PROTOCOL TITLE:

Immune response to pneumococcal vaccination in aging Renal Transplant Recipients

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INFB-019-17F

Date: October 23, 2019

1.0 Objectives / Specific Aims

Normal aging is characterized by changes in the immune system that result in increased susceptibility to infections, poor response to new vaccine antigens, loss of protection offered by previous vaccinations and decreased immune surveillance [1, 2]. Despite the chronic inflammation associated with immunosenescence, the aging population is living longer. Consequently, individuals >65 years of age, are the most rapidly growing population amongst those with end stage renal disease (ESRD) and account for more than 18% of renal transplant (RT) recipients [3].

The incidence of pneumococcal disease is significantly higher in both elderly [4] and those with RT [5] and the combination of these factors is likely additive, if not synergistic, for invasive pneumococcal disease (IPD). It is recommended that both elderly>65 and RT recipients be vaccinated with a regimen that includes both the 13-valent pneumococcal conjugate vaccine (PCV13) and the 23-valent pneumococcal polysaccharide vaccine (PPV23). However, small immunogenicity studies performed in the transplant populations have not shown superiority of a PCV containing regimen [6, 7]. Moreover, the addition of PCV to the pneumococcal vaccine regimen does not improve protective immunity in this population. Studies to date fail to elucidate the possible foundation of the disappointing immune responses to the PCV regimens, data essential for the development of a rational and effective next generation pneumococcal vaccine.

Specific Aim 1.

We will **define immune responses by measuring** serum antibody and functional antibody responses to PPS 14, 19A and 23F following PCV13 vaccination in RT recipients 65-75 years of age and compare these to: RT recipients 35-45 years of age and persons with DM/HTN but normal function 65-75 years of age to dissect out the age and RT components respectively. Healthy persons 35-45 and 65-75 years of age will be studied as age appropriate reference.

Specific Aim 2.

We will measure and characterize the antigen-specific B cell subset response following immunization with PCV13 in the RT recipients 65-75 years of age and compare them to each of the groups described in Specific Aim 1 using flow cytometry and fluorescently labeled PPS and monoclonal antibodies. These measures will be correlated with post-immunization functional antibody activity, a surrogate of protection.

Specific Aim 3.

We will measure TNFR expression by B cells following immunization with PCV13 in 65-75 year old RT and compare them to each of the groups described in Specific Aim 1. Gene expression, with focus on the B cell activating factor (BAFF) system, will be measured in PPS-specific and non-PPS specific B cells using single cell genomics and flow cytometry. These measures will be correlated with post-immunization functional antibody activity, a surrogate of protection.

Our central hypothesis is that the aging RT population responds poorly to PCV13 vaccination reflecting the combined effects of aging and RT. We postulate that both the number of memory B cells and expression of tumor necrosis factor (TNF) superfamily receptors, which play crucial roles in the response to pneumococcal polysaccharides (PPS), are deficient in the elderly RT population and contribute to poor pneumococcal vaccine responses.

We have developed fluorescently labeled PPS allowing us to study the nature and surface receptors of PPS-specific B cells. Our preliminary data demonstrate that RT recipients 1. Respond poorly to pneumococcal immunization as measured by antibody titer and functional antibody activity. 2. RT recipients and healthy elderly have lower absolute number of both IgM and switched memory B cells. 3. The number of PPS-specific IgM and switched memory B cells are significantly lower in RT recipients and 4. TACI and BAFF-R, members of the TNF superfamily receptors, expression is significantly lower in the PPS-specific memory B cells in the RT population versus healthy controls. The overall objective of this proposal is to characterize the immune response and explore possible mechanisms of poor vaccine responsiveness following immunization with PCV in the rapidly growing group of elderly with RT. As the RT population is a

heterogeneous group we will study only those in whom the underlying cause of renal failure is diabetes mellitus type 2 (DM2) and/or hypertension (HTN).

2.0 Background

Streptococcus pneumoniae is the most common bacteria isolated from the elderly with community acquired pneumonia [8]. The elderly are at high risk of pneumococcal infection, have an increased incidence of blood stream infection and a higher mortality rate compared to younger adults [4]. It is projected that between 2000 and 2040, the number of Americans ≥65 years of age will more than double. The rapid growth of the aging population has resulted in a significant increase in elderly individuals with end-stage renal disease (ESRD). In 2014, there were >300,000 older adults with ESRD, a seven-fold increase compared to the 1990's [3]. In addition, individuals with ESRD are living longer, disproportionally increasing the number of elderly with ESRD [9]. Moreover, older (>65 yrs) individuals now account for 18.4% of the 17,000 renal transplants performed in the US annually [3, 10] and are by far the fastest growing population requiring renal replacement therapy, i.e. dialysis or renal transplantation [11]. Although the renal transplant (RT) population is susceptible to a host of infections, S. pneumoniae is the most common bacterial respiratory pathogen and the incidence of invasive pneumococcal disease (IPD) is estimated to be 12.8 fold higher than in the general population [5]. Moreover, aging individuals with RT have two distinct risk factors for pneumococcal disease, namely age and RT accounting for the high incidence of disease in this population.

Accordingly, pneumococcal vaccination is recommended for all adults≥65 years of age and all RT recipients [12, 13]. In 2012 the vaccination recommendations by the Advisory Committee on Immunization Practices (ACIP) for these immune compromised adults, were changed to include vaccination with the 13-valent pneumococcal conjugate vaccine (PCV13) [12]. Although highly successful in infants, immunogenicity studies have overall failed to demonstrate superiority of PCV based regimens in older adults [14]. Similarly, a prime-boost regimen based on sequential immunization with PCV7 and the 23-valent pneumococcal polysaccharide vaccine, Pneumovax® (PPV23), has not demonstrated improved responses in RT and liver transplant populations [6, 7]. The RT population ≥65 years of age is rapidly growing and represents a unique subpopulation with combined immunodeficiencies related to aging [15] and immunosuppressive drugs related to transplant. Both aging and RT result in a marked increased risk of pneumococcal disease with decreased immune response to pneumococcal vaccination. The immune response to vaccination with PCV is poorly characterized in the aging and transplant populations, and may not be more effective than PPV23 alone, implying the need for alternate vaccine/adjuvant strategies. Moreover, studies characterizing and comparing antigen-specific B cell responses in well-defined RT populations are lacking.

We have developed directly labeled fluorescent PPS to enumerate and characterize PPS-specific B cells. This allowed the analysis of antigen-specific B cell responses to pneumococcal vaccination. The results of these studies demonstrated that the immune response to PPV in young adults consists predominantly of IgM memory B cells [16]. In contrast, in the elderly who are deficient in memory B cells, particularly IgM memory B cells [17], the predominant B cell phenotype in response to PPS consists of switched memory B cells (IgG/A+IgM-CD27+)[18]. HIV+ individuals, are also deficient in CD27+IgM+ B cells yet the predominant response consists of PPS-specific CD27+IgM+ B cells [19-22], albeit at significantly reduced numbers. Thus, the cause of poor pneumococcal vaccine responses varies according to study population.

Several investigators and our preliminary data show that like the elderly and HIV-positive, absolute number and percentage of memory B cells are significantly lower in stable RT recipients, at 6 months to 5 years post-RT, compared to healthy subjects [23, 24]. Moreover, low number and percentage of IgM memory B cells correlate with poor antibody responses to PPV23.

This may however not be the only cause for poor vaccine responses. The ability to isolate antigen-specific B cells allows us to explore other deficits and/or changes in surface markers and gene expression potentially implicated in poor vaccine responses. It has been well described that members of the TNFR family and

certain Toll like receptors (TLR) play a crucial role in the immune response to pneumococcal vaccination [25, 26]. As shown in preliminary data, we have found decreased TACI and BAFFR expression in PPS-specific B cells derived from RT recipients. Although the number of RT recipients studied is very small, it was a consistent finding and merits further study. Adjuvants that specifically upregulate TACI expression, such as CpG-ODN, could therefore effectively increase immune responsiveness to pneumococcal vaccines. This strategy has shown to increase immune and memory responses to the conjugate pneumococcal vaccine in HIV-positive persons [27]. Our central hypothesis is that the elderly RT population responds poorly to PCV13 reflecting combined effects of aging and RT that result in abnormalities in both number of memory B cells and TNF superfamily expression, which play crucial roles in the response to polysaccharide antigens. We will test this hypothesis by measuring PPS-specific antibody and opsonophagocytic antibody responses, PPS-specific B cell phenotypes and surface and gene expression of the TNFR with known role in response to pneumococcal vaccination and correlate these individually with antibody and functional antibody responses. This has important clinical implications as deficient TNFR expression can be modulated by the addition of adjuvants such as CpG-ODN.

Preliminary Data

Over the past 16 years, the PI's laboratory has studied the immune response to pneumococcal vaccination. All of these studies have been performed in humans. Initially these studies were focused on the elderly population. We defined immune response, functional antibody activity and antibody (V) gene usage in this population. We have developed unique directly labeled fluorescent pneumococcal polysaccharides (PPS). This has allowed us to focus on the antigen-specific B cell response. The PPS-specific immune response, including antibody response, functional activity and B cell phenotype analysis have been performed by us following pneumococcal polysaccharide vaccination (PPV23) and/or pneumococcal conjugate vaccine (PCV13), in young and elderly (>65 yrs) healthy persons as well as young (<50) and aging (50-65 yrs) HIV+ persons. These studies were published in Journal of Immunology, Journal of Infectious Diseases, Vaccine, PlosOne and the Journal of AIDS and Clinical Research . These studies have formed the basis for comparative analysis of the immune response following PCV13/PPV23 in elderly and HIV+ individuals. The results of these studies demonstrated unique B cell deficiencies in HIV and elderly. We are in a unique position to perform an in-depth analysis of the immune response to PCV13 in the aging renal transplant population, defining surrogates of efficacy and safety. Despite the recent change in vaccine recommendation, based on expert opinion, studies demonstrating superiority and safety of PCV has not been performed in an adequate manner in the past. In addition, we will explore the mechanism of poor responses, laying a foundation for development of more effective vaccines for this population of elderly.

3.0 Intervention to be studied (if applicable)

The intervention to be studied is the immune response to immunization with PCV13 or Prevnar®13. This FDA approved vaccine is given as part of the standard of care in Groups 1-4. The experimental part of the protocol in these groups will be blood draws at days 0, 7, 30 and years 1 and 2 in these groups.

In Group 5 two interventions will occur: 1. Immunization with the FDA approved PPV23 or Pneumovax® 23 and 2. PCV13 a FDA approved vaccine. In addition, blood samples will be obtained at days 0, 7, 30 year 1 and year2.

Both PPV23 and PCV13 are FDA approved vaccines for use in humans. PCV13 is recommended for all individuals enrolled in Groups 1-4. It is not recommended as routine part of care in Group 5 enrolled individuals however it has never shown to be harmful in this group of individuals.

4.0 Study Endpoints (if applicable)

The primary endpoint of this study is: quantify the antibody titers in mg/mL and opsonophagocytic antibody titer calculated as serum dilution, number of polysaccharide specific B cells, and absolute number of cells/mL induced by vaccination with PCV13.

Secondary endpoint of the study is to describe differences in gene expression of 56 genes to be determined by single cell PCR and comparing these between groups.

The primary safety endpoint of the study is measured as minimal versus moderate local side effect. Minimal side effect measured as no impairment in activity. Moderate local side effect is a side effect affecting use of the extremity for less than 24 hours.

5.0 Inclusion and Exclusion Criteria/ Study Population

Age ranges were chosen based on previous published and unpublished studies performed in our laboratory indicating that a significant decrease in pneumococcal vaccine response starts to appear at age 65 and that prior to age 50 the response is similar to individuals 20-30 age of years, i.e. optimal range. We chose the 35-45 age group specifically, and not 20-30, as renal transplants recipients with cause of renal failure is type II diabetes or hypertension are well represented in the MUSC transplant population.

Individuals will be screened for eligibility depending on the group they are recruited for:
Group 1 Older RT group (Age 65-75)
Group 2 Younger RT group (Age 35-45)
The elderly (65-75 years) and young (35-45 years) renal transplant recipient groups 1 and 2 seen at the MUSC and VA transplant clinics whose end-stage renal disease was caused by hypertension (HTN) and/or DM II who are at least 1 year post-transplant and due for their PCV 13 vaccination will be asked to participate in the study. Those that do not wish to participate in the study will receive the clinical standard of care.

Group 3 Individuals with DM II and/or HTN age 65-75.
Individuals with the diagnosis DM II and/or HTN due for routine visits at the general internal medicine or endocrinology clinic at the Ralph H Johnson VA Medical Center will be screened for normal (Glomerular filtration rate≥60) versus abnormal (GFR<60) who are due for their standard of care PCV13 vaccine will be asked to participate.

Group 4: 65-75 years old without DM2 or renal impairment who are due for their standard of care vaccination with PCV13.

Group 5: Healthy persons 35-45 years of age without DM or renal impairment willing to participate in a study to receive PPV23, if they have not received this previously or/and PCV13, at least 1 year after PPV23 vaccination.

Inclusion/Exclusion criteria

Inclusion criteria are group specific. HBV, HCV and HIV testing are not necessary in the RT groups as all RT recipients are tested prior to transplant.
Exclusion criteria are either applicable to all groups or group specific. Therefore we have listed the exclusion criteria applicable to ALL groups first. Group specific criteria are listed under each group

Exclusion Criteria common to all groups
1. Previous immunization with PCV13.

2. Pregnancy, no contraceptive practice in women of childbearing age, or breastfeeding
3. Known anaphylaxis, hypersensitivity or "bad allergic reaction" to the pneumonia vaccine. This does not include egg allergy or previous Guillan Barre syndrome.
4. Those who received blood products or gamma globulin within 3 months.
5. Inability to comprehend or sign the informed consent form
7. Previous/present illness that may affect immune response to the vaccine (previous pneumococcal Disease, removal of the spleen, auto-immune disease such as lupus or rheumatoid arthritis, end-stage liver disease, or cancer)
8. Significant abnormalities (3xULN and all those considered to be critical values) in CBC, chemistries including glucose.
9. HIV, HBsAg or HCV positivity
10. Receipt of PPV23 within 1 year

Groups 1 (65-75 yrs) and 2 (35-45 yrs) Renal Transplant populations

Inclusion Criteria:
Age range; RT 35-45 or 65-75 years old,
End stage renal disease cause either DM2 and/or hypertension (HTN)
Renal transplant >12 months ago
We will not restrict volunteers with respect to gender, ethnic or racial group.

Exclusion criteria:
1. Medications that are known to affect immune function (chemotherapy, anti-TNFα agents) with the exception of anti-rejection medication.
2. Episode of acute rejection within the last 6 month period
The inclusion/exclusion criteria will be determined by chart review and pregnancy test for females of child bearing potential.

Group 3: Diabetic/hypertensive 65-75 year old controls
Inclusion Criteria
65-75 years old with DM2 and/or HTN.
Previous immunization with PPV23 >1 year prior
Willingness to be tested for HIV, HBV and HCV
"normal kidney function" defined as glomerular filtration rate (GFR) of 60% or above
We will not restrict volunteers with respect to gender, ethnic, or racial group.

Exclusion Criteria
1. Medications that are known to affect immune function (chemotherapy, anti-TNFα agents).

The inclusion/exclusion criteria will be determined by chart review.

Group 4: Healthy Control 65-75 yr old

Inclusion Criteria
65-75 years old without DM2. May have high blood pressure (systolic>140 and/or diastolic>90) as long as it is well controlled (systolic<140 and/or diastolic <90) and has not affected kidney function.
Previous receipt of PPV23 > 1 year prior
Willingness to be tested for HIV, HBV and HCV
We will not restrict volunteers with respect to gender, ethnic, or racial group.

Exclusion Criteria
6. Medications that are known to affect immune function (chemotherapy, anti-TNFα agents).
The inclusion/exclusion criteria will be determined by chart review and pregnancy test for females of child bearing potential.

We will not restrict volunteers with respect to gender, ethnic, or racial group.

Group 5: Healthy Control 35-45 yr old
Inclusion Criteria
35-45 years old without DM2. May have high blood pressure (systolic>140 and/or diastolic>90) as long as it is well controlled (systolic<140 and/or diastolic <90) and has not affected kidney function.
Willingness to be tested for HIV, HBV and HCV and filling out a medical questionnaire that will include diabetes screening.
We will not restrict volunteers with respect to gender, ethnic, or racial group.

Exclusion Criteria
6. Medications that are known to affect immune function (chemotherapy, anti-TNFα agents).
The inclusion/exclusion criteria will be determined by chart review and pregnancy test for females of child bearing potential.

Diversity: we will attempt to mimic the local renal transplant recipient population consisting of a disproportionately high number of males (>60%) of African-American decent, ±50%. This gender and race pattern of distribution is consistent with the patient population of the Ralph H. Johnson VA Medical Center outpatient clinics, where we will be recruiting most of our control populations (Groups 3-5). We therefore anticipate a diverse population as a result of eligibility at the recruitment sites.

Children will NOT be included in this study as this study is uniquely aimed at defining the immune response to PCV13 in ADULTS. The immune response to PCV13 is age-dependent and therefore completely different in children, as is the recommended vaccine frequency.

6.0 Number of Subjects

275

7.0 Setting

The MUSC Renal Transplant clinic is located on the 9th floor of the Rutledge Tower. The RT recipients at the Ralph H. Johnson VA Medical Center are followed at the Nephrology outpatient clinics on the first floor. Nephrologists, physician assistants, pharmacists and nursing personnel are present at both facilities. These individuals are well trained in the administration of vaccines.
The study and consent and HIPAA forms will be explained to the potential participant by the study staff in a private room
Blood draws will be performed by staff trained in phlebotomy or phlebotomists at the facility laboratories. The group 5, healthy volunteers, 35-45 years of age will be asked to come to the P.I.'s laboratory at the Strom Thurmond Building Room 411 and study, consent and HIPAA explained in private by the study personnel. Either PPV23 or PCV13 will be administered by Dr. Westerink. Blood samples will be obtained at the MUSC laboratory.

Study Sites

1.The MUSC Renal Transplant clinic is located on the 9th floor of the Rutledge Tower
2.The nephrology clinic at the Ralph H. Johnson VA Medical Center
3. the P.I.'s laboratory at the Strom Thurmond Building Room 411

8.0 Recruitment Methods

- Potential study participants will be recruited from Ralph H. Johnson Veterans Affair Medical Center and Medical University of South Carolina during the appointment with their treating physician. Flyers will be posted in the waiting room areas of the clinics and attending physicians and clinic nurses will be asked to keep this study in mind and mention availability of these studies to their patients when ordering PCV13 for them.
- Potential study subjects will be explained the purpose of the study and how their information was obtained (from a treating physician or a chart review); further the eligibility criteria will be explained and what involves in participating in the study: procedures, estimated time of commitment, any reasonably foreseeable risks, benefits, and compensation. In a similar manner, healthy volunteers will be recruited through flyer advertisement on MUSC campus as well as in VA.
- Potential study subjects will be identified by reviewing medical records and by physician's recommendations.
- Flyers will be used to recruit study subjects as well as broadcast emails for MUSC and VA employees and volunteers.

9.0 Consent Process

Informed Consent will be obtained in a private room from all participants at either:
1. The MUSC Renal Transplant clinic is located on the 9th floor of the Rutledge Tower
Or 2.The nephrology clinic at the Ralph H. Johnson VA Medical Center
Or 3. the P.I.'s laboratory at the Strom Thurmond Building Room 411

Consent will be sought of **competent adults** who express interest in the study. The purpose of the study, the study details regarding gathering health information and obtaining blood samples, the potential benefit and risks involved, including risk of blood draw and vaccination, will be explained in detail and in layman terms, as well as the non-standard of care explained to the potential subject (i.e. blood draws for groups 1-4, vaccination protocol and blood draws for group 5). The subject will be encouraged to ask questions and they will be asked to answer questions regarding essential parts of the study to ensure proper understanding of the study requirements. We will explicitly explain that the study is completely voluntary, and they can withdraw from the study at any time. Additionally we will explain that participation is independent of the medical care the subject receives at the Ralph H Johnson VA medical center or MUSC.
Consent will be obtained as described above in the presence of the P.I. or study coordinator. The potential volunteer will be given written information, and opportunity for questions will be provided. Volunteers will be questioned concerning the study to assure complete understanding.

The consent will only be obtained from the participant him or herself and not from a surrogate.

There will be no waiting period between consent and beginning of the study

The study and consent form will be reviewed in depth and after each separate section the participant will be asked to summarize the section in his or her own words to ensure understanding.

Vulnerable populations, i.e. employees at the P.I.'s institution will be recruited, however they will ONLY be recruited if they have
1. no personal relationship with the PI
2 are not directly employees of the PI or a division where they could be vulnerable to coercion or undue influence.

3. it will be made clear to all these participants that participation is strictly voluntary
4.recruitment will only be performed in an in personal mass email

10.0 Study Design / Methods

The overall objective of this proposal is to characterize the immune response and explore possible mechanisms of poor vaccine responsiveness following immunization with PCV in the rapidly growing group of elderly with RT. As the RT population is a heterogeneous group we will study only those in whom the underlying cause of renal failure is diabetes mellitus type 2 (DM2) and/or hypertension (HTN). There will be 5 study groups as outlined above.
Volunteers from all 5 study groups will be recruited as above. All individuals from study groups 1-4 who meet the study criteria will receive Prevnar13® on day 0. Group 5 volunteers will be recruited during the first year of the study and those who have not previously received PPV23 will receive PPV23 on day 0 and PCV13 on day 366. Blood samples will be obtained as outlined below and results obtained in various groups will be compared.

Timing of blood samples

Peripheral blood samples will be collected at day 0 (pre-immune), day 7, and day 30 (4 weeks post-PPV23) for groups 1-4 and at days 366, 373 and 396 for group 5. In addition, day 5, and 10 blood samples will be obtained from a limited (n=10) number of elderly RT participants to determine the optimal time point for circulation of PPS-specific B cells. Yearly blood samples will be obtained thereafter for serum antibody and OPA analyses to test longevity of antibody and OPA responses. Samples from days 0, 30 and yearly samples will be used for antibody titers and opsonophagocytic assays. Samples from day 0 (day of vaccination with PCV13) and day 7 will be used for flow cytometric analysis. The day 7 time point for isolation of antigen-specific B cells was specifically chosen as we have extensively studied temporal dynamics of antigen-specific B cell in peripheral blood samples (unpublished studies) and have found that the highest number of antigen-specific B cells are present in the peripheral circulation at day 6 and 7 with rapid (70+%) decline at day 8 in healthy young, elderly including those with DM2 and HTN, and HIV-positive. At day 30, a minimal number of PPS-specific B cells were present. Others have described similar findings [28, 29].
However, it is possible that day 7 may not be the optimal time point for the isolation of antigen-specific B cells in the RT populations. Therefore, we will obtain blood samples on days 5, 7, and 10 from the first 10 participants in the aging RT group to redefine optimal time point for antigen-specific B cell isolation in this population. The first 10 participants in Group 1 will be recruited from both MUSC and the VAMC. The optimal time point will be applied to **all** subsequent RT participants.
BAFF concentration will be measured at day 0.

All groups: All recruited subjects will agree to be tested for HIV by rapid HIV test, HBsAg and HCV if their status is not available in their health records. In addition they will agree to have CBC and Complete metabolic profile (CMP) and hemoglobin A 1c (HbA1c) should a recent (<6 months old) result not be available.

Groups 1-4

All individuals in groups 1-4 agree to

1. Receive PCV13 (Prevnar®13): this is STANDARD of care in all group 3 recruited and enrolled subjects
2. Experimental part of the protocol they will agree to:
   In the first 10 vaccine recipients in groups 1 : donate blood specimens: at 7 different times of up to 60 mL per visit: day 0, day of vaccination: at day 5, day 7, day 10 and at day 30 and yearly

thereafter up to 2 years. Samples drawn at days 5, 7 and 10 are used to determine the optimal time point of isolation of PPS-specific B cells.
Participants 11-40 in group 1 and all Group 2: will donate blood samples at 5 different time points of up to 60 mL per visit: day 0, day of vaccination: at optimal time point being **either** day 5 **or** day 7 **or** day 10 and at day 30 and yearly thereafter up to 2 years.
The optimal time point of isolation of PPS-specific B cells post-vaccination in both young and elderly healthy and diabetic populations had been determined by us in previous studies, however this may vary in the transplant population. We will therefore test this in the first 10 RT subjects enrolled in both groups 1 and 2. The first 10 participants in Group 1 will be recruited from both MUSC and the VAMC.

Groups 3 and 4: There will be 5 visits: blood draws of up to 60 mL per visit: day 0, day of vaccination: at day 7 and at day 30 and yearly thereafter up to 2 years.

Group 5

1. Receive PPV23 (Pneumovax®) on day 0 of enrollment if they have never received PPV23. This is to assure that all participants in the study have been immunized with PPV23 at least 1 year prior to receiving PCV13.
2. Receive PCV13 (Prevnar®13) on day 366
2. Experimental part of the protocol they will agree to:
   All vaccine recipients donate blood samples up to 60mL per visit: at 5 different times: day 366 day 373, 396 and yearly thereafter up to 3 years, i.e. referred to years 1, 2 and 3.

In group 5, healthy 35-45 year olds, PPV23 and PCV 13 may not be routine part of care (in those without any underlying disease or tobacco use) and vaccination with PCV13/PPV23 is not part of routine care in any of these individuals, **however neither vaccination regimen is contra-indicated in these age groups and is NOT associated with a higher rate of adverse events exceeding vaccination in other age groups.** In fact, significant benefit may be derived from either vaccine regimen.

Visit #	Experimental part of protocol	Standard of care action	Time commitment
1	Enrollment Blood draw	PCV13	60 min
2-6 or 7	Report side effects Blood draw	-	30 min

Table 1. Groups 1-4. First 10-12 subjects enrolled

Visit #	Experimental part of protocol	Standard of care action	Time commitment
1	Enrollment Blood draw	PCV13	60 min
2-4 or 5	Report side effects Blood draw	-	30 min

Table 2. Groups 1 Subjects 11-55 enrolled and all of Group 2,3 and 4

Visit #	Experimental part of protocol	Standard of care action	Time commitment

1	Enrollment PPV23	-	60 min
2	PCV13 Blood draw	-	40 min
3-6 or 7	Report side effects Blood draw	-	30 min

Table 3. Group 5. All enrolled unless previously vaccinated with PPV23 in which case PPV23 will not be administered and enrollment will take place at "visit 2" with time commitment of 60 minutes.

The vaccine(s) administered to the participants are FDA approved vaccinations and vaccination protocols. The standard and recommended dose of vaccine will be administered either by a qualified nurse or physician and both vaccines are considered low risk. In groups 1-4 PCV13 will be administered per standard of care and is not part of the experimental protocol. In group 5, both PPV23 and PCV 13 are not routine part of care and are part of the experimental protocol. These vaccines have however been extensively studied in this and other populations and are considered low risk and 70-80% protective against pneumococcal disease [30].
The risk associated with blood draws is minimal.

The data to be collected will be stored using an excel spread sheet. The data include:
-cause of end stage kidney disease if applicable
Date of kidney transplant
Complete blood count (CBC), comprehensive chemistry profile including renal and liver functions, HbA1c, serum albumin as measure of nutrition, HbsAg, HCV and HIV test
Pre- and post-immunization serum antibody titers and opsonophagocytic antibody titers to PPS14, 19A and 23F in ug/mL
IgM and switched memory B cell number and percentage
PPS+ B cell number and percentage
Serum BAFF concentration
Gene expression of the genes listed below in Table 4

#	GENE SYMBOL	GENE TITLE	PROTEIN FUNCTION
		Apoptosis	
1	Bcl-2	B-cell lymphoma 2	Apoptotic suppressor
2	Bcl-Xl (BCL1)	B-cell lymphoma-extra large	Apoptotic suppressor
3	Mcl-1	Induced myeloid leukemia cell differentiation protein Mcl-1	Apoptotic suppressor
4	BAX	BCL2-associated X protein	Pro-apoptotic function
5	BAD	BCL2-associated agonist of cell death	Pro-apoptotic function
6	BIM (BCL11)	BCLS2-interacting mediator of cell death	Pro-apoptotic function
7	FAS	Fas cell surafce death receptor	Apoptosis signaling
8	BIK	BCL2-interacting killer	Pro-apoptotic function
9	TNFSF15	Tumor necrosis factor alpha	Activates NFkB and MAPK, promotes activation of of caspases and apoptosis
10	Siva1	Siva 1 apoptosis inducing factor	CD27 binding protein; pro-apoptotic
		Cytokines and receptors	
11	IL-1B	Interleukin1beta	Inflammatory mediator, plays a role in cell differentiation, proliferation and apoptosis
12	IL-6	Interleukin 6	Inflammation and maturation of B cells
13	IL-7	Interleukin 7	Plays a role in B cell development
14	IL-8	C-X-C motif chemokine ligand	Chemokine, mediator of the inflammatory response
15	IL-10	Interleukin 10	Immune regulator, inhibits certain cytokines; cell survival and proliferation
16	IFN-γ receptor	Interferon gamma receptor	Innate and adaptive immune response
17	IFN-γ	Interferon gamma	Innate and adaptive immune response
18	TNF-α	Tumor necrosis factor alpha	B cell function
19	TLR1	Toll like recptor 1	Innate immune response
20	CCR2	C-C motif chemokine recepto· 2	Mediates chemotaxis
		Transcription factors	
21	IRF4	Interferon regulatory factor 4	Lymphocyte specifc trabscriptional activator that regulates TLR signaling
22	BACH2	BTB domain and CNC homolcg 2	Transcriptional repressor, expressed thought B cell differentiaton; major target Blimp1=Prdm1
23	PRDM1	PR domain zinc finger protein 1	Controls plasma cell differentiation
24	XBP1	X-box binding protein 1	Controls late events of plasma differentiaton
25	Bcl6	B-cell CLL/Lymphoma 6	Transcription repressor required for GC formation antibody affinity maturation
26	Pax-5	Paired box-5	TF B cell lineage specific proteins
27	IKZF3	Zinc finger protein Aiolos	TF B cell proliferation and differentiation
28	SPIB	Spi-B Transcription factor	B cell development and antigenic stimulation
29	FOXO1	Forkhead box o1	Plays a role in b cell differentiation

#	GENE SYMBOL	GENE TITLE	PROTEIN FUNCTION
		Lymphocyte signaling	
30	CBLB	E3 ubiquitin-protein ligase	Negatively regulates B cell Ag receptor signaling
31	FCRL2	Fc receptor like 2	Expressed in memory B cells in humans
32	CD1D	CD1D	Antigen presentation
33	CD200	OX2 membrane glycoprotein	Controls inflammation; immunsupression
34	LAIR1	Leukocyte associated immunoglobulin like receptor 1	Inhibitor of B cell mediated signaling
35	CD72	B cell differentiation antigen CD72	Negative regulator of B cell responsivness
36	RFTN1	Raft linking protein	B cell antigen receptor mediated signaling
37	LCP2	Lymphocyte cytosolic protein 2	B cell receptor signaling
38	GCET2 (GCSAM)	Germinal center associated signaling and motility	B cell signaling and motility
39	CD38	CD38 molecule	Signal transduction
40	NT5E	5' nucleotidase ecto	CD73; promotes class switching exonucleases
41	CD39	CD39 molecule	Exonucleases
42	MS4A1	Membrane spanning 4-domain A1	Plays a role in T cell independent immune response
		Igs	
43	IgD	Immunoglobulin heavy constand delta	Effector phase of humoral immunity
44	IgA	Immunoglobulin alpha Fc receptor	Mediates immune response to pathogens
		Metabolism	
45	HKII	Hexokinase 2	Hexokinases phosphorylate glucose to produce glucose-6-phosphate (G6P)
46	PFKP	Phosphofructokinase	Catalyzes conversion of fructose 6-phosphate to fructose 1,6-biphosphate
47	ENO1	Enolase 1	Glycolitic enzyme
48	PGAM	Phosphoglycerate mutase	Enzyme that catalyzes step 8 in glycolisis
49	LDHA	Lactate dehydrogenase A	Enzyme that catalyzes the inter conversion of purivate and L-lactate with interconversio of NADH and NAD+
51	COXII	Cytochrome c oxidase subunit 2	Component of respiratory chain
52	CPT1	Carnitine palmitoyltransfere 1 a	Mitochondrial oxidation gene
53	GPT2	Glutamic-pyruvic transaminase	Encodes mitohondrial alanine transminase
55	ACTB	Actin beta	Conserved motif, ubiquitously expressed in all eukaryotic cells

Table 4. Gene expression to be studied by single cell PCR.

11.0 Specimen Collection and Banking (if applicable)

Specimens (i.e. blood samples) will be collected at the laboratories at MUSC or the Ralph H. Johnson VA Medical Center

- Blood samples will be collected at each visit
- The specimens will be labeled with a code that only study personnel can link back to a study subject. Researchers outside of this study will not be given a link between the code number and name or any other identifying information. Other information might include race, ethnicity, sex, medical history. Such information might be important for research or public health. It is possible that this information (including genetic information) might come to be associated with your racial or ethnic group.
- Specimen will be stored in the laboratory freezers with limited access; all associated data will be stored in the password protected server that can be accessed only by research personnel at MUSC. Blood samples obtained at MUSC will be stored in a locked minus 80°degree freezer in the Clinical Science building 927 while those obtained at the VAMC will be stored in the locked minus 80°degree freezer in the Strom Thurmond building 411.
- The blood samples and cells will be destroyed at the end of 10 years.
- Research personnel (PI, program study coordinator, graduate student, post-doctoral scholar, and a technician will) have access to specimen and associated data

- Research personnel (PI, program study coordinator, graduate student, post-doctoral scholar, and a technician will) will be responsible for receipt or transmission of the data or specimens.
- No specimens or associated data be sent to an outside facility

Specimen/Banking for Future Use

- If blood samples are used in future unrelated studies, prior approval will be obtained from the MUSC Institutional Review Board. This future research may be conducted by Dr. Westerink or by other researchers who obtain IRB approval for their research.
- The specimens will be labeled with a code that only study personnel can link back to a study subject. Researchers outside of this study will not be given a link between the code number and name or any other identifying information. Other information might include race, ethnicity, sex, medical history. Such information might be important for research or public health. It is possible that this information (including genetic information) might come to be associated with your racial or ethnic group.
- The blood samples and cells will be destroyed at the end of 10 years.
- Only study personnel will have access to specimens
- This research will not involve genetic studies.

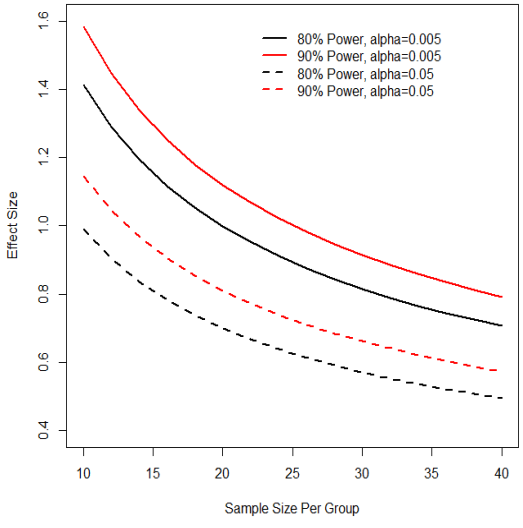
- Study participants may request at any time that their research samples be removed from storage and not be used for future research. Study participants may contact Dr. Westerink via written communication at the following address: : 135 Rutledge Avenue, Suite 1209, MSC752, Charleston, SC 29425 Once the request is received, and if the samples have not already been used for other research, they will be destroyed. If this request is not made, the specimens will be stored indefinitely or until completely used.

12.0 Data Management

Statistical Analysis Plan and Sample Size Justification for Aims 1 and 2

The primary outcomes in Aim 1 are serum antibody levels and functional antibody response measured using the OPA response at baseline and 30 days post-PVC_{13} vaccine measured in the five groups defined in Table 1. The primary outcomes in Aim 2 are absolute number and proportion of B-cells phenotype specific to PPS_{14}, 19A, and 23F at baseline and 7 days post-PVC_{13} vaccine measured in the same five groups. Comparisons between groups and within group between times for each aim will be evaluated using a linear mixed model (LMM) approach. **The models will include fixed effects for participant group (65-75 RT, 35-45 RT, 65-75 DM/HTN, 65-75 healthy, and 35-45 healthy) and for time (day 0 or 30) and a random subject effect to account** for repeated measures of antibody levels within participant. Model assumptions will be checked graphically and transformations will be considered when necessary. Comparisons for each outcome between groups and within group between times will be evaluated using a series of contrasts from the LMM. **We** are presenting power calculations for a range of assumptions to demonstrate that we anticipate having sufficient power to detect observed differences for all comparisons even if we fail to meet the recruitment target. For power calculations we estimated the effect sizes for sample sizes ranging between 10 to 40 participants per group measured at 2 times at 80% and 90% power using a two-sided test assuming within subject correlation of 0.5 and Bonferroni adjusted and unadjusted significance

Figure 7: Effect sizes for different sample sizes, power, and significance



levels of p = 0.005 and 0.05 respectively. We will also evaluate the correlation between serum antibody levels and B cell count with OPA response across all participant groups using an LMM approach where OPA response is the dependent variable and antibody level or B-cell count is the independent variable. The model will include a random subject effect to account for repeated measures in the same subject. We anticipate good correlation (between 0.6-0.99), thus the objective is to estimate the correlation and define the level of precision around that correlation. A sample size of 200 subjects, 40 per group, provides >80% power to detect weak correlations of r = 0.20 and allows us to estimate a 95% confidence interval around the correlation to within ± 0.14.

Statistical Analysis Plan and Sample Size Justification for Aim 3

Aim 3 will examine differences in expression of TNFR markers (TACI, BCMA, and BAFF-R) using single cell qPCR on a subset of 10 participants (5 responders and 5 non-responders) from each of the 5 groups described in Aims 1 and 2 evaluated at baseline and 7 days post-vaccination. Single cell qPCR allows us to not only evaluate differences in expression between groups but also allows us to estimate the within subject and within group heterogeneity with great precision**.** Each participant sample provides between 40-96 viable cells that will be evaluated for gene expression at the single cell level. Relative fold-change in expression between participants who are 65-75 RT, 35-45 RT, or 65-75 DM/HTN with respect to the relevant controls will be evaluated using a linear mixed model approach. The model will include fixed effects for participant group, response status, and time and a random subject effect to account for repeated measurements resulting from individual cells from the same subject samples. Comparisons of number of gene expression between groups and within group between times will be evaluated using a series of contrasts from the LMM. For comparisons within group between responders and non-responders, a sample size of 5 responders and 5 non-responders with $\geq$ 40 cell level measures at 2 time points provides 80% power to detect 2.7 fold-increase or a 0.60 fold-decrease in expression using a two-sided test assuming within subject correlation of 0.5 and a Bonferroni corrected significance level (up to 10 comparisons) of p= 0.005. For comparisons between groups, we will have 10 subjects per group allowing us to detect even smaller fold-changes in expression.

All electronic data will be kept on the VA secure V drive or R drive and on MUSC secure Z drive that only team members will have access to.

13.0 Provisions to Monitor the Data to Ensure the Safety of Subjects (if applicable)

All adverse events (AE) will be reported and monitored through the data safety monitoring plan. The AE's will be characterized by: intensity/severity; expectedness; relatedness; frequency; outcome; treatment or action taken.

Monitoring will be performed at each study visit by the study coordinator and/or P.I. and will occur at the Ralph H. Johnson VA Medical Center and MUSC and all AEs will be recorded on an event form with physician/nurses/coordinator notes attached. These will be reviewed by the safety monitoring committee The safety monitoring committee will include board certified Infectious Diseases specialists. Meetings will occur on a quarterly basis. Dr. Westerink, the P.I. will arrange for the meetings quarterly, make all materials available for review by the committee and be ultimately responsible for the meetings.

The study endpoints will be reporting and assessing safety of the vaccination and blood draw procedures and to ensure that these procedures are well within the acceptable complication risk associated with vaccination and blood draws.

All serious, unexpected, AND related adverse events will be reported to the MUSC IRB within 10 working days of learning of the event.

14.0 Withdrawal of Subjects (if applicable)

Subjects will only be withdrawn from the study for failure to appear at day 7 and day 30, the 2nd and 3rd visit of the study, at which times crucial blood samples are to be obtained. This may occur

voluntarily at the participant's discretion or due to unforeseen circumstances. Should this occur, no further study visits will be requested and no further visit payments will be made.

15.0 Risks to Subjects

Risks to subjects are minimal. This study involves immunization with an FDA approved vaccines which are standard of care for all study groups included with the exception of the young healthy group. Small volume blood draws will be performed at various time points before and after vaccination as outlined above. The risks associated with blood draws is minimal and may result in minor hematomas at the blood draw site and/or bleeding. The blood samples, lymphocytes and cDNA will **not** be marked with donor identifiable data. The risk of breach of confidentiality is minimal, all staff associated with the project have completed appropriate training in human subject research regulations and policies (including the Health Information Portability and Accountability Act (HIPAA).

We will obtain IRB approval at the VA/ MUSC in compliance with Federal Regulations, state and local laws and institutional policy governing human subject research. Following informed consent, all volunteers who choose to participate in this research will complete a questionnaire to evaluate their overall health and eligibility. The vaccination protocol and blood draw schedule will be explained in detail. The risks of blood draws and vaccination with the conjugated pneumococcal polysaccharide vaccine will be explained and the intent to isolate their lymphocytes for the use in flow cytometry and other molecular biology experiments for further study will be outlined as part of the informed consent process prior to any specimens being obtained.

Possible side effects of blood draws (approximately 60 mL/visit) may be the risk of pain, small bruises and bleeding, or infection. All blood draws will be done by experienced technicians or an experienced medical doctor under sterile conditions with proper measures to prevent risks and pain. There are no long term risks associated with blood draws.

Vaccination with the pneumococcal vaccine is linked with side effects that are usually considered minor side effects. In adults aged 18 years and older, the most commonly reported solicited adverse reactions (>5%) PCV13, or Prevnar were pain at the injection site (>50%), fatigue (>30%), headache (>20%), muscle pain (>20%), joint pain (>10%), decreased appetite (>10%), injection site redness (>10%), injection site swelling (>10%), limitation of arm movement (>10%), vomiting (>5%), fever (>5%), chills (>5%), and rash (>5%). The most common adverse reactions, reported in >10% of subjects vaccinated with PPV 23 or Pneumovax in clinical trials, were: injection-site pain/soreness/tenderness (60.0%), injection-site swelling/induration (20.3%), headache (17.6%), injection-site erythema (16.4%), asthenia and fatigue (13.2%), and myalgia (11.9%). Sometimes an allergic reaction can occur but this is very rare. The side effects are seldom more severe and may consist of fever, redness at the site of injection with lymph node swelling in the armpit which may last up to 3 days. This occurs rarely. It should be noted that pneumococcal vaccination (PCV13) is recommended for all RT recipients and all individuals >65 years of age where it is considered standard of care. All individuals in groups 1-4 will already have received PPV23 as part of their routine health care. Although both PPV23 and PCV13 vaccines are FDA approved , two vaccine regimen is not routine as standard of care in healthy HIV negative persons <65 although it is safe, so this vaccination is experimental in this use. Thus as healthy young control group, 35-45 years of age, an essential part in this study for comparator reasons, we will immunize with PPV23 a year before immunizing them with PCV13 so that all groups have received both PPV23 followed by PCV13. Again, a step absolutely necessary and safe, to generate interpretable data from this study.

All paperwork containing donor identifiable data is held in a locked file cabinet in the office of the PI. Each donor is assigned a random number and all samples resulting from that donor (blood, serum, cDNA) are labeled with that number. No donor identifiable data is used to mark samples. All staff who have completed appropriate IRB and HIPAA training and are aware of the importance of maintaining donor confidentiality.

The risk of pneumococcal vaccination adverse side effects is minimal. In the rare event that an adverse reaction of greater magnitude then local reaction occurs, each volunteer is provided with a contact telephone number to reach research personnel who will see and potentially treat the adverse reaction as appropriate. The most common of these occasional adverse reactions consists of low grade fever, malaise and swollen lymph nodes, treatable with 2 days of prednisone. Adverse events associated with blood draws are rare and most commonly consist of prolonged bleeding treated with the application of local pressure.

All adverse events will be monitored and recorded. Monitoring will take place at every visit by the study coordinator and the P.I.

16.0 Potential Benefits to Subjects or Others

The benefits of participation in the study will consist of;

1. Individual benefit: The protection offered by the vaccination protocol is thought to be beneficial to each participant although the degree of protection will vary from person to person. Pneumococcal vaccination is however recommended to all over 50 years of age and some studies show that the addition of PCV13 is beneficial and results in a better protection against pneumonia. The majority of the participants will receive PCV13 regardless of participation in the study as this is part of standard of care for individuals in groups 1-4. The vaccine costs in group 1-4 will be charged to the patient's insurance as pneumococcal vaccination is standard of care.
2. The ultimate goal of the study is to define the degree of protection against pneumococcal disease induced by the pneumococcal conjugate 13 valent vaccine in the aging RT population. Does the a The experimental data, i.e. antibody titers, opsonophagocytic titers, B cell characteristics and single cell PCR results will be stored on laboratory computers and individual notebooks however this data will only be identified by numerical identifier. NO patient identifiers will be used on laboratory specimen or data at ANY time during the study. All experimental data will be confirmed by performing each experiment in duplicate. All computers require ID specific log-in. At no time is patient information associated with the data except at the time of analysis by the investigator if additional clinical information is needed. The results of the study will clarify and increase our understanding of both the aging and young RT recipients and particularly identify the potential cause of poor or deficient responses. The results of these studies may benefit not only the 20% of the RT population over 65 years of age but also the younger RT recipients.

The risks involved in this study are truly minimal and therefore based on the potential personal benefit to the enrolled volunteers and the overall knowledge gained, reasonable.

17.0 Sharing of Results with Subjects

The data obtained during this study will NOT be shared on an individual basis with others. De-identified study results will be used for publication purposes.

18.0 Drugs or Devices (if applicable)

The PCV13 and PPV23 vaccines to be administered to participants in Group 5, healthy volunteers 35-45 years of age will be kept in a **dedicated refrigerator with thermometer, alarm and lock in the P.I.'s laboratory in Strom-Thurmond Building room 411**. A log will be kept of inventory and every vaccine dispensed by study personnel.

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