Non-inferiority study of adjuvanted vs. high dose flu vaccine in residents of long term care

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Title: Non-inferiority study of adjuvanted vs. high dose flu vaccine in residents of long term care

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Introduction/background

Influenza and pneumonia are the most common infection-related and vaccine-preventable causes of hospitalization and death. 90% of influenza-related deaths occur among adults aged >65 years. Over 2 million Americans reside in nursing homes, skilled nursing facilities, or long term residential care facilities and this number is expected to expand significantly in the coming decades according to the CDC. These are among the highest risk and debilitated seniors that may acquire influenza. Two influenza vaccines are now available that are FDA approved specifically for persons over age 65: the newly approved adjuvanted seasonal influenza vaccine (Fluad) and the high dose (HD) vaccine that was approved in 2009 and is increasingly being used. The absolute or relative clinical advantage to long-term care residents of Fluad vs. HD over standard dose (SD) vaccine remains unclear from existing immunologic and clinical evidence.

For Year 2, we will be adding a 3rd vaccine substudy, recombinant FLUBLOK. FLUBLOK is a newer FDA approved influenza vaccine that has a higher amount of HA in the formulation than SD, and has a unique feature of not being produced in eggs. None of the three vaccines have been compared head-to-head and an industry-sponsored study is unlikely to be conducted to evaluate relative efficacy or effectiveness due to uncertainty as to whether manufacturers will gain additional market advantage while risking uncovering relative inferiority.

Both Fluad, HD, and FLUBLOK vaccine have been shown to be more effective than SD vaccine in trials conducted in different cohorts. HD vaccine and FLUBLOK are more expensive than SD vaccine, and Fluad is less expensive than HD vaccine. As an adjuvanted vaccine, Fluad has been shown to have higher levels of heterologous immunity to drifted influenza strains than non-adjuvanted (SD) vaccine - another potential advantage of Fluad over HD vaccine, which is non-adjuvanted. This project's rationale and innovation derive from its ability to determine the best use of these two vaccines in the setting of one of the most vulnerable elderly populations - those living in nursing homes (NH) and other various long-term care institutions.

Increased response to HD over SD influenza vaccine in geriatric adults

In 2009, HD influenza vaccine consisting of 4 times the antigen of SD vaccine was licensed for use in persons over the age of 65. In studies prior to its approval, the anti-influenza titers as determined by HAI were significantly higher in the HD vaccine group than the SD group (Falsey et al., 2009).

Clinical efficacy of HD vaccine has been demonstrated in a large clinical trial (n=31,989) in which HD vaccine was 24.3% better at preventing laboratory confirmed influenza than SD vaccine overall (DiazGranados et al., 2014). A retrospective cohort study of 2.5 million Medicare recipients demonstrated that HD was 22% more effective than SD at prevention of probable influenza based on diagnosis codes and treatment and 22% more effective at prevention of influenza hospital admissions in older adults (Izurieta et al., 2015). A VA-wide retrospective study using the national VA database only showed benefit of HD over SD in veterans age 85 and older (Richardson et al., 2015), but notably employed a differently defined clinical endpoint than the other clinical efficacy studies (hospitalizations with a primary diagnosis of pneumonia or influenza without any requirement of lab confirmation or treatment regimen). We ran a pilot study in NHs and found the HD vaccinated group were 30% less likely to have any hospitalization compared with the SD group (adjusted relative risk 0.701; 95% CI: 0.543, 0.905; p=0.006) in an A/H3N2 dominant season (unpublished).

Increased response of Fluad over SD influenza vaccine in geriatric adults

Fluad has not been tested in a large RCT with laboratory confirmed documentation of prevention of disease as HD vaccine has been. There are, however, multiple studies that support greater protective efficacy from Fluad than SD. Mannino et al showed in a large cohort study conducted in Italy over 3 seasons that the risk of hospitalization from influenza or pneumonia was 25% lower in patients receiving Fluad relative to SD vaccine (Mannino et al., 2012). This difference in protection is very similar to that observed in two of the HD vs. SD studies discussed above. Other studies focusing on nursing home patients including VanBuynder et al demonstrated in a case control study that Fluad was superior to non-adjuvanted vaccine in protection from disease (Van Buynder et al., 2013), and Iob et al found that Fluad was superior in effectiveness to the conventional vaccine, reporting protection rates of 80% vs. 57% respectively (Iob et al., 2005).

Increased Efficacy of FLUBLOK in adults

Dunkle et at performed a large RCT enrolling individuals age 50 and older comparing FLUBLOK and SD vaccine (Dunkle, et al, 2017). They found the probability of influenza-like illness was 30% lower with FLUBLOK than with SD Quadravalent vaccine (95% confidence interval, 10 to 47; P = 0.006) and satisfied prespecified criteria for the primary noninferiority analysis and an exploratory superiority analysis of FLUBLOK over SD.

Justification/rationale/significance of the study

Scientific Rationale

As the primary endpoint we are using pre- to post-vaccine changes in HAI titers to compare seroconversion rates and post-vaccination HAI titers to calculate the ratio of the geometric mean titers in the two treatment groups. HAI is an in vitro bioassay that determines a subject's serum levels of anti-influenza antibodies. The FDA uses this as a standard immunogenicity assay for licensure. We will follow the guidelines set out in the FDA guidance document discussing non-inferiority immunogenicity studies. As additional methods to assess immunogenicity we are adding an assessment of anti-NA by performing NA inhibition assays (NAI) and SVN assays. Dunning et al recently supported the use of NAI and SVN assays as a correlate for protection in a trial of geriatric subjects (Dunning et al., 2016). Memoli in a healthy human challenge model showed that NAI is more predictive of protection and reduced disease than HAI (Memoli et al., 2016).

In the large HD vaccine clinical efficacy trial (n=31,989) one third of subjects also had immunogenicity data that allowed looking for correlations of immune assays with protection (DiazGranados et al., 2014). Their conclusions were that HAI and other immune assays are potential correlates of influenza vaccine protection in older adults, and that the protective thresholds for the HAI assay in the elderly appear consistent with those previously described for younger adults, provided the assay virus matches the circulating virus (Dunning et al., 2016).

Significance

Data compiled by CDC in 2011-2012 showed that there were 1,383,700 residents in NHs. Also about 4,742,500 patients received services from home health agencies, and 1,244,500

patients received services from hospices, collectively accounting for much of the frailest in the US. Overall, these provider sectors served over 8 million people annually (2013). Our study will focus on residents in long term care (LTC) facilities, but the findings of our study are highly relevant to persons frail enough to require such services in all of settings where the vast majority are at least 65 years old and thus appropriate for Fluad or HD, influenza vaccines licensed for this age group.

The SD influenza vaccine has diminished efficacy in the older population with the more debilitated LTC residents being among the worst responders yet with the highest mortality. Deaths due to pneumonia and influenza and chronic lung disease were 20 times higher among NH residents compared to community residents (Menec et al., 2002). **The current availability of two vaccines specifically for the elderly that both appear to work better than SD vaccine begs the question: is the newer and less-costly Fluad vaccine non-inferior or even superior to HD vaccine?** The proposed study aims to initially address non-inferiority as this is feasible in the clinical trial R01 grant structure and a critical first step to obtain head-to-head data from the same trial, cohort and vaccine years. Our proposed study itself may provide direct guidance on vaccine usage or inform a future trial assessing actual superiority should that be appropriate based on the results of our study.

HD vaccine is increasingly used by older Americans despite its greater cost over the SD vaccine and no preferential recommendation by the Advisory Committee on Immunization Practices (ACIP), the CDC committee responsible for making the vaccine recommendations for the U.S.. A finding of non-inferiority in the primary endpoint would provide a strong rationale to consider using Fluad over HD that could result in some cost avoidance across large long-term care system in the U.S. We are not powered for a superiority analysis but in a non-inferiority trial if the findings are substantial enough they may show superiority.

In the normal seasonal setting, influenza strains drift antigenically and therefore vary from year to year. The CDC's prediction many months before the vaccination season sets the composition for the next season's vaccine, but does not always correctly anticipate the exact strain match that eventually actually circulates. We have Medicare claims data and modeling in the NH population that there is a significant increase in death and hospitalization in bad match over good match years particularly when A/H3N2 predominates (Pop-Vicas et al., 2015). In those mismatched years in particular, heterologous immunity or immunity to other non-exact match strains becomes much more important if the vaccine is going to provide any benefit that season. Fluad is an adjuvanted vaccine that has been shown to have a more broad-based or heterologous immunity than SD vaccine that is not adjuvanted (Ansaldi et al., 2008; Frey et al., 2014). HD is also not adjuvanted. Broad based immunity is especially desirable for A/H3N2 immunity as that has had 4 different circulating strains in the last 5 years while circulating A/H1N1 has been the same for 5 years; i.e., vaccine mismatch is more likely with the A/H3N2 circulating strain. A/H3N2 is associated with the majority of influenza hospitalizations and death among the elderly (Centers for Disease and Prevention, 2010).

For the recombinant FLUBLOK arm, there is emerging data that indicates that the method of producing the vaccine may be highly clinically significant. There are important egg-induced mutations in the production process of the recent A/H3N2 strains. The egg-produced vaccines induce antibodies to A/H3N2 that have substantially less blocking ability to wild type circulating A/H3N2 than do antibodies elicited by FLUBLOK, that is produced from a wild type sequence without mutations (Zost et al). If this deleterious issue of egg-adapted mutations continues, there may be a major push in the future toward non-egg-produced vaccines such as

FLUBLOK, and away from egg-produced vaccines such as both HD and AD. This could result in a change of production methods that shy away from egg-based vaccines. This supports the need and significance for having head to head comparative studies of FLUBLOK, HD and AD.

Purpose, including specific aims and/or hypotheses

Hypothesis: Adjuvanted flu vaccine, Fluad, is not immunologically inferior to HD influenza vaccine in older persons living in long-term care.

Objectives:

Primary:

To determine if Fluad is immunologically non-inferior to HD flu vaccine in long term care residents over age 65.

As the primary endpoint we will evaluate the hemagglutinin inhibition (HAI) titer rise following vaccination to assess overall differences and seroconversion rates (4-fold rise in antibody titer) and post-vaccination geometric mean HAI titer of at least 40 in the treatment groups. HAI is an *in vitro* bioassay testing subjects' sera for specific anti-influenza antibodies to each strain in the vaccine. The FDA uses this as the standard immunogenicity assay for licensure. We will follow the guidelines set out in the FDA guidance document on non-inferiority immunogenicity studies for the analysis plan. We will also perform neuraminidase inhibition (NAI) assays and serum virus neutralization (SVN) assays there are recent data that NAI and SVN assays may be a better correlate of protection than HAI.

Secondary:

Aim 2: Secondary objective: To determine if Fluad has greater heterologous immunity than HD vaccine.

HAI, NAI, SVN assays will be performed with heterologous A/H3N2 strains to determine if Fluad has an increased breadth of both B and T cell responses as would be predicted from an adjuvanted vaccine.

Aim 3: Pilot clinical objective: To determine if Fluad has similar protective efficacy to HD vaccine**.** We will perform a record review and obtain blood 2-4 weeks after the influenza season is over. From a record review and speaking with the Director of Nursing, we will obtain dates and diagnoses of influenza, hospitalizations, any "influenza like illness" (ILI), and other illnesses, diagnostic tests, and hospitalizations during the study participation period. From the remote blood sample, we will determine if there is serologic evidence of influenza infection with a 4-fold titer rise beyond the post-vaccine titers. In exploratory analysis we will compare efficacy of prevention of influenza infection of Fluad vs. HD using the record review and laboratory assessments. We will also be able to examine the concordance in this long term care population between record review and titer rise methods as evidence of infection that may aid in future study design.

Aim 4: Pilot objective: To compare the immunogenicity of recombinant flu vaccine (FLUBLOK) to the AD and HD influenza vaccines.

Study design including population to be studied, recruitment procedures and available resources

We propose a non-inferiority randomized clinical trial to enroll 500 Long Term Care residents age 65 and older to receive either Fluad or HD vaccine at 1:1 ratio. Blood will be sampled pre- and post-vaccine and post-influenza season and coded for blinded laboratory analysis.
The FLUBLOK pilot objective will be a substudy of the current AD Fluad vs. HD Fluzone study (UH IRB 10-27-29), and subjects will be consented on a separate FLUBLOK ICF.

Population: 500 subjects age 65 and older living in long-term care facilities in Northeast Ohio area. Up to an additional 120 subjects will be added to FLUBLOK substudy.
50:50 male:female ratio.
Study Duration: 3 years
Subject Duration: estimated 6-8 months with patient contact

Recruitment procedures
We will recruit from approximately 40 community long term care facilities in Northeastern Ohio, approximately 20 facilities per year. Since this is a two-year study, we anticipate that we may have other facilities that will join the study for the second year, as well as some changes in the facilities. If any new facilities join the study, we will notify the IRB of the changes.
We have received agreement currently form Seqirus (maker of adjuvanted vaccine), and are talking with Sanofi Pasteur (maker of high dose vaccine), to donate regular dose flu vaccine for use by employees at any participating LTC facility. This will be used as an incentive to the facility to agree to let us be a recruitment site. The vaccine will be sent directly from the manufacturer as a donation to the employee health team at the LTC site, so that we do not have to be involved in the handling, prescribing, or monitoring of the vaccine. If a LTC facility agrees to allow us to enroll, we will provide the manufacturer with the name and contact at the facility. We do not believe this is a coercive relationship with the long term care facility because they will get the vaccine once they agree to participate for the season, regardless of whether we recruit one or many from their site. We will also give all participating facilities an $1000 participation stipend, to cover any administrative and staff costs that participation in the study may incur. The nursing and administrative staff (administrator, DON, ADON, Social Worker, Unit Nurses) at the long term care facility support the study in the following ways; they review the census with the study team during our initial meeting and prescreening, and help identify those residents that meet study inclusion criteria, and those residents that are not eligible for recruitment due to study exclusions. Additionally, the LTC administrative and nursing staff (Social Worker, Director of Nursing, Assistant Director of Nursing, Unit Nurses) helps the study team identify which residents can self-consent, and which ones utilize a LAR for their health care decisions. The LTC facility nurses and social workers, also helps the study team to identify residents (location), discusses medical conditions of the potentially eligible residents to determine if they are still eligible for recruitment or self-consenting, discusses medical conditions with the study team of consented residents to determine if they are still eligible at the time of vaccination, reviews the enrolled residents' medical conditions with the study staff at the end of study participation to determine if they had the influenza virus and/or other medical conditions that the study will be documenting for the study. The long term care facility also supports the study on the day of vaccination, by providing a nurse who helps identify consented residents. With regards to the administrative support, a designated nurse, the Director of Nursing, or the administrator at the LTC facility is in touch with the study staff throughout the duration of the study. The study coordinator sends regular updates, as to which residents

consented onto this study. This is important so that the LTC facility nurse doesn't consent a resident for their own flu vaccine, when the resident and/or LAR already consented onto the study. The LTC facility staff is not involved in any recruitment activities for the study, but lends support to the study team with regards to medical information and updates, and on vaccination and blood work days.

Consenting will take place at various long term care facilities in Northeastern Ohio and with an IRB approved written Consent Form, which we will review with either the resident or the LARs on the phone. We will speak to the LTC Administrator, Director of Nursing, Assistant Director of Nursing, Social Worker, and Unit Nurses to determine if a resident can make their own health care decisions and consent for their routine care, or if a Legally Authorized Representative (LAR) is needed. If the nursing staff deems that the resident can consent for themselves, we will approach them directly to initiate the consenting process. The consent process should take approximately 60 minutes, with ample time devoted to reviewing the Consent Form in detail, and our study staff asking the resident several questions about the study to confirm they have an understanding. If the resident is not able to understand the study, we will proceed to the LAR consent. If the nursing staff (LTC Administrator, Director of Nursing, Assistant Director of Nursing, Social Worker, and Unit Nurses) deems that the resident cannot physically or mentally give informed consent, as documented in their facility medical record, we will send an IRB approved Study Consent Form, Letter to LARs, LTC Facility Letter of Support, Study Brochure, and addressed stamped envelope to these potential subjects' LAR. We will follow up with a phone call to review the Study Consent Form and if they would like to enroll their family member onto the study, we will ask them to appropriately sign the Consent Form and send it back to us, in the provided envelope. If a resident is physically and mentally able to sign an Assent Form, the study staff will review the Assent Form with the resident. If a subject is determined to be physically or mentally not capable of signing any documents, (as documented in the medical records and confirmed by the Director of Nursing, Assistant Director of Nursing, Social Worker, and/or Unit Nurse), then we will waive Assent and will file the medical documentation along with the LAR consent. LARs will be mailed a copy of the signed Consent Form for their records, and we will provide a copy to the nursing administration at the LTC facility. The Consent Form will have study contact information, so they can withdraw consent at any time.

This process will begin months before the anticipated vaccination time frame, to allow plenty of time for mail correspondence, phone calls, and consenting.

The research team will make sure that the residents and LARs understand that participation is voluntary, and if they choose not to enroll onto the study, they will receive the standard of care flu vaccination that the LTC facility provides.

For year 2 of the study, we will be modifying the recruitment plan as follows:
The study team will meet with the LTC Administrator, Director of Nursing, Assistant Director of Nursing, Social Worker, and Unit Nurses to review the study, sign a Letter of Agreement to collaborate on the study, and narrow down the census to those residents who meet the study's inclusion/ exclusion criteria. From there, the study team and Director of Nursing, Assistant

Director of Nursing, Social Worker, and/or Unit Nurses will come up with a list of residents who are possibly eligible for recruitment onto the study. For those residents who need an LAR, a pre-recruitment letter from the LTC facilities administration will be sent out. This letter would give an overview of the study, state that the LAR will be receiving more details about the study in a future mailing from the study team, and also state that if the family member prefers NOT to receive any communication from the study team, please call the LTC facility to let them know. The study team will suggest to the LTC facility nursing team and administrator to also pre-communicate about the study through an email and /or newsletter that will let the residents and family members know about the study in advance of the study team's mailing.

Recruitment for the FLUBLOK pilot arm for Year 2:
Any long term care facilities that participated on Year 1 of the study can participate on Year 2, as long as we do not repeat subjects. For the FLUBLOK substudy, we can recruit from those subjects that participated in Year 1 and received either the adjuvanted Fluad or the HD Fluzone. Since enrolled subjects from Year 1 cannot participate for a 2nd year on the Fluad vs. HD objective, these previously enrolled subjects would be eligible for recruitment on the FLUBLOK substudy, and therefore, we will use our previously described screening and recruitment methods to consent these subjects for enrollment to receive the FLUBLOK vaccine. We will consent these subjects on a separate FLUBLOK ICF. We may also recruit FLUBLOK subjects from new long term care facility sites that did not previously participate on the study, and we will use the same previously described consenting methods, and consent with the FLUBLOK ICF. If we hit our recruitment goals on the primary endpoint (Fluad vs HD Fluzone objective), then any additional long term care residents that we may recruit will not be randomized to receive Fluad or HD Fluzone, but rather will be enrolled in the pilot Aim 4 FLUBLOK arm. These residents will be consented on another ICF, that was modified specifically for FLUBLOK.

Inclusion criteria:

- $\geq$ 65 years old
- Able to obtain consent from subject or legally authorized representative (subject to provide assent if cognitively/physically able to do so).
- Able to participate throughout the study period.

Exclusion criteria:
This is the exclusion criteria for admission to the study (not for the 2nd and final blood draws)

- Recent illness (within 30 days) severe enough to require hospitalization or physician-directed outpatient pharmacotherapy.
- Administration of immunomodulatory agents (e.g. oral corticosteroids except prednisone $\leq$ 10 mg daily, cyclosporine, and biologics (DMARDS) for Rheumatologic/Dermatologic conditions) in the last 3 months.
- Cancer requiring treatment in the past three years, except for non-melanoma skin cancers or cancers that have clearly been cured or carry an excellent prognosis including prostate cancer.

- Myocardial infarction, major heart surgery (i.e. valve replacement or bypass surgery), stroke, deep vein thrombosis or pulmonary embolus in the past 4 months.
- Allergies or history of significant adverse reactions to any component of influenza vaccine including egg protein and latex or after a previous dose of any influenza vaccine.
- History of Guillian-Barré Syndrome within 6 weeks of a prior influenza vaccine.

Study procedures

The intervention is randomization to one of the two FDA approved seasonal influenza vaccines (Fluad(AD) and FLUZONE HD (HD)) at 1:1 ratio. The HD vaccine will serve as the control, and the newly FDA-approved AD will be the comparator. We will obtain 20 ml of blood up to 2 weeks prior to the vaccine, or right before the vaccine is given. The second blood draw will be 4 weeks +/- 3 working days post vaccine and the third blood draw of 10 ml will be performed 2-4 weeks after the influenza season is over. The post-season draw may be spread over a two week window because the entire cohort of subjects will need to be drawn during this window, and it may take as long as two weeks to achieve that at all the facilities.

For the FLUBLOK substudy, we will follow the same blood draws and schedule. The subjects recruited to receive the FLUBLOK vaccine will be recruited with the FLUBLOK ICF, and will not be part of the randomization process.

The study will obtain blood work and give a study flu vaccine to consented residents starting in late September, and the third and last blood work will be obtained at the end of the flu season, typically in April. Each subject will then be enrolled as long as 8 months if they are enrolled in September and the flu season lasts until April.

We will obtain the following PHI in the prescreening: name, birthdate, race, ethnicity, gender, facility name and resident location, and whether a resident can self-consent or needs to use the LAR method of consenting. If a resident cannot self-consent, we will obtain the name, mailing address, phone numbers, and relationship of an LAR. We may also collect contact information on a secondary contact/secondary LAR, when appropriate, during the pre-screening process. Some residents may have two LARs/POAs listed in the medical record.

After a resident is consented and found still eligible, they will be randomized using the UH based study database, RedCap, to receive one of the two licensed study influenza vaccines. Subjects will be assigned a donor code at that time that does not indicate which vaccine they have received so the lab analysis can remain blinded. Study staff will bring enough supply of each vaccine for either type of randomization and maintain the cold chain according to pharmacy policy so unused vaccine can be returned to the UH monitored study refrigerator. Vaccine will be given by research nurse or physician study staff or the clinical nurse at the long term care facility, since it is a standard of care vaccine that would have been given by that nurse even if they are not in the study. The first blood draw will be up to 2 weeks prior to the vaccine, or right before the vaccine is given. All blood draws will be obtained by the study research nurse.

Then at 4 weeks +/- 3 days we will return and draw the second blood draw. The third blood draw will be obtained 2-4 weeks after the influenza season is over, based on the CDC

surveillance in our region for that season when rates are near the offseason baseline. Typically that means the final blood draw will be sometime in April or early May of each season.

Resources

The NIH grant is for 3 years and have nearly a 500K budget per year. Based on our experience we have adequate funds to do the study. We have worked with Long Term care facilities in the past with the same team and anticipate being able to reach our target recruitment and enrollment goals.

Risks and discomforts and how minimized

Potential Risks

Vaccine risks

Enrolling in this study does not add additional risks beyond normal standard of care as far as the vaccine is concerned, since the subjects should be receiving one of the FDA approved vaccines though the course of their routine health care.

These are the side effects of the vaccine that are listed on the package inserts. They were mostly mild in severity.

FLUAD vaccine: most common (≥ 10%) local (injection site) adverse reactions were injection site pain (25%) and tenderness (21%). The most common (≥ 10%) systemic adverse reactions observed in clinical studies were muscle aches (15%), headache (13%) and fatigue (13%).

FLUZONE High Dose vaccine: most common injection-site reaction was pain (>30%); the most common solicited systemic adverse events were muscle aches, malaise, and headache (>10%).

FLUBLOK: In adults 50 years of age and older, the most common (≥10%) injection site reactions were tenderness (34%) and pain (19%); the most common (≥10%) solicited systemic adverse reactions were headache (13%) and fatigue (12%).

A Federal law, called the Genetic Information Nondiscrimination Act (GINA), effective May 21, 2010, 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 generally 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 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 Federal law does not protect you against genetic discrimination by companies that sell life insurance, disability insurance, or long-term care insurance.

All health insurance companies and group health plans must follow this law by May 21, 2010. All employers with 15 or more employees must follow this law as of November 21, 2009.

Genetic testing will NOT be performed on your samples, however, the laboratory analysis performed will use part of the genetic materials inside of your cells called RNA. RNA is one of the substances that your body makes from your DNA which contains the genetic information. Genetic information is unique to you and your family, even without your name or other identifiers. Study staff will follow procedures to prevent people who work with your genetic samples from being able to discover it belongs to you. However, new techniques are constantly being developed that may in the future make it easier to re-identify genetic data, so we cannot promise that your genetic information will never be linked to you.

All blood sample will be identified by a code number, and all other identifying information will be removed. Identifying information that links the blood sample to the enrolled subject will be kept in the secure study database, RedCap, housed on the UH server.

Analysis from the blood samples will be made publicly available to qualified researchers through a controlled access web site. This data will not be connected with the subject's name, birthdate, or medical record number.

Privacy risk
Participation in this study will involve a loss of privacy, but information about the subject will be handled as confidentially as possible. The research records will be labeled with a code number. This study does not focus on PHI factor that are often considered controversial or sensitive. There is no alternative to obtain this type of data and comparison if done in humans. There are minimal risks above standard of care so we feel the study is justified and the risk has been minimized with only small blood draws to do the study beyond what is normal standard of care.

Compensation for injury
The subject will have to pay for costs to any research related injury. The sponsor is not responsible for this. We have adopted this language that the IRB recommended for our consent form.
If injury occurs as a result of the subject's involvement in this research, medical treatment is available from University Hospitals or another medical facility but the subject's medical insurance will be responsible for the cost of this treatment. A research injury is an injury that happens as a result of taking part in this research study. If a subject is injured by a medical treatment or procedure that they would have received even if they weren't in the study, that is not considered a "research injury". There are no plans for payment of medical expenses or other payments, including lost wages, for any research related injury. To help avoid injury, it is very important to follow all study directions.

Benefits to subjects

The subject may directly benefit from participating in this study because they will be receiving a flu shot. This is one of the shots that they should have normally received through their routine recommended healthcare at the LTC facility, so this is not necessarily an additional benefit since these vaccines are the commercially purchased vaccines. Their participation may aid in deciding which of these two vaccines is optimal and would be recommended in the future. The subject will receive up to $60 for participation in the study.

Costs to the subject

There are no costs to the subjects.

Alternative(s) to participation

The alternative is to not participate and continue to receive the normal care at The LTC facility.

Payment to the subjects (include both reimbursement and incentives)

Subjects will receive $20 for the completion of each blood draw, which will be deposited either into their Resident Account or the Family Activity Council Account at the Long Term Care facility. There are no expenses.

Plan for obtaining informed consent

We will follow the policy of the LTC facility administrative and nursing staff (LTC Administrator, Director of Nursing, Assistant Director of Nursing, Social Worker, and Unit Nurses) as an initial guide for obtaining consent directly from the resident or determining if we will need to use the LAR method of consent.
We will speak to the DON, ADON, Social Worker and nursing staff on each unit of the facility to determine if a resident can make their own health care decisions and consent for their routine care, or if a Legally Authorized Representative (LAR) is needed. If the nursing staff deems that the resident can consent for themselves, we will approach the resident in a private location to initiate the consenting process. We will review the current IRB approved ICF with the resident, asking them several questions about the study to confirm they have an understanding. We will ensure that they can explain the purpose of the study and ask the resident questions about the study to confirm that they have an understanding of what the study entails. The consent process should take approximately 60 minutes. If the resident appears to not understand the scope of the study, we will proceed to the LAR method of consenting.

If the nursing staff (Director of Nursing, Assistant Director of Nursing, Social Worker, and Unit Nurses) deems that the resident cannot physically or mentally give informed consent, as documented in their facility medical record, we will send an IRB approved Study Consent From, Letter to LARs, facility Letter of Support, Study Brochure, and addressed stamped envelope to these potential subjects' LARs. We will follow up with a phone call to review the Study Consent Form and if they would like to enroll their family member onto the study, we will ask them to appropriately sign the Consent Form and send it back to us, in the provided envelope. If a resident is physically and mentally able to sign an Assent Form, the study staff will review the Assent Form with the resident. If a subject is determined to be physically or mentally not capable of signing any documents (as documented in their nursing home record and confirmed with either the DON, ADON, or Unity nurse), then we will waive Assent and will file the medical documentation along with the LAR signed written Consent Form. LARs will be mailed a copy of the signed Consent Form for their records, and we will give a copy to the nursing administration at the LTC facility. The Consent Form will have study contact information, so they can withdraw consent at any time.

Waiver of written Assent will not impact subject's safety or rights, as the vaccine being administered is approved by the FDA and administered to all LTC patients per standard of care. If a subject refuses the research blood draw, a later attempt will be made. Subjects who refuse the research blood draw will still remain in the study for data collection.

Provisions for subjects from vulnerable populations

This study examines the responses of influenza vaccine in Long Term Care residents. The whole premise of the study is to study this population that is more impacted by influenza and as a result, stands to benefit the most from using the most effective flu vaccine. If the person cannot consent for themselves we will use the Legally Authorized Representative (LAR) and an Assent form. All of the subjects are residents of the LTC facility. As a result, the LTC facilities have an assessment and clinical practice plan for each resident if they are able to consent for their routine care or if the use of an LAR is needed. We will follow the LTC facility and nursing team's initial lead (speaking with the LTC Administrator, Director of Nursing, Assistant Director of Nursing, Social Worker, and Unit Nurses). If a resident uses an LAR to make his/her healthcare decisions, we will not even attempt to consent a resident directly. We will use the LAR method of consenting. If a resident is deemed able to make his/her own healthcare decisions and consent for themselves, we will approach the LTC resident and initiate a self-consenting process. In the process of consenting, we will review the consent and ask questions to assure the resident's understanding. If our study staff members feel that the subject is not able to understand the consent form and the study, we will then proceed to the LAR method for consenting or just not enroll the subject, if LAR consenting is not possible.

For illiterate or blind residents, we will have a witness present who will sign the witness line on the informed consent, if a subject would like to enroll. It is beyond the scope and budget of our study to have consents in multiple languages and access to translators, therefore we will not include this population. In our experience in this population this occurs very infrequently.

Plans for the subjects at the end of the protocol

At the end of the study, enrolled subjects will continue to receive their normal standard of care that the LTC facility provides.

Data safety monitoring plan or Data safety monitoring board or committee

Prior to writing the IRB protocol Dr. Canaday had Dr. Shorer at NIAID review the application. He said that a DSMB is not required. So we do not plan to have a DSMB. Here is the rationale for our case we made to him. The study is low risk -- all subjects are receiving a standard of care FDA approved seasonal influenza vaccination, a risk they would incur as residents of the facility even if they were not participants in the study. The new risks from the study are access to HIPAA data and blood sampling on three occasions, all low-risk activities and none traditionally requiring additional DSMB oversight.

The PI will review the data collected in both the pre-screening process and the study once a month during the duration of the study to make sure all data collection is in compliance with approved methods.

Plan to monitor adverse events

Subjects will have external monitoring while in the study as they are all long-term care residents. The LTC facilities are required to report any vaccine associated adverse events and we will have them cc us if any are reported. These vaccines are well tolerated and have very few severe adverse side effects reported. The sites will therefore notify us if there are any SAE in the 4 weeks between the first two blood draws. We will then report immediately any SAE to the IRBs. Virtually all SAE that would occur in this population would be vastly more likely due to issues related to their age and co-morbidities rather than vaccine related. Also there is already a mechanism in place for vaccine adverse event reporting (VAERS). Since both vaccines are part of FDA-approved and ACIP-recommended routine care, additional oversight and monitoring above standard mechanisms is not warranted. As the study is still formally a clinical trial we will still monitor the events reported through the established mechanism. Our LTC sites will be instructed to notify us with any events to subjects. We will also be on site at week 4, at the minimum, for the second blood draw and for an in person assessment as well.
The LTC sites will be visited at the follow-up blood draw at 4 weeks unless some SAE evaluation would require a visit earlier. The vaccine is part of what would normally be routine care at the LTC sites, so they should not need extensive specific monitoring.

How will data (electronic and hardcopy files) be maintained

Primary data sheets and paperwork in hard copy will be stored in a locked room and lock drawer. Any electronic files that have any link of subject PHI and study code will be stored on password protected files. The study will be using the secure research database, RedCap, which is housed on the UH server. Only the PI and the authorized study staff will have access to the link between the PHI and study code. The de-identified data for the study will be used for publication of the results of the objectives. The data will also be loaded onto ClinicalTrials.gov.

How long will research data be stored by the PI after study closure

Records will be stored for 3 years after closure of the study.

Subject privacy

Subject confidentiality is held strictly in trust by the participating investigators, their staff, and the sponsor(s) and their agents. The records will be viewed in a private room and protected against viewing by any person not on study team. There will be no discussion outside closed doors regarding any information pertaining to data collected. All samples will be labelled with the deidentified code right on site to protect their confidentiality.

Data/Sample confidentiality plan

We will establish a study code when subjects are enrolled. This will happen by all study staff that are authorized to enroll subjects. The samples will be obtained by all study staff trained and authorized to obtain samples. The samples will be labeled on site with the study code and date only. All PHI will be stored on the secure study database, RedCap, and lab data will then only be linked to the code. Data will be entered directly into RedCap, housed on the UH server. If any source documents are collected, or study data is collected on a data sheet, they will be stored

either in the locked offices of PI David Canaday, BRB 1022, Study Coordinator Sabina Rubeck, BRB 1035, or in the office of Nurse Coordinator Beth Bednarchik, at the UH CRC. All PHI collected by the study, as well as study consent forms, will be stored in one of the aforementioned offices. The study code for each enrolled resident is assigned through the secure study database, RedCap. Enrolled participants are identified through their study code, and are also randomized to receive one of the two study vaccines through the RedCap study database. The blood samples will only be labeled with the participant's study code and date of collection. Only IRB authorized study personnel will have access to the RedCap database, as well as to the source documents.

Data/Sample security plan

As is mentioned in the previous section, source documents such as data collection tools (if used), and Consent Forms will be stored in a locked room, in a locked cabinet. Most data will be entered directly into the secure study database, RedCap, housed on the UH server. If any source documents are collected, or study data is collected on a primary data sheet, they will be stored in a locked cabinet, either in the locked offices of PI David Canaday, BRB 1022, Study Coordinator Sabina Rubeck, BRB 1035, or in the office of Nurse Coordinator Beth Bednarchik, at the UH CRC. All PHI collected by the study, as well as study consent forms, will be stored in one of the aforementioned offices. Only the PI, and authorized members of the study team who are listed on the protocol will have access to the RedCap database, as well as any PHI.
All blood samples are coded, and therefore can be accessed by non-study staff. The samples will be stored primarily at the CWRU lab BRB 1001.

Any electronic files that have any link of subject PHI and study code will be stored on password protected files. The coded data for the study will be used for publication of the results of the objectives. The data will also be loaded onto ClinicalTrials.gov. We will keep the raw data as long as is dictated by our IRB.

Data analysis plan

Study Outcome Measures

As the primary endpoint we are using pre- to post-vaccine changes in hemagglutinin inhibition assay (HAI) titers to compare seroconversion rates and post-vaccination HAI titers to calculate the ratio of the geometric mean titers in the two treatment groups. HAI is an in vitro bioassay that determines a subject's serum levels of anti-influenza antibodies. The FDA uses this as the Gold Standard immunogenicity assay for licensure. We will follow the guidelines set out in the FDA guidance document discussing non-inferiority immunogenicity studies. SVN and NAI assays will be evaluated using the same methods.

Sample Size Considerations

Due to the clinical importance of the A/H3N2 flu strain, we will focus this power analysis on the A/H3N2 strain. Between 1976 and 2007, the CDC estimates that on average three times more people died from influenza during A/H3N2-predominant seasons than in non-A/H3N2 predominant seasons (Centers for Disease and Prevention, 2010). Even the current A/H1N1 strain, which has circulated since 2009 as the pandemic 2009 A/H1N1 strain, is a less important

cause of morbidity and mortality in the elderly attributed to sustained pre-existing cross-reactive antibody titers to older strains that offer some protection (Hancock et al., 2009).

We used the criteria and comparisons proposed by FDA for a non-inferiority immunogenicity trial (FDA, 2007). They focus on changes in influenza antibody titers (our primary endpoint). The study should be adequately powered to assess the co-primary endpoints for HAI antibodies 1) post-vaccination geometric mean titer (GMT), and 2) seroconversion rates. Seroconversion is a 4-fold rise in GMT pre- to post-vaccination as long as pre-titer at least 1:10 and in pre-titer <1:10 for seroconversion the titers must achieve ≥1:40.

To demonstrate non-inferiority in such a study:
• The upper bound of the two-sided 95% CI on the ratio of the GMTs (GMT HD vaccine/GMT Fluad) should not exceed 1.5.
• The upper bound of the two-sided 95% CI on the difference between the seroconversion rates (Seroconversion HD vaccine – Seroconversion Fluad) should not exceed 10%.

This power calculation will address both of those primary endpoints. No trials directly compare adjuvanted flu vaccine (Fluad) to HD flu vaccine or give sufficient detail in the same vaccination season to allow use of exactly the same flu strains. As a result, we must use the GMT and seroconversion data from two large trials each over 3500 subjects that compared Fluad or HD to the SD vaccine and that reported both GMT and seroconversion data (Falsey et al., 2009; Frey et al., 2014). Calculations were performed in R using the TrialSize package and power simulations were performed to confirm the results. The numbers below are the per-group sample sizes required for 80% power.

strain	Titer analysis	Seroconversion analysis
H1N1	24	23
H3N2	243	144
B strain	571	168

To power the A/H3N2-specific analysis, we require 243 per vaccine group or 486 subjects total. With this sample size, we can test non-inferiority in A/H1N1 and A/H3N2 with 80% power. Assuming our observed titers are similar to those from published studies, this sample size will be sufficient to demonstrate superiority of Fluad in A/H1N1. In order to achieve this sample size, we will overenroll by 15%. This degree of over enrollment is informed by prior clinical trial experience and is expected to be adequate given the short time between blood draws and the inclusion criteria of the subjects' expectation, willingness, and ability to have the three blood draws obtained.

For the FLUBLOK pilot studies as there are not sufficient preliminary data to perform a power calculation. That is one of the key reasons why we are adding this pilot aim to prepare for future studies.

Participant Enrollment and Follow-Up

We will enroll 620 subjects over the 2 seasons of recruitment and enrollment. To minimize withdrawal from the study, recruiters will aim to identify subjects who have a high likelihood of still being a LTC resident present in the same facility for the second blood draw. This will be an important criterion for inclusion into the study. This is generally the case with LTC residents because length of stay is on the time scale of months to years rather than days to weeks.

Analysis Plan

The FDA guidelines (listed above) for demonstrating non-inferiority for a seasonal flu vaccine dictate the analysis of the co-primary endpoints. The ratio of the geometric mean titers will be assessed using tests of means on the log-transformed titers. The upper limit of the 95% CI of the difference (HD – Fluad) in the mean log titer will be compared to log(1.5). The difference in the seroconversion rates will be assessed with a two-sample proportion test. The upper limit of the 95% CI of the difference (HD – Fluad) in the seroconversion rates will be compared to 0.1. These analyses will be performed on HAI titers of all three strains obtained from studies of pre- and post vaccination serum.

For practical recruitment purposes we have to run the study over 2 years to recruit sufficient numbers of the full cohort for A/H3N2 analysis based on the sample size calculation. At the conclusion of our study, we can summarize the A/H3N2 HAI titers and seroconversion for each year separately; but our non-inferiority hypothesis is powered based on combining the years to include the total enrolment for the A/H3N2 analysis. Since we will recruit enough subjects each year due to small number of subjects needed to power the A/H1N1 analysis, we will perform and report separate analysis for each year studied as well as combining the data. For the B strain we are underpowered and will combine the two years for this exploratory result.

We will perform analyses on the other assays (NAI and SVN) on samples obtained at pre- and post- vaccination from all 3 strains. We will run HAI on all 3 strains for the post-season blood draw for Aim 1 as a secondary analysis for differences in the ability of titers to hold in one vaccine vs. the other for the length of the season. We will also run the NAI on the A/H3N2 only (for feasibility to keep assay number reasonable) on the post-season sample to assess if there are differences in maintaining anti-NA titers between the two vaccines. Any statistical tests performed for these assays are not powered to detect superiority or non-inferiority while preserving our study-wise error rate and will be considered exploratory in nature.

Blood draws will be analyzed for humoral and cell mediated immune assays to determine flu-specific cells and antibody titers and immune status. Cells may be analyzed by RNAseq analysis to correlate aging immune responses and immune responses to the vaccines. We will not be making any DNA or doing any DNA gene analysis. No identifiable raw RNAseq files will be released that could be transcribed back to DNA for gene analysis purposes.

Analysis of FLUBLOK will be performed using the HAI and SVN assays as above and compared to the adjuvanted and high dose vaccines. NAI assays will not be used for this vaccine as it does not contain HA. If the pilot data are not powered by full non-inferiority studies we will also use Mann-U non-parametric tests to study differences in the mean responses between the vaccine groups.

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