Title: Systems Biology of Zoster Vaccine Recombinant, Adjuvanted

NCT#: NCT04047979

Date: Oct/22/2021

HUMAN IMMUNOLOGY PROJECT CONSORTIUM (HIPC)

PROTOCOL NUMBER: HIPC-VAX2-007

LONG TITLE

Systems Biology of Zoster Vaccine Recombinant, Adjuvanted.

SHORT TITLE

Systems Biology of Zoster Vaccine.

V5.0 /10/22/2021

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Confidentiality Statement

The information contained within this document is not to be disclosed in any way without the prior permission of the Protocol Chair, or the Division of Allergy, Immunology and Transplantation, National Institute of Allergy and Infectious Diseases of the National Institutes of Health.

INVESTIGATOR SIGNATURE PAGE	
Protocol: HIPC-VAX2-007	**Version/Date:** V 5.0/10/22/2021
Site Principal Investigator: Nadine Rouphael, M.D.	
Title: Systems Biology of Zoster Vaccine Recombinant, Adjuvanted.	
Study Sponsor: The National Institute of Allergy and Infectious Diseases (NIAID)	
INSTRUCTIONS: The Principal Investigator should print, sign, and date at the indicated location below. The original should be kept for your records and a copy of the signature page sent to DAIT RMC.	
I confirm that I have read the above protocol in the latest version. I understand it, and I will work according to the principles of Good Clinical Practice (GCP) as described in the United States Code of Federal Regulations (CFR) – 45 CFR part 46 and 21 CFR parts 50, 56, and 312, and in the International Conference on Harmonization (ICH) document *Guidance for Industry: E6 Good Clinical Practice: Consolidated Guidance* dated April 1996. Further, I will conduct the study in keeping with local legal and regulatory requirements. As the site Principal Investigator, I agree to carry out the study by the criteria written in the protocol and understand that no changes can be made to this protocol without the written permission of the IRB and NIAID. _____ Nadine Rouphael, M.D _____ **Site Principal Investigator (Print)** _____ _____ **Site Principal Investigator (Signature)** **Date**	

Protocol Synopsis

Title	Systems Biology of Zoster Vaccine Recombinant, Adjuvanted
Short Title	Systems Biology of Zoster Vaccine
Clinical Phase	Mechanistic study
Number of Sites	The Hope Clinic (Emory Vaccine Center), 500 Irvin Court, Suite 200, Decatur, GA 30030
IND Sponsor/Number	Not applicable
Study Objectives	Primary Objective: To determine the innate immune signatures after each dose of Zoster vaccine, recombinant, adjuvanted, in two cohorts, 50-60 and ≥70 years of age Secondary Objective: To assess the safety of Zoster vaccine recombinant, adjuvanted. Exploratory Objectives: • To assess the specificity, magnitude, kinetics or phenotype of VZV-specific (gE and other antigens) T cells of the Zoster vaccine recombinant, adjuvanted. • To assess the magnitude, kinetics of VZV-specific B cells of Zoster vaccine recombinant, adjuvanted. • To identify innate immune signatures that correlate with the magnitude of the adaptive immune responses to the Zoster vaccine recombinant, adjuvanted. • To determine the differences in specificity, magnitude, kinetics or phenotype of VZV-specific (gE and other antigens) T cells to the Zoster vaccine recombinant, adjuvanted between adults ≥ 70 and 50-60 years of age. • To determine the differences in magnitude, kinetics of VZV-specific B cells between adults ≥ 70 and 50-60 years of age to the Zoster vaccine recombinant, adjuvanted. • To collect stool for microbiome analysis.
Study Design	Single center, open label mechanistic study in which older adult subjects will receive Zoster vaccine recombinant, adjuvanted. Blood samples will be collected at D0 (pre-vaccination) and D1, D3, D7, D14, D30, D60 (second dose of vaccination), D61, D63, D67, D74, D90, and D180 post vaccination to study innate and adaptive immunity responses. An optional visit at 9 months and beyond

	(D270+) after the first visit may occur. Blood draw and information about concomitant medication, receipt of vaccinations and any SAE may be collected. Subjects will be asked to report and record local or systemic AEs for 7 days post each vaccination. Unsolicited AEs will be reported for 30 days post each vaccination and any SAE for the duration of the study.
Primary Endpoint(s)	• Differences in innate immune signatures between D0, D1, and D7 and each dose of Zoster vaccine recombinant, adjuvanted, in both age cohorts.
Secondary Endpoint(s)	• Differences in related adverse events and serious adverse events between each dose of Zoster vaccine recombinant, adjuvanted, in both age cohorts.
Exploratory Endpoint(s)/Outcome(s)	• The changes in transcriptomics, metabolomics and immune cell populations between D0 (before vaccination) and D1, D3, D7, D14 and D30 after each dose of Zoster vaccine recombinant, adjuvanted, in both age groups. • The changes in the phenotype of T cell responses and the kinetics of these changes between D0 (before vaccination) and D7, D14 and D30 after each dose of Zoster vaccine recombinant, adjuvanted, as well as D180 and D270+ (optional visit) in both age groups. • The changes in the magnitude of activated B cells, antibody secreting cells and memory B cells and the kinetics of these changes between D0 (before vaccination) and D7, D14 and D30 after each dose of Zoster vaccine recombinant, adjuvanted, as well as D180 and D270+ (optional visit) in both age groups. • The changes in VZV serology between D0 (before vaccination) and D14 and D30 after each dose of Zoster vaccine recombinant, adjuvanted, as well as D180 and D270+ (optional visit) in both age groups. • The changes in specificity, magnitude and phenotype of VZV-specific (gE and other antigens) T cells and the kinetics of these changes between D0 (before vaccination) and 7, 14, 30 days after each dose of Zoster vaccine recombinant, adjuvanted, and at D180 and D270+ (optional visit) in both age groups. • The changes in magnitude of VZV-specific B cells and the kinetics of these changes between D0 (before vaccination) and 7, 14, 30 days after each dose of Zoster vaccine recombinant, adjuvanted, and at D180 and D270+ (optional visit) in both age groups.

	• The collection of stool at baseline for microbiome analysis
Accrual Objective	N=60 subjects receiving 2 doses of Zoster vaccine recombinant, adjuvanted separated by 60 days including: • N=30 subjects between the ages of 50-60 years. • N=30 subjects age 70 years and above.
Study Duration	24 - 27 months (18 months accrual + 9 months of study participation, with an optional visit at 9 months and beyond after the first vaccination).
Treatment Description	Not applicable
Inclusion Criteria	1. Subject must be able to understand and provide informed consent. 2. Adults aged 50-60 years, or community dwelling adults aged 70 years and above.
Exclusion Criteria	1. Inability or unwillingness of a subject to give written informed consent or comply with study protocol. 2. Receipt of immune products: • Receipt of blood products within 6 months prior of the first dose of the study Zoster vaccine or expected receipt through 6 months after vaccination with the second dose of the study Zoster vaccine^. • Receipt of any vaccine within 4 weeks prior to vaccination with any of the two doses of the study Zoster vaccine or expected receipt within 4 weeks after vaccination with any of the two doses of the study Zoster vaccine^. • Receipt of any Zoster or varicella vaccines at any time prior to study entry. 3. Subject taking any non-topical antiviral therapy with activity against herpes viruses, including but not limited to acyclovir, famciclovir, valacyclovir, and ganciclovir 3 days prior to each vaccination or 14 days after^. 4. Prior history of shingles. 5. Presence of certain **comorbidities or immunosuppressive states** such as: • Chronic medical problems including (but not limited to) insulin-dependent diabetes, severe (at the discretion of the investigator or study physician) heart, lung, liver, or kidney diseases; auto immune diseases; severe gastrointestinal diseases; and uncontrolled hypertension.

	• Impaired immune function or chronic infections including (but not limited to) HIV, hepatitis B or C, tuberculosis, organ transplant, cancer, current and or expected receipt of chemotherapy, radiation therapy, steroids [i.e., ≥ 20 mg of prednisone given daily or on alternative days for 2 weeks or more in the past 90 days^^); (nasal (less than 1mg/day of fluticasone equivalent inhaled corticosteroid is allowable) and topical steroids are allowed)], antitumor necrosis factor agents, or any other immunosuppressive therapy, anatomic or functional asplenia, congenital immunodeficiency. 6. Conditions that could affect the safety of the subjects such as: • Severe reactions to prior vaccinations. • History of anaphylactic/anaphylactoid reaction to any component of the vaccines. • History of bleeding disorders. 7. Any acute illness, including any fever (≥ 100.4 F [≥ 38.0C], regardless of the route) within 3 days prior to study entry^. 8. Social, occupational, or any other condition that in the opinion of the investigator might interfere with compliance with the study and vaccine evaluation. 9. Alcohol or drug abuse and psychiatric conditions that in the opinion of the investigator would preclude compliance with the trial or interpretation of safety or endpoint data. 10. Use of investigational drugs within 12 months of participation. 11. Past or current medical problems or findings from physical examination or laboratory testing that are not listed above, which, in the opinion of the investigator or study physician, may pose additional risks from participation in the study, may interfere with the subject's ability to comply with study requirements or that may impact the quality or interpretation of the data obtained from the study. 12. Women of childbearing potential. **Notes:** ^An individual who initially is excluded from study participation based on one or more of the time-limited exclusion criteria (e.g., acute illness, receipt or expected receipt of live or inactivated vaccines) may be considered for enrollment once the condition has resolved as long as the subject continues to meet all other entry criteria. ^^Subjects receiving ≥ 20 mg/day of prednisone or its equivalent daily or on alternate days for more than 2 weeks may enter the study after therapy has been discontinued for more than 3 months.

Study Stopping Rules	**The study may be prematurely terminated for the following reasons:** - If Shingrix® is recalled by the manufacturer **The study may be placed on hold for expedited ISM review for the following reasons:** - A death of a participant, which is possibly or definitely related to the study vaccine - The occurrence of one study vaccine-related and unexpected SAE or Grade 4 AE
Participant Stopping Rules	**Participants may be prematurely terminated from the study for the following reasons:** 1. The participant elects to withdraw consent from all future study activities, including follow-up. 2. The participant is "lost to follow-up" (i.e., no further follow-up is possible because attempts to reestablish contact with the participant have failed). 3. The participant dies. 4. The Investigator no longer believes participation is in the best interest of the participant or that the study procedures, if conducted, risk compromising the scientific integrity of the study.

Study Contacts: Participating Centers

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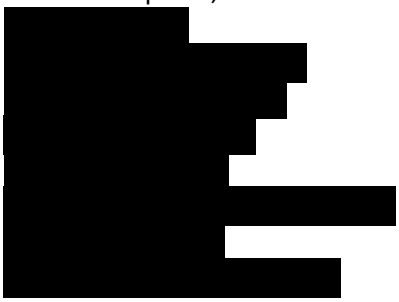

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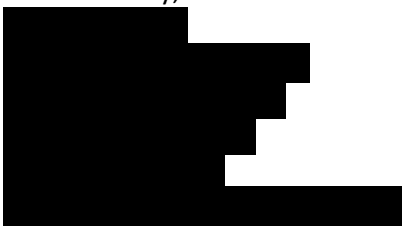

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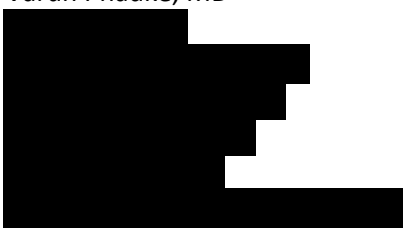

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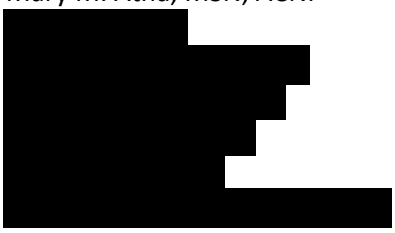

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Table of Contents

Glossary of Abbreviations

CFR	Code of Federal Regulations
CRF	Case Report Form
CTCAE	Common Terminology Criteria for Adverse Events
DAIT	Division of Allergy, Immunology, and Transplantation
FDA	Food and Drug Administration
GCP	Good Clinical Practice
ICH	International Conference on Harmonization
IND	Investigational New Drug
IRB	Institutional Review Board
ISM	Independent Safety Monitor
MOP	Manual of Procedures
NIAID	National Institute of Allergy and Infectious Diseases
PI	[Site] Principal Investigator
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SAR	Suspected Adverse Reaction
SOP	Standard Operating Procedure
SUSAR	Serious Unexpected Suspected Adverse Reaction
VZV	Varicella Zoster Virus
HIV	Human Immunodeficiency Virus
HZ	Herpes Zoster
PHN	Postherpetic Neuralgia
GSK	GlaxoSmithKline
HIPC	Human Immunology Project Consortium
AE	Adverse Event
GRITS	Georgia Registry of Immunization Transactions and Services
RNA	Ribonucleic Acid
miRNA	MicroRNA
mRNA	Messenger Ribonucleic Acid
SAM	Significance Analysis of Microarrays
FDR	False Discovery Rate
ELISA	Enzyme-Linked Immunosorbent Assay

HLA	Human Leukocyte Antigen
CPT	Cell Preparation Tube
CD4/CD8	Cluster of Differentiation 4/8
IgA/G/M	Immunoglobulin A/G/M
gE	Glycoprotein E of Varicella
CFR	Code of Federal Regulations
CRF	Case Report Form
CTCAE	Common Terminology Criteria for Adverse Events
DAIT	Division of Allergy, Immunology, and Transplantation
FDA	Food and Drug Administration
GCP	Good Clinical Practice
ICH	International Conference on Harmonization
IND	Investigational New Drug
IRB	Institutional Review Board
MOP	Manual of Procedures
NIAID	National Institute of Allergy and Infectious Diseases
PI	[Site] Principal Investigator
SAP	Statistical Analysis Plan
SAR	Suspected Adverse Reaction
SOP	Standard Operating Procedure
SUSAR	Serious Unexpected Suspected Adverse Reaction

1. Background and Rationale

1.1. Background and Scientific Rationale

Varicella zoster virus (VZV), a member of the Herpesviridae family, is known to cause a wide spectrum of disorders in an individual's lifetime, including chicken pox (varicella), herpes zoster (shingles) and central nervous system disease (myelitis, meningitis, encephalitis, etc.). Primary infection with VZV causes chicken pox, a self-limited viral exanthema of childhood leading to fever and disseminated skin lesions. Due to its neurotropic properties, VZV has the ability to remain latent in neurons of the dorsal root ganglia, autonomic ganglia and cranial nerves after primary infection, and potentially reactivate later in life causing herpes zoster (HZ).[1] Reactivation is due to a decreased VZV-specific cell-mediated immunity[2], and risk factors include older age and an immunocompromised state (HIV, lymphoma, leukemia, bone marrow transplant, solid organ transplant, and immunosuppressive medications, etc.). Other risk factors include: depression, Caucasian race, female sex, physical trauma, prior history of HZ, diabetes mellitus, and family history (particularly first-degree relatives).[3,4,5]

HZ results in a dermatomal rash and both neuropathic and nociceptive pain, occurring mostly in older individuals and the immunocompromised.[6] The incidence of HZ has been rising in the past several decades. The lifetime risk of HZ has been shown to be 20-30% in the general population, increasing after 50 years of age, and reaching 50% at age 85 years. The recurrence rate is also rising, occurring in 6.4% of immunocompetent individuals and even higher in the immunocompromised.[6] After resolution of the vesicular rash, individuals with HZ may experience postzoster sequelae, such as postherpetic neuralgia (PHN), with an incidence ranging from 5 to 30% noted in different studies. PHN occurs in 50% of individuals over 85 years of age[7-8] and is considered a debilitating consequence of HZ because it severely affects the quality of life of individuals.[9] Other sequelae of HZ are: secondary bacterial infections (with *Staphylococcus* or *Streptococcus*) and dermatologic complications (postzoster granulomatous dermatitis, dermatophytosis, lymphoplasmacytoid lymphoma, T-cell lymphoma, pseudolymphoma, etc…[10]). In addition, some studies indicate that in individuals older than 50 years, the risk for stroke or myocardial infarction is increased at 3 to 12 months after an episode of HZ compared to the general population, due to vasculopathy resulting from VZV in intracerebral and coronary arteries.[6] In addition, current data shows that more than 1 million cases of HZ occur every year in the Unites States, costing the health system around 5 billion USD.[11] For all the reasons listed above, having an effective vaccine against herpes zoster is an important public health measure.

Recently, the FDA approved a new herpes zoster vaccine, Zoster Vaccine Recombinant, Adjuvanted (Shingrix®)[12], developed by GlaxoSmithKline (GSK). The Advisory Committee on Immunization Practices (ACIP) prefers its use over the previously recommended live attenuated Zostavax®[13]. Shingrix® is a recombinant, adjuvanted vaccine, given in two doses, and recommended for prevention of herpes zoster in adults aged 50 years and older. Shingrix® reduces the incidence of HZ by 97.2% in a study done with more than 14,759 subjects aged 50 years and above who received two doses of the vaccine (at 0 and 2 months). This is in comparison to the previously approved HZ vaccine, Zostavax®, a live-attenuated vaccine approved by the FDA in 2006, which reduced the incidence of HZ and PHN by 51.3% and 66.5% respectively in a study of more than 38,000 adults aged 60 years and older.[14] Since Zostavax® is a live-attenuated vaccine, its use is contraindicated in immunocompromised individuals, who are usually at higher risk of having HZ than the general population. However, Shingrix® can be administered to these individuals, which gives it an advantage over the live-attenuated vaccine.

In our study, we would like to further investigate the immune response to Shingrix® vaccine, in order to understand better the factors that make this vaccine more efficient than the live-attenuated vaccine. In this context, systems biology

approaches offer a new approach to describe the global architecture of the immune response to Shingrix®. Systems biology is a biology-based interdisciplinary study field that focuses on complex interactions in biological systems, using a new holistic perspective. Systems biology offers several so-called "Omic" technologies that can provide unbiased and rich information on the physiological state of organisms through their molecular profiles. These systems biological approaches are likely to be of value in identifying molecular signatures that are induced rapidly after vaccination and that correlate with, and predict, the later development of protective immune responses. Furthermore, the predictive signatures would highlight new correlates of protective immunity and stimulate the formulation and validation of new hypotheses on the biological mechanisms by which innate immune responses modulate vaccine-induced immunity and protection.[15] This information would be useful in better understanding this new vaccine and compare it to the previous one. Accordingly, a key goal of the present proposal is to identify molecular signatures that predict the immune response to vaccination with Shingrix® in people above 50 years of age, and to obtain greater mechanistic insight underlying age-related immunological defects if they exist.

In summary, the proposed study offers an unprecedented opportunity to capture the global architecture of immune responses to Shingrix® in two age groups: those between the ages of 50 and 60, and those who are 70 years of age and above. This is likely to have a major impact in three areas: (i) the elucidation of molecular signatures or biomarkers that predict vaccine immunogenicity or efficacy and will thus be relevant from a public health perspective. (ii) such molecular signatures may illuminate the molecular defects responsible for potential sub-optimal immunity in the elderly population. iii) will help compare the immune responses to vaccination with the two available HZ vaccines, Shingrix® and Zostavax®, since a similar study using systems biology was done previously by our group on Zostavax® vaccine.[16]

1.2. Rationale for Selection of Investigational Product or Intervention

The vaccine used in the study will be Shingrix® manufactured by GSK (United Kingdom) and approved by the FDA on October 20th, 2017. It is the second licensed herpes zoster vaccine, approved for use in people above the age of 50. Shingrix® is considered generally safe (see Sections 4.1-4.3 for safety details) and effective for prevention of HZ.

Shingrix® was chosen to probe the differences in vaccine-induced innate and adaptive immune responses in healthy individuals aged 50-60 years, and those who are 70 years of age and above and to better understand the role of the adjuvant.

1.3. Preclinical Experience

Section not applicable as this is an FDA-approved vaccine.

1.4. Clinical Studies

The protective efficacy of Shingrix® was established by two main studies:

Study 1 was a randomized, placebo-controlled, observer-blind clinical study conducted in 18 countries. Randomization was stratified by age: 50 to 59 years, 60 to 69 years, 70 to 79 years, and ≥80 years. Subjects were followed for the development of HZ and PHN for a median of 3.1 years (range 0 to 3.7 years). The primary efficacy analysis population included 14,759 subjects aged 50 years and older who received two doses (at 0 and 2 months) of either Shingrix® (n=7,344) or placebo (n=7,415) and did not develop a confirmed case of HZ within 1 month after the second dose. Confirmed cases were determined either by Polymerase Chain Reaction (PCR) or by a Clinical Evaluation Committee (10.6%). It was found that compared to placebo, Shingrix® significantly reduced the risk of developing HZ by 97.2% in subjects 50 years and older. In a descriptive analysis, vaccine efficacy against HZ in subjects aged 50 years and older was

93.1% in the fourth year post-vaccination. No cases of PHN were reported in the vaccine group compared with 18 cases in the placebo group.

Study 2 was a randomized, placebo-controlled, observer-blind clinical study conducted in 18 countries. Randomization was stratified by age: 70 to 79 years and ≥80 years. Subjects were followed for a median of 3.9 years (range 0 to 4.5 years). The primary efficacy analysis population included 13,163 subjects aged 70 years and older who received two doses (at 0 and 2 months) of either Shingrix® (n=6,541) or placebo (n=6,622) and did not develop a confirmed case of HZ within 1 month after the second dose. In a descriptive analysis, vaccine efficacy against HZ in subjects 70 years and older was 85.1% in the fourth year after vaccination. Among all subjects aged 70 years or older, 4 cases of PHN were reported in the vaccine group compared with 28 cases in the placebo group. Vaccine efficacy against PHN was 85.5%; the benefit of Shingrix® in the prevention of PHN can be attributed to the effect of the vaccine on the prevention of HZ.

The pooled analysis combined results from Study 1 and 2 for subjects 70 years and older, with a total of 8,250 and 8,346 subjects who received Shingrix® and placebo, respectively. It was shown that compared to placebo, Shingrix® reduced the risk of developing HZ by 91.3% in subjects 70 years and older.

2. Study Hypotheses/Objectives

2.1. Hypotheses

The efficacy of the recombinant, adjuvanted herpes zoster vaccine (Shingrix®) is high. We hypothesize that early innate signatures of vaccination should correlate with responses to, and predict the immunogenicity of, Shingrix® in older adults, independently of age.

2.2. Primary Objective

- To determine the innate immune signatures after each dose of Zoster vaccine recombinant, adjuvanted, in two cohorts, 50-60 and ≥ 70 years of age.

2.3. Secondary Objective

- To assess the safety of Zoster vaccine recombinant, adjuvanted.

2.4. Exploratory Objectives

- To assess the specificity, magnitude, kinetics or phenotype of VZV-specific (gE and other antigens) T cells of the Zoster vaccine recombinant, adjuvanted.
- To assess the magnitude, kinetics of VZV-specific B cells of Zoster vaccine recombinant, adjuvanted.
- To identify innate immune signatures that correlate with the magnitude of the adaptive immune responses to the Zoster vaccine recombinant, adjuvanted.
- To determine the differences in specificity, magnitude, kinetics or phenotype of VZV-specific (gE and other antigens) T cells to the Zoster vaccine recombinant, adjuvanted between adults ≥ 70 and 50-60 years of age.

- To determine the differences in magnitude, kinetics of VZV-specific B cells between adults ≥ 70 and 50-60 years of age to the Zoster vaccine recombinant, adjuvanted.

- To collect stool for microbiome analysis.

3. Study Design

3.1. Description of Study Design

This is a single center, open label mechanistic study in which older adult subjects will receive Zoster vaccine recombinant, adjuvanted (Shingrix®). The study cohort will consist of 60, generally healthy older adults who will receive two doses of Shingrix®: 30 subjects between the ages of 50 to 60 years, and 30 subjects who are 70 years of age or above (shown below).

Enrollment*:*

N=60	50-60 year olds	N= 30	Shingrix®
	≥70 year olds	N= 30	Shingrix®

Once enrolled, subjects can withdraw from the study anytime. Subjects will be screened for health status by history and a targeted physical examination on the day of potential enrollment. Collected data will include demographic information, medical history, list of current medications and vaccination history. Subject will also receive a Shingrix® Vaccination Information Sheet.

Vaccination will occur on D0 after eligibility criteria have been confirmed and baseline blood has been obtained. Following administration of Shingrix®, symptoms and signs will be assessed in the clinic for at least 15 minutes after inoculation. Subjects will be followed for safety and immune responses after vaccination. Even though herpes zoster vaccine is administered as standard of care and is considered safe in subjects above 50 years of age, subjects will be asked to maintain a memory aid to record temperature and solicited systemic and local adverse events (AEs) for 7 days post each vaccination. The subject will record oral temperature around the same time each day with a thermometer provided by the clinic. The subject will use a ruler to measure the size of any localized reactions that may occur. The memory aid will be reviewed when the subject will return to the clinic within the first week after vaccination and examination of the injection site will be performed during these visits.

Additionally, unsolicited AEs will be reported for 30 days post each vaccination and SAEs for the duration of the study. During all visits, assessment of concomitant medications will be obtained and a targeted physical examination will be performed only if indicated, based on review of health status.

Blood samples will be collected at D0 (pre-vaccination) and D1, D3, D7, D14, D30, D60 (second dose of vaccination), D61, D63, D67, D74, D90, D180 and D270+ (optional visit) post vaccination to study innate and adaptive immunity responses. Samples from the different cohorts will be labeled to preserve the blind of the laboratory staff running the assays during the first phase of the analysis. Stool samples will be collected at baseline to assess the microbiome.

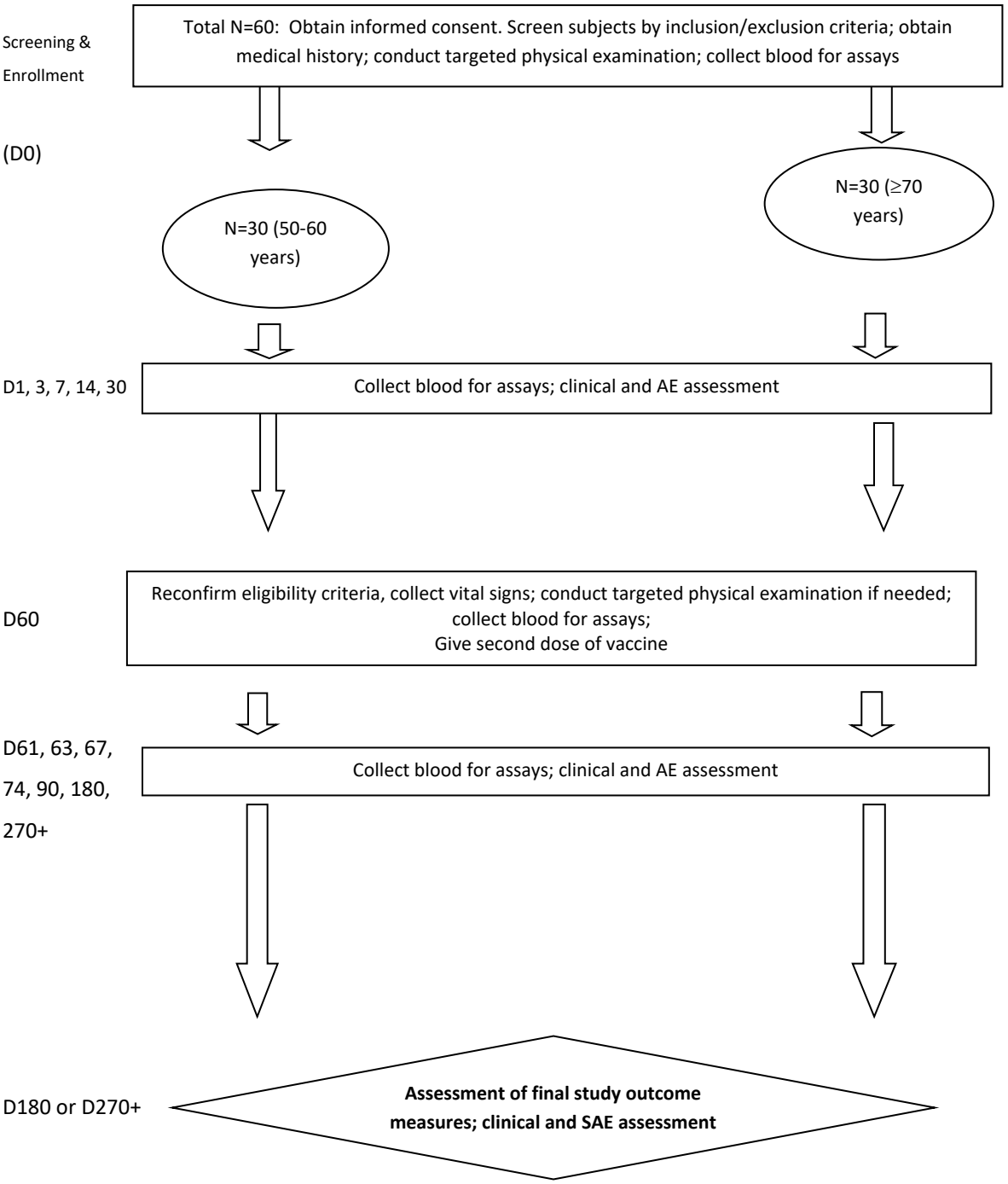

Figure 1: Study design

3.2. Primary Endpoint(s)/Outcome(s)

- Differences in innate immune signatures between D0, D1, and D7 and each dose of Zoster vaccine recombinant, adjuvanted in both age cohorts.

3.3. Secondary Endpoint(s)/Outcome(s)

- Differences in related adverse events and serious adverse events between each dose of Zoster vaccine recombinant, adjuvanted, in both age cohorts.

3.4. Exploratory Endpoint(s)/Outcome(s)

- The changes in transcriptomics, metabolomics and immune cell populations between D0 (before vaccination) and D1, D3, D7, D14 and D30 after each dose of Zoster vaccine recombinant, adjuvanted, in both age groups.

- The changes in the phenotype of T cell responses and the kinetics of these changes between D0 (before vaccination) and D7, D14 and D30 after each dose of Zoster vaccine recombinant, adjuvanted, as well as D180 and D270+ (optional visit), in both age groups.

- The changes in the magnitude of activated B cells, antibody secreting cells and memory B cells and the kinetics of these changes between D0 (before vaccination) and D7, D14 and D30 after each dose of Zoster vaccine recombinant, adjuvanted, as well as D180 and D270+ (optional visit), in both age groups.

- The changes in VZV serology between D0 (before vaccination) and D14 and D30 after each dose of Zoster vaccine recombinant, adjuvanted, as well as D180 and D270+ (optional visit), in both age groups.

- The changes in specificity, magnitude and phenotype of VZV-specific (gE and other antigens) T cells and the kinetics of these changes between D0 (before vaccination) and 7, 14, 30 days after each dose of Zoster vaccine recombinant, adjuvanted, and at D180 and D270+ (optional visit), in both age groups.

- The changes in magnitude of VZV-specific B cells and the kinetics of these changes between D0 (before vaccination) and 7, 14, 30 days after each dose of Zoster vaccine recombinant, adjuvanted, and at D180 and D270+ (optional visit), in both age groups.

- Microbiome parameters in stool collected at baseline. These will be correlated to the immune responses to the Zoster vaccine to assess whether the stool microbiome may be related, and possibly influence immune responses.

3.5. Stratification, Randomization, and Blinding/Masking

60 subjects (n=30: 50-60 years old, n=30: ≥70 years old) will be enrolled in the study and will receive two doses of Zoster Vaccine Recombinant, Adjuvanted (Shingrix®) at D0 and D60. There will be no randomization, study participant or clinical study personnel blinding, or masking.

3.5.1. Procedure for Unblinding/Unmasking

Not applicable for clinic staff. Laboratory staff will be blinded as to the age group that each participant belongs to

4. Selection of Participants and Clinical Sites/Laboratories

4.1. Rationale for Study Population

Older adults (50-60 years old and ≥70 years old) who have no history of HZ and are naïve to previous HZ vaccination will be enrolled to receive two doses of Shingrix®. Subjects will receive vaccination during the ACIP-recommended window for HZ vaccination (at 0 and 2 months), but separated in time from all other vaccines (influenza, pneumococcal, etc.). There will be no intentional recruitment of any specific racial groups.

4.2. Inclusion Criteria

1. Subject must be able to understand and provide informed consent.
2. Adults aged 50-60 years, or community dwelling adults aged 70 years and above.

4.3. Exclusion Criteria

1. Inability or unwillingness of a subject to give written informed consent or comply with study protocol.
2. Receipt of immune products:
    - Receipt of blood products within 6 months prior of the first dose of the study Zoster vaccine or expected receipt through 6 months after vaccination with the second dose of the study Zoster vaccine^.
    - Receipt of any vaccine within 4 weeks prior to vaccination with any of the two doses of the study Zoster vaccine or expected receipt within 4 weeks after vaccination with any of the two doses of the study Zoster vaccine^.
    - Receipt of any Zoster or varicella vaccines at any time prior to study entry.
3. Subject taking any non-topical antiviral therapy with activity against herpes viruses, including but not limited to acyclovir, famciclovir, valacyclovir, and ganciclovir 3 days prior to each vaccination or 14 days after^.
4. Prior history of shingles.
5. Presence of certain co morbidities or immunosuppressive states such as:
    - Chronic medical problems including (but not limited to) insulin-dependent diabetes, severe (at the discretion of the investigator or study physician) heart, lung, liver, or kidney diseases; auto immune diseases; severe gastrointestinal diseases; and uncontrolled hypertension.
    - Impaired immune function or chronic infections including (but not limited to) HIV, hepatitis B or C, tuberculosis, organ transplant, cancer, current and or expected receipt of chemotherapy, radiation therapy, steroids [i.e., $\geq$ 20 mg of prednisone given daily or on alternative days for 2 weeks or more in the past 90 days^^); (nasal (less than 1mg/day of fluticasone equivalent inhaled corticosteroid is allowable) and topical steroids are allowed)], antitumor necrosis factor agents, or any other immunosuppressive therapy, anatomic or functional asplenia, congenital immunodeficiency.
6. Conditions that could affect the safety of the subjects such as:
    - Severe reactions to prior vaccinations.
    - History of anaphylactic/anaphylactoid reaction to any component of the vaccines.

- History of bleeding disorders.

7. Any acute illness, including any fever (≥ 100.4 F [≥ 38.0C], regardless of the route) within 3 days prior to study entry^.
8. Social, occupational, or any other condition that in the opinion of the investigator might interfere with compliance with the study and vaccine evaluation.
9. Alcohol or drug abuse and psychiatric conditions that in the opinion of the investigator would preclude compliance with the trial or interpretation of safety or endpoint data.
10. Use of investigational drugs within 12 months of participation.
11. Past or current medical problems or findings from physical examination or laboratory testing that are not listed above, which, in the opinion of the investigator or study physician, may pose additional risks from participation in the study, may interfere with the subject's ability to comply with study requirements or that may impact the quality or interpretation of the data obtained from the study.
12. Women of childbearing potential.

Notes:

^An individual who initially is excluded from study participation based on one or more of the time-limited exclusion criteria (e.g., acute illness, receipt or expected receipt of live or inactivated vaccines) may be considered for enrollment once the condition has resolved as long as the subject continues to meet all other entry criteria.

^^Subjects receiving ≥ 20 mg/day of prednisone or its equivalent daily or on alternate days for more than 2 weeks may enter the study after therapy has been discontinued for more than 3 months.

4.4. Selection of Clinical Sites/Labs

The study will be conducted at The Hope Clinic, the clinical arm of the Emory Vaccine Center at Emory University.

Address:
500 Irvin Ct, Suite 200
Decatur, Georgia 30030

Samples may be processed at the following laboratories:

Bali Pulendran, Stanford University, California
Alex Sette, La Jolla Institute, California
Rafi Ahmed, Emory University, Atlanta, GA

5. Known and Potential Risks and Benefits to Participants

5.1. Risks of Investigational Product or Intervention as cited in Investigator Brochure or Package Insert

In clinical trials involving a pooled cohort of 29,305 subjects above 50 years of age who received at least one dose of Shingrix® (n=14,645) or saline (n=14,660) administered to a 0- and 2-month schedule, the following adverse reactions have been seen:

- Local adverse events: pain (78%), redness (38.1%), swelling (25.9%)

- General adverse event: myalgia (44.7%), fatigue (44.5%), headache (37.7%), shivering (26.8%), fever (20.5%), and gastrointestinal symptoms (17.3%).

The incidence of solicited local and general symptoms was lower in subjects aged 70 years and older compared with those aged 50 to 69 years.

The majority of solicited local adverse reactions and general adverse events seen with Shingrix® had a median duration of 2 to 3 days.

Headache and shivering were reported more frequently by subjects after Dose 2 (28.2% and 21.4% respectively) compared with Dose 1 (24.4% and 13.8% respectively). Grade 3 solicited general adverse events (headache, shivering, myalgia and fatigue) were reported more frequently by subjects after Dose 2 (2.3%, 3.1%, 3.6% and 3.5% respectively) compared with Dose 1 (1.4%, 1.4%, 2.3% and 2.4% respectively).

5.2. Risks of Investigational Product or Intervention cited in Medical Literature

No other data exists in the medical literature on risks of vaccination with Shingrix®, since it is a newly approved vaccine by the FDA (October 20th, 2017).

5.3. Risks of Other Protocol Specified Medications

We do not anticipate the use of any other medication; however, should anaphylactic or hypersensitivity reactions occur, epinephrine (1:1000) and diphenhydramine injections are readily available at the Hope Clinic during vaccine use.

Epinephrine injection can be associated with high blood pressure, arrhythmia, lightheadedness, nervousness, restlessness, tremor, shortness of breath and diaphoresis. The frequency of these side effects is not defined. Diphenhydramine injection may be required to treat possible allergic reactions and its use can be associated with low blood pressure, arrhythmia, confusion, dizziness, sedation, restlessness, diarrhea, nausea and urinary retention. The frequency of these side effects is also not defined.

When facing a medical emergency, the clinic staff will follow the institutional SOP by calling 911 first. If needed, the subject will be transferred to Emory University Emergency Department for further care.

Subjects are allowed to receive acetaminophen or nonsteroidal anti-inflammatory drugs (NSAIDs) if they experience a moderate to severe local or systemic side effects after vaccine administration. The use of these medications will be recorded as concomitant medications.

5.4. Risks of Study Procedures

The potential risks to subjects are those associated with an intramuscular injection and of blood drawing.

Intramuscular injections may cause temporary localized redness, pain or tenderness, swelling, bruising, itching, warmth, or induration at the injection site.

Blood sample collection involves transient discomfort and may cause fainting, which is managed by having the subject lie down prior. The blood draw site may bruise, and this can be ameliorated by holding pressure to this site following the blood draw. The sites of blood draw are potential sites of infection, but this risk is made very unlikely by the use of sterile technique.

5.5. Potential Benefits

Individuals who receive this vaccine will likely gain protection against the development of HZ as this is the recommended vaccine for HZ prevention.

6. Investigational Agent /Device/Intervention

6.1. Investigational Agents/Devices/Interventions

6.1.1. Investigational Agent

The investigational agent used in this study is Shingrix®, a zoster vaccine recombinant, adjuvanted, recently approved by the FDA for prevention of herpes zoster (shingles) in adults aged 50 years and older. It is given in two doses (0.5 mL each): at 0 and 2 to 6 months.

Shingrix® will be donated by the manufacturer (GSK, Brentford, United Kingdom). Subsequent storage and transport of vaccine from the site's pharmacy to the vaccination center will strictly follow the most updated conditions specified in the Shingrix® package insert for *Handling and Storage.* Shingrix® will be administered within the labeled indications and within ACIP-recommended windows for administration.

6.1.1.1. Formulation, Packaging, and Labeling

Shingrix® (Zoster Vaccine Recombinant, Adjuvanted) is a sterile suspension for intramuscular injection. The vaccine is supplied as a vial of lyophilized recombinant varicella zoster virus surface glycoprotein E (gE) antigen component, which must be reconstituted at the time of use with the accompanying vial of AS01B adjuvant suspension component. The lyophilized gE antigen component is presented in the form of a sterile white powder. The AS01B adjuvant suspension component is an opalescent, colorless to pale brownish liquid supplied in vials.

The adjuvant suspension component is AS01B which is composed of 3-*O*-desacyl-4'-monophosphoryl lipid A (MPL) from *Salmonella minnesota* and QS-21, a saponin purified from plant extract *Quillaja saponaria* Molina, combined in a liposomal formulation. The liposomes are composed of dioleoyl phosphatidylcholine (DOPC) and cholesterol in phosphate-buffered saline solution containing disodium phosphate anhydrous, potassium dihydrogen phosphate, sodium chloride, and water for injection.

After reconstitution, each 0.5-mL dose is formulated to contain 50 mcg of the recombinant gE antigen, 50 mcg of MPL, and 50 mcg of QS-21. Each dose also contains 20 mg of sucrose (as stabilizer), 4.385 mg of sodium chloride, 1 mg of DOPC, 0.54 mg of potassium dihydrogen phosphate, 0.25 mg of cholesterol, 0.160 mg of sodium dihydrogen phosphate dihydrate, 0.15 mg of disodium phosphate anhydrous, 0.116 mg of dipotassium phosphate, and 0.08 mg of polysorbate 80. Shingrix® does not contain preservatives. Each dose may also contain residual amounts of host cell proteins (≤3.0%) and DNA (≤2.1 picograms) from the manufacturing process.

Shingrix® is supplied as 2 components: A single-dose vial of lyophilized gE antigen component (brown cap) and a single-dose vial of adjuvant suspension component (blue-green cap) (packaged without syringes or needles).

6.1.1.2. Dosage, Preparation, and Administration

Shingrix® is prepared by reconstituting the lyophilized varicella zoster virus glycoprotein E (gE) antigen component with the accompanying AS01B adjuvant suspension component. The reconstituted vaccine should be an opalescent, colorless to pale brownish liquid.

Two doses (0.5 mL each) administered intramuscularly according to the following schedule: A first dose at Month 0 followed by a second dose administered anytime between 2 and 6 months later. The preferred site for intramuscular injection is the deltoid region of the upper arm.

Storage before Reconstitution:

Adjuvant suspension component vials: Store refrigerated between 2° and 8°C (36° and 46°F). Protect vials from light. Do not freeze. Discard if the adjuvant suspension has been frozen.

Lyophilized gE antigen component vials: Store refrigerated between 2° and 8°C (36° and 46°F). Protect vials from light. Do not freeze. Discard if the antigen component has been frozen.

Storage after Reconstitution:

After reconstitution, administer Shingrix® immediately or store refrigerated between 2° and 8°C (36° and 46°F) and use within 6 hours. Discard reconstituted vaccine if not used within 6 hours. Do not freeze. Discard if the vaccine has been frozen.

6.2. Drug Accountability

Under Title 21 of the Code of Federal Regulations (21CFR §312.62) the investigator will maintain adequate records of the disposition of the investigational agent, including the date and quantity of the drug received, to whom the drug was dispensed (participant-by-participant accounting), and a detailed accounting of any drug accidentally or deliberately destroyed.

Records for receipt, storage, use, and disposition will be maintained by the study site investigational drug services. A drug-dispensing log will be kept current for each participant. This log will contain the identification of each participant and the date and quantity of drug dispensed.

All records regarding the disposition of the investigational product will be available for inspection.

All unused vaccine will be destroyed by the Emory Investigational Drug Service according to the pharmacy SOP.

6.3. Assessment of Participant Compliance with Investigational Agent

Not applicable.

6.4. Toxicity Prevention and Management

Unless the vaccine used in the study is recalled by the manufacturer, there will be no modification for the study vaccine during the study.

6.5. Premature Discontinuation of Investigational Agent

Study therapy may be prematurely discontinued for any participant for any of the following reasons:

- Inability to obtain the subject's blood on multiple occasions.
- Subject does not meet the eligibility criteria anymore.
- Development of an anaphylactic or severe allergic reaction after administration of the first study vaccine.

Study therapy may also be prematurely discontinued for any participant if the investigator believes that the study treatment is no longer in the best interest of the participant.

7. Other Medications

7.1. Concomitant Medications

7.1.1. Protocol-mandated

Not applicable.

7.1.2. Other permitted concomitant medications

Subjects are allowed to use acetaminophen or nonsteroidal anti-inflammatory drugs (NSAIDs) if they experience a moderate to severe local or systemic side effects after vaccine administration.

7.2. Prophylactic Medications

Not applicable.

7.3. Prohibited Medications

In accordance with exclusion criteria, subjects expected to receive prohibited medications (**see Section 4.3**) will be considered non-eligible for the study. All medications, therapies or vaccines administered to study subjects after study entry will be documented at each visit.

7.4. Rescue Medications

Should an anaphylactic or hypersensitivity reaction occur, epinephrine (1:1000) and diphenhydramine intramuscular injections are readily available at clinic sites. If needed, the site will initiate emergency procedures as per their institution policy. If needed, participant will be transferred to the Emory University Hospital Emergency Department.

8. Study Procedures

Shingrix® vaccine will be administered in two doses (0.5 mL each, at D0 and D60) intramuscularly in the deltoid region of the arm. Aseptic technique will be used.

Blood will be drawn at D0 (pre-vaccination), and at D1, D3, D7, D14, D30, D60, D61, D63, D67, D74, D90 D180 and optional D270+ post vaccination. Stool will be collected at baseline D0 (pre-vaccination).

A targeted physical examination will be performed when deemed necessary.

A memory aid will be completed for symptom assessment (local and systemic) from Day 0 to Day 7 and from D60 to D67.

8.1. Enrollment

The research study will be explained in lay terms to each potential research participant. The potential participant will sign an informed consent form before undergoing any study procedures. Once the informed consent has been signed, the participant is considered enrolled in the study and will be assigned a unique participant number.

Enrollment will be completed within 18 months. The duration of participation for each subject is 6 months, with an optional visit at 9 months and beyond after the first visit.

8.2. Screening Visit (D -28 to 0) (Optional Visit)

The purpose of the screening period is to confirm eligibility to continue in the study. The screening visit will last around 1 – 1 1/2 hours.

The following procedures and assessments will be conducted to determine participant eligibility:

1. Collect demographic information
2. Assess inclusion/exclusion criteria
3. Collect medical history, current medications and vaccination history
4. Record vital signs
5. Targeted physical examination if needed based on symptomatology and medical history
6. Provide subject with stool collection kit and instructions and collect stool if possible

8.3. Vaccination Visit (D0 - first dose of vaccination)

The purpose of the screening period is to confirm eligibility to continue in the study.

The following procedures and assessments will be conducted to determine participant eligibility:

1. Review inclusion/exclusion criteria
2. Review medical history, current medications and vaccination history
3. Record vital signs
4. Targeted physical examination if needed based on symptomatology and medical history
5. Draw blood tubes for analysis (pre-vaccination) and collect stool.
6. Administer first dose of Shingrix vaccine (0.5 mL) intramuscularly in the deltoid region of the preferred arm. Aseptic technique will be used.
7. Observe subjects for at least 15 minutes for any immediate hypersensitivity reactions.
8. Record vaccination in the Georgia Registry of Immunization Transactions and Services (GRITS).
9. Provide subjects with memory aid and other study related materials (thermometer and ruler to measure the size of any localized reactions that may occur) to record daily temperature and potential systemic and local side effects.

The visit will last around 1 ½ to 2 hours. No randomization is performed since all subjects, if eligible and wish to proceed with the study, will receive the first dose of the Shingrix® vaccine.

The Screening and Vaccination Visits may be combined into one visit if all activities can be completed in one visit. The combined screening/baseline visit will last around 2 – 2 ½ hours.

Subjects will be encouraged to take their oral temperature around the same time each day. Subjects will be instructed on how to use the memory aid and how to measure and record reactogenicity prior to leaving the clinic. Subjects will be instructed to notify the study center by telephone if they develop any severe reactions following vaccination. If the investigator deems the reaction severe enough, she/he will give further instructions on the proper course of action, including a return to the clinic for immediate evaluation if appropriate.

8.4. Study Visits or Study Assessments

8.4.1. Scheduled Study Follow-up Visits

All subjects will return for follow-up visits on D1, D3, D7 (+/-1), D14 (+/-2), D30 (+/-7), D60 (second dose of vaccine), D61, D63, D67 (+/-1 from second vaccination), D74 (+/-2 from second vaccination), D90 (+/-7 from second vaccination), D180 (+/-14 from first vaccination), and an optional visit past 9 months (D270 +) from first vaccination. These visits will last 30 minutes.

At these visits, study personnel will:

1. Review current health status
2. Record change in medications or receipt of other vaccination (on optional later visit).
3. Note any health changes since last visit
4. Perform a physical examination and vital signs (only if indicated, based on review of health status).
5. Draw blood tubes for analysis
6. Ask subjects to report local and systemic AEs developing the day of any blood draw.
7. Inquire about SAEs.
8. Review memory aid information with subject and examine the vaccine site (on Days 1, 3, 7, and Days 61, 63, 67).

8.4.2 Second Vaccination Visit (Day 60)

This visit will take approximately 2 hours. At this visit, study personnel will:

1. Review current health status.
2. Record change in medications.
3. Review eligibility criteria
4. Note any health changes since last visit.
5. Record vital signs
6. Perform a physical examination (only if indicated, based on review of health status).

7. Draw blood tubes for analysis.
8. Ask subjects to report local and systemic AEs developing the day of any blood draw.
9. Inquire about SAEs.
10. Administer second dose of Shingrix® vaccine.
11. Observe subject for at least 15 minutes for any immediate hypersensitivity reactions.
12. Record vaccination in the Georgia Registry of Immunization Transactions and Services (GRITS).
13. Provide subjects with memory aid and other study related materials (thermometer and ruler) to record daily temperature and potential systemic and local side effects.

Subjects will be encouraged to take their temperature around the same time each day. Subjects will be instructed on how to use the memory aid and how to measure and record reactogenicity prior to leaving the clinic. Subjects will be instructed to notify the study center by telephone if they develop any severe reactions following vaccination. If the investigator deems the reaction severe enough, she/he will give further instructions on the proper course of action, including a return to the clinic for immediate evaluation if appropriate.

8.5. Unscheduled Visits

If concerns arise between regularly scheduled visits, participants should be instructed to contact study personnel and may be asked to return to the study site for an “unscheduled” visit.

Subjects may also be asked to return for an unscheduled visit if phlebotomy is unsuccessful or if insufficient blood is collected at a prior visit. At this visit, study personnel may review their memory aid, review the vaccination site if within 14 days of vaccination, list of medications, and note any other AEs or SAEs since last visit. Blood may be drawn. Physical examination will be performed only if indicated, based on review of health status. Subjects may be also asked to report local and systemic AEs and any blood draw-related AEs or SAEs. These visits will last about 1 hour.

Early Termination Visit

At this visit, study personnel may review the memory aid with the subject (if within 7 days of vaccination) review the vaccination site, list of medications, and note any other AEs or SAEs since last visit. Blood may be drawn. Physical examination will be performed only if indicated, based on review of health status. Subjects will be also asked to report local and systemic AEs and any blood draw-related AEs. These visits will last about 1 hour.

Missed Vaccination Visit

Subjects that may have missed the second dose of the study vaccine due to the COVID-19 pandemic, may be asked to return for missed vaccination visit, if they have not received the second dose from an outside source. Subjects that agree to return will be reconsented. During the missed vaccination visit the same procedures and assessments will be followed as outlined for the second vaccination visit at Day 60. Following the second vaccination the subject will return for regular follow up visits scheduled within the specified time limits.

8.6. Visit Windows

Study visits should take place within the time limits specified below:

D0 (first dose of vaccination) D1, D3, D7 (+/-1), D14 (+/-2), D30 (+/-7), D60 (+7) (second dose of vaccine), D61, D63, D67 (+/-1 from second vaccination), D74 (+/-2 from second vaccination), D90 (+/-7 from second vaccination), D180 (+/-14 from first vaccination, for each scheduled visit. An optional visit at any time point past 9 months (D270 +) from first vaccination may also take place.

This is also indicated on the Table of Events.

9. Mechanistic Assays

1. Expression of cytokines and chemokines via FACS. This assay will detect the expression profile of cytokines and chemokines to determine the innate response to vaccination.
2. Analysis of innate immune responses using FACS to assess numbers of activated innate cell populations. This assay will detect innate cell population activation including DC subsets, monocyte subsets, and NK cells, since these cell subsets are known to play crucial roles in immune regulation.
3. Molecular signatures of vaccination using RNA-seq on 2 mL of whole blood. This assay will determine the changes in mRNA and miRNA expression in whole blood. Results will be interpreted with use the method Significance Analysis of Microarrays (SAM) to determine change over baseline.
4. Microbiome Analysis: Analysis of microbiome diversity in the stool samples will be performed by Illumina MiSeq V4 16S Sequencing. Sample preparation will involve quantification and normalization of genomic DNA, PCR amplification of V4 hypervariable region of the bacterial 16S rDNA gene, PCR product quantification and library construction. Illumina MiSeq V4 16S sequencing and Bioinformatics analysis will involve V4 hypervariable region paired-end amplicon sequencing (MiSeq: 2x250 cycles). Filtering and trimming of all sequencing data will be done and mapped to taxonomic databases to identify bacterial composition in samples.
5. Levels of VZV-specific IgG/IgA/IgM antibody titers in the plasma by ELISA. This antibody assay will be used to quantify the levels of VZV-specific IgG and IgA in the plasma. Positive results will be assessed by 2X the negative control wells.
6. Number of VZV-specific IgG/IgA/IgM plasmablasts via ELISPOT. This cellular assay will be used to quantify the number of VZV-specific and total IgG/IgA/IgM antibody secreting cells in the blood. Positive results will be assessed by > 5 spots per million PBMCs.
7. Number of plasmablasts via flow cytometry. This cellular assay will be used to quantify the number of total plasmablasts in the blood.
8. Number of VZV-specific memory B cells in the blood via memory B cell assay. This cellular assay will be used to quantify the number of VZV-specific memory B cells in the blood. Positive results will be assessed by 2X the negative control wells.
9. In vitro stimulation and intracellular cytokine staining by flow cytometry. This cellular assay will be used to quantify the number of VZV-specific CD4 and CD8 T cells in the blood. Positive results will be assessed by either CD4+CD3+IFNg+CD40L+ or CD8+CD3+IFNg+CD40L+ after stimulation with VZV infected cell lysate or VZV-derived peptides.
10. Number of activated T cells in the blood via flow cytometry. This cellular assay will be used to quantify the number of VZV-specific CD4 and CD8 T cells in the blood using activation markers such as CD38, HLA-DR and Ki-67.

11. Phenotype of activated CD4 T cells in the blood via flow cytometry. This cellular assay will be used to characterize and quantify different CD4 T cell subsets.
12. Number and cross-reactivity of HLA-A2/ILI-specific CD8 T cells in the blood via flow cytometry. This cellular assay will be used to quantify the number and characterize the cross-reactivity of HLA-A2/ILI-specific CD8 T cells in the blood using MHC-I tetramer staining.
13. Flow cytometric sorting of blood plasmablasts and cloning of immunoglobulins to generate monoclonal antibodies. This cellular assay will be used to generate VZV-specific monoclonal antibodies to assess their avidity and protective capacity as well as the overall breadth of the immunoglobulin repertoire.

10. Biospecimen Storage

1. Plasma will be collected from CPT tubes, aliquoted in sterile 1.5 ml microcentrifuge tubes and placed in a -20^{o}C freezer that is monitored by an electronic monitoring alarm that makes a loud noise if the temperature is warmer than -10^{o}C. The freezer temperature is monitored daily.
2. PBMCs will be collected from CPT tubes, cryopreserved and placed in an automatic fill liquid nitrogen freezer. This freezer has an audible alarm if the liquid nitrogen level or temperature is low. The freezer temperature is monitored daily.
3. Stool will be aliquoted from the specimen collection container into new cryovials and will be placed in a -80^{o}C freezer that is monitored by an electronic monitoring alarm that makes a loud noise if the temperature is warmer than -60^{o}C. The freezer temperature is monitored daily.

11. Criteria for Participant and Study Completion and Premature Study Termination

11.1. Participant Completion

Efforts are made to encourage participants to attend all clinic visits. To achieve the primary end points of the study, we will need to have longitudinal data on participants who should be able to complete, within the required windows, at least the following visits: D1, D3 and D30 after vaccination. The minimum amount of blood needed on the critical visits stated above to fulfill the primary endpoints is: 1 CPT tube.

11.2. Participant Stopping Rules and Withdrawal Criteria

Participants may be prematurely terminated from the study for the following reasons:

1. The participant elects to withdraw consent from all future study activities, including follow-up.
2. The participant is “lost to follow-up” (i.e., no further follow-up is possible because attempts to reestablish contact with the participant have failed).
3. The participant dies.
4. The Investigator no longer believes participation is in the best interest of the participant or that the study procedures, if conducted, risk compromising the scientific integrity of the study.

11.3. Participant Replacement

Participants who withdraw or are withdrawn will not be replaced if they have received at least one dose of the study vaccination.

11.4. Follow-up after Early Study Withdrawal

If a participant is withdrawn from the study for any reason, the participant may be asked to complete a final visit and/or final assessments. At this visit, study personnel may review the memory aid with the subject (if within 7 days of vaccination) review the vaccination site, list of medications, and note any other AEs or SAEs since last visit. Blood may be drawn. Physical examination will be performed only if indicated, based on review of health status. Subjects will be also asked to report local and systemic AEs and any blood draw-related AEs. These visits will last about 1 hour.

11.5. Study Stopping Rules

The study may be prematurely terminated for the following reasons:

- If Shingrix® is recalled by the manufacturer

The study will be placed on hold (for both enrollment and vaccine dosing) for expedited ISM review for the following reasons:

- A death of a participant, which is possibly or definitely related to the study vaccine
- The occurrence of one study vaccine-related and unexpected SAE or Grade 4 AE

12. Assessment of Safety

12.1 Overview

This section defines the types of safety data that will be collected under this protocol and outlines the procedures for appropriately collecting, grading, recording, and reporting those data. Adverse events that are classified as serious according to the definition of health authorities must be reported promptly (per Section 12.5, Reporting of Serious Adverse Events and Adverse Events) to DAIT/NIAID. Appropriate notifications will also be made to site principal investigators, Institutional Review Boards and health authorities.

Information in this section complies with ICH Guideline E2A: Clinical Safety Data Management: Definitions and Standards for Expedited Reporting, ICH Guideline E-6: Guideline for Good Clinical Practice, 21CFR Parts 312 and 320, and applies the standards set forth in the National Cancer Institute (NCI), Common Terminology Criteria for Adverse Events (CTCAE), Version 5.0 : http://ctep.cancer.gov/reporting/ctc.html.

12.2 Definitions

12.2.1 Adverse Event (AE)

Any untoward or unfavorable medical occurrence associated with the subject's participation in the research, whether or not considered related to the subject's participation in the research (modified from the definition of adverse events in the 1996 International Conference on Harmonization E-6 Guidelines for Good Clinical Practice) (from OHRP "Guidance on Reviewing and Reporting Unanticipated Problems Involving Risks to Subjects or Others and Adverse Events (1/15/07)" http://www.hhs.gov/ohrp/policy/advevntguid.html#Q2)

For this study, an adverse event will include any untoward or unfavorable medical occurrence associated with:

- **Study vaccine regimen**: Shingrix® will be administered 0.5mL intramuscularly on Day 0 and Day 60. Solicited AEs will be collected beginning from each vaccination though Day 7, unsolicited AEs will be collected through 30 days after each vaccination. Serious adverse events will be collected through the completion of the study.

- **Study mandated procedures**: Adverse events associated with intramuscular injections or blood draws will be collected for 24 hours after study-mandated injections and phlebotomies.

12.2.1.1 Suspected Adverse Reaction (SAR)

Any adverse event for which there is a reasonable possibility that the investigational drug caused the adverse event. For the purposes of safety reporting, 'reasonable possibility' means there is evidence to suggest a causal relationship between the drug and the adverse event. A suspected adverse reaction implies a lesser degree of certainty about causality than adverse reaction, which means any adverse event caused by a drug (21 CFR 312.32(a)).

12.2.2 Unexpected Adverse Event

An adverse event or suspected adverse reaction is considered "unexpected" if it is not listed in the package insert or is not listed at the specificity, severity or rate of occurrence that has been observed.

12.2.3 Serious Adverse Event (SAE)

An adverse event or suspected adverse reaction is considered "serious" if, in the view of either the investigator or Sponsor (DAIT/NIAID), it results in any of the following outcomes (21 CFR 312.32(a)):

1. Death.
2. A life-threatening event: An AE or SAR is considered "life-threatening" if, in the view of either the investigator or Sponsor (DAIT/NIAID), its occurrence places the subject at immediate risk of death. It does not include an AE or SAR that, had it occurred in a more severe form, might have caused death.
3. Inpatient hospitalization or prolongation of existing hospitalization.
4. Persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions.
5. Congenital anomaly or birth defect.
6. Important medical events that may not result in death, be life threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed above.

Elective hospitalizations or hospital admissions for the purpose of conduct of protocol mandated procedures are not to be reported as an SAE unless hospitalization is prolonged due to complications.

12.3 Grading and Attribution of Adverse Events

12.3.1 Grading Criteria

The study site will grade the severity of adverse events experienced by the study subjects according to the criteria set forth in the National Cancer Institute's Common Terminology Criteria for Adverse Events (CTCAE) (version 5.0). This document (referred to herein as the NCI-CTCAE manual) provides a common language to describe levels of severity, to analyze and interpret data, and to articulate the clinical significance of all adverse

events. The NCI-CTCAE has been reviewed by the Principal Investigator and has been deemed appropriate for the subject population to be studied in this protocol.

Adverse events will be graded on a scale from 1 to 5 according to the following standards in the NCI-CTCAE manual version 5.0:

Grade 1 = mild adverse event.

Grade 2 = moderate adverse event.

Grade 3 = severe and undesirable adverse event.

Grade 4 = life-threatening or disabling adverse event.

Grade 5 = death.

Events grade 1 or higher will be recorded on the appropriate AE case report form paper CRF for this study.

For grading an abnormal value or result of a clinical evaluation, a treatment-emergent adverse event is defined as an increase in grade from baseline or from the last post-baseline value that doesn't meet grading criteria. Changes in grade from screening to baseline will also be recorded as adverse events, but are not treatment-emergent. If a specific event or result from a given clinical or laboratory evaluation is not included in the NCI-CTCAE manual, then an abnormal result would be considered an adverse event if changes in therapy or monitoring are implemented as a result of the event/result.

12.3.2 Attribution Definitions

The relationship, or attribution, of an adverse event to the study therapy regimen or study procedure(s) will initially be determined by the site investigator and recorded on the appropriate AE paper case report form (AE/SAE paper CRF). Final determination of attribution for safety reporting will be determined by DAIT/NIAID. The relationship of an adverse event to study therapy regimen or procedures will be determined using the descriptors and definitions provided in Table 12.3.2.

For additional information and a printable version of the NCI-CTCAE manual version 5.0, consult the NCI-CTCAE web site: http://ctep.cancer.gov/reporting/ctc.html.

Table 12.3.2 Attribution of Adverse Events

Code	Descriptor	Relationship (to primary investigational product and/or other concurrent mandated study therapy or study procedure)
UNRELATED CATEGORY		
1	Unrelated	The adverse event is clearly not related: there is insufficient evidence to suggest a causal relationship.
RELATED CATEGORIES		
2	Possible	The adverse event has a reasonable possibility to be related; there is evidence to suggest a causal relationship.

3	Definite	The adverse event is clearly related.

12.4 Collection and Recording of Adverse Events

12.4.1 Collection Period

Adverse events will be collected from the time of vaccination until 30 days after each vaccination or after the participant prematurely withdraws (without withdrawing consent) or is withdrawn from the study. SAEs will be collected until a participant completes the study.

12.4.2 Collecting Adverse Events

Adverse events (including SAEs) may be discovered through any of these methods:

- Observing the subject.
- Interviewing the subject and using the memory aid.
- Receiving an unsolicited complaint from the subject.

12.4.3 Recording Adverse Events

Throughout the study, the investigator will record adverse events and SAEs as described previously (Section 12.2, Definitions*)* on the appropriate paper case report form (AE/SAE paper CRF) regardless of the relationship to study therapy regimen or study procedure.

Once recorded, an AE/SAE will be followed until it resolves with or without sequelae, or until the end of study participation, or until 30 days after the subject prematurely withdraws (without withdrawing consent)/or is withdrawn from the study, whichever occurs first.

12.5 Reporting of Serious Adverse Events and Adverse Events

12.5.1 Reporting of Serious Adverse Events to DAIT/NIAID

This section describes the responsibilities of the site investigator to report serious adverse events to the DAIT/NIAID Medical Monitor and Project Manager. Timely reporting of adverse events is required by 21 CFR and ICH E6 guidelines.

Site investigators will report all serious adverse events via email to the DAIT/NIAID Medical Monitor and Project Manager (see Section 12.2.3, Serious Adverse Event*)*, regardless of relationship or expectedness, within 24 hours of discovering the event.

For SAEs, all requested information on the SAE paper CRF will be provided. Unavailable details of the event will not delay submission of the known information. As additional details become available, the SAE paper CRF will be updated and re-submitted.

12.5.2 Reporting to the FDA

Not applicable – this study is not conducted under an IND/IDE.

12.5.3 Reporting of Adverse Events to IRBs/IECs and to VAERS

The Protocol Chair shall report adverse events, including expedited reports, in a timely fashion to the IRB in accordance with applicable regulations and guidelines. Since this is an FDA-approved vaccine, vaccine-related

adverse events will also be reported to VAERS at https://vaers.hhs.gov/index, in accordance to VAERS guidelines.

12.6 Pregnancy Reporting

Not applicable.

12.7 Reporting of Other Safety Information

An investigator shall promptly notify the site IRB as well as the DAIT/NIAID when an "unanticipated problem involving risks to subjects or others" is identified, which is not otherwise reportable as an adverse event.

12.8 Review of Safety Information

12.8.1 Medical Monitor Review

The DAIT/NIAID Medical Monitor shall review and make decisions on the disposition of all SAEs received from the protocol investigator (See Sections 12.5.1, Reporting of Serious Adverse Events to DAIT/NIAID).

In addition to receiving individual SAE reports within 24 hours, the DAIT/NIAID Medical Monitor and Project Manager shall receive quarterly reports from the protocol investigator compiling new and accumulating information on AEs and SAEs recorded by the study site on appropriate paper CRFs.

12.8.2 Independent Safety Monitor (ISM) Review

12.8.2.1 Planned Reviews

The Independent Safety Monitor shall review safety data at least yearly. Data for the planned safety reviews will include, at a minimum, a listing of all reported AEs and SAEs.

The ISM will be informed of an Expedited Safety Report by the Protocol Chair and the DAIT/NIAID Medical Monitor in a timely manner.

12.8.2.2 *Ad hoc* ISM Reviews

In addition to the pre-scheduled planned safety monitoring, the ISM may be called upon by the DAIT/NIAID Medical Monitor for *ad hoc* reviews. The ISM will review event(s) that potentially impact(s) safety at the request of the Protocol Chair or DAIT/NIAID. In addition, the following events will trigger an *ad hoc* comprehensive ISM Safety Review:

A death of a participant, which is possibly or definitely related to the study vaccine (also a study hold - enrollment and vaccine dosing- rule)

The occurrence of one study vaccine-related and unexpected SAE or Grade 4 AE (also a study hold – enrollment and vaccine dosing- rule)

The occurrence of a Grade 3 or higher vaccine-related and unexpected SAE in 2 or more of the study participants

After review of the data, the ISM will make recommendations regarding study conduct and/or continuation.

13. Statistical Considerations and Analytical Plan

13.1 Overview

This is a single center, open label study to assess the immune responses of the Zoster Vaccine Recombinant, Adjuvanted, in different age cohorts of older adults using a systems biology approach. A total of N=60 subjects will be recruited to receive 2 doses of Zoster vaccine recombinant, adjuvanted, separated by 60 days. 30 subjects are between the ages of 50-60 years, and 30 subjects are in the age group of 70 years and above.

13.2 Endpoints

Primary Endpoint

- Differences in innate immune signatures between D0, D1, and D7 and each dose of Zoster vaccine recombinant, adjuvanted, in both age cohorts.

Secondary Endpoint

- Differences in related adverse events and serious adverse events between each dose of Zoster vaccine recombinant, adjuvanted, in both age cohorts.

Exploratory Endpoints

- The changes in transcriptomics, metabolomics and immune cell populations between D0 (before vaccination) and D1, D3, D7, D14 and D30 after each dose of Zoster vaccine recombinant, adjuvanted, in both age groups
- The changes in the phenotype of T cell responses and the kinetics of these changes between D0 (before vaccination) and D7, D14 and D30 after each dose of Zoster vaccine recombinant, adjuvanted, as well as D180 and D270+ (optional visit) in both age groups.
- The changes in the magnitude of activated B cells, antibody secreting cells and memory B cells and the kinetics of these changes between D0 (before vaccination) and D7, D14 and D30 after each dose of Zoster vaccine recombinant, adjuvanted, as well as D180 and D270+ (optional visit) in both age groups.
- The changes in VZV serology between D0 (before vaccination) and D14 and D30 after each dose of Zoster vaccine recombinant, adjuvanted, as well as D180 and D270+ (optional visit) in both age groups.
- The changes in specificity, magnitude and phenotype of VZV-specific (gE and other antigens) T cells and the kinetics of these changes between D0 (before vaccination) and 7, 14, 30 days after each dose of Zoster vaccine recombinant, adjuvanted, and at D180 and D270+ (optional visit) in both age groups.
- The changes in magnitude of VZV-specific B cells and the kinetics of these changes between D0 (before vaccination) and 7, 14, 30 days after each dose of Zoster vaccine recombinant, adjuvanted, and at D180 and D270+ (optional visit) in both age groups.
- Baseline stool microbiome alpha and beta diversity parameters.

13.3 Measures to Minimize Bias

This is an open-label mechanistic study. No randomization will be conducted.

13.4 Analysis Plan

Data from the study will be analyzed using the statistical software R. High-throughput data will be analyzed using appropriate methods, either by stand-alone software or modules in leading statistical software, e.g. the Bioconductor in the R framework. For cytokine data, missing values will be dealt with using multiple imputations. For gene expression and metabolomics data, nearest neighbor method and local least squares may be used to fill missing values. Unsupervised learning techniques such as PCA, PLSDA, clustering, and factor analysis will be used for the visualization and identification of global patterns. For descriptive endpoints, we will generate summary statistics, and visualize the data using histograms and boxplots when applicable. For the identification of innate signatures, the two age groups will be analyzed separately.

13.4.1 Analysis Populations

We will use all subjects who receive at least the first dose of the vaccine, and have measurements at days 0 and day 30 after the vaccination in the data analysis.

13.4.2 Primary Analysis of Primary Endpoint

We will use paired t-test or Wilcoxon signed-rank test to compare each immune parameter (innate immune responses) between D1 and D7 after first dose of vaccine, between D61 and D67 after second dose of vaccine, and D0 and D60 (at each dose of Zoster vaccine). The choice of the test will depend on the normality of the data, as judged by normal Q-Q plots.

13.4.3 Supportive Analyses of the Primary Endpoint(s)/Outcome(s)

N/A

13.4.4 Analyses of Secondary and Other Endpoint(s)/Outcome(s)

AE rates in each group will be summarized using frequency tables. Between-group comparison will be conducted using Fisher's exact test.

13.4.5 Analyses of Exploratory Endpoint(s)/Outcome(s)

Exploratory Endpoint 1. The innate immune response signatures are identified from traditional immune parameters, gene expression measured by RNA-seq experiment, and metabolomics data measured by LC/MS. The innate signatures will be identified for the two age groups separately. The traditional immune parameters, genes, and metabolites that change significantly over baseline after immunization are considered innate signatures.

Traditional markers will be tested using paired t-test or Wilcoxon test against D0, and significant markers will be considered part of the innate immune signature.

RNA-seq data will be preprocessed using Bowtie and Tophat, and FPKM (fragments per kilobase of exon model per million mapped reads) values will be calculated. Gene level comparison of D1, D4, D14 and D30 vs D0 will be conducted using DESeq to find the transcriptomics signature of vaccination.

Metabolomics data will be processed using packages apLCMS, XCMS, and xMSanalyzer and generate data tables containing accurate mass *m/z*, retention time(s), intensity, coefficient of variation, and

related descriptive characteristics, including minimal information standards for metabolomics data. SAM(8) and similar methods will be used to detect differential abundance of features between D1, D4, D14 and D30 vs D0 to select metabolic innate signatures.

After each vaccination, the innate signatures that are correlated with the D30 antibody titers are considered correlation signatures of the innate immune responses. The correlation will be assessed using Spearman's correlation and the associated p-values.

Exploratory Endpoint 2. We will use paired t-test or Wilcoxon test to compare each immune parameter (changes in phenotype of T cell responses and the kinetics of these changes) between D7, D14 and D30 after vaccination and D0 for each dose of Zoster vaccine.

Exploratory Endpoint 3. We will use paired t-test or Wilcoxon test to compare each immune parameter (changes in the magnitude of activated B cells, antibody secreting cells and memory B cells and the kinetics of these changes) between D7, D14 and D30 after vaccination and D0 for each dose of Zoster vaccine.

Exploratory Endpoint 4. We will use paired t-test or Wilcoxon test to compare VZV serology between D14, D30, D180 and D270+ (optional visit) after vaccination and D0 for each dose of Zoster vaccine.

Exploratory Endpoint 5. We will use paired t-test or Wilcoxon test to compare each immune parameter (changes in specificity, magnitude, and phenotype of VZV-specific T cells and the kinetics of these changes) between D7, D14, D30, D180 and D270+ (optional visit) after vaccination and D0 for each dose of Zoster vaccine.

Exploratory Endpoint 6. We will use paired t-test or Wilcoxon test to compare each immune parameter (changes in magnitude of VZV-specific B cells and the kinetics of these changes) between D7, D14, D30, D180 and D270+ (optional visit) after vaccination and D0 for each dose of Zoster vaccine.

Although our statistical methods will adhere to what is proposed herein, our experience with analyses of similar studies in the past has underscored the need for flexibility and adaptability in trying different statistical approaches to arrive at the most informative results. As such, we may use alternative approaches such as the gene set enrichment analyses, or other approaches, in an *ad hoc* basis.

13.4.6 Descriptive Analyses

The log2 antibody titers of each group at each time point will be summarized and presented using histograms and strip charts for visual comparison. AE rates in each group will be summarized by tabulation.

13.5 Interim Analyses

Not applicable.

13.6 Statistical Hypotheses

We hypothesize that certain molecular signatures (immune markers, expression of genes, miRNAs, and metabolites), are associated to, and can predict the innate and adaptive immune response to the vaccine.

13.7 Sample Size Considerations

For sample size consideration, we will use the alpha level of 0.01 for the immune parameters. Given less than 50 immune parameters will be tested, this alpha level translates to an expected count of false positives of <1, which is an acceptable level. For the gene expression data and for sample size consideration only, we will use the alpha level of 0.0005 to offset the effect of multiple testing. This means an average of one false positive for every 2000 null genes tested; given ~20,000 genes will be measured, this alpha level translates to an expected 10 false positives. We expect >100 genes will be truly differentially expressed . Thus among all genes called differentially expressed, the proportion of false positives will be <10%, which is an acceptable level in gene expression studies. We note this consideration is for sample size analysis alone. In actual data analysis false discovery rate (FDR) will be used.

For the traditional immune parameters, with 30 samples in a treatment group, we can reject the null hypothesis of equal means with 80% power if the true effect size (mean difference/sigma) is 0.66, using a two-sided paired t-test and the alpha level of 0.01. For the gene expression, the actual data analysis will be performed using the false discovery rate. For simplicity, for sample size consideration, we will consider the simpler case of a paired t-test. With 30 samples in a treatment group, we can reject the null hypothesis of equal means with 80% power if the true effect size (mean difference/sigma) is 0.88, using a two-sided paired t-test and the alpha level of 0.0005.

For unpaired test, with sample size of 30 per group, at the alpha level of 0.01, we can reject the null hypothesis of equal means with 80% power if the true effect size ((mean0-mean1)/sigma, assuming equal variance) is 0.91 using an unpaired two-sided t-test.

For a paired t-test, with the sample size of 30, at the alpha level of 0.01, we can reject the null hypothesis of no change with 80% power if the true effect size (mean difference/sigma) is 0.66.

For correlation analysis, with 30 samples in the treatment group, at the alpha level of 0.01, we can reject the null hypothesis of zero correlation with 80% power if the true correlation is 0.57 using a two-sided test for Pearson's correlation coefficient.

14. Identification and Access to Source Data

14.1. Source Data

Source documents and source data are considered to be the original documentation where subject information, visits consultations, examinations and other information are recorded. Documentation of source data is necessary for the reconstruction, evaluation and validation of clinical findings, observations and other activities during a clinical trial.

14.2. Access to Source Data

The site investigators and site staff will make all source data available to the DAIT/NIAID as well as to relevant health authorities. Authorized representatives as noted above are bound to maintain the strict confidentiality of medical and research information that may be linked to identified individuals.

15. Protocol Deviations

15.1. Protocol Deviation Definitions

Protocol Deviation – The investigators and site staff will conduct the study in accordance to the protocol; no deviations from the protocol are permitted. Any change, divergence, or departure from the study design or procedures constitutes a protocol deviation. As a result of any deviation, corrective actions will be developed by the site and implemented promptly.

Major Protocol Deviation (Protocol Violation) - A Protocol Violation is a deviation from the IRB approved protocol that may affect the subject's rights, safety, or well-being and/or the completeness, accuracy and reliability of the study data. In addition, protocol violations include willful or knowing breaches of human subject protection regulations, or policies, any action that is inconsistent with the NIH Human Research Protection Program's research, medical, and ethical principles, and a serious or continuing noncompliance with federal, state, local or institutional human subject protection regulations, policies, or procedures.

Non-Major Protocol Deviation - A non-major protocol deviation is any change, divergence, or departure from the study design or procedures of a research protocol that does not have a major impact on the subject's rights, safety or well-being, or the completeness, accuracy and reliability of the study data.

15.2. Reporting and Managing Protocol Deviations

The study site principal investigator has the responsibility to identify, document and report protocol deviations as directed by the study Sponsor. However, protocol deviations may also be identified during Quality Assurance review or during other forms of study conduct review.

A protocol deviation is any noncompliance with the clinical trial protocol, GCP, or protocol specific MOP requirements. The noncompliance may be either on the part of the subject, the site principal investigator, or other study personnel. Upon determination that a major protocol deviation has occurred, the study staff will a) notify the site Principal Investigator, b) notify the NIAID/DAIT Project Manager and c) will complete a Protocol Deviation form. Major protocol deviations will be determined in conjunction with the NIAID/DAIT Medical Monitor. It is the responsibility of the Protocol Chair and other study personnel to use continuous vigilance to identify and report major deviations within 5 working days of identification of Major Protocol Deviation, or within 5 working days of the scheduled protocol-required activity. Non-Major Protocol Deviations will be summarized and reported to DAIT every 3 months.

As a result of deviations, corrective actions are to be developed by the site and implemented promptly.

These practices are consistent with ICH E6:

4.5 Compliance with Protocol, Sections 4.5.1, 4.5.2, and 4.5.3

5.1 Quality Assurance and Quality Control, Section 5.1.1

5.20 Noncompliance, Sections 5.20.1, and 5.20.2.

All protocol deviations, as defined above, must be addressed in study subject data collection forms. A completed copy of the Protocol Deviation Form must be maintained in the Study File, as well as in the subject's chart.

Protocol deviations must be sent to the local IRB/IEC per their guidelines. The site principal investigator and other study personnel are responsible for knowing and adhering to their IRB requirements.

16. Ethical Considerations and Compliance with Good Clinical Practice

16.1. Statement of Compliance

This clinical study will be conducted using good clinical practice (GCP), as delineated in *Guidance for Industry: E6 Good Clinical Practice Consolidated Guidance*, and according to the criteria specified in this study protocol. Before study initiation, the protocol and the informed consent documents will be reviewed and approved by the IRB. Any amendments to the protocol or to the consent materials will also be approved by the IRB before they are implemented.

16.2. Informed Consent Process

The consent process will provide information about the study to a prospective participant and will allow adequate time for review and discussion prior to his/her decision. The principal investigator or designee listed on the Investigator of Record form will review the consent and answer questions. The prospective participant will be told that being in the trial is voluntary and that he or she may withdraw from the study at any time, for any reason. All participants will read, sign, and date a consent form before undergoing any study procedures. Consent materials will be presented in participants' primary language. A copy of the signed consent form will be given to the participant.

The consent form will be revised when important new safety information is available, the protocol is amended, and/or new information becomes available that may affect participation in the study.

16.3. Privacy and Confidentiality

A participant's privacy and confidentiality will be respected throughout the study. Each participant will be assigned a unique identification number and these numbers rather than names will be used to collect, store, and report participant information. Site personnel will not transmit documents containing personal health identifiers (PHI) to the study sponsor or their representatives.

17. Publication Policy

The Human Immunology Project Consortium (HIPC) policy on the publication of study results will apply to this trial.

18. References

1. Mueller, N. H., et al. (2008). "Varicella zoster virus infection: clinical features, molecular pathogenesis of disease, and latency." Neurol Clin 26(3): 675-697, viii.
2. Yawn, B. P. and D. Gilden (2013). "The global epidemiology of herpes zoster." Neurology 81(10): 928-930.
3. Marra, F., et al. (2016). "Increasing incidence associated with herpes zoster infection in British Columbia, Canada." BMC Infect Dis16(1): 589.
4. Schmader, K. (2016). "Herpes Zoster." Clin Geriatr Med 32(3): 539-553.
5. Marin M, Harpaz R, Zhang J, et al. Risk factors for herpes zoster among adults. Open Forum Infect Dis 2016;3(3):ofw119.
6. John, A. R. and D. H. Canaday (2017). "Herpes Zoster in the Older Adult." Infect Dis Clin North Am 31(4): 811-826.
7. Kawai, K., et al. (2014). "Systematic review of incidence and complications of herpes zoster: towards a global perspective." BMJ Open 4(6): e004833.
8. Friesen, K. J., et al. (2017). "Cost of shingles: population based burden of disease analysis of herpes zoster and postherpetic neuralgia." BMC Infect Dis 17(1): 69.
9. Rullan, M., et al. (2017). "Efficacy of gabapentin for prevention of postherpetic neuralgia: study protocol for a randomized controlled clinical trial." Trials 18(1): 24.
10. Ferenczi, K., et al. (2015). "Herpes zoster granulomatous dermatitis: histopathologic findings in a case series." J Cutan Pathol 42(10): 739-745.
11. McLaughlin, J. M., et al. (2015). "Estimated Human and Economic Burden of Four Major Adult Vaccine-Preventable Diseases in the United States, 2013." J Prim Prev 36(4): 259-273
12. Shingrix® package insert. Revised October 2017.
13. Zostavax® package insert. Revised 2006.
14. Oxman, M. N., et al. (2005). "A vaccine to prevent herpes zoster and postherpetic neuralgia in older adults." N Engl J Med 352(22): 2271-2284.
15. Querec, T. D., et al. (2009). "Systems biology approach predicts immunogenicity of the yellow fever vaccine in humans." Nat Immunol 10(1): 116-125.
16. Li, S., et al. (2017). "Metabolic Phenotypes of Response to Vaccination in Humans." Cell 169(5): 862-877 e817.
17. Langmead, B., et al. (2009). "Ultrafast and memory-efficient alignment of short DNA sequences to the human genome." Genome Biol 10(3): R25.
18. Trapnell, C., Pachter, L. and Salzberg, S.L. (2009) TopHat: discovering splice junctions with RNA-Seq. *Bioinformatics*, 25, 1105-1111.
19. Anders, S. and Huber, W. (2010) Differential expression analysis for sequence count data. *Genome Biol*, 11, R106.

20. Yu, T., Park, Y., Johnson, J.M. and Jones, D.P. (2009) apLCMS--adaptive processing of high-resolution LC/MS data. *Bioinformatics*, 25, 1930-1936.

21. Yu, T. and Peng, H. (2010) Quantification and deconvolution of asymmetric LC-MS peaks using the bi-Gaussian mixture model and statistical model selection. *BMC bioinformatics*, 11, 559.

22. Smith, C.A., Want, E.J., O'Maille, G., Abagyan, R. and Siuzdak, G. (2006) XCMS: processing mass spectrometry data for metabolite profiling using nonlinear peak alignment, matching, and identification. *Anal Chem*, 78, 779-787.

23. Uppal, K., Soltow, Q.A., Strobel, F.H., Pittard, W.S., Gernert, K.M., Yu, T. and Jones, D.P. (2013) xMSanalyzer: automated pipeline for improved feature detection and downstream analysis of large-scale, non-targeted metabolomics data. *BMC bioinformatics*, 14, 15.

24. Tusher, V.G., Tibshirani, R. and Chu, G. (2001) Significance analysis of microarrays applied to the ionizing radiation response. *Proc Natl Acad Sci U S A*, 98, 5116-5121.

Appendix A. Severity Scale for Local Vaccine Reactions (local reactogenicity events)

	INJECTION SITE REACTIONS		
	Grade		
	1	2	3
Swelling/ Induration/	Mild induration, able to move skin parallel to plane (sliding) and perpendicular to skin (pinching up)	Moderate induration, able to slide skin, unable to pinch skin; limiting instrumental activities of daily living	Severe induration, unable to slide or pinch skin; limiting arm movement limiting self-care activities of daily living
Redness/ erythema	Asymptomatic or mild symptoms; intervention not indicated	Moderate; minimal, local; limiting age-appropriate instrumental activities of daily living	Severe but not immediately life-threatening; hospitalization or prolongation of existing hospitalization indicated; disabling; limiting self-care activities of daily living
Pain/tenderness	Mild	Moderate pain; limiting instrumental activities of daily living	Severe pain; limiting self-care activities of daily living

Note:

Instrumental Activities of Daily Living refer to preparing meals, shopping for groceries or clothes,

using the telephone, managing money, etc.

Self-care Activities of Daily Living refer to bathing, dressing and undressing, feeding self, using the

toilet, taking medications, and not bedridden

Appendix B. Adverse Events Grading

Vital Signs	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially Life Threatening (Grade 4)
Fever*				
(°C)	38.0- 38.4	38.5- 38.9	39.0- 40.0	>40.0
(°F)	100.4- 101.1	101.2- 102.0	102/1- 104.0	>104.0

*Oral temperature; no recent hot or cold beverages or smoking

Appendix C. Severity Scale for Generalized Vaccine Reactions (systemic reactogenicity events)

Systemic (General)	**Mild (Grade 1)**	**Moderate (Grade 2)**	**Severe (Grade 3)**	**Potentially Life Threatening (Grade 4)**
Nausea/vomiting	No interference with activity or 1 – 2 episodes/24 hours	Some interference with activity or > 2 episodes/24 hours	Prevents daily activity, requires outpatient IV hydration	ER visit or hospitalization for hypotensive shock
Abdominal Pain	No interference with activity	Some interference with activity	Significant; prevents daily activity	ER visit or hospitalization
Diarrhea	2 – 3 loose stools or <400 gms/24 hours	4 – 5 stools or 400 – 800 gms/24 hours	6 or more watery stools or >800gms/24 hours or requires outpatient IV hydration	ER visit or hospitalization
Headache	No interference with activity	Repeated use of non-narcotic pain reliever >24 hours or some interference with activity	Significant; any use of narcotic pain reliever or prevents daily activity	ER visit or hospitalization
Fatigue	No interference with activity	Some interference with activity	Significant; prevents daily activity	ER visit or hospitalization
Myalgia	No interference with activity	Some interference with activity	Significant; prevents daily activity	ER visit or hospitalization
Shivering	No interference with activity	Some interference with activity	Significant; prevents daily activity	ER visit or hospitalization

Note:

Instrumental Activities of Daily Living refer to preparing meals, shopping for groceries or clothes,

using the telephone, managing money, etc.

Self-care Activities of Daily Living refer to bathing, dressing and undressing, feeding self, using the

toilet, taking medications, and not bedridden

APPENDIX D. SCHEDULE OF EVENTS

Day	**D -28 – D0**	**D0**	**D1**	**D3**	**D7 +/- 1**	**D14 +/- 2**	**D30 +/- 7**	**D60 + 7**	**D61**	**D63**	**D67 +/- 1***	**D74 +/- 2***	**D90 +/- 7***	**D180+/- 14****	**D270+*****
Visit	**Screen -ing**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**
Informed Consent & HIPPA***	X														
Verify Eligibility Criteria	X	X						X							
PID assignment	X														
Demographic and Medical History (including medication and vaccine history).	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Concomitant Medications	X[%]	X[%]	X	X	X	X	X	X	X	X	X	X	X	X	X
Vital signs (Oral Temperature, Pulse Rate, and BP)	X	X	(X)	(X)	(X)	(X)	(X)	X	(X)	(X)	(X)	(X)	(X)	(X)	(X)
Height and Weight	X														
Focused physical exam (only if indicated based on review of health status)	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Vaccination		X						X							
Assessment of health status 15 minutes after administration of study vaccine		X						X							
Memory Aid Review			X	X	X				X	X	X				
Instruction given to study subjects on safety events and concomitant medications****		X	X	X	X	X	X	X	X	X	X	X	X		
Blood draw for Innate Assays and Adaptive Assays		X	X	X	X	X	X	X	X	X	X	X	X	X	X
Stool collection	X	X													
Adverse Events		X	X	X	X	X	X		X	X	X	X	X		
Serious Adverse Events		X	X	X	X	X	X	X	X	X	X	X	X	X	X

Footnotes:

[%]All current medications and medications taken within 30 days prior to day 0 of the study.
* Windows here are in relation to the second (D60) vaccination: D67 (+/-1 from second vaccination), D74 (+/-2 from second vaccination) and D90 (+/-7 from second vaccination).
** D180 +/- 14 days from first vaccination,

***Prior to study procedures
*****D270+, an optional visit at 9 months and beyond, after the first visit may occur.
Any Adverse Event (including – but not limited to- vaccine reactions and local or systemic reactogenicity events) of grade 2 or higher severity or serious adverse event (SAE) occurring after vaccination while the subject is still at the clinical site will be recorded and reported.
**** Instruction includes the following:
1. Subjects will be provided (on D0 and D60 only) with a written description of local and systemic vaccine reactions of mild, moderate and severe intensity and instructed to call the site to report reactogencity events of grade 3 severity within 1 week following vaccination.
2. Subjects will also be instructed to promptly call the site if he/she develops any of the following:
• Illness or treatment from a physician or emergency department; and/or hospitalization due to any illness throughout the entire duration of the study;
• Any adverse event that limits self-care activities of daily living (e.g. bathing, dressing and undressing, feeding self, using the toilet, taking medications, and not bed ridden) even if he/she decides not to seek medical care; or
• He/she starts/stops medications during enrollment in the study.