COLORADO MULTIPLE INSTITUTIONAL REVIEW BOARD
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Project Title: Persistence Of Protection Conferred By Shingrix Against Herpes Zoster In Older Adults
NCT04169009
COMIRB approval date: 09MAR2022

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Version Date: March 8, 2022

I. Hypotheses and Specific Aims

Hypotheses

1) Shingrix (RZV) recipients will have a lower rate of varicella-zoster virus (VZV) DNAemia after a live intradermal (ID) VZV challenge (vOka vaccine) than Zostavax (ZVL) recipients because of higher and/or more rapid VZV-specific T cell responses.
2) After a live VZV ID challenge, RZV recipients will have the same rate of VZV DNAemia whether the challenge was performed <1 or >5 years after vaccination; whereas ZVL recipients will have lower rates of VZV DNAemia if they are challenged <1 year after vaccinations compared with >5 years.in t
3) The peak memory VZV and/or gE Th1 responses to RZV mediate protective immune responses conferred by RZV against VZV DNAemia after VZV ID challenge; early responses may also play a role

Specific Aims/Study Objectives

Primary Objective 1. To compare the rate of vOka DNAemia within 7 days after ID vOka administration in adults who received RZV or ZVL >5y before the challenge.

Primary Objective 2. To compare the rate of vOka DNAemia after ID vOka challenge at <1y after RZV or ZVL vaccination and at >5y after vaccination.

Secondary Objective 3. To identify the characteristics of the immune responses to RZV that predict the immunologic control of vOka DNAemia after ID vOka challenge at <1y and at >5y after vaccination.

Note: This will include studies to further define epitope-specific immune responses, and will measure transcript profile and epigenetic changes in immune cells following vaccination.

II. Background and Significance

Background - Herpes zoster (HZ) is a frequently severe infection caused by varicella-zoster virus (VZV). 60% of the annual 1.2 million US cases occur in people ≥50 years of age, although immune compromise is another risk factor for HZ (1,2). Common to these two risk factors is a significant decline, to a critical level, of VZV T-cell mediated

immunity (VZV-CMI) initially stimulated by the primary VZV infection (childhood varicella) (3-5). Varicella also results in the lifelong persistence of VZV in sensory ganglia. Sporadic reactivation of VZV occurs, but such events typically remain subclinical because of VZV-CMI. However, when an adequate VZV-CMI response to reactivation is absent, propagation of VZV in the ganglion continues and spreads down the sensory nerve to cause the characteristic signs and pain of HZ (6). A live attenuated HZ vaccine (ZVL) has been available since 2006 to prevent this, but its efficacy for preventing HZ is 51% in the 1st 3y after immunizations and drops sharply after 5y, and efficacy decreases with the age of the vaccinee (7. Shingrix (RZV), a recombinant VZV glycoprotein E (gE) adjuvanted vaccine, which became available late in 2018, is remarkably superior, with 97% overall efficacy against HZ. RZV protection declines little over time (8). The efficacy of RZV is unique compared with any approved vaccines for older adults and any candidate vaccines for herpesviruses. The magnitude and persistence of RZV immunogenicity represent a paradigm shift in vaccinology that warrants granular studies.

Significance - Central to understanding the superiority of RZV is determining the timing, magnitude, and nature of the systemic and local immune events at the site of VZV reactivation and replication. *Since the milieu of sensory ganglia cannot be directly evaluated, this proposal will use intradermal (ID) administration of live attenuated VZV vaccine (vOka strain)* to simulate reactivated VZV replication in the skin.

Ageing is associated with increasing frailty and multiple concomitant medical conditions, which are compounded by an increasing incidence of infections as a consequence of immune senescence. The resulting societal burden is evident from increased hospitalization and medical costs among older people (9). This is clearly demonstrated by HZ, where the incidence, severity, hospitalization, and complications increase dramatically after the 6th decade of life (10). This problem will intensify in the future, since there are currently 110 million people in the US over 50y of age and there will be 32 million people ≥80y in 2050 (11). Understanding how a vaccine for older people successfully limits a common infection (namely HZ) in ageing individuals may be applicable to the general problem of improving the immunogenicity of other vaccines for older people. Specifically, this proposal determines how a vaccine shapes the early immune response to a live attenuated vOka VZV challenge and limits VZV infection at the challenge site. Local intracellular vOka antigen production and DNAemia resulting from the challenge provide endpoints for this new model to understand the effect of early post-reactivation immune responses on VZV replication.

III. Preliminary Studies/Progress Report

The following preliminary studies have been completed in support of this proposal:

1. We submitted the results of a 1 year study (so far in year 4 of a 5 year study) to *Nature Vaccine* that determined that RZV generates superior VZV-specific T cell memory responses compared to ZVL. All of the immunologic assays needed for the proposed trial have been validated and appear in our published reports (12).

2. We published methods and findings that demonstrated that VZV DNAemia after a vOka skin challenge correlates with the differentiation and magnitude of VZV-CMI responses (13).
3. We published on the methods, safety, and immunogenicity of ID vOka (14). 4. Experiments in preparation for this proposal that have been completed include:

a. Measures of plasma biomarkers after different modes of administration of ZVL and RZV.
b. Measures of selected T cell and antigen-presenting cell responses after ZVL and RZV.
c. Discovery of CD4+ and CD8+ epitopes that will be used for tetramer synthesis for the advanced immunologic assessments proposed.
d. Skin specimens have been studied at the Human Immune Monitoring Shared Resource at the Anschutz campus using the 7-color Vectra 3 instrument. This methodology will be used to study local responses to the ID vOka challenge. We were able to detect VZV and immune cell phenotypes on these skin biopsies.

IV. Research Methods

A. Outcome Measures/Study Endpoints

Primary Endpoint **-** Rate of VZV DNAemia after ID vOka challenge.

Secondary Endpoint – Duration and magnitude of VZV DNAemia and rate of VZV RNAemia. VZV- and gE-specific T cell and other immune cell responses in blood and at the site of ID vOka challenge; cytokines levels in blood and at the site of ID vOka inoculation; transcriptomics of immune cells in blood and skin biopsies; immunohistochemistry of skin biopsies of the ID vOka challenge.

Exploratory Endpoint –After RZV: Tetramer analysis of the T cell responses; T cell epigenome.

B. Description of Population to be Enrolled

Inclusion Criteria

1. Subject able to understand and provide informed consent.
2. Age ≥ 50 years
3. Ambulatory and in generally good health except for common morbidities of older people;
4. Women of non-childbearing potential meet at least 1 of the following:
    a. Achieved postmenopausal status, defined as cessation of regular menses for at least 12 consecutive months
    b. Have undergone hysterectomy and/or bilateral oophorectomy
5. **Part A, Cohorts 1 and 2** – Documented evidence of immunization with ZVL (Cohort 1; n=35) or RZV (Cohort 2; n=35) at least 5 years previously;

6. **Part B, Cohorts 3 and 4** - HZ vaccine naïve - Cohort 3 (n=35) will be vaccinated with ZVL at study enrollment, **or** Cohort 4 (n=35) will be vaccinated with RZV at study enrollment.

Exclusion Criteria

1. Inability or unwillingness of a participant to give written informed consent or to comply with study protocol.
2. Prior history of HZ;
3. Known history of hypersensitivity to ZVL or vaccine components in all study candidates and to hypersensitivity to RZV or vaccine components in Cohort 4.
4. Known neomycin contact allergy or other ZVL components such as gelatin.
5. Untreated anemia in Cohorts 1 and 2.
6. Blood products received in the 3 months prior to study enrollment or planned for the subsequent week for Part A; Part B requires the same exclusion but extends it to the week after the vOka challenge, which is 6 months after completing ZVL or RZV administration.
7. Any confirmed or suspected immunosuppressive or immunodeficient condition resulting from disease (e.g., malignancy, HIV), or immunosuppressive/cytotoxic therapy.
8. Use, or anticipated use, of immunosuppressants or other immune-modifying drugs during the period starting 30 days prior to ID ZVL challenge through 7 days after. This includes chronic administration of corticosteroids (>14 consecutive days of prednisone at a dose of ≥ 20 mg/day [or equivalent], long–acting immune-modifying agents, or immunosuppressive/cytotoxic therapy (e.g., medications used during cancer chemotherapy or to treat autoimmune disorders). *Topical, ophthalmic, intra-articular, or inhaled/nebulized steroids are allowed.*
9. Concomitant vaccine received within 2 (inactive) or 4 (live) weeks prior to the study for subjects in all Cohorts, and during the first week of the study. For subjects in Cohorts 3 and 4, no concomitant vaccine received within 2 (inactive) or 4 (live) weeks prior to ID vOka and for the following 7 days.
10. Women of child-bearing potential.
11. Pregnancy or breast-feeding.
12. Participation in a concurrent clinical study in which the subject will be exposed to an investigational product (drug or vaccine) during the period starting 7 days before the first dose of study vaccine through the completion of the study.
13. Antivirals with activity against VZV, including acyclovir, valacyclovir, ganciclovir, valganciclovir, foscarnet, cidofovir, brincidofovir and penciclovir within 7 days of the ID ZVL challenge.
14. 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, may pose additional risks from participation in the study, may interfere with the participant's ability to comply with study requirements or that may impact the quality or interpretation of the data obtained from the study.

C. Study Design and Research Methods

Part A; Cohorts 1 and 2

Seventy subjects with documented evidence of a HZ vaccine at least 5 years previously, divided equally between recipients of ZVL (Cohort 1) or RZV (Cohort 2) - 35 in each group - will be enrolled. Participation will consist of 4 visits over the course of 7-10 days in which blood is drawn, a challenge dose of vOka is given, and a skin biopsy is obtained.

ID vOka challenge live attenuated VZV (as 1 dose of licensed ZVL; Zostavax) will be prepared by mixing 0.35 ml of accompanying diluent with the vaccine and injecting 2 side-by-side intradermal doses of 0.125ml each, for a total of 0.25 ml (about ¾ of the normal dosage or 13,857 plaque forming units) intradermally in the non-dominant deltoid region, using a NanoPass MicronJet600 device, on D0. Blood (60 ml) will be drawn prior to vaccination (D0), and at Days 1, 3, and 7. A skin biopsy (4 mm punch biopsy) will be done at the injection site, on the first 20 subjects (up to 25, if replacements needed) in each vaccine group; five each of these 20 will be biopsied on Day 0, 1, 3, or 7 in the order in which the participants enter the study. The Day 0 biopsy will be done on the contralateral arm in the deltoid region prior to vaccination. Initially every subject enrolled will be required to do the biopsy; this is reflected in the consent. The consent form will be amended to remove references to the biopsy procedure once the number of subjects needed for the biopsies is reached.

Subjects in the ZVL group will be offered RZV vaccine (to comply with ACIP recommendations for shingles immunization), to be administered beginning ≥ 60 days after Day 0. If subject wishes to receive the RZV vaccine, 2 additional visits will be required at Day 60 and Day 120, increasing the duration of participation up to 4 months. Subjects will not be reimbursed for these visits but will receive the vaccine free of charge.

Schedule of Events – Cohorts 1 & 2

Cohorts 1 and 2 **ZVL/RZV > 5y previously**	**Visit 1** Day 0	**Visit 2** Day 1	**Visit 3** Day 3	**Visit 4** Day 7 (+3)	**Phone** + 48 hour after V4	**Visit 5** Day 60 (+30)	**Visit 6** Day 120 (+30)
Informed consent, review of eligibility criteria	●						
Medical history and current medications	●					●[3]	●[3]
Blood draw (60ml)	●	●	●	●			
Pre-vaccination body temperature	●					●[3]	●[3]
Vaccination (ID vOka challenge)	●						
Punch biopsy of ID challenge site[1]	●[1,2] ,or	●[1] ,or	●[1] ,or	●[1]			
Vaccination – Dose 1 RZV[3]						●[3]	
Vaccination – Dose 2 RZV[3]							●[3]
AE assessment	●	●	●	●	●		

[1]40 subjects (20 in each vaccine group) with 5 each at one of the time points identified
[2]Contralateral site only
[3] If prior ZVL

Recruitment – There are very few people in the world who had RZV administered 5 years previously. These subjects will come from an experiment done here (COMIRB #13-3192) in which we compared immune responses to RZV and ZVL. The 4y post-vaccination samples are being collected this year, and the 5y sampling will be completed between Nov 2019 and Mar 2020. When these older vaccinees complete 5y after immunization, they will constitute a unique population eligible for our proposal. To secure participation for this proposal, we will contact participants in our current trial (who had consented to be contacted about future clinical trials).

Part B; Cohorts 3 and 4 – No Previous HZ vaccine

Part B measures VZV DNAemia and systemic VZV-specific immune responses after ID vOka challenge in individuals who received HZ vaccines 6 months prior to the injection.

Cohort 3 (n=35) subjects will be vaccinated with ZVL at enrollment; blood will be obtained at Days 0, 1, 3, 7 and 30 days after. Five - six months after receiving ZVL subject will be challenged with vOka administration (as described in Part A) followed by visits 1, 3, and 7 days later at which blood is drawn. Total of 9 to 11 visits.

These subjects will also be offered RZV vaccine (to comply with ACIP recommendations for shingles immunization) to be administered ≥ 60 days after vOka challenge. If subject wishes to receive the RZV vaccine, 2 additional visits will be required 2 beginning 2 months after Visit 6, increasing the duration of participation up to 10 months. Subjects will not be reimbursed for these visits but will receive the vaccine free of charge.

Schedule of Events – Cohort 3

Cohort 3 – No Previous HZ Vaccine (to be admin ZVL)	**Visit 1**	**Visit 2**	**Visit 3**	**Visit 4**	**Phone**	**Visit 5**	**Visit 6**	**Visit 7**	**Visit 8**	**Visit 9**	**Phone**	**Visit 10**	**Visit 11**
	Day 0	**Day 1**	**Day 3**	**Day 7**	**Day 9 (+3)**	**Day 30 (+7)**	**Day 150 (+44)**	**Day 151**	**Day 153 (+2)**	**Day 157 (+10)**	**+48 hours after V9**	**60 Days after V6 (+30)**	**60 days after V10 (+30)**
Informed consent, review of eligibility criteria	●												
Medical history and current medications	●						●						
Blood draw (60ml)	●	●	●	●		●	●	●	●	●			
Pre-vaccination body temperature	●						●						
Vaccination – Zostavax	●												
Vaccination (ID vOka challenge)							●						
AE assessment	●	●	●	●	●	●	●	●	●	●	●		
Vaccination – Dose 1 RZV[1]												●[1]	
Vaccination – Dose 2 RZV[1]													●[1]

[1]If subject wishes to receive RZV

Cohort 4 (n=35) subjects will be enrolled, have blood (60ml) drawn, and will be administered RZV (2 doses –D0, D60) prepared and administered in accordance with the package insert, in the clinic. Participation will consist of 10 visits over the course of approximately 9 months. Blood will be drawn at Day 0, Days 61, 63, 67, and 90. These subjects will receive the challenge dose of vOka 6 months later at Day 240, with blood drawn, then drawn again at Days 241, 243, 247. Participation will consist of 10 visits over the course of approximately 8.5 months.

Schedule of Events – Cohort 4

Cohort 4 – No Previous HZ Vaccine (to be admin RZV)	**Visit 1**	**Phone**	**Visit 2**	**Visit 3**	**Visit 4**	**Visit 5**	**Visit 6**	**Visit 7**	**Visit 8**	**Visit 9**	**Visit 10**	**Phone**
	Day 0	**Day 7 (+3)**	**Day 60 (+14)**	**Day 61**	**Day 63**	**Day 67 (+3)**	**Day 90 (+7)**	**Day 240 (+14)**	**Day 241**	**Day 243**	**Day 247 (+3)**	**+48 hours after V 5, 6, 10**
Informed consent, review of eligibility criteria	●											
Medical history and current medications	●		●					●				
Blood draw (60ml)	●		●	●	●	●	●	●	●	●	●	
Pre-vaccination body temperature	●		●					●				
Vaccination – Dose 1 RZV	●											
Vaccination – Dose 2 RZV			●									
Vaccination (ID vOka challenge)								●				
AE assessment	●	●	●	●	●	●	●	●	●	●	●	●

D. Description, Risks, and Justification of Procedures and Data Collection Tools

Risks

Zostavax- The risks are those associated with a licensed vaccine for people 50 years and older. Subjects will be given the relevant Vaccine Information Sheet (VIS).

ID vOka challenge (as 1 dose of licensed ZVL; Zostavax) –The intradermal route was previously studied in ~200 subjects without a noteworthy safety signal (13). The device for ID administration is licensed and was used in a prior study (COMIRB #11-1210). Some subjects will be receiving ZVL for the second time. There is no recommendation for a second dose of ZVL, but we completed a study (COMIRB #10-1461) in which 200 participants safely received a second dose of ZVL. Furthermore, ZVL is recommended after prior herpes zoster, which is analogous to re-exposure (as with our ID challenge) to the virus of herpes zoster. Very rarely, a brief, painless blistery rash limited to the site of injection may develop.

Shingrix – This vaccine will be administered according to the package insert. The risks are those associated with a licensed vaccine for people 50 years and older. Subjects will be given the Vaccine Information Sheet (VIS).

As with all vaccines or drugs, rarely an allergic reaction could occur – a rash, hives or even difficulty breathing.

Risk of receiving multiple shingles vaccines – two doses of RZV have been safely administered to prior ZVL recipients, and also to individuals who had prior HZ. This was done prior to licensure and mentioned in the ACIP recommendation, indicating no concern with an aberrant immune response in this setting.

Blood draws – 60 ml of blood will be taken at each blood draw for a total of 240 ml in 1 week for subjects in Cohorts 1, 2, 3 and 4. Subjects in Cohort 3, receiving ZVL, will have a total of 540 ml drawn over the course of the study. Subjects in Cohort 4, receiving RZV, will have a total of 600 ml drawn, over the course of the study. Neither will have more than 240 ml drawn in a week. The risks of phlebotomy are minimal and include pain, swelling, bruising, or infection around the vein, although this is very uncommon.

Punch biopsy – The skin biopsy will be taken at the ID vOka challenge site (except for Day 0 participants, in which case it will be done on the contralateral arm). The area will be numbed; then a punch biopsy device will be pressed into the skin removing a small circle of skin.

Risks include local pain, potential for infection, and scarring at punch site, and possible allergic reaction to the numbing medicine. This procedure is being overseen by a dermatologist skilled in this procedure. This procedure is important because it will indicate what the local immune response to the herpes zoster virus is in people who previously received either ZVL or RZV.

Genetic testing – In order to do epitope mapping and to create tetramers we will do HLA typing of white blood cells.

Benefits

There is no known benefit from this study, but this research may influence future decisions made by participants concerning booster doses of HZ vaccine. It may also led to improved vaccines for older persons. ID ZVL may further enhance protection. This is likely, but is unstudied. Some participants may receive RZV, which is recommended to prevent herpes zoster.

E. Safety Reporting

For this study, adverse events (AE) will be collected for 48 hour after each study procedure and for 7 days after vOka administration. Additionally, AEs will be collected for 7 days after administration of ZVL to Cohort 3, and RZV to Cohort 4 participants. Participant will be asked at each visit: “Have there been any significant changes to your health since we saw you last? New medications? Hospitalizations?”

An adverse event will include any untoward or unfavourable medical occurrence associated with:

- **Study intervention**: ID vOKA – AEs starting within 7 days after injection and Grade ≥ 3 will be recorded and followed until resolution. ZVL – Subjects in Cohort 3 receiving ZVL on Day 0 will be followed for AEs for 7 days. RZV – Subjects in Cohort 4 receiving RZV on Day 0 and Day 60 will also be followed for AEs for 7 days after each injection. Any Grade ≥ 3 will be recorded and followed until resolution.
- **Study mandated procedures**: blood draw, skin biopsies and injections – Any AE, Grade ≥ 3, occurring within 48 hours at the site of vOKA injection, skin biopsy or venipuncture will be recorded and followed until resolution. (This excludes optional RZV vaccination for participants in Cohorts 1 and 3.)

Adverse events will be graded on a scale from 1 to 5 according to the following:

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.

The PI will review AEs on a continuous basis during the study. Both Zostavax and Shingrix are FDA-approved vaccines that have been extensively studied. While no unusual vaccine-related SAEs are anticipated, we propose the following halting rules for risk management. The PI will responsible for responding to vaccine-related serious adverse events. Reporting will be to the manufacturers of each vaccine, the IRB, and to the NIH Medical Monitor (as required).

Attribution of Events

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

Any SAE considered possibly, probably, or definitely related will result in halting of the study while an analysis of the event is undertaken. Any halting of the study and the circumstances involved will be reported to the NIH Medical Monitor within 24 hours. An Independent Safety Monitor (ISM), who is an Infectious Disease physician not

associated with the study, will review any SAE considered possibly, probably, or definitely related, prior to discussion with the research team. When sufficient information is available, the PI, the ISM, and the NIH Medical Monitor, will determine if and when the study can proceed with additional subjects and under what conditions.

Death or serious injury caused by the Nanopass device and device malfunctions will be reported to the manufacturer as per memo of understanding between the manufacturer and the FDA.

Any subjects who experiences an SAE considered related after the first dose of Shingrix vaccine will not be given a second dose.

F. Potential Scientific Problems

1. Recruitment will take considerable effort. Potential challenges include the invasive procedures (punch biopsy) involved, and finding persons in the community who've never received HZ vaccination.
2. DNAemia may not be absolutely equivalent to viremia.
3. Early events in skin may not mimic those in ganglionic environment.
4. vOka challenge may not be similar to natural infection with VZV. Immune response to each may be different.

G. Data Analysis Plan

An independent bio-statistician will review data prior to submission for publication.

For analysis of kinetics of DNAemia**:**

For sample size estimation, we assumed an incidence of viremia of 86% in the ZVL group and a ≥2-fold reduction in the RZV group. The 2-fold reduction is a conservative estimate, considering that the efficacy is 2.9-fold higher 5y after RZV vs. ZVL. With 19 participants per group, we have 80% power to detect a 2-fold difference in viremia with □=0.05. However, a larger sample size is needed to achieve the 2ary objectives. 35 participants per group will allow us to have sufficient power in correlation analyses of our primary outcome measure with 2 measures of the immune responses, which may represent clusters of several analytes that group together using PCA or other dimensionality reduction method. For the primary objective, logistic regression models will be fit using VZV DNAemia defined in Section 13.2. as a dependent variable and vaccine group as the primary independent variable and adjusted for potential confounders such as age at vaccination, age at ID vOKA challenge, sex, and/or race as appropriate. For all regression models fit to the data, 95% confidence intervals will be reported based on Wald statistics in conjunction with appropriate asymptotic normal distributional theory. Secondary analyses will include the duration of DNAemia defined as the last day after ID vOKA administration when the VZV DNA PCR

Relative change in percentages between groups	Total sample size (50% of total in ZVL group and 50% in SRX group)
50%	70
65%	60
80%	45
90%	25

was positive, the magnitude of VZV DNAemia, defined as the peak viral load, and the occurrence of VZV RNAemia defined as ≥1 VZV RT-RNA PCR positive result during the first week after the ID vOKA challenge.

For analysis of soluble factors and phenotypic markers:
We will generate descriptive tables of response and compare soluble factors and phenotypic markers between the 2 groups using multivariate analysis methods. We will use Hotelling's T2 test for the flow cytometric and soluble factors. We will adjust the outcome measures for potential confounders and redo the T2 test. If necessary, outcome measures will be log-transformed in order to better satisfy the assumptions of the T2 method. Cross sectional pairwise comparisons of gene expression will be made at 0, 1, 3 and 7d between groups using the DESeq2 package. Genes differentially expressed at day 0 will be filtered out at subsequent time points to avoid confounders unrelated to the intervention. Differentially expressed genes of interest at 1, 3 or 7d will be validated by RT-qPCR and used as input for downstream gene ontology pathway analysis. We will use the lists from each time point to investigate trends in gene expression over time (e.g. Is gene A upregulated at all time points?). Due to the large number of tests, False discovery rate (FDR) will be controlled at the level of 0.05.

For analysis of soluble markers and relationship to transcriptomic results:
We will generate descriptive tables of vOka replication and immune responses from all parameters by vaccine groups. We will use generalized linear models (GLM) to identify correlates between local vOka replication and viremia and between local and systemic APC, NK and Tconv, soluble markers and gene expression. Correlations will be examined between biopsies and systemic data collected at the same, preceding, and following time points. We will adjust for age and gender as appropriate and use FDR p-values.

For analysis of systemic and local immune responses that limit viremia**:**
With the different biological measurements, the major analytical goal will be to determine biomarkers that serve as intermediates in the pathway between virus challenge with DNAemia. With all the matched measurements (transcriptomic, soluble marker, VZV-CMI T cell responses) on the same subjects, we will use mediation analysis methods (15) in order to evaluate the mediation potential of these markers. As a primary analysis, we will create a composite mediation score based on the following linear combination: $V_i = \sum_{m=1}^{M} \beta_m X_{mi}$, where β_m denotes an association between molecule m with the exposure, and X_{mi} is the measurement on molecule m for subject i, i=1,…,n. Upon standardizing V, we then can test for mediation using the approach in Vittinghoff et al. (16). Based on the available n = 70, we performed power analyses based on the Vittinghoff et al. approach to evaluate power for mediation of the composite marker. We assumed that the probability of outcome is 56% based on our preliminary data and that we are testing at a significance level of 0.05. Using the powerMediation package in R (https://cran.r-project.org/web/packages/powerMediation/index.html), with the given sample size, we will have at least 80% power of identifying mediating effects of V that

are at least 1.96 on the log odds ratio scale based on the proposed extended Sobel test given in Vittinghoff et al. (16).

Sample size: Sample size is 70. Calculation for predictability of this sample size is described above.

H. Summarize Knowledge to be Gained

This project is designed to determine why RZV is a superior vaccine compared to ZVL, and to understand why it is uniquely effective in protecting elderly people. This will be accomplished by understanding the timing, magnitude, and nature of the systemic and local immune events at the site of VZV reactivation and replication. *Since the milieu of sensory ganglia cannot be directly evaluated, this proposal will create a model for this purpose, which will be intradermal administration of live attenuated VZV vaccine (vOka) strain* to simulate reactivated VZV replication in the skin. Both systemic and local responses to this challenge will be determined. Understanding how a vaccine for older people successfully limits a common infection (namely HZ) in ageing individuals may be applicable to the general problem of improving the immunogenicity of other vaccines for older people.

I. Future Studies

Serum plasma and white blood cells will be archived for future studies of immune responses to VZV.

J. References

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