performed in accordance with the Duke Regional Biocontainment Laboratory Virology Unit's fully optimized and approved SOP (RVUSOP004 Influenza HI of Serum Samples). Briefly, test samples will be assayed by HAI as duplicate 2-fold dilution series starting at 1:10. Serum dilutions are then incubated with a concentration of virus verified to possess a known potential for red blood cell (RBC) agglutination. The presence of virus-specific antibodies is visualized via incubation of the virus-serum mixture with a RBC solution; the endpoint titer for a given dilution series is then expressed as the reciprocal of the final dilution in which complete HAI is observed. By convention, seronegative samples are defined as having an endpoint HAI titer < 40 and seropositive samples as having an endpoint titer of ≥1:40; and seroconversion as a 4-fold change in endpoint titer relative to pre-immunization baseline or a change from <10 to ≥1:40 [37].

7 STATISTICAL CONSIDERATIONS

In collaboration with the Boston Medical Center and Cincinnati sites, the research team at Duke will oversee the statistical analysis. Data will reside on a secure Duke server maintained by Duke Health Technology Solutions (DHTS). For the study, a database will be developed and a data set for the study without personal identifiers will be made available to the CDC upon request. Duke statisticians will develop a comprehensive Statistical Analysis Plan. The summary points of the analysis plan are presented below.

The null hypothesis is the allV3 H3N2 seroconversion rate is inferior to IIV3-HD seroconversion rate.

Ho: allV3 H3N2 rate - IIV3-HD H3N2 rate ≥ 0.1 (10%)

The alternative hypothesis is the allV3 H3N2 seroconversion rate is non-inferior to IIV3-HD H3N2 seroconversion rate.

Ha: allV3 H3N2 rate - IIV3-HD H3N2 rate < 0.1 (10%)

The upper bound of the one-sided binomial confidence interval of the difference will be used to make this assessment.

7.1.6 Secondary Objective 1

- To compare the proportions of local and systemic reactions after aIIV3 and IIV3-HD in the full study population and by age-group (65-79 years and ≥80 years) (other than moderate/severe injection site pain in the full study population)

The proportions in the full study population will be conducted as for Primary Objective 1 using a non-inferiority test to determine if allV3 is non-inferior to IIV3-HD with a 5% non-inferiority margin. These secondary objectives will be conducted with a one-sided alpha at the 0.01 level to adjust for multiple comparisons. It is recognized that with 12 different assessments at the alpha 0.01 level (given the events are truly independent) we are allowing an 11.4% chance of making a type I error.

The age-group assessments will be assessed using standard two-sided 95% confidence boundaries of the difference in proportions between the groups. No formal statistical testing will be implemented for the age-group comparisons.

7.1.7 Secondary Objective 2

- To describe and compare changes in health-related quality of life after aIIV3 and IIV3-HD in the full study population and by age-group

Each HRQOL instrument produces a summary score to measure health-related quality of life. The change in score from pre-vaccination (baseline, Visit 1) to post-vaccination (Day 3, Visit 2) time points will be compared within and between the two groups.

The Late-Life Disability and Function Instrument and Late-Life Disability and Function Instrument-Computer Adapted Test (Year 1 Only)

The function and disability sections scale raw scores are transformed to a 0 (worst health) to 100 (best health) scale. The changes from baseline will be assessed within vaccine group using a paired t-test for the function and disability outcomes. If normality assumptions are not met, the testing will be performed with a Wilcoxon signed-rank test. The Mann-Whitney U test (Wilcoxon rank-sum test) will be used to compare the difference scores from baseline between the two vaccine groups for the function and disability outcomes.

EQ-5D-5L and Visual Analogue Scale (VAS)

The EQ-5D-5L responses are converted to a Utility Index that ranges from -0.109 (worst health) to 1.0 (best health) using the US specific value sets (http://www.euroqol.org/fileadmin/user_upload/Documenten/Excel/Crosswalk_5L/EQ-5D-5L_Crosswalk_Value_Sets.xls). The EQ VAS has a range of 0 (worst health) to 100 (best health). The changes from baseline will be assessed within vaccine group using a paired t-test for the index values and VAS. If normality assumptions are not met, the testing will be performed with a Wilcoxon signed-rank test. The Mann-Whitney U test (Wilcoxon rank-sum test) will be used to compare the difference scores from baseline between the two vaccine groups for the index values and VAS.

These health-related quality objectives will be conducted with a two-sided alpha at the 0.01 level to adjust for multiple comparisons. It is recognized that with 12 different assessments at the alpha 0.01 level (given the events are truly independent) we are allowing a 11.4% chance of making a type I error.

The statistical tests described above will also be performed for the subset (N=176) of subjects 80 or older. This testing will be considered exploratory with an alpha level of 0.05 with no alpha adjustment.

7.1.8 Secondary Objective 3

To compare serum hemagglutination inhibition (HAI) antibody titers after aIIV3 and IIV3-HD for each of the three influenza vaccine strains contained in the respective vaccine for that season in the full study population and by age-group (except for seroconversion for the H3N2 strain in the full study population)

Responses to each influenza antigen will be analyzed, with the exception of seroconversion for H3N2, which is analyzed in Primary Objective 1. The proportion of subjects with seroprotection (pre- and post-immunization) seroconversion (4-fold rise from baseline) in the two treatment groups will be presented along with 95% exact binomial confidence intervals. A 95% confidence interval of the difference in proportions between the treatment groups will also be presented.

The GMTs for each influenza antigen, including H3N2, and 95% confidence boundaries will be presented for both treatment groups. A 95% confidence interval of the difference in GMTs between the treatment groups will also be presented. This information will also be prepared for the subset (N≥176) of subjects 80 or older.

7.1.9 Sensitivity Analyses

For Primary Objective 1 a sensitivity analysis will be performed on the following sub-group of the Full Analysis Population: subjects who provided complete/informative symptom diary information for injection-site pain (i.e., eight days of completed pain field data on diary or graded pain if present) and have no specified protocol violations as described in the Manual of Operations. This a supporting safety analysis.

7.1.10 Exploratory Objectives

The analysis for the exploratory objectives will be detailed in the comprehensive Statistical Analysis Plan.

7.2 Data Management

The novel Vanderbilt-designed resource developed specifically for online collection of research information, the Research Electronic Data Capture (REDCap) platform (https://projectredcap.org/), will be used to design study forms, including the reaction forms, and short customized questionnaires to collect information from study subjects. REDCap provides: 1) a streamlined process for rapidly building a database; 2) an intuitive interface for collecting data, with data validation and audit trail; 3) automated export procedures for seamless data downloads to common statistical packages; 4) branching logic, file uploading, and calculated fields; and 5) a quick and easy protocol set-up. This system will be used by Duke for data management. All electronic linkages will fulfill regulations for protection of human subjects and requirements to minimize the risk of breach of confidentiality.

All study-related documents containing protected health information, e.g. enrollment logs, case report forms, diaries (Appendix E) completed by study participants, will be maintained in secure research offices at Duke, Boston University, and Cincinnati Children's Hospital, which are accessible to research staff only.

The study team will utilize a secure, encrypted, file transfer method for sharing study documents and data with the CDC. No personal identifiers will be included in any shared documents or datasets.

7.2.1 Research Electronic Data Capture (REDCap)

REDCap (http://project-redcap.org/), assists with the collection and management of data for diverse clinical and translational research studies. REDCap was designed around the concept of giving research teams an easy method to specify project needs and rapidly develop secure, web-based applications for collection, management and sharing of research data. REDCap accomplishes these key functions through use of a single study metadata table referenced by presentation-level operational modules. Based on this abstracted programming model, databases are developed in an efficient manner with little resource investment beyond the creation of a single data dictionary. The concept of metadata-driven application development is well established, and the critical factor for successful data collection lies in creating a simple workflow methodology allowing research teams to autonomously develop study-related metadata in an efficient manner. Both products include secure institutional data hosting and include full audit-trails in compliance with Health Insurance Portability and Accountability Act (HIPAA) security requirements. The REDCap Consortium is comprised of 647 active institutions. The REDCap currently supports 68,000 projects with over 89,000 users spanning numerous research focus areas across the consortium. The current project will use this software application for the design of electronic forms to collect information from study participants, to link the baseline data, sample collection date, and laboratory results in an automated database family, to perform data cleaning and data quality assurance efficiently, and to design an analytical dataset for the analysis of the project data.

Data will be entered into the REDCap database by members of the study team from Duke, Boston Medical Center, and Cincinnati Children's Hospital using the paper case report forms utilized to record data collected as part of study procedures. Study investigators will be responsible for assuring that all paper records are securely stored according to the requirements of their IRBs. The study investigators will be responsible for assuring the accuracy of the data entered from the paper forms into REDCap. Only the assigned identifiers will be used in REDCap. Therefore, personal health identifiers will not appear in the REDCap database.

In order to perform data cleaning and data quality assurance efficiently, numerous built-in filters and checks for consistency of the data including range and limit checks, branching logic and pull down menus to limit choices for categorical variables to a pre-specified list will be implemented and performed automatically to minimize data entry error. The data will be randomly sampled and checked against source records on a regular basis. The data and related analytical datasets will also be stored at the lead and contributing sites with secured password-protected computers.

7.3 Role of the CDC Investigators in the Project

This study is funded by a CDC contract with Duke University and Boston University as Task Orders in the CISA Project Contract. The Duke University PI (Ken Schmader) will oversee the study in partnership with the Boston University PI (Elizabeth Barnett). Boston University has a subcontract with Cincinnati Children's Hospital (PI Elizabeth Schlaudecker). CDC staff will collaborate with both sites to develop the protocol, conduct the study, ensure the study is aligned with US Department of Health and Human Services (CDC) public health priorities, and analyze the data and disseminate the results. CDC may receive access to coded data not containing any directly identifying information.

8 HUMAN SUBJECTS

8.1 Human Subjects Involvement, Characteristics, and Design

Duke, Boston University, and Cincinnati Children's Hospital investigators will be responsible for submitting the protocol, informed consent (Appendix A), diaries (Appendix E), recruitment letters (Appendix M), flyers (Appendix N), and any written or verbally conveyed materials (Appendix O) specific to this project to their institutional review boards. CDC staff will be responsible for submitting materials to the CDC for Human Subjects review and approval.

To facilitate subject recruitment at the practices, we will request a waiver of consent and HIPAA authorization for ascertainment (identification, selection) and/or recruitment of potential subjects while recording identifiable private health information (PHI) prior to obtaining the subject's consent. This information will be obtained from review of the electronic scheduling and medical record systems in the clinics in order to determine eligibility for study enrollment. We will review only the minimum amount of information necessary to determine eligibility, i.e. date of birth, medical and surgical history, and recent laboratory test results. The PHI collected prior to consent will be used to recruit and screen only. Use of PHI in this manner involves no more than minimal risk to subjects and no information will leave the study sites.

Requests for continuing review, when required, will be submitted at each engaged institution in accordance with institutional procedures. Protocol deviations or concerns about study integrity will be reported promptly to the overseeing IRB or CDC in accordance with institutional requirements.

8.2 Sources of Material

Medical history and immunization history will be obtained from the medical record and from patient report. Demographic information will be obtained from the medical record and patient report. Subjects will record solicited adverse reactogenicity events and any medical intervention sought on study days 1-8 on the symptom diary (Appendix E). Diary information will be reported to the study team during a telephone call. The research staff will assess one or more of the following: weight, height, temperature, blood pressure, and pulse.

8.3 Potential Risks and Benefits

aIIV3 or IIV-HD3 are FDA-licensed vaccines approved for use in adults ≥65 years old. Both vaccines are standard clinical practice and recommended by the CDC. Participants will be provided with the CDC Vaccine Information Statement (VIS) for IIV (https://www.cdc.gov/vaccines/hcp/vis/vis-statements/flu.pdf).

IIV risks include minor problems such as soreness, redness, swelling, or pain where the shot was given, hoarseness, sore, red or itchy eyes, cough, fever, aches, headache, itching, fatigue, all of which usually occur within 1-2 days of vaccination and are self-limiting. Some people get severe pain in the shoulder and have difficulty moving the arm where a shot was given. This happens very rarely. Syncope (fainting) can occur in association with administration of injectable vaccines. Sitting or lying down for about 15 minutes can help prevent fainting, and injuries caused by a fall, as recommended in the ACIP General Recommendations on Immunization[1]. Subjects should inform their doctor should they feel dizzy, or have vision changes or ringing in the ears. More serious problems including a small increased risk of Guillain-Barré Syndrome estimated at 1 or 2 additional cases per million people vaccinated. This is much lower than the risk of severe complications from influenza infection, which can be prevented by IIV[38]. In addition, any medication can cause a severe allergic reaction, or anaphylaxis, which is estimated at ~ 1 in one million doses of IIV administered[39].

Risks of blood drawing include pain, swelling, bleeding, or bruising at the site where the blood sample is collected. Subjects may also experience dizziness or fainting. There is a small risk of infection around the vein where the blood was collected. Each study subject will be asked to have up to 3 blood samplings with the total volume not to exceed 30mL over approximately 6 month period of time. Participants who return for repeat vaccination in the second year will be asked to have 2 additional blood sampling with the volume in the second year not to exceed 20 mL.

As with any licensed vaccine, protection may not occur in 100% of vaccinated persons.

An additional risk of study participation is the potential for loss of confidentiality.

8.4 Adequacy of Protection Against Risks

Appendix L: Perceptions of the Vaccination Experience

10. Mild to severe dementia as determined by the Mini-Cog tool and the Rowland Universal Dementia Assessment Scale (RUDAS)
11. Substance use that could interfere with study compliance
12. Receipt of any inactivated licensed vaccine within 2 weeks, or live attenuated licensed vaccine within 4 weeks prior to enrollment in this study, or planning receipt of any vaccines during the 42 days post-vaccination period (including pneumococcal vaccines)
13. Receipt of Shingrix (Zoster Vaccine Recombinant, Adjuvanted) or HEPLISAV-B (Hepatitis B Vaccine (Recombinant), Adjuvanted) vaccine within 6 weeks prior to enrollment in this study, or planning receipt of Shingrix or HEPLISAV-B during the 42 days post-vaccination period.
14. Anyone who is already enrolled or plans to enroll in another clinical trial with an investigational product within 28 days of vaccine receipt. Co-enrollment in observational or behavioral intervention studies are allowed at any time while enrollment in a clinical trial involving an investigational product (other than vaccine) may occur after 30 days following vaccine receipt.
15. Hearing loss determined by the investigators to prevent successful communication over the phone
16. Any condition which, in the opinion of the investigators, may pose a health risk to the subject or interfere with the evaluation of the study objectives.
17. Anyone who is a relative or subordinate of any research study personnel.

4.3 Recruitment

Participants ≥65 years of age will be recruited from several sources at Duke University Medical Center (DUMC), Boston Medical Center (BMC), and Cincinnati using varying techniques. Study investigators will enroll at least 440 persons including at least 20% adults ≥ 80 years of age at Duke over two seasons (~220 participants per season), ~340 persons including at least 20% adults ≥ 80 years of age at Boston over two seasons (~170 per season), and ~ 100 persons including at least 20% adults ≥ 80 years of age at Cincinnati in 2018-2019 only. During 2017-2018 at Duke only, a subset of approximately 100 subjects will be assigned to receive an additional blood draw at day 181 (Section 5.2.1).

The general techniques for how subjects will be recruited include the following: Study staff, including PIs and study nurses, will approach their patients in clinic directly about the study during clinic visits; notify other health care professionals in their health system about the study via letters and flyers for potential referrals; notify potential subjects about the study via study registries and recruitment service programs; letters and talks to senior groups in various venues including Senior Centers and Continuing Care Retirement Communities; advertising in newspapers; flyers posted at senior locations; letters and talks to potential referral sources; and letter campaigns to older adults in the surrounding catchment area. More specific mechanisms to DUMC, BMC, and Cincinnati are described below.

At DUMC potential subjects will be approached via the following mechanisms: 1) Older participants of previous vaccine studies who have agreed to enroll in future studies; 2) Duke Center for Aging Human Subjects Registry, a unique long-standing registry of over 3000 individuals who volunteer for human studies; 3) Croasdaile and The Forest at Duke continuing care retirement communities. Medical care for these communities is provided by the Duke Division of Geriatrics, overseen by Dr. Schmader; 4) Duke's "Aging" network of senior centers; senior activities; and referrals from professionals who work with seniors cultivated via the work

- Obtain information on preferred method of contact for follow-up (telephone or email reminder), and obtain contact info for caregiver/significant other
- Obtain demographic data
    - Age, gender, race/ethnicity, language spoken, contact information, education, insurance payer, employment status, living alone or not
- Obtain medical history including chronic conditions, hearing and sensory impairment, chronic pain
- Obtain concomitant medications, including use of statin medications.
- Obtain influenza immunization history for the previous two seasons (identify whether vaccine used was FLUAD, Fluzone-High Dose, some other inactivated influenza vaccine, or unknown, and indicate if this information came from the patient or their chart)
- Obtain vital signs including oral temperature, blood pressure, and pulse; and height and weight in order to calculate body mass index (BMI)
- Obtain baseline health-related quality of life assessments prior to vaccination (Section 5.5)
- Obtain answers to first two questions of perceptions of the vaccination experience questionnaire
- Perform pre-vaccination baseline immediate reactogenicity assessment (Appendix D)
- Obtain one tube of blood (~10 mL) prior to vaccination for serologic analysis (Section 5.7.1)
    - If unable to draw blood, subject will remain in study
    - If less than 1 mL of processed serum is collected, it is a protocol deviation
- Randomize study participant to aIIV3 or IIV3-HD administration (Section 5.2.1)
    - Subjects in the second year of the repeat vaccination sub-study will be assigned to receive the same vaccination product that they received the first year (using the FDA-formulation for the year 2 season)
- Administer assigned study products – Unblinded trained, licensed staff will administer either aIIV3 or IIV3-HD as described in Section 5.3.1. Ensure participants receive inactivated influenza Vaccine Information Sheets (VIS) during visit. (https://www.cdc.gov/vaccines/hcp/vis/vis-statements/flu-largetype.pdf) Participants and study staff doing follow-up evaluations are to remain blinded.
- Dispense symptom diary (Appendix E), paper version of EQ-5D-5L quality of life tool, oral digital thermometer with large display, and injection site measurement tool with predetermined local reaction measurement scales. **Review instructions for use of thermometer, injection site reaction measurement tool, diary completion, and EQ-5D-5L. Encourage participants to complete diary at the same time every evening. Should a subject misplace the study-provided thermometer, any oral thermometer can be used for symptom diary reporting.**
- Assess for immediate injection site pain using the Faces Pain Scale (Appendix F), at ≥ 15 minutes after vaccination
- At ≥ 15 minutes after vaccination assess for any immediate reactogenicity symptoms and other adverse events using the assessment form used for baseline reactogenicity (Appendix D)
- Confirm preferred method of contact for follow-up (telephone or email reminder)
- Confirm date of next appointment

Study Days 1 – 8

Participants complete symptom diary form and FACES pain scale (injection site pain) on days 1-8 and complete the paper version of EQ-5D-5L on Day 3.

different moments in time. The measure is designed for self-completion by respondents. It is simple, straightforward, take only a few minutes to complete and can easily be completed by older adults. The instrument was designed to reduce respondent burden while achieving standards of precision for purposes of group comparisons involving multiple health dimensions. It has been widely used throughout the world in many different studies, including randomized controlled clinical trials, vaccine studies, and health-related quality of life studies in older adults.

5.5.2 Vaccination Specific Measure of HRQOL

An important issue with the proposed health status instruments is that they are generic measures of HRQOL. Therefore, the instruments are sensitive to anything that occurs in a participant's life and are not specific to vaccine reactogenicity We will employ a measure that relies on the concept of vaccine reactogenicity specific interference with activities and impact on HRQOL [34,35].

The vaccination reaction specific measure will consist of specific instructions and interference with daily living items using a five-category word response as "not at all"; "a little"; "somewhat"; "a lot"; or "completely" (Appendix K). The participant would be asked to "circle the response that best describes how these problems from your flu shot have made things harder to" followed by key functional activities for older adults [34,36].

5.5.3 Perceptions of the Vaccination Experience and Methods of Adverse Event Monitoring (Not Applicable for Repeat Vaccination Sub-Study)

The participant's perception of the vaccination experience and methods of adverse event monitoring will be collected with a questionnaire that includes their preferences, values and knowledge about vaccination. The participant's perceptions about their participation in the vaccine clinical trial will be assessed with a questionnaire as well (Appendix L).

5.6 Health Care Utilization

Participants will be asked to report health care utilization including: telephone calls to the medical provider for medical advice, e-mail portal, electronic health record, clinic visits, urgent care visits, emergency department visits and hospital admissions occurring through day 43 according to the schedule in Table 1 above. The reason for health care use will also be obtained. Electronic or paper health records will be obtained and reviewed to confirm reports of clinic visits, urgent care visits, emergency department visits and hospital admissions. Health care utilization and the reason for health care utilization will be recorded on the symptom diary.

5.7 Biospecimens Collection & Handling

5.7.1 Serum

Blood specimens will be collected during study visits as described in Table 1.
All blood samples (≈10 mL) will be collected into serum separator tubes and processed as follows:

- Allow blood to clot at room temperature for at least 30 minutes while standing upright in a rack.
- Centrifuge tube within 8 hours of collection at 1100 to 1300 RCF(g) for 10 minutes.
    - Gently remove the vacutainer stopper avoiding serum contamination with red blood cells. Using a single-use pipette, transfer 1.0 mL aliquots of serum (top layer) into 1.0mL or 1.8 mL cryovials, up to 5 cryovials are expected. If less than 1 mL of processed serum is collected, it is a protocol deviation

7.1 Analysis Plan

Should an interim safety analysis be required, the alpha level will be adjusted to assure the overall type I error is maintained at the one-sided alpha 0.025 level for the primary outcome of non-inferiority.

7.1.1 Sample Size

Safety: Based on data from prelicensure studies [4,5,7], we assume that 5% of older adults have moderate/severe injection-site reactions after aIIV3 or IIV-HD. We have selected a clinically meaningful non-inferiority margin of 5%. Statistical calculations, without consideration of drop-out, show that with an alpha of 0.025 (one-sided), we would need 668 total subjects (334 subjects in each group across all study sites) to have at least 80% power be able to demonstrate that the proportion of moderate/severe pain was non-inferior after aIIV3 vs. IIV3-HD. Enrollment in this study shall occur during two influenza seasons (2017-18 and 2018-19).

Immunogenicity: Based on data from prelicensure studies [4,5,7], we conservatively estimate that 50% of older adults demonstrate seroconversion after vaccination with aIIV3 or IIV3-HD for the H3N2 strain. We have selected a clinically meaningful non-inferiority margin of 10%. Statistical calculations, without consider of drop-out, show that with an alpha of 0.025 (one-sided), we would need 780 total subjects (390 subjects in each group across all study sites) to have approximately 80% power to able to demonstrate that the seroconversion rate after aIIV3 was non-inferior to IIV3-HD.

The total number of subjects needed for this study was derived based on 4 factors: 1) Sample size needed for adequate statistical power to test the safety hypothesis; 2) sample size needed for adequate statistical power to test the immunogenicity hypothesis; 3) potential for subject drop-out; and 4) feasibility of dividing subjects across 2 sites. Based on these considerations, the study aims to enroll at least 880 subjects to assess both the safety and immunogenicity endpoints. The study aims to enroll at least 720 to test only the safety hypothesis.

7.1.2 Analysis Populations

Full Analysis Population:

- For Primary Objective 1 and Secondary Objective 1, the primary analysis population will be the Full Analysis Population; defined as all subjects who are randomized, vaccinated, and provide at least one day of complete data on the symptom diary.
- For Primary Objective 2 and Secondary Objective 2, the primary analysis population will be the Full Analysis Population; defined as all subjects who are randomized and vaccinated.

Immunogenicity Population:
For Primary Objective 3 and Secondary Objective 3 the primary analysis will be for the Immunogenicity Population; defined as subjects who received vaccine, provide baseline and Visit 4 blood draws of acceptable volume and quality within the protocol-defined time frame with

8.4.1 Protections against Risk

To decrease the possibility of infection at the site of blood drawing, the area on the arm above the vein where blood will be taken will be prepped with 70% isopropyl alcohol antiseptic prior to venipuncture.

Subjects will be counseled on possible side effects following vaccination and followed closely during the 8 days post-vaccination for assessment of moderate to severe local or systemic reactogenicity. Subjects will be evaluated and cared for as described in the Unscheduled Visit section above. All subjects will be monitored in a sitting or lying position for 15 minutes following vaccinations to help prevent fainting, and injuries caused by a fall. Subjects with a prior history of severe allergic reaction after a previous dose of any influenza vaccine, or to a vaccine component, including egg protein, will be excluded from study enrollment. Data Safety monitoring, as described above (Section 5.4.3 and Appendix H), shall also be done.

The study team will provide documentation to the participant and primary care provider regarding receipt of influenza vaccine without specification of whether it was high dose or adjuvanted vaccine to preserve blinding.

If a participant's care requires the identity of the vaccine received, blinding will be broken for that patient. At the end of the study, the participants and providers will receive documentation about which vaccine the participant received.

Every effort possible will be made to keep information about participants confidential. Computerized participant information will be kept in password-protected files on secured servers. Paper case report forms will be kept in locked files belonging to the study personnel. Any publications resulting from this work will not contain any identifiable participant information.

8.4.2 *ClinicalTrials.gov Requirements*

The project is registered on ClinicalTrials.gov (NCT # NCT03183908).

8.5 Human Subjects

In obtaining and documenting informed consent, the Investigator and study team will comply with the applicable regulatory requirements, Good Clinical Practices, and ethical principles. The written informed consent form must be signed and dated by the study participant prior to initiation of any study activities.

8.5.1 Vulnerable Subjects Research

This study proposes to include subjects with mild cognitive impairment. All potential subjects will undergo cognitive assessment to ensure they are capable of providing consent. Mild cognitive impairment is a common age-related condition that is defined by the presence of short-term memory impairment that does not interfere the individual's ability to perform activities of daily living or affect other areas of cognition, including judgment and independent decision-making. Therefore, persons with mild cognitive impairment have the capacity to make decisions about their health care choices, including influenza vaccination, and participation in research studies. Influenza vaccination is recommended for these individuals. The benefits and burdens of the proposed study apply equally to these individuals as to persons without mild cognitive impairment.

Appendix M: Recruitment Letter

Dear Sir or Madam,
The Duke Division of Geriatrics and the Duke Vaccine and Trials Unit are conducting an influenza vaccine research study. We are contacting you because as an older adult you or someone you know may be interested in this study. Every eligible study participant will be randomized (like flipping a coin), to receive an injection of either FLUAD™, an adjuvanted inactivated influenza vaccine, or Fluzone® High-Dose, inactivated influenza vaccine. An adjuvant is a substance added to a vaccine to increase the immune response. Both vaccines are currently licensed and approved in the United States for older people.

Vaccines can help prevent infection and disease. Vaccines work by causing the body to make proteins called antibodies that fight infection. When you get flu vaccine (sometimes called a flu shot), your immune system makes antibodies against the flu virus.

The **purpose** of this research study is to determine if there is a difference in older people for side effects following vaccination, and also to compare the immune response to the two flu vaccines. Previous studies have shown that both vaccines provide protection from the flu in older people.

What is involved with this study?

- Come in for **2 visits**: baseline and day twenty-nine. You will receive the vaccine at the baseline visit
- Have about **2 teaspoons** of blood taken from your arm at each visit
- Have your temperature, blood pressure and pulse measured at each visit
- Complete post vaccination assessments and questionnaires
- Receive **3 telephone calls** following your vaccination to review questionnaires
- Receive payment for your time and travel

If you would like to participate, please call the study team at 919-660-7581 or 919-668-8728.
Sincerely,

Kenneth Schmader, M.D.,
Principal Investigator
Chief, Division of Geriatrics
Department of Medicine

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