

Title: Effects of Chronic Viral Infection on Immune Response to Zoster Vaccination

NCT Number: NCT02590068

Last Approval Date: 05/15/2019

12.0 Objectives and Rationale

12.1 * Overview

Briefly state the ***purpose of this study***. Give enough background and rationale to provide both scientists and lay members of the IRB and ACCTS with the basis for exposing human participants to the risks involved.

Zoster vaccine (Zostavax, Merck) is recommended for the prevention of herpes zoster (HZ) reactivation (shingles; painful blistering rash along the cranial, thoracic or lumbosacral dermatomes). It is recommended for individuals > 50 years without an underlying immune deficiency (HIV, malignancies, immunosuppression and transplantation). In non-immunocompromised individuals, zoster vaccine decreases HZ reactivation by 51-61%. In the U.S., 99.5% of adults >40 years have been infected with Varicella zoster virus (VZV) and are at risk of herpes zoster virus reactivation and its complications (acute or chronic PHN, osteonecrosis, herpes zoster ophthalmicus with visual impairment, increased risk of blindness and a four-fold risk of cerebral vasculitis-associated stroke). Chronic infections with persistent mycobacterial, helminthes and viruses are associated with increased susceptibility to other pathogens and decreased vaccination efficacy. Although chronic Hepatitis C virus (HCV) infection is not considered a clinically immunosuppressed state, it has been associated with persistent immune activation and decreased immune response to vaccination. Zostavax is routinely administered to chronic HCV patients. However, at present, no other study has documented the immune responses elicited by Zostavax in this population. This study aims to identify the innate and adaptive immune signatures elicited by zoster vaccination in individuals chronically infected with HCV as compared to healthy volunteers. Methods to be Results from this study could identify targets of potential vaccine optimization for this population and increase available knowledge on host immune response to chronic viral infections.

12.2 * Engaging Stakeholders: Describe any plans to engage other stakeholders (Scientists, practitioners, patients, advocacy groups, etc.) for hypothesis generation, or feasibility purposes.

The PI along with other members of the Rice laboratory were instrumental in the design of this study. Biostatisticians at the Rockefeller University provided additional statistical evaluations for the Clinical and Translational Science Center.

The team will identify multiple members of the patient community, as well as outreach and harm-reduction workers with experience referring HCV infected volunteers to clinical research, in order to engage patients in the design of the study, including aspects of recruitment, conduct, retention, and the analysis and sharing of results. Patients and other will be invited to face-to-face meetings with the research team and support staff (or teleconference if preferred), and will be compensated for their time /effort. Patient stakeholders engaged in this way are not expected to enroll into the study; any exploration of their enrollment would be made separately, free of undue influence

12.3 * Hypothesis

Describe the ***research hypothesis*** in a single sentence.

We hypothesize that individuals with chronic HCV infection will demonstrate impaired innate and adaptive immune responses following zoster vaccination.

12.4 * Aim(s)

Indicate how you will **address the hypothesis** (e.g., to compare groups, to estimate a parameter, to ascertain feasibility). Since the sample size determination is usually based on the primary aim only, the primary aim should be sufficient to justify the study.

AIM 1: Characterize the adaptive immune response to zoster vaccination in chronic HCV infection

Zoster vaccine will be administered to healthy and HCV-infected individuals. Volunteers serum, PBMCs, and plasma will be collected at screening, days 0, 3, 7, 14, and 42 and batched for analysis to limit variations. In order to fully evaluate the immune signature generated by the zoster vaccine, we will determine the frequency and functionality of specific PBMC populations using flow cytometry and ELISpot assays. We aim to compare the data generated from this study to other published human system immunology and/or vaccination studies. To achieve this aim, our research group has adapted a flow cytometry panel designed to limit variations in standardizing human immunophenotyping. This flow cytometry panel is also currently being applied to the HBV vaccine study (CRI-0844). We will analyze intracellular cytokine staining and expression of cell surface markers on immune cells. IFN-gamma ELISpot assay has been used in older HZ vaccine studies to evaluate T-cell immune response post-vaccination. Commercially available VZV-derived peptides pools will be used to stimulate PBMCs in the ELISpot assay. VZV-specific antibody production post-immunization would also be assessed using commercially available VZV-ELISA kits.

AIM 2. Characterize the innate immune response to zoster vaccination by RNA-Seq analysis

RNA-seq will be used to evaluate innate immune activation by evaluating the changes to the expression levels of interferon-stimulated genes pre- and post-vaccination. In this pilot project, RNA will be isolated with using commercially available PAXgene tubes to immediately stabilize intracellular RNA profile and traditional homogenization of isolated volunteer PBMCs in Trizol prior to cryopreservation. RNA-seq sample preparation and sequencing will be performed at the Rockefeller University Genomics Resource center. The Rosenberg research laboratory, with their extensive experience with RNA-seq will perform analysis of this data. Genome-wide mRNA expression profiles will be compared between the 2 arms of the study, healthy and chronically HCV infected volunteers. Gene expression levels will be quantified in relation to a reference standard of External RNA Control Consortium (ERCC) controls – a calibrated panel of defined RNA molecules at defined concentrations that will be “spiked in” to each library preparation to serve as a universal reference across samples.

12.5 * Primary Outcome(s)

Indicate which **variable(s)** will be assessed to judge the primary specific aim. Give measurement units, if applicable.

We will measure the following parameter:

- **Serum zoster antibody levels by gpELISA expressed in (units/ml)**

12.6 * Secondary Outcome(s)

Indicate which **additional variable(s)** will be assessed to judge the secondary outcome(s). Give measurement units, if applicable.

- **Interferon-stimulated gene (ISG) expression in PBMCs expressed as fold-change (FCH) above baseline**
- **Serum biomarkers of innate immune activation (expressed in %)**
- **Responder cell frequency: VZV specific CD4+ T cells post-vaccination (expressed in %)**
- **Interferon-gamma response by ELISpot expressed as number of spot-forming cells per 10^6 PBMCs**

12.7 * Methods and Procedures

Please provide a description of the laboratory and clinical analyses and procedures that will be performed. Include the role of external collaborators and consultants when appropriate. Please refer to Help text for Guidance.

This study is an open-label protocol divided into the healthy versus chronic HCV volunteer arms. The study will be advertised in local newspapers and different online resources. Potential participants identified with the aid of the Bellevue Hospital Center Hepatitis clinic and other local physicians, will be asked to call 1-800-RUCARES (1-800-782-2737), if they are interested in the study.

Our aim is to screen and enroll 20 age-matched (\pm 5 years) and gender-matched individuals between 50 and 60 years of age into each arm of the study. Individuals recruited into the study must be HIV and hepatitis B negative, without a history of vaccination within the past month, no history of immunosuppressive therapy, active malignancies or herpes zoster reactivation. Baseline screening tests (HIV ELISA, hepatitis B anti-surface and core antibodies, hepatitis C antibody, viral load and genotyping, complete blood counts and a comprehensive metabolic panel) will be obtained prior to enrolling in the study. HCV viral load and genotyping: will be determined by clinical laboratory analysis. Patient blood samples will be sent to a CLIA certified reference laboratory for analysis.

The initial protocol was written in the summer of 2015, prior to the availability of the influenza vaccine for the current 2015 – 2016 season. It would be unethical to have interested participants delay receipt of their influenza vaccination prior to enrolling in this study. Also, a 3-month delay after participants' influenza vaccination would delay the progress of the clinical study, as we have already secured some funding for the study.

In addition, receipt of vaccination 4 weeks prior to entry into a zoster vaccination study has a precedent, namely the landmark study by Oxman et al. The FDA's approval of a license for use of Zostavax in 2006 cited this study¹.

Reference:

1. Oxman, M. N. *et al.* A vaccine to prevent herpes zoster and postherpetic neuralgia in older adults. *N Engl J Med.* 2005 Jun 2;352(22):2271-84.

In this study there will be a total of seven study visits (Screen I and II, Days 0, 3, 7, 14, 42). All enrolled volunteers will be given a single 0.65 mL dose of the live attenuated zoster vaccine (Zostavax, Merck & Co, Inc) on day 0 with follow-up visits on days 3, 7, 14, and 42. The most frequent adverse reactions, reported in subjects vaccinated with Zoster vaccine, were headache and injection-site reactions. Additional adverse effects have not been reported in individuals chronically infected with Hepatitis C virus. All women of child-bearing potential are required to use a highly effective form of contraception such as condoms, birth control pills, birth control injections, or intrauterine devices for three months following the vaccination. Any adverse reactions observed in women of child-bearing potential should be reported to: To report SUSPECTED ADVERSE REACTIONS or exposure during pregnancy or within three months prior to conception, contact Merck Sharp & Dohme Corp., a subsidiary of Merck & Co., Inc., at 1-877-888-4231 or VAERS at 1-800-822-7967 or www.vaers.hhs.gov.

At the Day 0 and Week 6 visit we will collect approximately 121 mL of blood during and at the other visits (Days 3, 7, and 14) we will collect 49 or 53 ml of blood respectively for research and clinical laboratory monitoring. Non-fasting blood samples will be drawn during the initial screening visit. Subsequent laboratory blood samples will be fasting for screening visit II and days 0, 3, 7, 14 and 42. Research blood will be separated into PBMCs, plasma and serum by the study PI. All samples will be stored in cryopreserved aliquots until batched assays prior to described assays.

HCV persistence has been associated with HCV-specific-CD8+T-cell exhaustion and bystander CD8+T-cell dysfunction. HCV-specific CD8+T-cell exhaustion is characterized by diminished *ex vivo* polyfunctionality, upregulation of negative co-stimulatory cell modulators, decreased cellular proliferation, interferon production and cytotoxicity. Effective immune responses to vaccination include the activation and expansion of antigen-specific memory B-cells and T-cells following antigen stimulation. Chronic HCV has been associated with impaired immune response to vaccination against Hepatitis A virus, influenza and most prominently Hepatitis B virus with 40-60% in chronic HCV versus 90-95% in healthy volunteers. Studies on HBV-vaccination of chronic HCV patients have identified impaired specific and non-specific CD4+T-cell immune in non-responders to HCV infection.

Flow cytometry, ELISpot and ELISA analyses will be performed on PBMCs to determine B-cell and T-cell proliferation and polyfunctionality. we will determine the frequency and function of specific PBMC populations using flow cytometry and ELISpot assays. For flow cytometry, we will utilize an antibody panel designed to limit variations in standardizing human immunophenotyping to assess intracellular cytokine staining and expression of cell surface markers on immune cells. IFN-gamma ELISpot assay has been used in older HZ vaccine studies to evaluate T-cell immune response post-vaccination. We will utilize commercially available VZV-derived peptide pools will be used to stimulate PBMCs in the ELISpot assay. VZV-specific antibody production post-immunization would also be assess using commercially available VZV-ELISA kits. A multiplex Luminex assay targeting TNF-alpha, IFN-gamma, IL-2, IL-4, IL-17 and other cytokines will be performed on plasma samples. Flow cytometry and multiplex cytokine assays will be performed with the assistance of the Translational Technology Core Laboratory Resource center.

Chronic HCV infection is associated with persistent innate immune activation and dampened cellular immune responses. Interferons (IFNs) are key mediators of the antiviral innate immune response, initiating expression of interferon-stimulated genes (ISGs) with numerous host protective effector functions. In chronic HCV, high pre-therapy expression of ISGs and persistent activation of the innate immune system negatively predict the response to IFN-based therapies and failure of viral clearance. Innate immune response will be determined by RNA-seq pre- and post-vaccination. RNA will be isolated using commercially available PAXgene tubes to immediately stabilize intracellular RNA profile, in addition to the traditional homogenization of isolated volunteer PBMCs in Trizol prior to cryopreservation. Blood will be collected into PAXgene tubes and isolated PBMCs will also be collected into TRIZOL for RNA isolation and RNA-seq analysis. RNA isolated from PBMC will be prepared by the Genomic Resource center and sequenced for genome-wide mRNA expression profiling. Genome-wide mRNA expression profiles will be compared between the two arms of the study (healthy and chronically HCV infected volunteers). Gene expression levels will be quantified in relation to a reference standard of External RNA Control Consortium (ERCC) controls – a calibrated panel of defined RNA molecules at defined concentrations that will be “spiked in” to each library preparation to serve as a universal reference across samples.

PBMC samples may also be analyzed by single cell resolution InDrop RNA-Seq (REFERENCE PMID: 26000487), established for immune response profiling in the Rosenberg laboratory. Within a microfluidic device, individual cells and individual hydrogel “barcode beads” are co-encapsulated in water-in-oil droplets, in which reverse transcription tags nascent cDNA with cell-specific barcode sequences. After droplet breakage, library preparation and high throughput sequencing, reads can be assigned to individual cells by barcode for gene expression profiling. Using this approach, sufficient sampling can be achieved with relatively low input cell numbers and rare cells (i.e. dendritic cells, antigen-specific lymphocytes) are represented in resulting datasets.

In order to define the immune functions engaged by Zoster vaccination at single cell resolution, longitudinal PBMC samples will be analyzed by InDrop RNA-Seq. Data analysis will focus on first classifying cells by type (e.g. T cells, B cells, NK cells, classical monocytes, dendritic cells, etc.) using multidimensional clustering methods as implemented in the Seurat tool (REFERENCE PMID: 25867923). Next, differential gene expression patterns active in different cell subsets will be assessed over time, and the heterogeneity of these patterns will be evaluated at single cell resolution. We expect these results to complement bulk RNA-Seq results by providing greater detail on specific cell subsets and their respective transcriptional networks and response dynamics.

Klein AM, Mazutis L, Akartuna I, Tallapragada N, Veres A, Li V, et al. Droplet Barcoding for Single-Cell Transcriptomics Applied to Embryonic Stem Cells. *Cell*. Elsevier; 2015;161: 1187–1201. doi:10.1016/j.cell.2015.04.044

Satija R, Farrell JA, Gennert D, Schier AF, Regev A. Spatial reconstruction of single-cell gene expression data. *Nat Biotechnol*. 2015. doi:10.1038/nbt.3192.

With the May 2019 IRB continuing review submission, Dr. Jegede has been replaced by Dr. Rice as the Principal Investigator of this study. Dr. Jegede has been listed as External Personnel and will not conduct any further data analysis or be involved with any protocol-related activities.

With the May 13, 2019 amendment, Dr. Rosenberg, a Guest Investigator in the Rice laboratory, will conduct the data analysis and review of the stored research samples collected in this study.

12.8 * Data Analysis

Describe method(s) of data analysis. Include the role of external collaborators as appropriate.

The primary analysis for the set of primary outcomes will search for statistical evidence of impairment in the innate and adaptive immune response in chronic-HCV subjects compared to age-matched Healthy Controls by testing the hypotheses that the variation from baseline to day 42 will differ between these two groups. To achieve this goal, we will apply two-sided paired t-tests and/or Wilcoxon tests for each one of the outcomes. Additionally, as a secondary analysis, we will fit a mixed-effects model that will use group (HCV – HC) and time (0,3,7,14, and 42 days) as fixed effects and a random intercept for each subject. This modeling strategy allows to infer about differences across time points, and also within time points, while controlling for multiple testing. Further evaluation of the immune signature generated by the zoster vaccine will be done with flow cytometry and ELISpot assays and qualitative comparisons with other

human system immunology and /or vaccination studies will be carried out. For the secondary outcomes, the analysis for the ISG expressions as measured by RNA-seq counts will be processed in *limma* package in R and the same formulation for mixed-effects model described previously will be applied to verify differences at gene-wise level. Serum biomarkers of immune activation will be compared across groups with paired t-tests and Wilcoxon test for each time point.

12.9 * Explain the rationale for the choice of statistical measures and the number of participants proposed for the study, including the power calculations when applicable.

A sample of n=40 (20 HCV / 20 HC) individuals provides 80% power at 1.6% (after Bonferroni correction for 3 outcomes) significance to detect minimum differences in biomarkers variation from baseline to day 42 as 1.08 standard deviations when applying two-sided paired t-tests. Since there is no preliminary data showing the degree of variability in the primary outcomes for the chronic HCV population, this pilot study will be valuable for future power analysis. To test for gene-wise differences between healthy and HCV-infected groups for a single time point, a sample size of n=20 individuals in each groups provides 80% power at 5% significance to detect effect sizes greater than 1.5 FCH (Fold-Changes), assuming a coefficient of variation as 40% and a depth of coverage greater than 20. Calculation was performed using the library RNASeqPower in R software version 3.2.1.

12.10 * Will samples be coded?

Yes No

If Yes, Please describe coding scheme consistent with GCP. If samples will not be coded, please provide justification for this proposed departure from GCP practice.

All samples will be coded with unique identifiers based on study number assigned to each individual upon study enrollment. Study volunteers' HIPAA protected information will not be associated with any research data. All computers used to store and analyze research data will be password protected. All data will be backed-up into the system maintained by Information Technology Service at the Rockefeller University.

If available, upload the Data and Sample Sharing Management Plan approved by RU IT.

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