WU 445: Longitudinal tracking of bone marrow plasma cell responses to licensed human vaccines

Version 1 18JULY2025

STATEMENT OF COMPLIANCE

The study will be carried out in accordance with Good Clinical Practice (GCP) as required by the following:

- U.S. Code of Federal Regulations applicable to clinical studies (45 CFR 46)
- 21 CFR 312
- ICH GCP E6
- Completion of Human Subjects Protection Training
- NIH Clinical Terms of Award

SIGNATURE PAGE

The signature below constitutes the approval of this protocol and the attachments and provides the necessary assurances that this clinical study will be conducted according to all stipulations of the protocol, including all statements regarding confidentiality, and according to local legal and regulatory requirements and applicable US federal regulations and ICH guidelines.

Signed: _____ Date_____

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WU 445: Longitudinal tracking of bone marrow plasma cell responses to licensed human vaccines

PROTOCOL SUMMARY

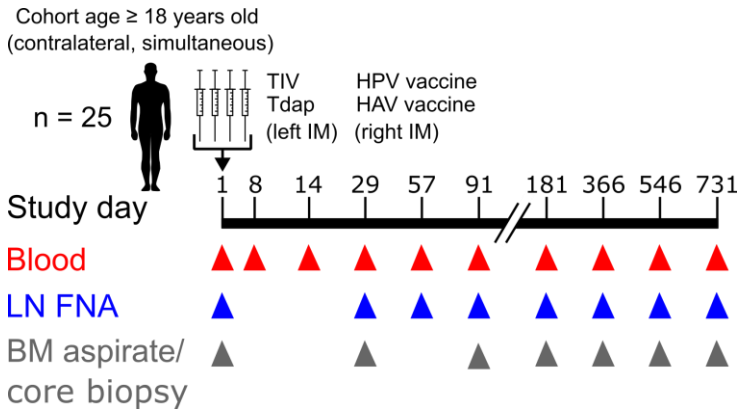
Participants: Persons willing to receive TIV, Tdap, HPV, and HAV vaccinations, ≥18 years old, and undergo sample collections.

Study Site: Infectious Disease Clinical Research Unit (ID CRU) Washington University School of Medicine 620 South Taylor, St. Louis MO 63110

Study Duration: 2 years

Number of participants: 25 with BMA, optional FNA and BMCB assessments
Participant Participation Duration: 24 months

Description of Intervention: Participants will receive four licensed vaccines administered intramuscularly at a single visit: trivalent inactivated influenza vaccine (TIV) and the tetanus, diphtheria and acellular pertussis vaccine (Tdap) in the left arm, and the nonavalent HPV vaccine (HPV) and hepatitis A (HAV) vaccines in the right arm. No investigational products will be used. All participants will undergo peripheral blood draws and bone marrow aspirates at scheduled intervals over two years to assess vaccine-induced immune responses. Optional procedures include ultrasound-guided lymph node fine needle aspirates (FNA) and bone marrow core biopsies (BMCB) for spatial and cellular analysis of immune responses.



OBJECTIVES AND HYPOTHESIS:

Objectives:

Primary

Define the frequency, durability, and transcriptional profiles of antigen-specific bone marrow plasma cells (BMPCs) following simultaneous vaccination with TIV, Tdap, HPV, and HAV in healthy adults.

Secondary

1. Compare BMPC responses elicited by recall (TIV, Tdap) versus primary (HAV) or partially primed (HPV) vaccines.
2. Evaluate transcriptional heterogeneity of BMPCs across different vaccine platforms.
3. Assess germinal center responses and their association with BMPC formation.
4. Determine the impact of age on BMPC induction and plasma cell niche characteristics.
5. Identify lymph node features associated with long-lived BMPC generation.
6. Examine how vaccine type influences spatial organization of BMPCs in the bone marrow.

Hypothesis:

Vaccines differ in their ability to generate long-lasting antibody-producing cells in the bone marrow. We hypothesize that recall vaccines (TIV, Tdap) will produce different bone marrow plasma cell responses compared to primary or partially primed vaccines (HAV, HPV), and that age may influence the strength and durability of these responses.

BACKGROUND INFORMATION AND SCIENTIFIC RATIONALE

Influenza virus vaccine

Annual influenza virus epidemics by subtype A viruses (H1N1 and H3N2), and/or influenza B viruses have been shown to cause substantial morbidity and mortality, exacting an average of 36,000 deaths and more than 200,000 hospitalizations in the US alone[1].

To combat this important public health threat, two types of inactivated influenza vaccines have been approved by the FDA: 1) a trivalent, inactivated vaccine (TIV); and 2) a quadrivalent inactivated vaccine (QIV). The TIV vaccine contains the hemagglutinin (HA) and neuraminidase (NA) proteins corresponding to the three influenza viruses predicted to be in circulation during the influenza season (two A strains and one B), and the quadrivalent type contains the HA and NA for an additional B strain. These vaccines induce predominantly HA-specific and NA-specific antibodies that are capable of virus neutralization. Antibodies to HA are critical to provide immune protection to the host against influenza virus infection, as this protein plays a key role in virus entry to initiate the replication cycle in the cell[2].

Currently, the Advisory Committee on Immunization Practices (ACIP) of the CDC recommends that everyone aged 6 months or older in the United States receive the influenza vaccine on an annual basis[3]. The recommendation for annual vaccination, regardless of previous vaccination history, is in part due to the constantly changing nature of the influenza strains in circulation. Through the processes of antigenic drift and antigenic shift, the viral HA protein is constantly changing to evade the antibody response[4]. This requires that the formulation of the influenza vaccine is updated each season[5]. However, when the vaccine formulation remains unchanged from year to year, re-vaccination is still recommended because antibody levels decline over the course of a year.

In recent years it has become apparent that a significant fraction of the human antibody response to influenza targets highly conserved neutralizing epitopes on the virus. This raises the possibility that an optimally designed vaccine could elicit a broad immunity to the virus. If long-lived antibody responses could be generated against these neutralizing epitopes through immunization, it could eliminate the need for yearly re-vaccination. The development of such a vaccine will therefore require an understanding of the factors that lead to the generation of long-lived antibody responses.

Fluarix (GlaxoSmithKline) is a commercially available seasonal influenza virus vaccine prepared from influenza viruses propagated in embryonated chicken eggs. Each of the influenza viruses is produced and purified separately. After harvesting the virus-containing fluids, each influenza virus is concentrated and purified by zonal centrifugation using a linear sucrose density gradient solution containing detergent to disrupt the viruses. Following dilution, the vaccine is further purified by diafiltration. Each influenza virus solution is inactivated by the consecutive effects of sodium deoxycholate and formaldehyde leading to the production of a "split virus." Each split inactivated virus is then suspended in sodium phosphate-buffered isotonic sodium chloride solution. The vaccine is formulated from the 3 split inactivated virus solutions. Fluarix is standardized according to U.S. Public Health Service (USPHS) requirements for the influenza virus season and is formulated to contain 45 mcg HA per 0.5-mL dose, in the recommended ratio of 15 mcg HA of each of the following 3 strains: H1N1, H3N2 and, B-Victoria lineage. Each 0.5-mL dose also contains octoxinol-10 (Triton X-100) ≤0.085 mg, α-tocopheryl hydrogen succinate ≤0.1 mg, and polysorbate 80 (Tween 80) ≤0.415 mg. Each dose may also contain residual amounts of hydrocortisone ≤0.0015 mcg, gentamicin sulfate ≤0.15 mcg, ovalbumin ≤0.05 mcg, formaldehyde ≤5 mcg, and sodium deoxycholate ≤50 mcg from the manufacturing process.

Tetanus/diphtheria/acellular pertussis vaccine

The tetanus/diphtheria/acellular pertussis vaccine (Tdap) is a combination vaccine that protects against three potentially life-threatening bacterial diseases: tetanus, diphtheria, and pertussis (whooping cough)[6].

Tetanus (also known as lockjaw) is a serious, often fatal disease caused by an extremely potent neurotoxin produced by *C. tetani*. Protection against disease is due to the development of neutralizing antibodies to tetanus toxin[7]. A serum tetanus antitoxin level of 0.01 IU/ml measured via a neutralization assay is considered the minimum protective level.

Diphtheria is an acute toxin-mediated disease caused by toxigenic strains of *C. diphtheriae*. Protection against disease is due to the development of neutralizing antibodies to diphtheria toxin. A serum diphtheria antitoxin level of 0.01 IU/ml is the lowest level giving some degree of protection.

Pertussis, known commonly as whooping cough, is a highly contagious respiratory tract infection[8]. Although it initially resembles an ordinary cold, whooping cough may eventually turn more serious, particularly in infants. Since the early 1980s, there has been an overall trend of an increase in reported pertussis cases. Pertussis is naturally cyclic in nature, with peaks in disease every 3 to 5 years. But for the past few decades, peaks got higher and overall case counts went up. The acellular pertussis vaccines (DTaP and Tdap) used now may not protect for as long as the whole cell vaccine (DTP) doctors used to use. Throughout the 1990s, the United States switched from using DTP to using DTaP for babies and children. Whole cell pertussis vaccines are associated with higher rates of minor and temporary side effects such as fever and pain and swelling at the injection site. Serious neurologic adverse reactions, including chronic neurological problems, occurred rarely among children who had recently received whole cell vaccines. Studies have inconsistent results about whether the vaccine could cause chronic neurological problems.

However, public concern in the United States and other countries led to a concerted effort to develop a vaccine with improved safety. Due to these concerns, along with the availability of a safe and effective acellular vaccine, the United States switched to acellular pertussis vaccines (DTaP). Immunity to pertussis infection is known to decrease within a decade of completing the childhood vaccination series[9]. In 2005, the ACIP recommended booster vaccination with Tdap for all adolescents and adults[10].

There are two Tdap vaccines used in the United States: Adacel® (Sanofi Pasteur) and Boostrix® (GlaxoSmithKline). The current ACIP recommendation is to give one dose of Tdap to any adult who has not previously received it[11].Revaccination is recommended every 10 years due to waning immunity.

Each 0.5-mL dose of Adacel® contains 5 Lf tetanus toxoid, 2 Lf diphtheria toxoid, and acellular pertussis antigens (2.5 µg detoxified PT, 5 µg FHA, 3 µg pertactin, 5 µg FIM). Other ingredients per 0.5-mL dose include 1.5 mg aluminum phosphate (0.33 mg aluminum) as the adjuvant, ≤5 µg residual formaldehyde, <50 ng residual glutaraldehyde, and 3.3 mg (0.6% v/v) 2-phenoxyethanol (not as a preservative).

Each 0.5-mL dose of Boostrix® contains 5 Lf of tetanus toxoid, 2.5 Lf of diphtheria toxoid, 8 µg of inactivated PT, 8 µg of FHA, and 2.5 µg of pertactin. Each 0.5-mL dose contains aluminum hydroxide as adjuvant (not more than 0.39 mg aluminum by assay), 4.5 mg of sodium chloride, ≤100 µg of residual formaldehyde, and ≤100 µg of polysorbate 80 (Tween 80).

Human papilloma virus vaccine

Human papilloma virus (HPV) is a DNA virus and one of the most common sexually transmitted agents. Over 40 HPV types can infect the genitals, mouth, and throat in women and men, 15 genotypes are associated with cervical cancer, and one type (type 16) accounts for 95% of HPV-positive oropharyngeal carcinomas[12]. Identifying HPV as the cause of cervical cancer[13] resulted in efforts to develop an HPV vaccine[14]. Expression of viral capsid proteins results in the formation of empty viral-like particles (VLPs) that resemble infectious virus[14] and are capable of generating neutralizing antibody responses[15]. Since the initial discovery of how to produce VLPs, several commercial vaccines have been developed and licensed. The first was Gardasil® (Merck), a quadrivalent vaccine targeting HPV-6, HPV-11, HPV-16 and HPV-18. A bivalent vaccine, Cervarix® (GlaxoSmithKline), targets HPV-16 and HPV-18. A nonavalent vaccine, Gardasil 9® (Merck) was licensed in 2014 targeting HPV-6, HPV-11, HPV-16, HPV-18, HPV-31, HPV-33, HPV-45, HPV-52 and HPV-58[16]. All three vaccines utilize VLPs produced in eukaryotic cells.

Prophylactic HPV vaccination has been extremely effective at reducing HPV infections and disease worldwide[17]. The vaccine is highly immunogenic, with virtually all vaccinees seroconverting, and induces neutralizing antibody titers that can persist up to 10 years post vaccination[15,18,19]. Although HPV vaccines were initially licensed as a three dose schedule, the World Health Organization updated their recommendation in 2014 to a two dose schedule for adolescent girls aged 9-14 years old following studies demonstrating the effectiveness of this schedule compared to three doses in adults[20], and further evidence suggests even a single dose can be effective at protecting against HPV[21,22].

The ACIP currently recommends children and adults aged 9 through 26 years receive HPV vaccination[23]. HPV vaccination is routinely recommended at age 11 or 12 years; vaccination can be given starting at age 9 years. Catch-up HPV vaccination is recommended for all persons through age 26 years who are not adequately vaccinated. Catch-up HPV vaccination is not recommended for all adults aged >26 years. Instead, shared clinical decision-making regarding

HPV vaccination is recommended for some adults aged 27 through 45 years who are not adequately vaccinated[24].

Each 0.5-mL dose of Gardasil 9® contains approximately 30 mcg of HPV Type 6 L1 protein, 40 mcg of HPV Type 11 L1 protein, 60 mcg of HPV Type 16 L1 protein, 40 mcg of HPV Type 18 L1 protein, 20 mcg of HPV Type 31 L1 protein, 20 mcg of HPV Type 33 L1 protein, 20 mcg of HPV Type 45 L1 protein, 20 mcg of HPV Type 52 L1 protein, and 20 mcg of HPV Type 58 L1 protein. Each 0.5-mL dose of the vaccine also contains approximately 500 mcg of aluminum (provided as AAHS), 9.56 mg of sodium chloride, 0.78 mg of L-histidine, 50 mcg of polysorbate 80, 35 mcg of sodium borate.

Hepatitis A vaccine

Hepatitis A virus (HAV) is a pathogenic, hepatotropic, RNA picornavirus transmitted by the fecal-oral route usually through person-to-person contact[25]. Unsanitary conditions typically contribute to the spread of the virus. The virus replicates in the liver, is transferred from bile to stool, and then can be spread by consuming contaminated food or beverages[26]. Infections in children under 6 years of age tend to be asymptomatic, while those in older children and adults are symptomatic[27]. Symptoms of acute illness include fever, malaise, anorexia, headache, and jaundice. HAV infection can result in fulminant hepatitis, severe liver injury complicated by the development of hepatic encephalopathy within 8 weeks of the onset of the jaundice. The case fatality rate of fulminant hepatitis is about 80%[27].

Improved standards of public health and the development of a HAV vaccine have greatly contributed to a decline in Hepatitis A infections, particularly in industrialized countries. While cyclic community-wide outbreaks continue to occur in the United States, the development of formalin-inactivated HAV vaccines in the 1990s has led to reduction in overall cases[26]. HAV vaccines are highly immunogenic, generating neutralizing antibodies in more than 94 percent of vaccinees one month after the first dose has been given, and essentially seroconverting all recipients after the second dose[28,29]. Seroconversion is defined as achieving a detectable and quantifiable postvaccination IgG anti-HAV level of ≥10 mIU/mL by standard assays[30,31]. Seroprotection is considered a surrogate of clinical protection and persists when IgG anti-HAV levels remain higher than the correlate of protection.

Currently licensed HAV vaccines in the United States include Havrix® (GlaxoSmithKline) and Vaqta® (Merck). The ACIP recommends HAV vaccination for adults likely to be at risk for HAV infection, including international travelers traveling to countries with endemic or high rates of HAV, persons using injection or noninjection illegal drugs, men who have sex with men, persons experiencing homelessness, persons with occupational health hazards, and older adults (> 40 years old)[32].

Vaqta® is licensed in two formulations. Persons aged ≥19 years should receive 50 units per dose in a 2-dose schedule. Vaqta® is an inactivated whole virus vaccine derived from hepatitis A virus grown in cell culture in human MRC-5 diploid fibroblasts. It contains inactivated virus of a strain which was originally derived by further serial passage of a proven attenuated strain. The virus is grown, harvested, purified, and formalin inactivated, and then adsorbed onto amorphous aluminum hydroxyphosphate sulfate. One milliliter of the vaccine contains approximately 50U of hepatitis A virus antigen, which is purified and formulated without a preservative. Each 1-mL adult dose contains 50U of hepatitis A virus antigen and adsorbed onto approximately 0.45 mg of aluminum provided as amorphous aluminum hydroxyphosphate sulfate, and 70 mcg of sodium borate as a pH stabilizer, in 0.9% sodium chloride.

Havrix® also is licensed in two formulations. Persons aged ≥16 years should receive 1,440 ELISA units per dose in a 2-dose schedule. Havrix® is a sterile suspension of inactivated virus for intramuscular administration. The virus (strain HM175) is propagated in MRC-5 human diploid cells. Each 1-mL adult dose of vaccine contains 1440 EL.U. of viral antigen, adsorbed on 0.5 mg of aluminum as aluminum hydroxide.

SCIENTIFIC RATIONALE

Efficacious vaccines depend on generating protective antibody responses. Protective antibody titers are the result of B cell responses coordinated in the draining lymph nodes. After vaccination, antigen-specific B cells present in lymph nodes recognize vaccine antigens, become activated, and receive co-stimulatory signals from CD4+ T cells. Some of these activated B cells will form germinal centers within the B cell follicles of lymph nodes. Germinal centers are microanatomical structures that facilitate the proliferation of antigen-specific B cells to expand their frequency, somatic hypermutation of their B cell receptors, and selection of mutated clones to increase the binding affinity of the subsequent antibodies produced by the immune system[33]. B cell clones which exit from the germinal center can differentiate into memory B cells, retained in circulation and capable of rapidly differentiating into antibody-secreting plasmablasts upon pathogen re-exposure[34,35], or bone marrow plasma cells, terminally differentiated effector cells capable of continuously maintaining durable antibody titers[36,37]. Generating both humoral effector cell types after vaccination is critical to establishing long-term protective immunity, but despite decades of research and the development of vaccines for many diseases it is still unclear what factors control the differentiation of memory B cells and bone marrow plasma cells. Additionally, it is not certain what factors maintain long-lived bone marrow plasma cells over short-lived cells which fail to establish durable antibody titers. Some vaccines, such as the measles, mumps, and rubella vaccine induce antibodies that persist for decades, while other vaccines such as seasonal influenza virus vaccine induce antibodies of limited duration[38,39]. Furthermore, influenza-specific bone marrow plasma cell frequencies increase 1 month after seasonal influenza virus vaccination, but decline 1 year later[40], suggesting current human influenza virus vaccine formulations have a limited capacity to induce long-lived protective responses. Understanding the factors that promote the differentiation and maintenance of long-lived bone marrow plasma cells would not only facilitate the development of influenza virus vaccines with longer protection but advance the capacity to produce effective vaccines against emerging pathogens. Additionally, understanding how different vaccine formulations (e.g. inactivated virus, recombinant protein, virus-like particles etc.) and antigens contribute to immune durability would improve both current and future vaccine design.

Studies in mice and humans have characterized many of the factors dictating the long-lived bone marrow plasma cell phenotype. Plasma cells home to the bone marrow under the direction of CXCR4 and are retained by VCAM1. Studies have suggested that bone marrow plasma cells differentiate later than memory B cells from germinal center reactions with highly mutated receptors[41,42]. Those clones preferentially selected for long-term maintenance may correspond to a $CD19^{lo}$ surface phenotype[43], but whether that phenotype is instructed within germinal centers or corresponds to cells differentiated from $CD19^{hi}$ cells is unclear. Frequencies of long-lived cells appear to increase with age in mice, suggesting an expansion of niche capacity with time[44]. Moreover, fluctuations in survival factors within the bone marrow niche, such as the tumor necrosis superfamily ligand APRIL, likely play a role in determining how effectively newly elicited plasma cells outcompete or displace resident bone marrow plasma cells from previous responses[45]. However, neither a model in which longevity is instructed within germinal centers nor a model in which new plasma cells displace old cells over time fully explains why some vaccines in humans are more effective at generating durable protective antibody titers[38].

We will employ our laboratory's extensive experience studying antigen-specific B cell responses in blood, axillary lymph nodes, and bone marrow[46–48] to investigate the factors contributing to heterogeneity within long-lived bone marrow plasma cells. We will compare vaccine responses in humans to the trivalent inactivated seasonal influenza virus vaccine (TIV), tetanus/diphtheria/pertussis vaccine (Tdap), human papilloma virus (HPV) vaccine, and Hepatitis A virus (HAV) vaccine to identify bone marrow plasma cells elicited by vaccines with variable persistent immunity alongside antigen-specific bone marrow plasma cells likely elicited from prior infections or vaccinations. In addition to clones specific for TIV, Tdap, HPV, and HAV, we will use a panel of antigen probes to isolate long-lived clones specific for historic seasonal influenza viruses, SARS-CoV-2, respiratory syncytial virus, measles, mumps, rubella, hepatitis B, varicella zoster virus, vaccinia virus, and polio virus. We will compare the transcriptional heterogeneity within antigen-specific bone marrow plasma cells to identify subsets corresponding to recently stimulated plasma cells versus long-lived cell types. Additionally, comparisons to clones within lymph node germinal centers and short-lived plasmablasts in blood will be used to identify factors likely dictating longevity and established within germinal center responses. Finally, core biopsies of bone marrow will allow for the investigation of survival factors regulating the bone marrow niche. The rapid plasmablast response at approximately 8 days post vaccination induced by the seasonal influenza virus vaccine[34] makes this an ideal vaccine response for comparing recently elicited short-lived plasmablasts and plasma cells to long-lived bone marrow plasma cells.

We will recruit participants ≥18 years old (n=25). TIV and Tdap will be administered in the left arm intramuscularly (IM); simultaneously, HPV vaccine and HAV vaccine will be administered in the right arm IM. We will sample a single lymph node at baseline on both sides and on days 29, 57, 91, 181, 366, 546, and 731 after vaccination. We will collect peripheral blood at baseline and days 8, 14, 29, 57, 91, 181, 366, 546, and 731 after vaccination. We will collect bone marrow aspirates to isolate antigen-specific bone marrow plasma cells and optional core biopsies to spatially assess the bone marrow plasma cell niche at baseline on days 29, 57, 91, 181, 366, 546, and 731 after vaccination. Tracking vaccine responses for 1-2 years will provide key data for evaluating durable humoral immunity. The administration of these four vaccines simultaneously allows us to address the following questions in the context of long-lived antibody titers:

1. Do recall responses to booster immunizations (TIV and Tdap) promote higher or lower frequencies of long-lived bone marrow plasma cells compared to primary responses without prior vaccination and infection (HAV vaccine) or prior vaccination (HPV vaccine)?
2. Do antigens associated with vaccines known for generating durable responses (Tdap, HPV vaccine, HAV vaccine) induce transcriptionally homogenous or heterogenous bone marrow plasma cells populations compared to vaccines known for generating waning immunity (TIV)?
3. Are the antigen-specific bone marrow plasma cells induced by TIV/Tdap/HPV vaccine/HAV vaccine transcriptionally similar/different from antigen-specific bone marrow plasma cells elicited from prior infections or vaccinations?
4. Does age affect the capacity to induce bone marrow plasma cells after vaccination and do the numbers of bone marrow plasma cell niches expand with age in humans?
5. Are there signatures of longevity instructed within germinal centers for bone marrow plasma cells in humans?
6. How do inactivated, split virus vaccines (TIV), recombinant protein adjuvanted vaccines (Tdap), inactivated adjuvanted virus vaccines (HAV vaccine), and viral-like particle vaccines (HPV vaccine) compare in their capacity to elicit both germinal center responses and long-lived bone marrow plasma cells?

POTENTIAL RISKS AND BENEFITS

Known Potential Benefits

There is potential benefit to participants. Participants will receive the annual trivalent influenza vaccine, as recommended by the CDC[3]. Vaccination will also likely generate an immune response against HPV, Hepatitis A, and toxins covered in Tdap. These infections carry a significant risk of morbidity and mortality. However, it is unclear if these vaccinations will provide protection outside of the FDA indicated populations. Participants will be given information about whether further vaccines (i.e. a second dose of HAV or HPV) would be required for full vaccination in the future. Understanding the immunological effects of differing vaccines on the immune response to vaccination may benefit society in generating better vaccines.

Known Risks

TIV

The influenza vaccine, including trivalent and quadrivalent inactivated formulations, is widely used and has a well-established safety profile. Common side effects include soreness, redness, or swelling at the injection site, along with mild systemic symptoms such as low-grade fever, headache, or muscle aches[49]. Rare adverse events include allergic reactions (e.g., urticaria, angioedema, or anaphylaxis), particularly in individuals with severe allergies to vaccine components such as egg protein[50]. Neurologic complications like Guillain-Barré syndrome have been reported rarely and may occur at a rate of approximately one to two additional cases per million doses administered[51]. The benefits of influenza vaccination in preventing severe illness and hospitalization significantly outweigh these minimal risks.

Tdap

The Tdap vaccine is generally safe and well tolerated. The most commonly reported side effects are mild and include pain, redness, or swelling at the injection site, as well as transient fever, headache, or fatigue[52]. Rare but more serious adverse events have been reported, including hypersensitivity reactions, brachial neuritis, and, infrequently, Guillain-Barré syndrome (GBS). However, available data do not establish a causal link between Tdap vaccination and GBS[53]. Severe allergic reactions such as anaphylaxis are very rare. Tdap should not be administered to individuals with a history of encephalopathy of unknown origin within seven days of a previous pertussis-containing vaccine.

HPV

The HPV vaccine, including the 9-valent formulation, has an excellent safety profile. Most adverse events are minor and self-limited, including pain, swelling, and erythema at the injection site, along with low-grade fever, headache, or dizziness[54]. Syncope, especially among adolescents, has been reported and is the most notable immediate adverse event; observation for 15 minutes post-vaccination is recommended to reduce the risk of injury from falls[55]. Serious adverse events are rare and have not been causally associated with HPV vaccination in large population-based studies[56]. The vaccine is not live and is safe for immunocompromised individuals.

HAV

The hepatitis A (HAV) vaccine is well tolerated and associated primarily with mild, short-term side effects. The most common adverse events include injection site tenderness, fatigue, and headache, usually resolving within 1–2 days[57]. Severe reactions such as anaphylaxis are exceedingly rare, and no consistent patterns of serious adverse events have been linked to

HAV vaccination[58]. The vaccine has a long record of safety across various populations, including children and immunocompromised individuals.

Blood collection
The blood collection procedure carries a risk of pain (typically mild pain and very temporary), vasovagal symptoms (e.g. temporary light headedness or low blood pressure), bleeding, bruising, redness or swelling and, very rarely, infection. These risks are very small, and phlebotomy will be performed by registered physicians, experienced clinical research nurses or accredited phlebotomists to minimize any potential risk or discomfort

BMA
The physical risks of bone marrow aspiration are pain and bruising that may last 1 to 3 days. Very rarely more serious side effects which include allergic reactions to lidocaine or damage to normal blood vessels, nerves or bone structures or localized infection at the area where the marrow aspirate occurs. The removal of 30 ml or less of marrow produces a temporary and mild anemia that may last one or two weeks. A small number of people experience vaso-vagal response including, lightheadedness, nausea, and transient hypotension. Bone marrow aspiration procedures will be performed by experienced and well trained personnel. Care will be taken to obtain these specimens in a safe and sterile manner.

BMCB
The physical risks of bone marrow core biopsy are similar to those of aspiration, including pain and bruising at the biopsy site that may last several days. Because the core biopsy involves removal of a small cylindrical sample of bone and marrow, patients may experience slightly more post-procedural soreness or discomfort. As with aspiration, more serious complications are rare but can include allergic reactions to local anesthetics, bleeding, infection, or inadvertent injury to surrounding blood vessels, nerves, or bone. A vasovagal reaction—such as lightheadedness, nausea, or a brief drop in blood pressure—may occur in a small number of individuals. The procedure does not typically result in significant or lasting changes in blood counts. All core biopsy procedures will be performed by experienced and well-trained personnel using sterile technique to minimize risks and ensure safety.

Axillary lymph node fine needle aspiration (FNA)
The procedural risks of FNA in the axillary lymph nodes are negligible. Lymph nodes are not prone to significant bleeding, and the FNA needles used are so small that they pose no significant risk of injury to sensitive structures. After the procedure, the puncture site on the skin is usually so small that it is difficult to identify and is covered with a Band-Aid. At most, participants may feel mild soreness in the armpit on the side of biopsy and mild swelling, redness, or bruising. Application of ice and oral, over-the-counter Tylenol or other analgesics may be helpful in alleviating these symptoms. There is a theoretical risk of infection at the skin puncture site. They should keep the area clean and dry. Participants should monitor their skin at the puncture site for inflammation (redness, tenderness, swelling) lasting beyond 24 hours or pus/discharge. If they notice such symptoms, they should contact their physician.

Lidocaine administration
Risk from administration of lidocaine can occur rarely and include cutaneous lesions, urticarial, edema or anaphylactoid reactions. Pruritus, burning, edema, erythema, purpura and bleeding may occur at the local injection site.

Financial risks
Financial risks are largely related to time for clinic visits and travel costs. Participants will not be charged for any of the services or procedures provided as a part of the study, although they may have charges for standard of care medical visits which may occur during the study or as a result of information obtained as part of the study.

Participants will be fully consented and made aware of the potential risks associated with their participation in the study and have the knowledge that they can discontinue the study at any time. Each participant will be monitored carefully and appropriately and will be instructed to contact the study team promptly upon noticing any complication or health issues. Should this occur, participants' health history will be reviewed by the study team

All participants asked to participate in the clinical studies will be advised that their participation in the studies is entirely voluntary, that they can choose discontinue participation at any time and that this will not negatively impact on their access to routine health care.

Impact on immunity to HAV and HPV
Participants will be made aware that participation in this study cannot guarantee adequate protection against Hepatitis A and Human Papilloma Virus. Per U.S. Centers for Disease Control guidelines, these vaccination series are typically given in multi-shot series and this study will only include a single vaccination.

Overall Risk/Benefit Assessment

The overall benefit/risk assessment for this clinical study is considered acceptable for the following reasons:

- These are FDA approved medications.
- Only study participants who meet all inclusion criteria and none of the exclusion criteria will be allowed to participate in this study.
- The selection criteria include adequate provisions to minimize the risk and protect the well-being of study participants in the study.
- Safety will be closely monitored throughout the study:
- After each study product administration, study participants will remain in the clinic and be closely observed by study staff for at least total 30 minutes post-vaccination or longer if deemed necessary by the Investigator, to monitor the development of any acute reactions.
- Any unsolicited, solicited local or systemic AEs will be documented during this period.
- The Investigator or the designee will document all AEs from the time of informed consent through Day 28. All SAEs or AEs leading to study discontinuation will be documented throughout the duration of the study.
- Any clinically significant abnormalities (including those persisting at the end of the study/early withdrawal) will be followed by the Investigator until resolution or until a clinically stable endpoint is reached.

Objectives:

Primary
Define the frequency, durability, and transcriptional profiles of antigen-specific bone marrow plasma cells (BMPCs) following simultaneous vaccination with TIV, Tdap, HPV, and HAV in healthy adults.

Secondary

1. Compare BMPC responses elicited by recall (TIV, Tdap) versus primary (HAV) or partially primed (HPV) vaccines.
2. Evaluate transcriptional heterogeneity of BMPCs across different vaccine platforms.
3. Assess germinal center responses and their association with BMPC formation.
4. Determine the impact of age on BMPC induction and plasma cell niche characteristics.
5. Identify lymph node features associated with long-lived BMPC generation.
6. Examine how vaccine type influences spatial organization of BMPCs in the bone marrow.

Outcome Measures

Primary
The frequency and durability of antigen-specific bone marrow plasma cells (BMPCs) measured at baseline and at days 29, 57, 91, 181, 366, 546, and 731 following vaccination.

Secondary
Frequency, severity, and causality of all adverse events.

STUDY DESIGN

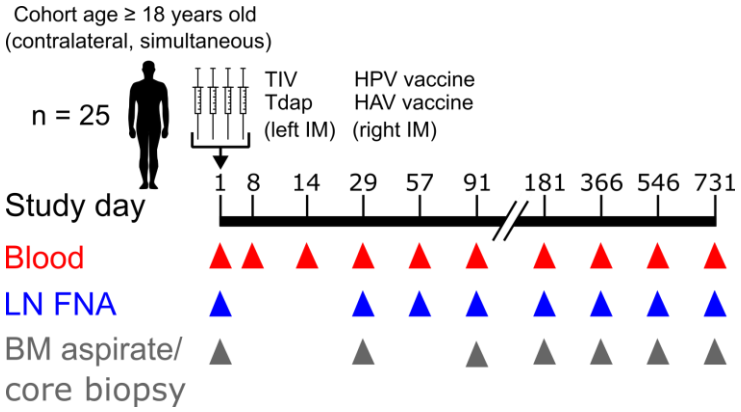
This is a single center, single cohort, mechanistic study in which 25 healthy participants will be recruited.

The immune responses in blood, bilateral draining lymph node (sampled by FNA of the lateral axillary lymph nodes), and bone marrow (sampled by BMA and BMCB) will be analyzed.

Blood samples will be collected at D1 (pre-vaccination) and on D8 (+/- 3 days), D14 (+/- 3 days), D29 (+/- 7 days), D57 (+/- 7 days), D91 (+/- 14 days), D181 (+/- 14 days), D366 (+/- 14 days), D546 (+/- 14 days), and D731 (+/- 14 days).

Lymph node sampling by FNA will be done at D1 (pre-vaccination) and on D29 (+/- 7 days), D57 (+/- 7 days), D91 (+/- 14 days), D181 (+/- 14 days), D366 (+/- 14 days), D546 (+/- 14 days), and D731 (+/- 14 days).

Bone marrow sampling by BMA/BMCB will be done on D1 (pre-vaccination) and on D29 (+/- 7 days), D91 (+/- 14 days), D181 (+/- 14 days), D366 (+/- 14 days), D546 (+/- 14 days), and D731 (+/- 14 days).



STUDY DRUGS

The vaccines used in this study are all FDA approved.

1. The investigation is not intended to be reported to the FDA as a well-controlled study in support of a new indication for use; nor intended to be used to support any other significant change in the labeling.
2. The investigation is not intended to support a significant change in the advertising for the product.
3. The investigation does not involve a route of administration or dosage level or use in a patient population or other factor that significantly increases the risks (or decreases the acceptability of the risks) associated with the use of the product.

This study will use four FDA-approved vaccines, all obtained from commercial manufacturers: Boostrix®, a combination vaccine containing tetanus toxoid, reduced diphtheria toxoid, and acellular pertussis components (0.5 mL intramuscular injection); HAVRIX®, a preservative-free hepatitis A vaccine (1 mL intramuscular injection); Gardasil® 9, a 9-valent human papillomavirus (HPV) vaccine (0.5 mL intramuscular injection); and a seasonal influenza vaccine, either Fluarix® (inactivated) or Flublok® (recombinant), each containing two A strains and one B strain (0.5 mL intramuscular injection), depending on first availability.

Study Population
Up to 25 males and non-pregnant females ages ≥18 will be enrolled from the existing population of adults residing in the St. Louis metropolitan area. We expect that many of these participants will be employees of Barnes Jewish Hospital or Washington University Medical School. Persons for whom the study team has a supervisory role will not be enrolled to avoid any potential for coercion. Study team members will not be allowed to participate. We plan to enroll 25 evaluable participants in the coming year, anticipating up to 20 enrolling in the optional FNA and BMCB assessments.

PARTICIPANT RECRUITMENT PLANS AND CONSENT PROCESS

Participants will be recruited by inviting prior participants, word of mouth, referrals and advertising through flyers and Volunteers for Health. Outreach may include the following: Fliers/ Posters/ Social media

1. Recruitment flier will be designed by study staff for use as a flier, poster, and email attachment for distribution to potential participants.
2. Washington University Volunteers for Health will use the Washington University Study Search website, email, and social media to disseminate approved recruitment materials to potential participants who have previously expressed interest in participating in research studies.

Once a potential participant is identified, the following will take place.
1. Potential participants will be invited to participate by screener
2. Screener will provide ICF, review ICF and study procedures with subject. If interested subject will be appointed for screening/preentry visit.
3. Study Team which will usually include the PI or sub-investigator meets subject, reviews consent with subject and any significant others, discussing in detail the nature, aims, duration, potential hazards of the study, and procedures to be performed during the study. All aspects of the study will be explained in lay language and questions answered. The study team will also explain that the participants are completely free to refuse to enter the study or to withdraw from it at any time. If acceptable to subject and team is confident that subject can offer informed consent ICF is signed and a copy is given to the subject.
The structure of the ID CRU helps minimize the possibility of coercion or undue influence during the consent process. Participants will have as much time as they desire to consider enrolling in the study, including:
An opportunity to thoroughly review the consent materials with knowledgeable members of the research team, and with family and/or friends as appropriate Sufficient time to have all of their questions answered. Any subsequent consent revisions will be handled in a similar fashion.

PARTICIPANT INCLUSION CRITERIA
1. Healthy participants over 18 years of age.
2. Able to understand and give informed consent.
3. Willing to receive TIV, Tdap, HPV, and HAV vaccinations
4. In stable health, as determined by medical history and targeted physical exam related to this history.
5. Willing to give BMA samples
6. For those willing to give FNA or BMCB samples, Willing to:
   give FNA specimens OR
   give BMCB specimens OR
   give both FNA and BMCB specimens

PARTICIPANT EXCLUSION CRITERIA
1. Has a history of severe allergic reaction to any component of the TIV, Tdap, HPV, or HAV vaccines, including allergic reactions to neomycin, yeast or prior severe reaction after vaccination including anaphylaxis or encephalopathy within 7 days of vaccination.

2. Has a current or previous diagnosis of immunocompromising condition to include human immunodeficiency virus, immune-mediated disease requiring immunosuppressive treatment, or other immunosuppressive condition.

3. Has received systemic immunosuppressants or immune-modifying drugs for > 14 days in total within 6 months prior to Screening (for corticosteroids ≥ 10 mg/day of prednisone equivalent) or is anticipating the need for immunosuppressive treatment at any time during participation in the study.

4. Is acutely ill or febrile (temperature >38.0 C [100.4F] less than 72 hours prior to or at the day 1 visit. Participants who meet this criteria may be rescheduled.

5. Currently has symptomatic acute or unstable chronic disease requiring medical or surgical care, to include significant change in therapy or hospitalization, at the discretion of the investigator.

6. History of excessive alcohol consumption, drug abuse, psychiatric conditions, social conditions or occupational conditions that in the opinion of the investigator would preclude compliance with the study.

7. Has received any vaccine ≤ 28 days prior to the injection (Day 1) or plans to receive a vaccine within 28 days before or after the study injection. These participants may be rescheduled.

8. Has received the 2025-2026 influenza trivalent, inactivated vaccine or quadrivalent, inactivated vaccine.

9. Has received any quadrivalent or trivalent inactivated influenza, Tdap, HPV, or HAV vaccines ≤ 180 days prior to the injection (Day 1).

10. Pregnant women and nursing mothers or women who are planning to become pregnant for the study duration.

11. Have donated blood, blood products or bone marrow within 30 days before study vaccination, plan to donate blood at any time during the duration of participant study participation, or plan to donate blood within 30 days after the last blood draw.

12. Any condition in the opinion of the investigator that would interfere with the proper conduct of the trial.

13. Coagulopathy (primary or iatrogenic) which would contraindicate bone marrow aspirate or core biopsy for participants willing to have those procedures done

Handling of Withdrawals

Participants are free to withdraw from the study at any time.

Participants may be taken off study without their consent if the study doctor determines that it is in the participant's best interest not to continue to participate in the study. In addition, the study doctor may remove a participant from study participation if the participant is unable to complete the required study procedures, or if the study is stopped by the institution, the sponsor, or the Food and Drug Administration (FDA) or other health authorities. If the participant is removed from the study, the principal Investigator or designee will contact the participant to discuss the study stopping procedures.

Participants who withdraw, or are withdrawn or terminated from the study, or are lost to follow-up after study enrollment visit will not be replaced. Participants who consented to the study but did not receive the vaccinations will be replaced. These participants will be considered as screen failures.

Concomitant Medications/Treatments

All concomitant medications and treatments will be recorded in the source document.

STUDY PROCEDURES

Procedures include vaccine and administration, draining lymph node fine needle aspirate, bone marrow aspirate and/or core biopsy, targeted physical examination, phlebotomy, signs/symptom assessment (local and systemic) and vaccination site evaluation.

We do not anticipate serious adverse events but will review each participant's study progress at study visits and request that participants inform study site of adverse events between visits. Any serious adverse events thought to be possibly related to the study will result in the formation of an ad hoc data safety committee consisting of a statistician and 2 physicians (at least one from infectious disease), all playing no part in the study to review and determine whether the study should continue in consultation with the IRB.

For all participants:
Prior to vaccination (Date of administration of the vaccination is considered Day 1)
Screening can be performed on the same day or up to 28 days prior to vaccination. Participants will be consented. Vital signs and medical history will be reviewed.

Vaccinations (Day 1)
Day 1 blood, bone marrow aspirations/CB, and FNA will be collected prior to administering vaccines. TIV and Tdap IM vaccine administration in the left arm. HPV and HAV IM vaccine administration in the right arm.

Post vaccination
Post vaccinations visits will occur at days 8, 14, 29, 57, 91, 18, 366, 546, and 731. Vital signs will be collected at screening and prior to vaccine administration. Vitals will be further collected at follow up visits if clinically indicated. A daily memory aid will be completed by participants days 1-7 after vaccination to collect solicited adverse reactions. All visits involve collection of any inter-current illnesses, adverse event solicitation and blood collection. Bone marrow aspirates/CB will occur at days 29, 91, 181, 366, 546, and 731.

For a subset of participants, fine needle aspiration from bilateral axillary lymph nodes will occur at days 29, 57, 91, 181, 366, 546, and 731.

Mandatory assays:

Bone Marrow Aspiration (BMA)

The subjects will be asked to lie in the prone position, the right or left posterior superior iliac crest region will be identified, and the area will be sterilely prepped. The region to be entered will be anesthetized with lidocaine. Following adequate anesthesia, a needle will be inserted into the iliac crest region and the bone marrow cavity will be penetrated. Up to 30 cc of bone marrow will be obtained by aspiration. After aspiration, the needle will be removed. After bone marrow is obtained, the site will be cleaned and bandaged and the subject will be instructed regarding care of the puncture site. The marrow aspirate will be placed in appropriate sterile collection tubes. The specimens will be labeled and transported to the laboratory of the requesting

investigator for further studies. Selected samples may be shared with other investigators or laboratories. No shared samples will contain any PHI or any information that may identify participants.

Optional assays:

Bone Marrow Core Biopsy (BMCB)

A core biopsy may be attempted if the participant gives informed consent for BMCB in addition to BMA. The positioning, sterile preparation, and numbing are all the same as described above for BMA. Prior to collecting the BMA, an approximately 1-2cm long core biopsy will be collected within the biopsy needle and removed in a collection cradle prior to preforming BMA using the same bone puncture site. BMCB and BMA may require separate bone punctures, but will most often be collected in a single puncture. After aspiration and biopsy, the needle will be removed and the core specimen expelled. The core specimen will be placed in sterile saline, labeled, and transported.

Ultrasound-Guided Fine Needle Aspiration (FNA)

FNAs will be taken from bilateral axillary lymph nodes. Procedures will be performed at Barnes Jewish Hospital or Washington University School of Medicine. . An appropriate lymph node is identified by the radiologist and measured according to the study protocol. The skin is marked. A timeout is performed. The skin is sterilized with antiseptic solution and draped. Using ultrasound guidance, up to 10 cc Lidocaine is administered via a 25 gauge needle for numbing from the skin surface to the lymph node. The participant may experience a mild stinging sensation with Lidocaine administration that wears off in a few seconds. An FNA pass is then made with a new 25-23 gauge needle. A pass involves advancement of the FNA needle into the cortex of the lymph node, after which it is pushed and pulled back and forth through the lymph node multiple times. Each forward push is a needle throw. Each pass consists of multiple throws (as many as 60) and usually lasts approximately 15 seconds. As throws are made, cells and fluid from the lymph node are collected inside of the needle. This process is repeated for a total of 6 passes, using 6 separate FNA needles. After each pass, the needle is removed and handed to a member of the research team who processes the sample. Additional passes beyond the routine 6 are sometimes but rarely necessary if the withdrawn material is scant. None of the passes are usually felt by the participant. The most uncomfortable aspect of the procedure is typically positioning, which involves the participant either laying on their back or on their side with their arm raised overhead. This position can be straining to the shoulder, and the raised arm often “falls asleep” during the procedure. The team can assist with repositioning and arm support if necessary for participant comfort. The entire procedure, from timeout to removal of the final needle, may last over 60 minutes depending on the size and visibility of the node and ease of approach. Additional time is required to identify and measure the most appropriate lymph node. This time varies widely according to how large the lymph nodes are and how easy they are to find, but may take up to 30 minutes. After the procedure, the skin is cleaned and a Band-Aid is applied.

SAFETY ASSESSMENTS

All study participants will be instructed to report any moderate to severe vaccine related side effects that occur within 28 days post-vaccination to the study coordinator. These include fever, headache, fatigue and muscle pain (myalgia). They will be instructed to call the study coordinator or clinician for any serious vaccine related side effects.

A serious adverse event (SAE) is defined as fatal or life-threatening experience resulting in death, hospitalization, significant disability, or congenital anomaly/birth defect.

All adverse events after vaccination will be graded according to the Vaccine Adverse Event Reporting System criteria. Study investigators will assess the adverse events as being definitely related, probably related, possibly related or unrelated to the vaccination. Any serious adverse events or unexpected events related to vaccination or study conduct will be reported to Washington University HRPO following the IRB guidelines. Adverse events will be collected via telephone contact or direct evaluation and through participant interviews at each study visit.

FINAL STUDY VISIT

All participants will be asked about their interest in donating blood in the future for vaccine related immunological studies.

LAB ASSAYS OR PROCEDURES

Research Labs

The blood will be processed to isolate peripheral blood mononuclear cells (PBMCs) and plasma collected in evacuated phlebotomy tubes.
Plasma from all time points will be separated from whole blood and will be frozen at -80^{o}C.
Plasma and PBMCs will be isolated on all study days.

Specimen Preparation, Handling, and Shipping

Blood samples for research testing will be labeled at the ID CRU with a unique identifier and will be transported to Dr. Ali Ellebedy' s lab BJC-IH 8th floor - Rm. 8020 via courier service in appropriate transport containers. FNA materials will be labeled with a unique identifier and will be transported to Dr. Ali Ellebedy's lab BJC-IH 8th floor - Rm. 8020 via courier service in appropriate transport containers. BMA and BMCB will be labeled with a unique identifier and will be transported to Dr. Ali Ellebedy' s lab BJC-IH 8th floor - Rm. 8020 via courier service in appropriate transport containers.

Future use of data and specimen

We may use the blood, lymph nodes, bone marrow, and data we are obtaining in this study for studies going on right now as well as studies that are conducted in the future. These studies may provide additional information that will be helpful in understanding the immune response or other diseases or conditions, including research to develop investigational tests, treatments, drugs or devices that are not yet approved by the U.S. Food and Drug Administration. It is unlikely that what we learn from these studies will have a direct benefit to participants. There are no plans to provide financial compensation to participants. Participants cede property rights to the use of blood, lymph nodes, bone marrow, and data.

Future research may also include genetic research. We may share participant's blood, lymph node biopsies, bone marrow biopsies, and data with other researchers. They may be doing research in areas similar to this research or in other unrelated areas. These researchers may be at Washington University, at other research centers and institutions, or industry sponsors of research. We may also share research data with large data repositories (a repository is a database of information) for broad sharing with the research community. Participants research

data placed in one of these repositories are only available to qualified researchers, who have received prior approval from individuals that monitor the use of the data.

Participant's blood, lymph nodes biopsies, and bone marrow biopsies will be stored without name or any other kind of link that would enable researchers to identify which sample(s) or data are a particular individual. Therefore, it will be available for use in future research studies indefinitely and cannot be removed

STATISTICAL CONSIDERATIONS

The data will be analyzed in consultation with biostatisticians in the Research Design and Biostatistics Group, Washington University ICTS. The hypotheses we are exploratory, and effect sizes are unknown; thus, adequate power analyses cannot be performed at present. The proposed enrollment numbers are designed to maximize use of the current infrastructure for lymph node and bone marrow biopsy to provide the most power to detect potentially relevant differences in immune responses between cohorts.

SOURCE DOCUMENTATION

All source documents will be kept at the ID CRU clinic in a locked high density filing system in a locked office. The building access is secured security officers during working hours and secure badge access at all other times. Offices with the ID CRU are all locked.

QUALITY CONTROL

All ICFs and a portion of the study charts (approximately 10%) will be audited annually by the ID CRU clinic quality management personnel per the ID CRU clinic SOPs.

ETHICS AND PROTECTION OF HUMAN SUBJECTS

Once the study is approved by the Washington University Human Research Protection Office (HRPO), recruiting and screening will begin for the study. All participants are required to read English, understand and sign the informed consent document.

DATA HANDLING AND RECORD KEEPING

The clinical data on the participants will be maintained at the ID CRU. ID CRU clinic in a locked file cabinet. The data on immunological assays will be de-identified and will only be identified with a unique code number and will be maintained in the laboratory of Dr. Ali Ellebedy. The links to the unique codes will be stored at the ID CRU clinic and will not be available to the laboratory personnel.

The study records will be maintained up to ten years after the completion of the study.

PUBLICATION POLICY

The PI and co-investigators will participate in manuscript preparation and submission to journals.

Schedule of Events

	Screening (up to 28 days prior to Day 1)	*Day 1 (date of vaccine administration)**	*Day 8 +/- 3 days*	*Day 14 +/- 3 days*	*Day 29 +/- 7 days*	*Day 57 +/- 7 days*	*Day 91 +/- 14 days*	*Day 181 +/- 14 days*	*Day 366 +/- 14 days*	*Day 546 +/- 14 days*	*Day 731 +/- 14 days*
Visit Number	***1***	***2***	***3***	***4***	***5***	***6***	***7***	***8***	***9***	***10***	***11***
Informed consent	X										
History	X	X	X								
Update Med HX, Meds	X	X	X	X	X	X	X	X	X	X	X
Vital Signs	X	X	X**	X**	X**	X**	X**	X**	X**	X**	X**
Bone Marrow aspiration (BMA)	X				X		X	X	X	X	X
Bone marrow core biopsy (BMCB)	X				X		X	X	X	X	X
Fine needle aspiration (FNA)	X				X	X	X	X	X	X	X
Blood Draw	X		X	X	X	X	X	X	X	X	X
Research Lab 10 ml CPT	80	80	80	80	80	80	80	80	80	80	80
Adverse Events		X	X***	X	X	X	X	X	X	X	X
Study Vaccine: TIV, Tdap, HPV, and HAV		X									

- ***Assessments for Day 1 should be performed prior to administration of vaccine**
- ** If clinically indicated**
- *** Solicited Adverse Reactions**

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