

Immune Response to Shingles Vaccination

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Study Protocol

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1 Background

There are two clinical illnesses caused by varicella zoster virus (VZV): chickenpox which typically occurs during childhood, and shingles (also known as zoster, or herpes zoster) which typically occurs in older adults. When an individual has their initial infection with VZV they develop chickenpox. Then, VZV becomes latent in the neurons on the dorsal root ganglia (DRG) which are collections of nerve cell bodies located near the spinal cord. Shingles occurs when VZV reactivates in the DRG and travels back down nerves to the skin. This can cause a painful rash on the skin or eye in the area supplied by the DRG.

Therapy for shingles is possible with oral antiviral medications sometimes given with corticosteroids; these are expensive and can have toxicities. Even after the shingles rash heals, a prolonged pain syndrome called post-herpetic neuralgia (PHN) occurs in a substantial proportion of persons; the risk of PHN increases with chronologic age.

The FDA has licensed a live attenuated VZV vaccine to prevent both chickenpox and shingles. The vaccine composition is the same for both indications. The dose varies: the dose for shingles prevention is 14 times higher than the dose for chickenpox prevention. In 1995, universal vaccination with Varivax (the brand name for the pediatric dose) became universally recommended in the US. In 2005, the results of a landmark randomized controlled clinical trial in the US, done in the Veteran's administration system, showed that administration of Zostavax (the brand name for the higher adult dose) lead to a moderate reduction in the incidence of both shingles and PHN when given to older adults. Currently, the Zostavax vaccine is FDA approved for immunocompetent adults 50 or older. ACIP recommends Zostavax at age 60 and most insurance companies follow this guideline by paying for Zostavax at age 60.

It is not clear how Zostavax works. It is generally thought that long term immune memory to VZV, set into place by childhood chickenpox, gradually wanes over time, and that a delicate equilibrium between the latent virus in neurons and the immune system finally tips in favor of the virus and the individual gets clinical shingles. Zostavax, being a live vaccine, is thought to be able to stimulate or re-stimulate a specific immune response to VZV, include the components of antibodies, T-cells with the CD4 marker, and T-cells with the CD8 marker. Because Zostavax contains all 70 proteins encoded by the VZV genome, it is thought that these immune components are stimulated quite broadly and that the vaccine elicits immune responses to many VZV proteins. To date, the research on this topic is simplistic and minimal. Our lab has developed systems to use before and after vaccine blood samples to measure in quantitative detail the CD4 and CD8 T cell immune response to each individual VZV protein.

2 Study Purpose

The overall purpose of this protocol is to study the immune responses to shingles vaccination.

3 Participant Selection

3.1 Inclusion Criteria

Men and women who meet all of the following criteria are eligible for inclusion in this study:

- 50 years of age or older
- History of chickenpox

3.2 Exclusion Criteria

Men and women who meet any of the following criteria are not eligible for this study:

- Previous vaccination with Zostavax or with the chickenpox vaccine.
- History of ever having had shingles.
- Been in close contact with a person who had chickenpox or shingles in the past 5 years.
- VZV seronegative
- Taking systemic suppressive regular doses of drugs with anti-VZV activity such as acyclovir, famciclovir, or valacyclovir. Episodic use is allowed. For Cohort 1: medication cannot be taken 24 hours prior to or 30 days after receiving Zostavax per CDC recommendations.
- HIV seropositive.
- Hepatitis C infection or active Hepatitis B infection.
- History of a life-threatening allergic reaction (anaphylactic/anaphylactoid reaction) to gelatin, neomycin, or any other component of shingles vaccine. Neomycin allergy manifested as contact dermatitis is not an exclusion.
- Has immunosuppression as a result of an underlying illness (e.g. leukemia, lymphoma or other malignant neoplasms) or treatment with immunosuppressive or cytotoxic drugs, or use of anticancer chemotherapy or radiation therapy.
- Has long-term use of oral or parenteral steroids (>7 days), or high-dose inhaled steroids (>800 mcg/day of beclomethasone dipropionate or equivalent) within the preceding 6 months (nasal and topical steroids are allowed).
- Women of child-bearing potential only: pregnant or planning to become pregnant 3 months post vaccination (Cohort 1 only).
- Donated blood in the past 8 weeks or planning to donate blood during the study
- Weighs less than 110 lbs
- Has any condition or medical history that would, in the opinion of the site principal investigator place the subject at an unacceptable risk of injury or render the subject unable to meet the requirements of the protocol.

Additional exclusions for skin biopsy:

1. Has an acute or chronic medical condition that, in the opinion of the investigator, would render biopsies unsafe
2. History of coagulopathy or taking medication that may cause bleeding (long term aspirin, heparin, coumadin)
3. History of keloid formation or excessive scarring
4. History of frequent cellulitis or boils (>3 episodes in past 2 years) requiring antibiotic therapy.
5. Allergy to lidocaine, silver nitrate, or mupirocin.

4 Study Procedures

4.1 Screening Visit

Study background and procedures will be explained. Potential participants will have a chance to ask questions and informed consent will be obtained. A separate consent form will be used for HSV testing.

Demographic, medical, and sexual history information will be obtained. Study specific inclusion/exclusion criteria will be evaluated. We will gather medical history specific to VZV infection, including history of chickenpox or shingles.

Blood will be drawn for HSV serology unless previous documentation is available. Blood will be drawn for laboratory testing of HIV, HepB, HepC, VZV and CMV serology. If HIV, HepB and HepC testing was performed in the last year and the participant was negative and is low risk, the test(s) need not be repeated based on medical history and clinical judgment.

If participants have a medical history and/or laboratory confirmation of HIV, Hepatitis C or active Hepatitis B infection, they will be referred to an appropriate provider, as needed, and will not be eligible for further participation. Subjects will be eligible regardless of the results of their tests for antibodies to HSV-1, HSV-2 and CMV.

4.2 Enrollment Visit (Day 0)

Eligible subjects will return for an Enrollment Visit. A blood sample (up to 110 ml) will be obtained for immunological assays and VZV research serology. Additional blood (up to 10 ml) will be obtained for HLA typing. After the blood sample is obtained, a standard dose of Zostavax will be administered. Participants will be provided with a copy of the CDC Shingles Vaccine Information Sheet.

4.3 Follow-up Visits

Participants will return for follow-up visits at Day 7-14, 28, and 180. Blood will be drawn for immunological assays and VZV research serology.

4.4 Skin Biopsy

Two 3-mm skin biopsies will be collected from the upper arm (contralateral to the vaccine injection) at Day 0 and again at Day 28. The biopsy will be performed prior to vaccination on Day 0.

4.5 Additional Visits

All participants may be asked to return for additional visits should the laboratory require extra blood samples for analysis. Up to 110 ml of blood may be collected during a visit for immunological assays and VZV research serology.

5 Risks & Benefits

5.1 Potential Risks

Phlebotomy: Blood drawing may cause discomfort and/or bruising. Rarely an infection may develop. Clinical discretion will be used when drawing blood from elderly (65+) participants. If necessary, blood draw volumes will be adjusted (50 ml minimum).

Zostavax vaccination: Mild reactions include redness, soreness, swelling, or itching at the site of the injection and headache. Serious adverse reactions include anaphylaxis. There is no documentation of a person getting chickenpox from someone who has received the shingles vaccine. Some people who get the shingles vaccine will develop a chickenpox-like rash near the place where they were vaccinated. There is a risk of disseminated infection in immunocompromised persons; therefore, they will be excluded from the study. There is a risk of transmission of the vaccine virus between vaccines and susceptible contacts. As with any vaccination, there may be side effects that are not known at this time.

Biopsy: The diameter of the biopsy will be equivalent to a very small straw. Anticipation of the procedure may cause anxiety. The biopsy itself may cause pain, even with the use of an anesthetic. Biopsies may cause a pressure or tugging sensation. Bleeding may also occur; in the case of excessive bleeding, silver nitrate will be applied. Infection and scarring are rare complications of biopsies. Biopsies will not be performed on face or neck.

Lidocaine (injectable, +/- epinephrine): Lidocaine administration is associated with temporary pain and burning. Skin biopsy may cause discomfort even when the area of the skin is numbed using lidocaine. Allergic reactions to lidocaine may occur, even in subjects with no history of allergic reactions. Examples of allergic reactions include wheezing or difficulty in breathing, lightheadedness or fainting, skin rash and itching. As with any drug there may be unknown side effects.

5.2 Potential Benefits

Subjects are not expected to benefit from participation. Society may benefit from an increased understanding of the cellular immune response and pathogenesis of varicella zoster virus and vaccination.

6 Clinical Procedures

6.1 Zostavax vaccination

ZOSTAVAX is a live attenuated virus vaccine indicated for prevention of herpes zoster (shingles) in individuals 50 years of age and older.

6.1.1 Recommended Dose and Schedule

ZOSTAVAX will be administered as a single 0.65-mL dose subcutaneously in the deltoid region of the upper arm.

6.1.2 Preparation for Administration

Use only sterile syringes free of preservatives, antiseptics, and detergents for each injection and/or reconstitution of ZOSTAVAX. Preservatives, antiseptics and detergents may inactivate the vaccine virus.

ZOSTAVAX is stored frozen and should be reconstituted immediately upon removal from the freezer.

When reconstituted, ZOSTAVAX is a semi-hazy to translucent, off-white to pale yellow liquid.

Reconstitution:

- Use only the diluent supplied.
- Withdraw the entire contents of the diluent into a syringe.
- To avoid excessive foaming, slowly inject all of the diluent in the syringe into the vial of lyophilized vaccine and gently agitate to mix thoroughly.
- Withdraw the entire contents of reconstituted vaccine into a syringe and inject the total volume subcutaneously.
- **ADMINISTER IMMEDIATELY AFTER RECONSTITUTION** to minimize loss of potency. Discard reconstituted vaccine if not used within 30 minutes. Do not freeze reconstituted vaccine.

6.1.3 Contraindications

Do not administer ZOSTAVAX to individuals with a history of anaphylactic/anaphylactoid reaction to gelatin, neomycin or any other component of the vaccine. Neomycin allergy manifested as contact dermatitis is not a contraindication to receiving this vaccine.

ZOSTAVAX is a live, attenuated varicella-zoster vaccine and administration may result in disseminated disease in individuals who are immunosuppressed or immunodeficient. Do not administer ZOSTAVAX to immunosuppressed or immunodeficient individuals including those with a history of primary or acquired immunodeficiency states, leukemia, lymphoma or other malignant neoplasms affecting the bone marrow or lymphatic system, AIDS or other clinical manifestations of infection with human immunodeficiency viruses, and those on immunosuppressive therapy.

Do not administer ZOSTAVAX to pregnant women. It is not known whether ZOSTAVAX can cause fetal harm when administered to a pregnant woman or can affect reproduction capacity. However, naturally occurring VZV infection is known to sometimes cause fetal harm. Therefore,

ZOSTAVAX should not be administered to pregnant women, and pregnancy should be avoided for 3 months following administration of ZOSTAVAX.

6.2 Skin Biopsy

Biopsies will be obtained by trained clinicians. Biopsies will be obtained from areas of the healed shingles rash and areas of normal skin. Biopsies will be performed only when participants are willing. Participants are free to refuse a biopsy at any visit. Biopsies will be performed on the trunk, buttocks, arms, or legs. Biopsies will not be performed on the face. Pre and post biopsy photographs will be taken for each biopsy.

3 mm punch biopsies will be performed as per the Biopsy SOP. Briefly, the skin/mucosal surface will be prepped with chlorhexidine or betadine. 1-5 cc lidocaine +/- epinephrine (1%) will be used to anesthetize the skin. A biopsy will be performed and placed into a 15mm tube with 6 ml of media. A supply of 15ml conical vials are kept in the lab refrigerator. The tube will be shipped with an ice pack to the Koelle lab.

Given the small size of the tissue obtained, sutures are not necessary. Pressure will be applied to the site for 5 minutes. If excessive bleeding persists, silver nitrate may be used to stop the bleeding. Participants will be advised to take over-the-counter Tylenol or ibuprofen for pain relief. These will be provided to the participants through the clinic free of charge as needed.

7 Non-Investigational Study Medications

Name	Dose	How administered
Zostavax®	Single 0.65ml dose	Subcutaneously in the deltoid region of the upper arm
Silver nitrate	One applicator, as needed for bleeding	Topical
Double antibiotic ointment	1-3 times daily, as needed	Topical
1% lidocaine	10mg/ml. Up to 5 ml per dose.	By injection

8 Safety

8.1 Adverse Events

An adverse event (AE) is defined as any untoward medical occurrence in a patient or a clinical investigation participant administered a pharmaceutical, which does not necessarily have a causal relationship with the treatment. Due to the specific setting in which this study is performed (outpatient research clinic), this study will focus on hospitalizations and other severe AE. Mechanisms of obtaining information on AE will rely primarily on biweekly visits to the clinic with a health care provider. The occurrence and severity of all AEs will be listed and graded according

to the NCI CTC criteria. For a complete listing, see <http://ctep.info.nih.gov/CTC3/ctc.htm>. The following information will be collected on all AEs experienced during this study:

- Name of the event: If the event is described in the NCI CTC, the Investigator should use that terminology. Otherwise, terminology that clearly describes the pathophysiology of the event and body system affected should be used.
- Onset Date
- Date of resolution
- Severity
 - If an event is not described on the NCI CTC and therefore not graded, the following grading will be used:
 1. Mild, easily tolerated by the patient, causing minimal discomfort and not interfering with everyday activities.
 2. Moderate, sufficiently discomforting to interfere with normal everyday activities
 3. Severe, prevents normal everyday activities
 4. Life-threatening, places the patient at immediate risk for death
- Relationship to study procedures/drug: All AEs will have a causality assessment performed at the time of reporting the event to document the Investigator's perception of causality. For the purposes of this study, causality will be assigned using the following criteria:
 - Related: The event cannot be attributed to the patient's underlying medical condition or other concomitant therapy and there is a compelling temporal association between the onset of the events and study drug administration that leads the Investigator to believe that there is reasonable chance of a causal relationship.
 - Remote: A relationship is not obvious but cannot be ruled out.
 - Not related: The patient's underlying medical condition or concomitant therapy can easily be identified as the cause of the event and there is no temporal relationship between the event and the study drug.

8.2 Serious Adverse Events

The Investigator is required to determine if each AE was a SAE. A SAE is any AE occurring at any dose of study drug that results in any of the following outcomes:

- Death
- A life-threatening adverse drug experience
- Inpatient hospitalization (hospitalizations for elective medical/surgical procedures, scheduled treatments, or routine check-ups are not SAE by these criteria)
- A persistent or significant disability / incapacity

Important medical events that may not meet any of the above criteria may be considered a serious adverse experience when, based upon appropriate medical judgment, it may jeopardize the patient or participant and may require medical or surgical intervention to prevent one of the outcomes listed above.

8.3 Reporting Procedures

Adverse events including local and systemic reactions not meeting the criteria for SAEs will be captured on the appropriate CRF. Information to be collected includes event description, date of onset, investigator assessment of severity, investigator assessment of relationship to study product, date of resolution of the event, seriousness, and outcome. The **intensity** of nonserious AEs can be assessed by a licensed clinician (i.e., physician, nurse, Nurse Practitioner, Physician Assistant). **Causality** of nonserious AEs can be assessed only by a clinician licensed to make medical diagnoses (ie, physician, Nurse Practitioner, Physician Assistant). All AEs occurring while on study will be documented appropriately regardless of relationship.

Any medical condition that is present at screening will be considered as baseline and will not be reported as an AE. If the severity of any pre-existing medical condition increases during the study period, then it will be recorded as an AE

All SAEs will be reported immediately to the Investigator.

SAE Alternate: A clinician from the Virology Research Clinic is available at any time by calling the operator at the Children's Hospital at 206-598-0924 and asking for the Virology Research Clinic Clinician on-call.

All SAEs will be:

- Recorded on the appropriate SAE report form.
- Followed through resolution by a study physician.

Timelines for submission of an SAE form are as follows:

- All deaths and life-threatening events regardless of relationship, will be recorded on the SAE form and the PI will be notified within 24 hr of identification of the SAE.

All SAEs determined to be related to study procedures will be reported to the Human Subjects Division of the University of Washington within 10-days of becoming aware of the event.

The SAE report will include the following information (as available)

- Patient ID
- Description of SAE (onset date, severity, causal relationship)
- Basic demographic information
- Outcomes attributed to the event
- Summary of relevant test results, laboratory data, and other relevant history
- The first and last dates of study drug administration
- Statement whether study drug was discontinued or schedule modified
- Statement whether the event abated after study drug was discontinued or schedule modified
- Statement whether the event recurred after reintroduction of the study drug if it had been discontinued.

8.4 Follow-up of Adverse Events

All AEs will be followed to adequate resolution or until considered stable.

All SAEs reported to the Virology Research Clinic will be relayed to the Human Subjects Division (HSD) of the University of Washington as required by HSD policy.

9 Data Analysis Plan

Positive response to VZV proteins in lymphoproliferation assays will be determined on a per-cell-line basis surpassing this threshold: at least $2.33 \times$ median absolute deviation of all negative-control antigen samples above the median response of those same samples. This calculation has a theoretical false-positive rate of 1.0%. VZV antigen wells will be scored positive only if both replicates exceed the threshold. ORFs synthesized as fragments (eg, ORF22) were scored positive once if any fragment was deemed positive.

Antibody titers, T-cell frequencies, and T-cell breadth will be compared between the different time points using the nonparametric Friedman's test for repeated measures. If significant, subsequent analysis of measures using Dunn's test identified which time points were different from prevaccine samples. Correlation analyses will be performed using the Spearman's test. Nonparametric tests will be used because the data are likely to be highly left skewed, thus did not satisfy assumptions of normality. Two-sided P values $<.05$ will be considered statistically significant. Statistical tests and graphs were generated using Prism (v7 or greater, GraphPad Software, La Jolla, California).

10 Human Subjects Protections

10.1 IRB Approval

Prior to the initiation of the study, the principal investigator will obtain written approval to conduct the study via the University of Washington Human Subjects Review Committee.

10.2 Informed Consent

The investigator or study coordinator will explain the purpose and nature of the study, including potential benefits and risks to the participant, to each potential participant before enrollment in the study. The participant must sign an informed consent form approved by the IRB before entering the study. All original informed consent forms must be retained by the principal investigator with the participant's records. The participant will receive a copy of the signed informed consent form.

Appendix A: Table of Procedures

Study Day	Screen	Day 0	Day 7 - 14	Day 28	Day 180	Additional Visits
Obtain Informed Consent	X					
Review Inclusion/Exclusion Criteria	X	X				
Medical History	X	X				
HIV Serology	X					
HSV Serology	X					
CMV Serology	X					
VZV Serology	X					
Hepatitis C Serology	X					
Hepatitis B Surface Antigen	X					
Blood (DNA) for HLA Typing		X				
Blood Draw for Immunological Assays		X (100)	X (100)	X (100)	X (100)	X (100)
VZV Research Serology		X	X	X	X	X
Vaccination		X ¹				
Skin Biopsy		X ²		X		

¹ Blood draw will occur prior to vaccination

² Biopsy obtained prior to vaccination