PROTOCOL TITLE

Systems Biology of 23-valent pneumococcal polysaccharide vaccine (PNEUMOVAX®23) and 13-valent pneumococcal conjugate vaccine (PREVNAR 13®).

Protocol Number

HIPC: VAX-003

Principal Investigator

Investigator: Nadine Rouphael

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Study Sponsor: DAIT/NIAID

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INVESTIGATOR SIGNATURE PAGE	
Protocol Systems Biology of 23-valent pneumococcal polysaccharide vaccine (PNEUMOVAX®23) and 13-valent pneumococcal conjugate vaccine (PREVNAR®13).	**Version/Date:** 7.0/03/30/2017
IND Number N/A	**Principal Investigator:** Nadine Rouphael, MD
Short Title: Systems Biology of PPV23 and PCV 13	
IND Sponsor: N/A	

INSTRUCTIONS: The Principal Investigator will print, sign, and date at the indicated location below. A copy should be kept in the investigator's records and the original signature page sent to the NIAID. After signature, please return the original of this form by surface mail to:

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I confirm that I have read the above protocol in the latest version. I understand it, and I will work according to principles of Good Clinical Practice (GCP) as described in the United States Code of Federal Regulations (CFR) 45 CFR part 46 and 21 CFR parts 50, 56, and 312 , and the International Conference on Harmonization (ICH) document "Guidance for Industry: E6 Good Clinical Practice: Consolidated Guidance" dated April 1996[1] and the spirit of the Declaration of Helsinki, Further, I will conduct the study in keeping with local, legal, and regulatory requirements.

As the Principal Investigator, I agree to conduct HIPC: VAX-003: *Systems Biology of 23-valent pneumococcal polysaccharide vaccine (PNEUMOVAX®23) and 13-valent pneumococcal conjugate vaccine (PREVNAR®13)*. I agree to carry out the study by the criteria written in the protocol and understand that no changes can be made to this protocol without written permission by the NIAID, the local IRB.

___NADINE ROUPHAEL, MD_____
Principal Investigator (Print)

_____ _____
Principal Investigator (Signature) Date

Synopsis

Title	Systems Biology of 23-valent pneumococcal polysaccharide vaccine (PNEUMOVAX®23) and 13-valent pneumococcal conjugate vaccine (PREVNAR®13) (HIPC: VAX-003).
Short Title	Systems Biology PPV23 and PCV13.
Rationale	PCV13 [13-valent pneumococcal conjugate vaccine (Prevnar®13)] induces better functional immune responses when compared to PPV23 [23-valent pneumococcal polysaccharide vaccine (Pneumovax®23)] in older naïve adults. We hypothesize that this is due to intrinsic defects in innate responses that could explain the poor immunogenicity of PPV23 when compared to PCV13. Therefore, we propose to extensively study innate and adaptive immune responses generated after administration of either pneumococcal polysaccharide or conjugate vaccines in older adults.
Clinical Phase	N/A
Mechanistic Study	Yes
IND Sponsor	N/A
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Participating Site(s)	The Hope Clinic of the Emory Vaccine Center, 500 Irvin Court, Suite 200, Decatur, GA 30030. The Hope Clinic is a community-based vaccine research clinic and

	is the clinical arm of the Emory Vaccine Center at Emory University.
Accrual Objective	N=88 (22 subjects (ages 25-40) and 66 subjects (ages 60-89))
Primary Study Objective	To identify innate immune signatures that correlate with the magnitude, quality and persistence of B cell responses after vaccination with either PPV23 or PCV13.
Study Design	Single center, mechanistic study in which healthy subjects will be will be stratified by age in 22 subjects (ages 25-40) and 66 subjects (ages 60-89) and randomized to receive PPV23 or PCV13 in 1:1 ratio. Blood samples will be collected on Days D0 (at enrollment) and D1, D3, D7, D14, D30, D180 and year 2-3.5 post vaccination to study innate and adaptive immune responses. Recording and reporting safety data will be as follows: • Any Adverse Event (including – but not only- vaccine reactions and local or systemic Reactogenicity Events) of grade 3 or higher severity or serious adverse event (SAE) occurring after vaccination while the subjects is still at the clinical site. • Local REs evaluation of grade 3 or higher severity occurring from Day 0 to Day 7 reported over the telephone by the participant and/or assessed at the clinic. • AEs of grade 3 or higher severity throughout D180 of the study (including -but not only – AEs linked to blood draws). • All SAEs throughout D180.
Primary Endpoints	1. Identification of generic innate immune signatures [Traditional immune parameters measurements + array-based gene expression] at days 1, 3 and 7 post vaccination with either PPV23 or PCV13 in the elderly. 2. Measurement of adaptive immune responses [Opsonophagocytosis assay (OPA) titers] at day 30 post vaccination with either PPV23 or PCV13 in the elderly. 3. Correlation of signatures of generic innate immune responses at days 1, 3 and 7 with adaptive immune responses [Opsonophagocytosis assay OPA titers] at day 30 post vaccination with either PPV23 or PCV13 in the elderly.
Secondary Endpoints	1. Comparison of the serotype-specific IgG levels at day 30 post vaccination with either PPV23 or PCV13 in the elderly. 2. Correlation of signatures of generic innate immune responses at days 1, 3 and 7 with serotype specific IgG levels at day 30 post vaccination with either PPV23 or PCV13 in the elderly. 3. Comparison of the serotype-specific avidity indexes in a subset

	of up to 6 vaccinees per vaccine group at day 180 post vaccination with either PPV23 or PCV13 in the elderly. 4. Correlation of signatures of generic innate immune responses at days 1, 3 and 7 with serotype specific avidity indexes in a subset of vaccinees at day 180 post vaccination with either PPV23 or PCV13 in the elderly. 5. Comparison of the adaptive immune responses [Opsonophagocytosis assay (OPA) titers] at day 30 post vaccination with either PPV23 or PCV13 in the elderly.
Exploratory Endpoints	1. Correlation of signatures of generic innate immune responses at days 1, 3 and 7 with adaptive immune responses [Opsonophagocytosis assay OPA titers specifically] at day 30 post vaccination with either PPV23 or PCV13 among subjects in different age strata. 2. Comparison of the kinetics and magnitude of select serotype specific plasmablasts (days 7, 14, 30(with day 30 on a subset of vaccinees only)) and memory B cells (days 30, 180) post vaccination with either PPV23 or PCV13. 3. Comparison of the plasmablast repertoire and monoclonal antibodies from plasmablasts in a subset of vaccinees per vaccine group at day 7 post vaccination with either PPV23 or PCV13. 4. Comparison of identified innate signature variables and correlation signature variables between PPV23 and PCV13 recipients. 5. Correlation of signatures of generic innate immune responses at days 1, 3 and 7 with adaptive immune responses [Opsonophagocytosis assay OPA titers and ELISA] at years 2-3.5 post vaccination with either PPV23 or PCV13. Note: This will include - but not only - a comparison of identified innate signature variables and correlation signature variables between elderly (60 to 89 yo) recipients of PCV13 and young (25-40yo) recipients of PPV23.
Inclusion Criteria	1. Able to understand and give informed consent. 2. Immunocompetent community dwelling subjects between the ages of ages of 25-40 and 60-89 years.
Exclusion Criteria	1. Prior vaccination with pneumococcal vaccine. 2. Receipt of any of the following products: a. Blood products within 3 months prior to study entry or expected receipt at any time after study entry*. b. Any live virus vaccines within 4 weeks prior to study entry or expected receipt within 4 weeks after study entry*.

	c. Any inactivated vaccine within 2 weeks or expected receipt within 2 weeks after study entry*. 3. Presence of co-morbidities or immunosuppressive states such as: o Chronic medical problems including (but not limited to) insulin dependent diabetes, severe heart disease, severe lung disease, severe liver disease, cerebrospinal fluid leaks, severe kidney disease, autoimmune diseases, severe gastrointestinal diseases and grade 4 hypertension per CTCAE criteria** . o Alcohol, drug abuse or psychiatric conditions that in the opinion of the investigator would preclude compliance with the trial or interpretation of safety or endpoint data. o Impaired immune function or known chronic infections including, but not limited, to known HIV, hepatitis B or C; organ transplant; immunosuppression due to cancer; current and/or expected receipt of chemotherapy, radiation therapy, steroids*** (i.e., more than 20 mg of prednisone given daily or on alternative days for 2 weeks or more in the past 90 days , or high dose inhaled corticosteroids**** or any other immunosuppressive therapies (including anti-TNF therapy), functional or anatomic asplenia and congenital immunodeficiency. 4. Conditions that could affect the safety of the volunteers such as: o Severe reactions to prior vaccinations. o An allergy to <u>any</u> component of the study vaccines (phenol, aluminum, CRM197 protein, succinic acid, Polysorbate 80). o History of Guillain-Barré syndrome. o History of bleeding disorders. 5. Volunteers with any acute illness* including, but not limited to, - fever (<u>≥</u> 100.4 F [<u>≥</u> 38 C], regardless of the route) within 3 days prior to study entry. 6. Volunteers with social conditions or occupational conditions or any condition that in the opinion of the investigator might interfere with compliance with the study and vaccine evaluation. 7. Pregnant or breast feeding or women expected to conceive within 30 days after vaccination ***** * An individual who initially is excluded from study participation based on one or more of the time-limited exclusion criteria (e.g., acute illness, receipt or expected receipt of live or inactivated vaccines) may be reconsidered for enrollment once the condition has resolved as long as the subject continues to meet all other entry criteria. ** Grade 4 hypertension per CTCAE criteria is defined as Life

	threatening consequences(e.g., malignant hypertension, transient or permanent neurologic deficit, hypertensive crisis) urgent intervention indicated. ***Subjects receiving ≥ 20 mg/day of prednisone or its equivalent daily or on alternate days for more than 2 weeks may enter the study after therapy has been discontinued for more than 3 months. ****High dose ICS is defined as: > 960 mcg/day of beclomethasone dipropionate or equivalent ***** Women of child-bearing potential (not surgically sterile via tubal ligation, bilateral oophorectomy or hysterectomy or who are not postmenopausal for ≥1 year) must agree to practice adequate contraception that may include, but is not limited to, abstinence, monogamous relationship with vasectomized partner, barrier methods such as condoms, diaphragms, spermicides, intrauterine devices, and licensed hormonal methods for 30 days before and 30 days after receiving PPV23 or PCV13.
Investigational Product(s)/ Intervention(s)	23-valent pneumococcal polysaccharide vaccine (PNEUMOVAX® 23 or PPV23) (Merck, Whitehouse Station, NJ). 13-valent pneumococcal conjugate vaccine (PREVNAR®13 or PCV13) (Pfizer, New York, NY).
Study Procedures	Vaccination, targeted physical examination, phlebotomy, urine pregnancy test.
Statistical Considerations	At the center of this study is the identification of the generic innate signatures and the correlation signatures of innate immune response. The generic innate signatures will be identified from both immune parameters measured by FACS/Luminex assays and array-based gene expression will be measured by microarray experiments. The generic innate signatures will be identified for the treatment groups separately. The traditional immune parameters and array-based gene expression that change after immunization are considered generic innate signatures. After vaccination, the innate signatures that are correlated with the adaptive immune responses (day 30 OPA titers, day 30 IgG levels, day 180 serotype specific avidity indexes, plasmasblasts) are considered correlation signatures of the innate immune responses.

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Glossary of Abbreviations

ACIP	Advisory Committee on Immunization Practices
AADCRC	Asthma and Allergic Diseases Cooperative Research Center
AE	Adverse Event
CFR	Code of Federal Regulations
CRF	Case Report Form
CTCAE	Common Terminology Criteria for Adverse Events
DAIT	Division of Allergy, Immunology, and Transplantation
DC	Dendritic Cell
ELISA	Enzyme-Linked ImmunoSorbent Assay
cGCP	Current Good Clinical Practice
GLP	Good Laboratory Practice
ICH	International Conference on Harmonization
IDE	Investigational Device Exemption
IL	InterLeukin
IM	IntraMuscular
ISM	Independent Safety Monitor (Physician who is independent from the study site and will, at minimum, review all SAEs to assess for possible changes to the overall risk of the study).
IND	Investigational New Drug
IRB	Institutional Review Board
IPD	Invasive Pneumococcal Disease
MCP1	Monocyte Chemoattractant Protein 1
MOP	Manual of Procedures
NCI	National Cancer Institute
NIAID	National Institute of Allergy and Infectious Diseases
NSAIDs	NonSteroidal Anti-Inflammatory Drugs
OPA	OpsonoPhagocytic Assay

PCV13	13-valent pneumococcal conjugate vaccine (Prevnar13®)
PI	Principal Investigator
PPV23	23-valent pneumococcal polysaccharide vaccine (Pneumovax®)
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SOP	Standard Operating Procedure.
TLR	Toll-Like Receptor
TNF	Tumor Necrosis Factor

BACKGROUND AND RATIONALE

1.1 Background

Pneumococcal diseases are a major public health problem, globally. According to the World Health Organization, invasive pneumococcal diseases (IPD) are responsible for 1.6 million deaths per year globally[2]. In the United States alone, there are four million cases of pneumonia each year and the pneumococcus is the most common agent leading to hospitalization[3]. Although all age groups may be affected, the highest rate of pneumococcal disease occurs in young children and in the elderly population. In addition, persons suffering from a wide range of chronic conditions and immune deficiencies are at increased risk.

Vaccination is the most effective way of preventing pneumococcal infections. Until December 2011, only one vaccine was licensed in the United States for adults: PNEUMOVAX®23, the 23-valent pneumococcal polysaccharide (PPV23) based on the 23 most common serotypes. With its wide serotype coverage, PPV23 is recommended for elderly and younger adults at high risk for pneumococcal infections[4]. The 23-valent vaccine accounts for at least 90% of pneumococcal blood isolates and at least 85% of all pneumococcal isolates from sites which are generally sterile as determined by ongoing surveillance of U.S. data. Despite the high valency of the vaccine and the good vaccine uptake in the US (>70%) among adults, they are still 24,000 cases per year of IPD leading to 4,500 deaths among adults>50 years[5]. Also PPV23 does not appear to reduce the much larger burden of nonbacteremic pneumococcal pneumonia with 364,000 cases each year among adults ≥ 65 years of age in the United States[4]. This is explained in part by the decrease in functionality (OPA activity and avidity) of antibodies in the elderly after PPV23[6].

The 7 and 13-valent pneumococcal conjugate vaccines (PCV7, PREVNAR®7 and PCV13, PREVNAR®13, respectively) have been licensed for use in the US for children since 2000 and 2010 respectively. The 7-valent conjugate vaccine has led to a marked reduction (~84 to 90%) in disease burden events linked to the 7 vaccine serotypes even in adults due to a "herd immunity" effect from universal vaccination in children <2 years old [5,7]. Since December 2011, PREVNAR®13 (PCV13) is licensed for adults 50 years and older. When PCV13 is given to older adults (age 60-64) who have never received PPV23, it induces opsonophagocytic antibody (OPA) titers to shared serotypes in both vaccines, and for most of those serotypes the immune

responses to PCV13 are significantly greater than those induced by PPV23[8]. A Phase IV trial, the Community Acquired Pneumonia Immunization Trial in Adults (CAPITA) is currently underway to evaluate the efficacy of PCV13 against vaccine type nonbacteremic pneumococcal pneumonia in adults ≥ 65 years of age who have never received pneumococcal vaccines in the past[9]. The trial enrolled approximately 85,000 adults in the Netherlands and efficacy results are expected in 2013.

A better understanding of the innate and adaptive immunologic mechanisms by which the elderly respond to both pneumococcal vaccines could determine the best vaccination strategies necessary to decrease the burden of disease associated with increase age. Defining the generic innate immune signatures of existing pneumococcal vaccines will allow more rapid and reliable evaluation of new vaccine candidates more suitable to populations at high risk for pneumococcal diseases particularly the elderly. Therefore additional early surrogates of protection are required to more accurately and rapidly evaluate and predict immune response after vaccination in the elderly.

In this context, systems biology approaches offer a new approach to compare the global architecture of the immune response between PPV23 and PCV13. Systems biology is a biology-based inter-disciplinary study field that focuses on complex interactions in biological systems, using a new holistic perspective. Systems biology offers several so-called "Omic" technologies that can provide unbiased and rich information on the physiological state of organisms through their molecular profiles. These systems biology approaches are likely to be of value in identifying molecular signatures that are induced rapidly after vaccination and that correlate with, and predict, the later development of protective immune responses. Such a strategy would be particularly useful when evaluating the efficacy or immunogenicity of suboptimal vaccines, or in identifying individuals who are likely to respond sub-optimally to vaccination, such as the elderly receiving PPV23 in particular. Furthermore, the predictive signatures would highlight new correlates of protective immunity and stimulate the formulation and validation of new hypotheses on the biological mechanisms by which such molecular signatures modulate vaccine-induced immunity and protection. Despite this promise in identifying molecular signatures, they have only recently been applied to vaccinology[10, 11].

Accordingly, a key goal of the present proposal is to identify molecular signatures that predict the sub-optimal immunogenicity of PPV23 when compared to PCV13 in the elderly. One of the

key systems biology technologies that will be employed are microarray analyses. Essentially, blood samples will be isolated at baseline (day 0 prior to vaccination), and at days 1, 3, 7, 14 post vaccination, and the RNA from the peripheral blood mononuclear cells isolated and analyzed for the expression of mRNAs. The microarray platform will be Affymetrix Human Genome U133 Plus 2.0 Array, similar to what we had used previously[10]**.** In our previous work on yellow fever vaccine[10], we identified a gene signature that correlates with and predicted YF-17D CD8+ T cell responses with up to 90% accuracy in an independent, trial. A distinct signature, including B cell growth factor TNFRS17, predicted then neutralizing antibody response with up to 100% accuracy. These findings highlight the utility of system biology approaches in predicting vaccine efficacy.

In summary the proposed study offers an unprecedented opportunity to capture the global architecture of immune responses to PPV23 versus PCV13 in the elderly. This is likely to have a major impact in two areas: (i) the elucidation of unique signatures of innate immune response that predict vaccine immunogenicity, and will thus be relevant from a public health perspective; (ii) such unique signatures of innate immune response may illuminate the immunologic defects responsible for sub-optimal immunity in the elderly population when using a polysaccharide vaccine when compared to a conjugate vaccine.

1.2 Rationale for Selection of Study Population

To be able to probe the immune system and better understand the innate and adaptive immune responses in older adults to pneumococcal vaccines, 88 healthy volunteers between the ages of 25-40 and 60-89 years will be randomized to receive either PPV23 or PCV13 in a 1:1 ratio. Both vaccines are approved for persons 50 and above. However, we elected to study older subjects (60 years and above) because functional antibodies decrease markedly with more advanced age. The younger group will include 22 subjects between the ages of 25-40 and will help understand the potential effect of age on immune responses to PCV13 and PPV23. Subjects will be recruited from the general population of metro Atlanta and enrolled at the Hope Clinic, located in Decatur, GA.

1.3 Investigational Product(s)/Intervention(s)

The vaccines used in the study will be PNEUMOVAX®23 manufactured by Merck (Whitehouse Station, NJ) and PREVNAR®13 manufactured by Pfizer (New York, NY). Subjects in the study will be administered 1 dose per label of PNEUMOVAX®23 or PREVNAR®13. Study vaccines will be purchased from the manufacturers or their distributors.

1.4 Rationale for Selection of Investigational Product(s)/Intervention(s) and Regimen

Both vaccines will be tested and compared to better understand the innate and adaptive immune responses of polysaccharide and conjugate pneumococcal vaccines in the elderly.

1.5 IMMUNE RESPONSE AGAINST PNEUMOCOCCUS AND SYSTEMS BIOLOGY EXPERIENCE

Immunity following pneumococcal disease is directed primarily against the capsular polysaccharide of the bacteria serotype involved. Protective immunity is mainly dependent upon type-specific, anti-capsular antibodies, although serological correlates of immunity are poorly defined. Capsular polysaccharides when incorporated alone into vaccines are recognized by the immune system as "T-cell independent antigens". The "T-cell independent" responses do not develop in children until 2 years of age. Although adequate IgG levels are obtained, the humoral response is characterized by a short-lived initial IgM response and the absence of a memory response[12]. In elderly, these "T-cell independent" responses elicit an adequate quantitative response (by ELISA) but a suboptimal qualitative response (measured by OPA and avidity) in vaccine recipients[6]. Thus, capsular polysaccharides alone are poorly immunogenic in the young and the elderly[13, 6].

The limited response to capsular polysaccharides has been overcome by coupling the polysaccharide to an immunogenic protein carrier, which can present the antigen as a T-cell dependent epitope. The glycoconjugate vaccines are "T-cell dependent antigens" and induce antibodies with good avidity [14] (47 to 76% of antibodies remaining bound to the antigen coated-ELISA well after addition of 0.05M sodium thiocyanate), maturation, isotype switching, as well

as memory formation [15]. Other major effects of these vaccines are prolonged mucosal antibody expression, interference with bacterial transmission, and herd immunity [5, 7]

Antibody mediated killing of *Streptococcus pneumoniae* (pneumococcus) by phagocytes is an important mechanism of protection of the human host against pneumococcal infections. It is thought that opsonophagocytic antibodies reflect *in vivo* mechanisms of defense against pneumococcal infection. Measurement of opsonophagocytic antibodies (functional antibody assay) using a standardized opsonophagocytic assay (OPA) is important for the evaluation of candidate vaccines[4] and a requirement for the licensure of new pneumococcal conjugate vaccine formulations in infant populations. The measurement of functional antibodies has been shown to better correlate with protection in infant populations and it is also likely to be a better indicator for elderly populations than the measurement of antibodies specific to the capsular polysaccharides by ELISA (antibody binding assay).

Although vaccination with PPV23 provides some protection against invasive pneumococcal disease in a healthy elderly population, there is evidence that vaccine efficacy declines with age [16], and with associated co-morbidities[4]. The decrease in efficacy could be associated with the impairment in the functional quality of the response. Even with advanced age, individuals seem to retain the ability to quantitatively respond to PPV (measured by ELISA). However, the ability to elicit a functional antibody response (measured by opsonophagocytic assay (OPA) is reduced with advanced age [6, 17].

In a randomized study comparing PCV13 to PPV23 in pneumococcal vaccine naïve subjects between the ages of 60 to 64 years, the GMTs for 10 of the 12 serotypes common to both vaccines were higher in the PCV13 group, and were statistically significant higher for 8 of the 12 serotypes[8]. In a previous randomized study comparing PCV7 to PPV23 in pneumococcal vaccine naïve subjects ≥70 years of age, OPA GMTs were significantly higher in the PCV7 group for 5 of the 7 common serotypes[18].

Data on innate immunity to PCV13 and to PPV23 are limited. Sen et al.[19] have shown that PPV23 contains both TLR2 and TLR4 ligands. Our preliminary in vitro data show that PPV23 signals via TLR2 and TLR9 and activates human CD11c+ myeloid DC precursors to secrete several cytokines and chemokines, including IL-6, IL-12p40, TNF and IL-8. Interestingly MCP-1, a chemokine involved in recruitment of many inflammatory cells including monocytes, was not

induced. In contrast, monocytes only produced IL-8 to PPV23. These results suggest that PPV23 induces distinct cytokine profiles from different subsets of antigen presenting cells.
A better knowledge of the innate immunity is crucial to understand the immunologic mechanisms of response to PPV23 and PCV13 vaccines.

1.5.1 Preclinical studies

N/A

1.5.2. Clinical studies

N/A

1.6 Risks

1.6.1 Risks of Investigational Product(s)/Intervention(s)

Pneumococcal polysaccharide vaccine is recommended for all adults aged ≥65 years and those adults aged 19-64 years with underlying medical conditions that put them at greater risk for serious pneumococcal infection [20]. The Advisory Committee on Immunization Practices (ACIP) will continue to review evidence as it becomes available to guide development of a recommendation regarding routine use of PCV13 in adults aged 50 years and older[21] . Eleven immunocompetent subjects between the ages of 25-40 will receive PPV23 without an indication. Safety concerns in this group are not anticipated on the basis of previous safety data generated in young adults vaccinated with pneumococcal vaccines with lower valency (6 and 12) which was used to support the licensure of PPV23[21a] .Additionally, the safety profile is not expected to differ between young adults without co-morbidities and adults with a medical indication to receive PPV23.
PNEUMOVAX®23, manufactured by Merck (Whitehouse Station, NJ), is indicated for vaccination against pneumococcal disease caused by those pneumococcal types included in the vaccine. The most common adverse experiences reported in >10% of subjects vaccinated with PNEUMOVAX®23 in clinical trials were: local reaction at injection site including pain/soreness/tenderness, erythema, swelling/induration and systemic reaction, mostly with asthenia/fatigue, myalgia and headache.

Other Adverse reactions identified during post approval use of PNEUMOVAX 23 include: Guillain-Barré syndrome, radiculoneuropathy, seizure, angioneurotic edema, cellulitis, nausea, vomiting, arthralgia, arthritis, limb mobility decreased, paresthesia, weakness, anaphylactoid reaction, lymphadenitis, serum sickness. Because these reactions are reported voluntarily from a population of uncertain size, it is not always possible to reliably estimate their frequency or their causal relationship to product exposure. [22].

PREVNAR®13, manufactured by Pfizer (New York, NY), is indicated in adults (50 years or older) for prevention of pneumonia and invasive disease caused by those pneumococcal types included in the vaccine. In adults aged 50 years and older solicited adverse reactions reported in >10% of subjects vaccinated with PREVNAR®13 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%) and limitation of arm movement (>10%)[23]. In one randomized study comparing safety of PCV13 to PPV23 in 60-64 years old previously unvaccinated, the frequency of local reactions was not statistically significant in both arms except for pain (more frequent in the PCV13 arm). Also the frequency of systemic reactions was not statistically different in both arms except for decrease in appetite, joint and muscle pains (more frequent in the PPV23 arm) [23]. In 6 studies comparing the safety of PCV13 to PPV23, SAE were similar among both groups (around 6% at 6 months post vaccination) [23].

Eleven immunocompetent subjects between the ages of 25-40 will receive PCV13 without an indication. The safety profile of PREVNAR13® in immunocompetent healthy adults aged 25-40 years -including both systemic and injection-site adverse events- is not expected to be different than in subjects ≥ 50 years old. Safety concerns in this group are not anticipated on the basis of previous safety data generated in young adults vaccinated with pneumococcal conjugate vaccines with lower valency (7). Multiple studies have been published on the use of PREVNAR7® in immunocompromised hosts in transplant recipients[23a] and HIV infected subjects [23b] with acceptable safety profile. In one study with 25 young adults non HIV infected between the ages of 29-46, side effects with PREVNAR7® were mild (98%) mostly involving local tenderness, malaise, and myalgia. These side effects occurred within 7 days after vaccination and none were severe or life-threatening [23b].

Revaccination with PPV23 is recommended by the ACIP for subjects with functional or anatomic asplenia and for persons with immunocompromising conditions[20], although, this population is not eligible for the current study. However, the ACIP recommends that those who received PPV23 before age 65 years for any indication should receive another dose of the vaccine at age 65 years or later if at least 5 years have passed since their previous dose.

Persons aged ≥65 years should be administered a second dose of vaccine if they received the vaccine ≥65 years previously and were aged <65 years at the time of primary vaccination. Revaccination with PNEUMOVAX®23 is considered safe although for subjects aged ≥ 65 years the overall injection-site adverse experiences rate was higher following revaccination (79.3%) than following primary vaccination (52.9%). The rate of vaccine-related systemic adverse experiences was also higher following revaccination (33.1%) than following primary vaccination (21.7%) in subjects ≥ 65 years of age[22]. With revaccination there is a potential risk of hyporesponsiveness with certain serotypes [24, 25]. In both of these longitudinal studies, patients were, of course, older at the time of revaccination than at first vaccination and so it is possible that some of the differences in the immune response to revaccination were due to an age-effect, rather than to an effect of prior receipt of pneumococcal polysaccharide vaccine.

Ortqvist et al. also show how the responses in the elderly are serotype specific, with some serotypes eliciting poorer responses than others[26]. A recent study by Manoff et al., show that both IgG antibodies and functional antibodies persist in persons older than 65 year above baseline concentrations five year after primary vaccination and revaccination[27]. Although the responses were slightly lower after revaccination there was no blunting of the responses to a second dose of the polysaccharide vaccine. Responses are markedly different in the elderly if the polysaccharide vaccine is given after the conjugate vaccine, where there is clear evidence of the lack of booster response and possible hyporesponsiveness regardless of the dose of conjugate vaccine used for priming [28, 29]. In contrast, immunologic hyporesponsiveness is not seen with PCVs, and repeated doses of PCVs can be given to without diminishment of the immune response [28, 30].

However, it should be noted that because the quantity of antibodies that correlate with protection against pneumococcal disease has not been clearly defined, it is unknown if the lower antibody levels seen on revaccination correlate with inferior protection. There is also the added advantage of increased serotype coverage by the PPV23. Currently, there are no

published recommendations from the ACIP regarding the subsequent use of PCV13 in immunocompetent adults previously vaccinated with PCV13.

1.6.2 Risk of Study Procedures

The discomforts of this study include having blood drawn, intramuscular (IM) injection, and possible allergic reactions to the components the pneumococcal vaccines administered in the study.

Drawing blood causes transient discomfort and may cause fainting. Bruising at the blood draw site may occur but can be prevented or lessened by applying pressure to the draw site for several minutes. Intramuscular injection also may cause transient discomfort. The use of aseptic technique will make infection at the site where blood will be drawn or where the vaccination is given extremely unlikely. Vaccines used in the study will be a single vial dose preventing the risk of over or under dosing and the potential risk of contamination when compared to multi-dose vials.

In the clinical trials of PNEUMOVAX® 23 [22], the most common local adverse events at the site of injection were pain/tenderness/soreness (60.0%), swelling/induration (20.3%), and erythema. The most common systemic adverse experiences were headache (17.6%), asthenia/fatigue (13.2%), and myalgia (11.9%). All of these adverse reactions were reported at a rate lower than 10% after receiving a placebo injection.

In the post-marketing experience of PNEUMOVAX®23, the following additional adverse reactions have been identified: Cellulitis, Malaise, Fever (>102°F), Warmth at the injection site, Decreased limb mobility, Peripheral edema in the injected extremity, Nausea, Vomiting, Lymphadenitis, Lymphadenopathy, Thrombocytopenia in patients with stabilized idiopathic thrombocytopenic purpura, Hemolytic anemia in patients who have had other hematologic disorders, Leukocytosis, Anaphylactoid reactions, Serum Sickness, Angioneurotic edema, Arthralgia, Arthritis, Paresthesia, Radiculoneuropathy, Guillain-Barré syndrome, Febrile convulsion, Rash, Urticaria, Cellulitis-like reactions, and Increased serum C-reactive protein. In the clinical trials of PREVNAR® 13 [23], the most common solicited local reactions were pain (69-88%), limitation of arm movement (24-41%), swelling (10-22%), and redness (12-20%). The most common systemic adverse experiences were generalized new muscle pain (47-62%), generalized aggravated muscle pain (16-32%), generalized new joint pain (16-32%), generalized aggravated

joint pain (14-26%), decreased appetite (15-25%), chills (20-24%), headache (50-66%), fatigue (51-63%).

1.6.3 Risk of Concomitant Medications, Prophylactic Medications and Rescue Medications

We do not anticipate the use of any other medication; however should anaphylactic or hypersensitivity reactions occur, an epinephrine (1:1000) and diphenhydramine injections are readily available at the Hope Clinic during vaccine use. Epinephrine injection can be associated with high blood pressure, arrhythmia, lightheadedness, nervousness, restlessness, tremor, shortness of breath and diaphoresis. The frequency of these side effects is not defined. Diphenhydramine injection can be associated with low blood pressure, arrhythmia, confusion, dizziness, sedation, restlessness, diarrhea, nausea and urinary retention. The frequency of these side effects is also not defined.

Subjects are allowed to use acetaminophen or NSAIDs if they experience a moderate to severe local or systemic side effects after vaccine administration.

1.7 Benefits

1.7.1 Benefits of Investigational Product(s)/Intervention(s)

Participants will have the benefit of receiving a licensed pneumococcal vaccine which may offer protection against pneumococcal disease. Also the knowledge gained from this study could facilitate the design of more effective pneumococcal vaccines for the geriatric population.

1.7.2 Benefits of Study Procedure(s)

None

2. OBJECTIVES

PCV13 induces better functional immune responses when compared to PPV23 in older naïve adults. We hypothesize that this is due to intrinsic defects in innate responses that could explain the poor immunogenicity of PPV23 when compared to PCV13. Therefore, we propose to extensively study innate and adaptive immune responses generated after administration of either pneumococcal polysaccharide or conjugate vaccines in older adults.

2.1 Primary Objective(s)

To analyze the generic innate immune signatures that correlate with the magnitude of the adaptive immune responses (opsonophagocytosis [OPA] titers) at day 30 in order to identify the correlation signatures of the innate immune responses to PPV23 and PCV13.

2.2 Secondary Objective(s)

To analyze the generic innate immune signatures that correlate with the persistence and the quality of the adaptive immune responses determined by serological assays to PPV23 and PCV13.

2.3 Exploratory Objective(s)

To compare the generic innate immune signatures that correlate with the magnitude of the adaptive immune responses (opsonophagocytosis [OPA] titers) in elderly and the young in the different age strata.

To compare the generic innate immune signatures that correlate with the persistence and the quality of the adaptive immune responses as determined by memory B cells, plasmablasts and monoclonal antibodies to PPV23 and PCV13.

3. STUDY DESIGN

This is a single center, mechanistic study in which 88 healthy subjects will be stratified by age with 22 subjects (ages 25-40) and 66 subjects (ages 60-89). and randomized to receive PPV23 or PCV13 in a 1:1 ratio.

Study volunteers will be recruited from the general population of metropolitan Atlanta. The study will be conducted at the Hope Clinic of the Emory Vaccine Center (Decatur, GA). The expected duration of subject participation is 6 months. An additional optional blood draw will be offered 2-3.5 years after enrollment.

3.1 Study Endpoints

3.1.1 Primary Endpoint(s)

3.1.1.1 Identification of generic innate immune signatures [Traditional immune parameters measurements + array-based gene expression] at days 1, 3 and 7 post vaccination with either PPV23 or PCV13 in the elderly.

3.1.1.2. Measurement of adaptive immune responses [Opsonophagocytosis assay (OPA) titers] at day 30 post vaccination with either PPV23 or PCV13 in the elderly.

3.1.1.3. Correlation of signatures of generic innate immune responses at days 1, 3 and 7 with adaptive immune responses [Opsonophagocytosis assay OPA titers] at day 30 post vaccination with either PPV23 or PCV13 in the elderly.

3.1.2 Secondary Endpoint(s)

3.1.2.1. Comparison of serotype specific IgG levels at day 30 post vaccination with either PPV23 or PCV13 in the elderly.

3.1.2.2. Correlation of signatures of generic innate immune responses at days 1, 3 and 7 that correlate to serotype specific IgG levels at day 30 post vaccination with either PPV23 or PCV13 in the elderly.

3.1.2.3. Comparison of the serotype specific avidity indexes in a subset of up to 6 vaccinees per vaccine group at day180 post vaccination with either PPV23 or PCV13 in the elderly.

3.1.2.4. Correlation signatures of generic innate immune responses at days 1, 3 and 7 that correlate to serotype specific avidity indexes in a subset of up to 6 vaccinees per vaccine group at day 180 post vaccination with either PPV23 or PCV13 in the elderly.

3.1.2.5. Comparison of the adaptive immune responses [Opsonophagocytosis assay (OPA) titers] at day 30 post vaccination with either PPV23 or PCV13 in the elderly.

3.1.3 Exploratory Endpoint(s)

3.1.3.1. Identification of correlation signatures of generic innate immune responses at days 1, 3 and 7 that correlate to adaptive immune responses [Opsonophagocytosis assay OPA titers specifically] at day 30 post vaccination among subjects in different age strata.

3.1.3.2. Comparison of the kinetics and magnitude of select serotype specific plasmablasts (days 7, 14, 30(with day 30 on a subset of vaccinees only)) and memory B cells (days 30, 180) post vaccination with either PPV23 or PCV13.

3.1.3.3. Comparison of the repertoire and monoclonal antibodies from plasmablasts in a subset of vaccinees per vaccine group at day 7 post vaccination with either PPV23 or PCV13.

3.1.3.4. Comparison of identified innate signature variables and correlation signature variables between PPV23 and PCV13 recipients.

3.1.3.5. Comparison of generic innate immune responses at days 1, 3 and 7 with adaptive immune responses [Opsonophagocytosis assay OPA titers and ELISA] at years 2-3.5 post vaccination with either PPV23 or PCV13.

Note: This will include - but not only - a comparison of identified innate signature variables and correlation signature variables between elderly (60 to 89 yo) recipients of PCV13 and young (25-40yo) recipients of PPV23.

3.2 Study Completion

This study will be considered "completed" when the primary and secondary objectives have been met. This includes the analysis of all the data required to meet the study objectives described above.

After the study is completed, the Principal Investigator (or co-PI) or Data Center will compile an abbreviated study reports. The study report will be reviewed by the ISM and submitted to the

local IRB and DAIT/NIAID. Alternatively, the publication of the study could be used for the purposes of this requirement.

4. SELECTION OF STUDY PARTICIPANTS

4.1 Inclusion Criteria

1. Able to understand and give informed consent.

2. Immunocompetent community dwelling subjects between the ages of 25-40 and 60-89 years.

4.2 Exclusion Criteria

1. Prior vaccination with pneumococcal vaccine.
2. Receipt of immune products
    a. Subjects received blood products within 3 months*.
    b. Subjects received of any live virus vaccines within 4 weeks prior to study entry or expected receipt within 4 weeks after study entry*.
    c. Subjects received of any inactivated vaccine within 2 weeks or expected receipt within 2 weeks after study entry*.
3. Presence of co morbidities or immunosuppressive states such as:
    - Chronic medical problems including (but not limited to) insulin dependent diabetes, severe heart disease, severe lung disease, severe liver disease, cerebrospinal fluid leaks, severe kidney disease, autoimmune diseases, severe gastrointestinal diseases and grade 4 hypertension per CTCAE criteria**.
    - Alcohol, drug abuse or psychiatric conditions that in the opinion of the investigator would preclude compliance with the trial or interpretation of safety or endpoint data.
    - Impaired immune function or known chronic infections including, but not limited, to known HIV, hepatitis B or C; organ transplant; immunosuppression due to cancer; current and/or expected receipt of chemotherapy, radiation therapy, steroids*** (i.e., more than 20 mg of prednisone given daily or on alternative days for 2 weeks or more in the past 90 days , or high dose inhaled corticosteroids**** or any other immunosuppressive therapies (including anti-

TNF therapy), functional or anatomic asplenia and congenital immunodeficiency.

4. Conditions that could affect the safety of the volunteers such as:
   - Severe reactions to prior vaccinations.
   - An allergy to **any** component of the study vaccines (phenol, aluminum, CRM197 protein, succinic acid, Polysorbate 80).
   - History of Guillain-Barré syndrome.
   - History of bleeding disorders.

5. Volunteers with any acute illness* including, but not limited to, fever (> 100.4 F [> 38 C], regardless of the route) within 3 days prior to study entry.

6. Volunteers with social conditions or occupational conditions or any condition that in the opinion of the investigator might interfere with compliance with the study and vaccine evaluation.

7. Pregnant or breast feeding or women expected to conceive within 30 days after vaccination *****

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

** Grade 4 hypertension per CTCAE criteria is defined as Life-threatening consequences (e.g., malignant hypertension, transient or permanent neurologic deficit, hypertensive crisis) urgent intervention indicated.

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

****High dose ICS is defined as: > 960 mcg/day of beclomethasone dipropionate or equivalent.

***** Women of child-bearing potential (not surgically sterile via tubal ligation, bilateral oophorectomy or hysterectomy or who are not postmenopausal for ≥1 year) must agree to practice adequate contraception that may include, but is not limited to, abstinence, monogamous relationship with vasectomized partner, barrier methods such as condoms,

diaphragms, spermicides, intrauterine devices, and licensed hormonal methods for 30 days before and 30 days after receiving PPV23 or PCV13.

4.3 Early Study Termination

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

a. The participant elects to withdraw consent from all future study activities, including follow-up.

b. The participant is considered by the PI to be "lost to follow-up" (i.e., no further follow-up is possible because attempts to reestablish contact with the participant have failed).

c. The participant dies.

d. The participant develops a medical condition or is started on new medication(s) not previously mentioned in the list of prohibited medications that, 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 of the data obtained from the study.

e. Blood not able to be drawn (for technical or other reasons) or participant not tolerating multiple blood draw attempts.

f. As deemed necessary by the PI or her designee for noncompliance or other reasons.

g. As deemed necessary by the PI after development related AE/SAE.

h. The participant meets the individual stopping rule -delineated in section 8.

Participants with early termination from this study may be replaced as needed to preserve the statistical power needed to prove the primary endpoint (Refer to section 9.1).

Note:

Up to the discretion of the PI, participants receiving prohibited medications before blood draw at day 30 (+/-7 days) or missing any of the blood draws required for the primary endpoint [meaning draws at days 1, 3, 7($\pm$1), and 30 ($\pm$7)] may still continue with the scheduled study blood draws required for secondary and exploratory study endpoints.

Subjects receiving prohibited medications before blood draw at day 30 (+/-7 days) or missing any of the blood draws required for the primary endpoint [meaning draws at days 1, 3, 7($\pm$1), and 30 ($\pm$7)], along with participants with early termination from this study for any reason may

be replaced as needed to preserve the statistical power needed to prove the primary endpoint. (Refer to section 9.1)

5. INVESTIGATIONAL PRODUCT(S)/INTERVENTION MATERIAL(S), OTHER STUDY PRODUCTS (CONTROLS/PLACEBOS)

5.1 Investigational Product(s)/Intervention(s)

The Emory Investigational Drug Service will purchase PNEUMOVAX® 23 from its manufacturer (Merck, Whitehouse Station, NJ) and PREVNAR®13 from its manufacturer (Pfizer, New York, NY) or their distributors. The Emory Investigational Drug Service will store the vaccines and will monitor temperatures of refrigerator containing the vaccines.

Refer to section 1.6, and applicable product labeling for known and potential risks to human participants associated with the investigational product(s) intervention(s).

5.2 Formulation, Packaging, and Labeling

PNEUMOVAX® 23 (Pneumococcal Vaccine Polyvalent) is a sterile, liquid vaccine for intramuscular or subcutaneous injection. It consists of a mixture of highly purified capsular polysaccharides from the 23 most prevalent or invasive pneumococcal types of *Streptococcus pneumoniae,* including the serotypes that most frequently cause invasive drug-resistant pneumococcal infections among children and adults in the United States.

23 Pneumococcal Capsular Types Included in PNEUMOVAX 23

Nomenclature	Pneumococcal Types
Danish	1 2 3 4 5 6B** 7F 8 9N 9V** 10A 11A 12F 14** 15B 17F 18C 19F** 19A** 20 22F 23F** 33F
** These serotypes most frequently cause drug-resistant pneumococcal infections[1]	

PNEUMOVAX 23 is manufactured according to methods developed by the Merck Research Laboratories. Each 0.5 mL dose of vaccine contains 25 micrograms of each polysaccharide type in isotonic saline solution containing 0.25% phenol as a preservative.

PNEUMOVAX 23 will be supplied as a single-dose vial of liquid vaccine.

PREVNAR 13®, Pneumococcal 13-valent Conjugate Vaccine (Diphtheria CRM197 Protein) is a sterile suspension of saccharides of the capsular antigens of *Streptococcus pneumonia* serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F, individually linked to nontoxic diphtheria CRM197 protein. Each serotype is grown in soy peptone broth. The individual polysaccharides

are purified through centrifugation, precipitation, ultrafiltration, and column chromatography. The polysaccharides are chemically activated to make saccharides, which are directly conjugated by reductive amination to the protein carrier CRM197, to form the glycoconjugate.
The individual glycoconjugates are compounded to formulate Prevnar®13. Potency of the formulated vaccine is determined by quantification of each of the saccharide antigens and by the saccharide to protein ratios in the individual glycoconjugates. Each 0.5 mL dose of the vaccine is formulated to contain approximately 2.2 µg of each of *Streptococcus pneumoniae* serotypes 1, 3, 4, 5, 6A, 7F, 9V, 14, 18C, 19A, 19F, 23F saccharides, 4.4 µg of 6B saccharides, 34 µg CRM197 carrier protein, 100 µg polysorbate 80, 295 µg succinate buffer and 125 µg aluminum as aluminum phosphate adjuvant.

Note: No additional labeling of the vaccines will be required

5.3 Preparation, Administration, and Dosage

PNEUMOVAX®23:
Vaccine vials will be stored at +2°C to +8°C (36°F to 46°F) and administered per label as below:
The vaccine will first be inspected visually for particulate matter and discoloration prior to administration and will not be used if particulate matter or discoloration is found. The vaccine is used directly as supplied. No dilution or reconstitution is necessary.
A single 0.5 mL dose of PNEUMOVAX®23 will be administered via the intramuscular route in the deltoid muscle.
PREVNAR®13:
Vaccine vials will be stored at +2°C to +8°C (36°F to 46°F) and administered per label as below:
The vaccine will first be inspected visually for particulate matter and discoloration prior to administration and will not be used if particulate matter or discoloration is found. The prefilled syringe will be shaken vigorously immediately prior to use to obtain a homogenous, white suspension in the vaccine container.
A single 0.5 mL dose of PREVNAR®13 will be administered via the intramuscular route in the deltoid muscle.

5.4 Accountability of Investigational Product(s)/Intervention(s)

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

Records for receipt, storage, use, and disposition will be maintained by the study site. A dispensing log will be kept current for each participant. This log will contain the identification of each participant, the name of the vaccine administered to the subject, the lot of vaccine received by the subject and the date and quantity of vaccine dispensed.

All records regarding the disposition of the investigational product(s)/intervention material(s) will be available for inspection by the site monitor, the health authorities, and DAIT/NIAID personnel.

5.5 Assessment of Compliance with Investigational Product(s)/Intervention Material(s)

Number of used vaccine vials will be tracked and reconciled by the nursing staff prior to sending the vaccine back to the Emory Investigational Drug Service.

5.6 Modification or Discontinuation of Investigational Product(s)/Intervention Material(s)

5.6.1 Modification of Investigational Product(s)/Intervention(s)

Unless the lots of PNEUMOVAX®23 or PREVNAR®13 used in the study are recalled by the manufacturers, there will be no discontinuation of administration of study vaccines.

5.6.2 Premature Discontinuation of Investigational Product(s)/Intervention(s)

Refer to sections 4.3 for possible causes of early study termination.

If a subject withdraws from the study for any reason, the subject will be then be censored from the study.

Refer to section 8.1.3 for safety follow-up after early study termination.

6. OTHER MEDICATIONS

6.1 Concomitant Medications

In accordance with exclusion criteria, subjects expected to receive prohibited medications (see section 6.4) will be considered non-eligible for the study. All medications, therapies or vaccines administered to study subjects after study entry will be documented at each visit.

6.2 Prophylactic Medications

Prophylactic medications will not be administered before vaccination or any study procedure.

6.3 Rescue Medications

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

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

Subjects are allowed to use acetaminophen or NSAIDs if they experience a moderate to severe local or systemic side effects after vaccine administration.

6.4 Concomitant Study Medications

All medications and vaccines received by study participants after administration of study vaccine should be reported to the study staff and recorded including (but not limited to) the following:

- blood products, chemotherapy, immunosuppressive therapy (including anti-INF therapy) and radiation therapy (administered at any time after study vaccination)

- inactivated vaccines (administered before the day 14 blood draw)

- live-attenuated vaccine (administered before the day 30 blood draw)

Any of the above medications can affect the innate and adaptive assay results and should not be used unless medically indicated.

Up to the discretion of the PI, participants receiving concomitant medications may still continue with scheduled study blood draws. Subjects receiving concomitant medications, along with participants with early termination from this study for any reason, may be replaced as needed to preserve the statistical power needed to prove the primary endpoint. (Refer to section 9.1.).

7. STUDY VISITS AND PROCEDURES

7.1 Enrollment and Randomization

This research study will be explained in lay terms to each potential research participant. The potential participant will sign an informed consent form before undergoing any study procedures. Participants who are deemed eligible for the study (see sections 4.1 and 4.2) will be enrolled and assigned a unique participant number. Approximately eighteen months are needed for enrollment. The duration of participation for each subject is approximately 6 months. An additional optional blood draw will be offered 2-3.5 years after enrollment.

Eighty eight subjects (22 subjects (ages 25-40) and 66 subjects (ages 60-89)) will be randomized to receive PPV23 or PCV13 in a 1:1 ratio.

7.2 Screening Visit(s)

Refer to 7.3

7.3 Baseline Visit(s)

Interested subjects will call recruiters for a telephone screening where the eligibility criteria of the subject will be reviewed. To confirm that participant has never received pneumococcal vaccine in the past, vaccination records may be requested from participant's physician after obtaining a release of information from the participant. If the subject is found to be eligible and continues to be interested in participating after reading the informed consent (emailed or mailed to him/her), an appointment will be scheduled.

Study staff will review with the subject the study informed consent form and will answer all questions related to the study. Once the subject signs the informed consent, a study participation number will be assigned and subject will be randomized to receive PPV23 or PCV13. The participant will be asked to provide demographic information and information related to his/her medical history including current medications and vaccination history to verify eligibility. The volunteer's vital signs will be recorded and a targeted physical exam will only be performed if needed based on findings during review of health status. For female volunteers of age bearing potential, a urine pregnancy test is obtained. Only females with a

negative urine pregnancy test will be enrolled in the study. Females of child bearing potential will be asked to use effective contraceptive method thirty days before and 30 days after vaccination. Blood for immunological assays will be drawn at this visit. For female participants aged 25-40 blood for immunological assays will be drawn after results of the urine pregnancy test are available and shown to be negative.

The participant will get the pneumococcal Vaccine Information Sheet and then receive 1 dose per label of either PNEUMOVAX®23 or PREVNAR®13 according results of randomization.

Any Adverse Event (including – but not only- vaccine reactions and local or systemic Reactogenicity Events) of grade 3 or higher severity or any SAE occurring after vaccination while the subjects is still at the clinical site will be recorded and reported.

The participant will be provided with a written description of what represents a local and systemic vaccine reaction of mild, moderate and severe intensity (Refer to Appendix 3 and Appendix 4). Subjects will be instructed to notify the study center by telephone if they develop any severe reactions within 1 week following vaccination.

At the end of the visit, the participant will also be instructed to promptly call the site if he/she develops any of the following:

- becomes sick or has been treated at the doctor's office or emergency department or has been hospitalized for any illness throughout D180 of the study;
- develops any adverse event that limits self-care activities of daily living (e.g. bathing, dressing and undressing, feeding self, using the toilet, taking medications, and not bed ridden) even if he/she decides not to get medical care; or
- starts or stops any medications/therapies during enrollment in the study.
- Becomes pregnant.

Refer to section 8.2.5 for safety data that must be recorded and reported.

Note: Subjects calling the site for any of the above will receive further instructions on the proper course of action, including a return to the clinic for immediate evaluation if appropriate.

The Baseline visit will last approximately 90 minutes.

7.4 Main Study Visit(s)

All participants will return for the study-related blood draws on Days 1, 3, 7(±1), 14(±2), 30 (±7) 180 (±14) and year 2-3.5 (optional visit) after immunization**.** These visits will last approximately 20 minutes.

On days 1, 3, 7, 14, 30 and 180 study personnel will review current health status including:

- Evaluation of local REs and systemic REs of grade 3 or higher severity developing after the last visit (only until Day 7 Visit).
- Any adverse event (AE) that limited self-care activities of daily living or serious adverse event (SAE) which may not have been reported by the participant by calling the site as directed at a prior visit.
- Any medications administered after vaccination.

At the end of the visits on days 1, 3 and 7, 14 and 30 the participant will be instructed to promptly call the site if he/she becomes sick or has been treated at the doctor's office or emergency department or has been hospitalized for any illness throughout D180 of the study. The participant will also be instructed to call the site if he/she develops any adverse event that limits self-care activities of daily living even if he/she decides not to get medical care, or starts/stops any medications/therapies.
Refer to section 8.2.5 for safety data that must be recorded and reported.

A focused physical exam will be conducted -if indicated- based on results of the review of health status as above.

At year 2-3.5 visit, participant will be asked about any major change in health status and if they have received any pneumococcal vaccine since last study visit.

7.5 Follow-up Visit(s)

Please refer to 7.4

7.6 Visit Windows

Study visits should take place within the time limits below:

D0, D1, D3, D7 (±1), D14 (±2), D30 (±7), D180 (±14) and year 2-3.5

7.7 Study Procedures

Refer to sections 7.3 and 7.4

7.8 Study Arm Assignment Procedures

7.8.1 Blinding and Randomization

In this study 88 healthy subjects 22 subjects (ages 25-40) and 66 subjects (ages 60-89)) will be randomized to receive PPV23 or PCV13 in a 1:1 ratio.
The randomization of the pre-filled vaccine syringes will be performed by the Emory Investigational Drug Service.

7.8.2 Securing Randomization Information

The information on and randomization is kept at the Emory Investigational Drug Service.

7.8.3 Requirements for Unblinding

HIPC: VAX-003 is an open study. Subjects will be made aware of which vaccine they are receiving at the time of vaccine administration and will be informed about:
1. the need for a second dose of vaccine depending on age at entry and randomization status.
2. the commitment of the investigators to inform subjects if any changes in recommendations for revaccination develop during subject's participation in the study.

7.8.4 Documenting an Unblinding

N/A

8. SAFETY PROCEDURES

8.1 Stopping Rules

8.1.1 Study Stopping Rules

Study enrollment will be suspended pending expedited review of all pertinent data by the institutional review board, the ISM, and the NIAID, if any of the following occurs:

- One unexpected SAE related to the vaccination
- Two unexpected AEs of grade 3 or higher severity of similar nature related to the vaccination
- Study vaccine is recalled by the manufacturer
- Occurrence of a case of Guillain-Barré Syndrome, radiculoneuropathy, anaphylactoid reaction, anaphylaxis or serum sickness considered related to vaccination

8.1.2 Individual Stopping Rules

Individual stopping rules include:

- Failure to receive a full dose of vaccine.

8.1.3 Follow-up after early study termination

Participants who are prematurely terminated from the study due to a related AE/SAE will be followed until resolution of the AE or until 30 days after a participant terminates from the study, whichever comes later.

Note:

Resolution of an AE is defined as the return to baseline status or as stabilization of the condition with the expectation that it will remain chronic.

8.1.4 Participant Replacement

Up to the discretion of the PI, participants receiving concomitant medications may still continue with scheduled study blood draws. Subjects receiving concomitant medications, along with participants with early termination from this study for any reason, may be replaced as needed to preserve the statistical power needed to prove the primary endpoint (Refer to section 9.1).

8.2 Adverse Events

This section defines the types of adverse events and outlines the procedures for appropriately collecting, grading, recording, and reporting them. Information in this section complies with ICH Guideline E2A: Clinical Safety Data Management: Definitions and Standards for Expedited Reporting and ICH E6: Guideline for Good Clinical Practice, and applies the standards set forth in the National Cancer Institute (NCI), Common Terminology Criteria for Adverse Events Version 4.0 [Published: May 28, 2009; revised version 4.03; June 14. 2010)]. These criteria have been reviewed by the study investigators and have been determined appropriate for this study population.

8.2.1 Definitions

8.2.1.1 Adverse Events

An adverse event (AE) is any occurrence or worsening of an undesirable or unintended sign, symptom, laboratory finding, or disease that is experienced during participation in the trial. An AE can therefore be any unfavorable and unintended sign, symptom, or disease temporarily associated with the use of a medicinal (investigational) Study Agent(s)/Intervention(s), whether or not related to the medicinal (investigational) Study Agent(s)/Intervention(s).

8.2.1.2 Adverse Events Associated with Study Vaccines and Study Procedures

The most common adverse reactions after vaccine administration are fully described in Section 1.6.1 .

Adverse events after blood draws may include:

- Fainting /Vasovagal events
- Bruising at puncture site larger than 2 cm diameter
- Bleeding from puncture site lasting more than 30 minutes
- Swelling at puncture site larger than 2 cm

8.2.2. Serious Adverse Event (SAE)

An adverse event or suspected adverse reaction is considered ‘‘serious’’ if, in the view of either the investigator or sponsor, it results in any of the following outcomes:

-Death

- A life-threatening adverse event

- Inpatient hospitalization or prolongation of existing hospitalization

- A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions

-A congenital anomaly/birth defect

Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

Note:

An adverse event or suspected adverse reaction is considered ''life-threatening'' if, in the view of either the investigator or sponsor, its occurrence places the patient or subject at immediate risk of death.

It does not include an adverse event or suspected adverse reaction that, had it occurred in a more severe form, might have caused death.

8.2.3. Unexpected Adverse Event or Adverse Reaction

An adverse event or suspected adverse reaction is considered ''unexpected'' if it is not listed in the product label information of PNEUMOVAX®23 nor PRENAR®13 or is not listed at the specificity or severity that has been observed.

For example, under this definition, hepatic necrosis would be unexpected (by virtue of greater severity) if the investigator brochure referred only to elevated hepatic enzymes or hepatitis. Similarly, cerebral thromboembolism and cerebral vasculitis would be unexpected (by virtue of greater specificity) if the investigator brochure listed only cerebral vascular accidents.

''Unexpected,'' as used in this definition, also refers to adverse events or suspected adverse reactions that are mentioned in the product label information as occurring with a class of vaccine or as anticipated from the pharmacological properties of the product but are not specifically mentioned as occurring with the particular drug under investigation.

8.2.4. Independent Safety Monitor

The ISM is a physician with relevant expertise whose primary responsibility will be to provide independent safety monitoring in a timely fashion and to provide recommendations regarding the safe continuation of the study.

The ISM is a physician independent from the study team and will evaluate adverse events, including SAE, against the known safety profile of the study product to assess for possible changes to the overall risk of the study. Contact information for the ISM and the NIAID Medical Officer is listed in page 3 of this protocol.

The ISM and the NIAID Medical Officer will communicate as needed to discuss any safety events of special interest developing during the study and when conducting the review of periodic reports of cumulative safety data. The study has provisions for a back-up to ensure that independent safety monitoring happens at all times during the study. Additional roles and responsibilities of the ISM are described in sections 8.2.7 and 8.3 below.

8.2.5 Collecting, Recording and Managing Adverse Events

8.2.5.1 Collecting and reporting Adverse Events

Adverse events occurring after the subject has signed the consent will be collected and reported as follows:

- Any Adverse Event (including – but not only- vaccine reactions and local or systemic Reactogenicity Events) of grade 3 or higher severity or SAE occurring after vaccination while the subjects is still at the clinical site.
- Local REs and systemic REs of grade 3 or higher severity occurring from Day 0 to Day 7 reported over the telephone by the participant and/or assessed at the clinic.
- AEs of grade 3 or higher severity throughout D180 of the study (including -but not only – AEs linked to blood draws).
- All SAEs throughout D180.

Adverse events may be identified during this study through any of these methods:

1. Examination of the participant during study visits.
2. Questioning the participant during study visits.

3. Receiving a safety report from the participant by phone or during a site visit at any time during the study

Note: Not all safety events identified may require recording and reporting

A complete recording of safety events in the CRF will include event term, date of onset and resolution/stabilization, assessment of severity, relationship to study vaccine or procedures/intervention(s) such as phlebotomy, expectedness, determination of whether the AE qualifies as serious, treatment required, action taken with study participation and outcome. AEs qualifying as SAEs also require a narrative of the event. Updates in safety events will be recorded as additional information becomes available.

8.2.5.2 Managing Adverse Events

The site investigator must apply his or her clinical judgment as to whether an AE is of sufficient severity to require that the participant immediately be removed from further treatment under the protocol. The investigator must institute any necessary medical therapy to protect a participant from any immediate dangers.

Severe related AEs and all SAEs will be followed until they are resolved.

Participants who are prematurely terminated from the study due to a safety event will be followed until resolution of the AE or until 30 days after a participant terminates from the study, whichever comes later.

Note:

Resolution of an AE is defined as the return to baseline status or as stabilization of the condition with the expectation that it will remain chronic.

8.2.6. Grading and Attribution

8.2.6.1. Grading criteria

Adverse events requiring reporting in this study will be graded according to the criteria set forth in the National Cancer Institute's Common Terminology Criteria for Adverse Events (Version 4.03; June 14, 2010).

Adverse events requiring reporting in this study if not listed in the NCI-CTCAE (Version 4.03; June 14, 2010) will be graded on a scale from 1 to 5 according to the following general guideline in the NCI-CTCAE manual):

Grade 1 Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated.

Grade 2 Moderate; minimal, local or noninvasive intervention indicated; limiting age-appropriate instrumental ADL*.

Grade 3 Severe or medically significant but not immediately life-threatening;
hospitalization or prolongation of hospitalization indicated; disabling;
limiting self-care ADL**.

Grade 4 Life-threatening consequences; urgent intervention indicated.

Grade 5 Death related to AE.

*Instrumental Activities of Daily Living (ADL) refer to preparing meals, shopping for groceries or clothes, using the telephone, managing money, etc.

**Self-care Activities of Daily Living (ADL) refer to bathing, dressing and undressing, feeding self, using the toilet, taking medications, and not bedridden.

Anaphylaxis is a disorder characterized by an acute inflammatory reaction resulting from the release of histamine and histamine-like substances from mast cells, causing a hypersensitivity immune response. Clinically, it presents with breathing difficulty, dizziness, hypotension, cyanosis and loss of consciousness and may lead to death.

Severity grading of anaphylaxis as per the NCI-CTCAE (Version 4.03; June 14, 2010) manual is as follows:

Grade 1=

Grade 2=

Grade 3= Symptomatic bronchospasm, with or without urticaria; parenteral intervention

indicated; allergy-related edema/angioedema; hypotension

Grade 4= Life-threatening consequences; urgent intervention indicated

Grade 5= Death

8.2.6.2. Definition of Attribution

The attribution of an adverse event to the study will be determined by the Principal Investigator or designated physician co/sub-investigator. The Principal Investigator or designee will record the determination of attribution on the appropriate adverse event or serious adverse event form.

For the purpose of this study, the attribution of an AE to PNEUMOVAX®23 or PREVNAR 13® will be determined using the descriptors in the following table:

Attribution of adverse events

Code	Descriptor	Definition (guidelines)
UNRELATED CATEGORY		
1	Unrelated	The adverse event is clearly not related to study. The event is completely related to an etiology other than the study product or study intervention (the alternative etiology must be documented in the study subject's medical record)
RELATED CATEGORIES		
2	Unlikely	The adverse event is doubtfully related to study and likely to be related to factors other than study product or study intervention.
3	Possible	The adverse event may be related to study. There is an association between the event and the administration of study product and there is a plausible mechanism for the event to be related to the study product; there may be also an alternative etiology, such as characteristics of the subject's clinical status and/or underlying disease
4	Probable	The adverse event is likely related to study. There is (1) an association between the event and the administration of study product or study intervention, (2) a plausible mechanism for the event to be related to the study product, and (3) the event could not be reasonably explained by known characteristics of the subject's clinical status and or an alternative etiology is not apparent
5	Definite	The adverse event is clearly related to study. There is (1) an

		association between the event and the administration of the study product or study intervention, (2) a plausible mechanism for the event to be related to the related to the study product, and (3) causes other than the study product have been ruled out and/or the event re-appeared on re-exposure to the study product

8.2.7. SAE Reporting Criteria and Procedures

The Principal Investigator will be notified by the study staff as soon as a staff member becomes aware of the SAE. In the absence of the Principal Investigator, a physician sub-investigator will be notified.

8.2.7.1. Notifying the Independent Safety Monitor and the NIAID Medical Officer of SAEs and pregnancy

The Principal Investigator will notify the ISM and the NIAID Medical Officer by email simultaneously of any related SAE within 24 hours of becoming aware of the event.

The investigator will be informed immediately of any pregnancy and will report all pregnancies within 24 hours to the ISM and the NIAID Medical Officer utilizing the SAE report form. This report is for tracking purposes only. All pregnancies that are identified during the study will be followed to conclusion and the outcome of each will be reported. The investigator will discuss with the participant and/or the treating physician the known possible risks of the investigational product(s) on the fetus. Monitoring of the participant will continue until the conclusion of the pregnancy, and a follow-up SAE report form detailing the outcome of the pregnancy will be submitted to the NIAID Medical Officer and Project Manager. Subject will not be terminated from the study if pregnancy occurred after day 30.

The investigator will also report within 24 hours to the ISM and the NIAID Medical Officer any of the following events:

- Guillain-Barré Syndrome

- Radiculoneuropathy

- Anaphylactoid reactions

-Anaphylaxis

- Serum Sickness

For non-related SAEs, the Principal Investigator will notify the ISM and the NIAID Medical Officer by email simultaneously of any related SAE within 7 days of becoming aware of the event.

The initial SAE CRF should be signed by the PI or co-PI and include as much information as possible. The SAE case report form will re-submitted and signed by the PI or co-PI to the ISM the NIAID Medical Officer with updated relevant medical information as needed until the event is considered closed by the ISM and the NIAID Medical Officer.

The study biostatistician, PI or study data manager will provide the ISM and the NIAID Medical Officer with cumulative periodic safety reports (tables and data listings of all AEs, including SAEs) during the conduct of the study. Resulting memorandums following evaluations of safety data will be sent to the IRB as needed in accordance with IRB rules and regulations.

8.2.7.2. Notifying the FDA

N/A

8.2.7.3. Notifying the Institutional Review Board

The Principal Investigator (or co-PI) will ensure the timely dissemination of SAE information, including SAEs requiring expedited ISM review and resulting memorandums following evaluations of safety data, to the IRB in accordance with IRB regulations and guidelines.

8.3. Protocol Deviations

Deviations occur when the Investigator, site study staff, or participants fail to adhere to protocol requirements or when there is non-adherence to (GCP) as delineated in ICH E6 (R1) guidelines. NIAID will not allow any waivers or planned deviations from the protocol.

Upon determination that a protocol deviation has occurred, the study staff will a) notify the Principal Investigator, b) notify the NIAID Project Manager(refer to investigator's signature

page for contact information) and c) will complete the Protocol Deviation form. NIAID may request discussion with the Principal Investigator and the ISM to determine the effect of the protocol deviation on the study participant and his/her further study participation, the effect of the protocol deviation on the overall study and corrective actions.

The Principal Investigator will complete and sign the Protocol Deviation form and submit it to the NIAID Medical Officer and Project Manager, to the ISM Monitor and to the site IRB, per IRB regulations.

Identification of protocol deviations will be done through the quality manager at the study site, or the NIAID project manager and/or Medical Officer. Reviewing and reporting of minor protocol deviations will be discussed at the Hope Clinic monthly meetings and among the Hope Clinic investigators. Major protocol deviations will be discussed with the PI, the ISM and NIAID study teams and will be promptly communicated to the regulatory agencies as needed.

All study deviations will be captured in monthly reports along with safety data and forwarded to the study sponsor and the ISM.

9. SAMPLE SIZE CALCULATIONS AND STATISTICAL PLAN

9.1. Sample Size and Power Calculations

The study is explorative. Not enough preliminary data exists to support accurate calculation of the sample size. However we conducted a sample size analysis based on hypothetical scenarios and knowledge of similar studies. Based on previous studies performed at the Hope Clinic and the Vaccine Research Trials Center, the rate of subjects that become ineligible during the study (i.e. do not meet exclusion/inclusion criteria anymore, lost to follow-ups, unable to have blood draws) is between 5 to 10%. Assuming 10% attrition rate, 88 subjects will be recruited and 1:1 randomized into the two treatment groups, resulting in an expected 80 subjects for data analysis. The sample size is on-par with similar studies. In the case that the sample size of subjects missing any of the 4 draws required for the primary endpoint [meaning draws at days 1, 3, 7($\pm$1), and 30 ($\pm$7)] is higher than 10% of the 88 subjects to be recruited for the study, we will recruit extra patients to maintain the target sample size of 80 subjects.

The randomization process will be detailed in the MOP. At the center of this study is the identification of generic innate signatures and finding their correlations with the adaptive immune response. The generic innate signatures will be identified from both traditional immune parameters measured by FACS/Luminex assays and array-based gene expression will be measured by microarray experiments. The generic innate signatures will be identified for different treatment groups separately. The traditional immune parameters and array-based gene expression that change after immunization are considered generic innate signatures. The traditional immune parameters and array-based gene expression that are correlated with the adaptive immune responses (day 30 OPA titers, day 30 IgG levels, day 180 serotype specific avidity indexes, plasmablasts also) are considered correlation signatures of the innate immune responses.

Both for sample size consideration and data analysis, we will use different stringency levels for the traditional immune parameters and microarray data, because the traditional immune parameters are measured with less noise, and a traditional immune parameter is more likely than an average gene to be associated with the immune response.

Primary Endpoint 1.

The innate signatures will be detected in each treatment group separately. A two-sided paired paired t-test will be used to test each immune parameter, in order to find immune parameters that change significantly over the baseline. As multiple immune parameters are studied, we use the alpha level of 0.01 to offset multiple testing. With 30 samples in each treatment group in the elderly, we can reject the null hypothesis of equal means with 80% power if the true effect size (mean difference/sigma) is 0.66, using a two-sided paired t-test and the alpha level of 0.01.

For the gene expression, again the innate signatures will be detected in each treatment group separately. The actual data analysis will be performed using the method Significance Analysis of Microarrays (SAM) with paired design. For sample size consideration, we will consider the simpler case of a paired t-test. As the number of genes tested is in the order of tens of thousands, we use the alpha level of 0.0005 to offset the effect of multiple testing. This means an average of one false positive for every 2000 null genes tested, which is an acceptable level as we expect >100 truly differentially expressed genes. In actual data analysis false discovery rate (FDR) will be used. With 30 samples in each elderly treatment group, we can reject the null hypothesis of equal means with 80% power if the true effect size (mean difference/sigma) is 0.88, using a two-sided paired t-test and the alpha level of 0.0005.

Primary Endpoint 3, Secondary Endpoints 2&4.

The correlation signatures will be identified for each treatment group separately. The innate signature markers/genes identified in the analysis of Primary Endpoint 1 will be used in this step for the identification of correlation signature. For the traditional immune parameters, with 30 samples in each elderly treatment group, at the alpha level of 0.01, we can reject the null hypothesis of zero correlation with 80% power if the true correlation is 0.57 using a two-sided test for Pearson's correlation coefficient.

For the gene expression data, the actual data analysis will be performed using SAM with quantitative outcome. For sample size consideration, we will consider the simpler case of testing the significance of Pearson's correlation coefficient. We use the alpha level of 0.005 to offset the effect of multiple testing. This means an average of one false positive for every 200 null genes tested, which is an acceptable level as we expect the number of innate signature genes under study will be in the hundreds. The alpha level used here is different from that in

Primary Aim 1, because the numbers of multiple tests being conducted are different (several hundreds v.s. tens of thousands in Aim 1). With 30 samples in each elderly treatment group, at the alpha level of 0.005, we can reject the null hypothesis of zero correlation with adaptive immune responses with 80% power if the true correlation is 0.60 using a two-sided test for Pearson's correlation coefficient.

9.2. Data Analysis

9.2.1. General considerations

The young treatment groups are of limited size. The main purpose of the young treatment groups is to provide some indication whether the elderly respond to the vaccines differently than the young. By themselves, the young groups do not have sufficient statistical power to generate reliable innate and correlation signatures. However when used in comparison against elderly groups, and by only considering biological pathways (collections of genes contributing to the same biological process) that are affected by the vaccine, they can provide some indication as to how different the response is in the elderly compared to the young. Thus for data analysis, the young treatment groups will be used only in exploratory endpoints. The primary and secondary endpoints will focus on the elderly groups.

The analyses will be based on population with critical time points met, defined as those who received vaccine per label and got the 4 draws required for the primary endpoint, i.e. draws at days 1, 3, 7 *(±1)*, and 30 *(±7)* within the required windows. Data from the study will be analyzed using the statistical software R[31] .High-throughput data will be analyzed using appropriate methods, either by stand-alone software or modules in leading statistical softwares, e.g. the Bioconductor in the R framework[32] .For cytokine data, missing values will be dealt with using multiple imputations. For microarray data, nearest neighbor method and local least squares may be used to fill missing values. Unsupervised learning techniques such as PCA, PLSDA, clustering, and factor analysis [33] will be used for the visualization and identification of global patterns. For descriptive endpoint (primary endpoint 2), we will generate summary statistics, and visualize the data using histograms and boxplots when applicable. For the identification of innate signatures, the two treatment groups will be analyzed separately.

Analyses of primary endpoints:

1. Identification of generic innate immune signatures [Traditional immune parameters measurements + array-based gene expression] at days 1, 3 and 7 post vaccination with either PPV23 or PCV13 in the elderly.

The innate signatures will be detected in each elderly treatment group separately. We will take the measurements on the day of vaccination as baseline. For traditional immune parameters, we will test if an immune parameter shows significant change over baseline at day 1, 3, and 7 after each vaccination, using a two-sided paired t-test. In addition, for each immune parameter, the fold-change over baseline will be calculated for every subject. The average (arithmetic mean) fold change over the 30 subjects will be combined with the test p-value in a selection criterion as appropriate. Based on previous studies similar in nature, the tentative selection criterion is p-value ≤ 0.01 and average fold change ≥ 3.

For the gene expression data, again the innate signatures will be detected in each elderly treatment group separately. We will use the method Significance Analysis of Microarrays (SAM) [34] with paired design to find differentially expressed genes over the baseline. False discovery rate (FDR) will be used as selection criterion. The tentative selection criterion is FDR ≤ 0.1.

2. Measurement of adaptive immune responses [Opsonophagocytosis assay (OPA) titers] at day 30 post vaccination with either PPV23 or PCV13 in the elderly.

The OPA titers will be presented as descriptions of the actual values separately in both study elderly populations (PPV23 and PCV13 recipients). Summary statistics, boxplots and histograms will be used to summarize the data.

3. Correlation of signatures of generic innate immune responses at days 1, 3 and 7 that correlate to adaptive immune responses [Opsonophagocytosis assay OPA titers] at day 30 post vaccination with either PPV23 or PCV13 in the elderly.

The correlation signatures will be detected in each elderly treatment group separately. The innate signature markers/genes identified in Primary Endpoint 1 will be used in this step for the identification of correlation signature.

For traditional immune parameters, we will calculate the correlation coefficient between the immune parameter and the adaptive immune response (day 30 OPA titers). The p-values associated with the correlation coefficients will be used to select traditional immune parameters

that are associated with the respective adaptive immune responses (day 30 OPA titers). The tentative selection criterion is p-value ≤ 0.01.

For gene expression data, we will use the method Significance Analysis of Microarrays (SAM) with quantitative outcome to identify genes that are significantly associated with the respective adaptive immune responses (day 30 OPA titers). False discovery rate (FDR) will be used as selection criterion. The tentative selection criterion is FDR ≤ 0.1.

Analyses of secondary endpoints:

1. Comparison of the serotype specific IgG levels at day 30 post vaccination with either PPV23 or PCV13 in the elderly.

Descriptive analysis, boxpots and histograms, will be conducted to present the data. Testing between the two elderly treatment groups will be conducted using unpaired t-test after proper data transformation. In the case that near-normality cannot be achieved with data transformation, we will resort to the Wilcoxon test.

2. Correlation of signatures of generic innate immune responses at days 1, 3 and 7 with serotype specific IgG levels at day 30 post vaccination with either PPV23 or PCV13 in the elderly.

The analysis will be conducted in each elderly treatment group separately. For traditional immune parameters, we will calculate the correlation coefficient between the immune parameter and the adaptive immune response (IgG levels at day 30 post vaccination). The p-values associated with the correlation coefficients will be used to select traditional immune parameters that are associated with the respective adaptive immune responses (IgG levels at day 30 post vaccination). The tentative selection criterion is p-value ≤ 0.01.

For gene expression data, we will use the method Significance Analysis of Microarrays (SAM) with quantitative outcome to identify genes that are significantly associated with the respective adaptive immune responses (IgG levels at day 30 post vaccination). False discovery rate (FDR) will be used as selection criterion. The tentative selection criterion is FDR ≤ 0.1.

3. Comparison of the serotype-specific avidity indexes in a subset of up to 6 vaccinees per vaccine group at day 180 post vaccination with either PPV23 or PCV13 in the elderly.

Descriptive analysis, boxpots and histograms, will be conducted to present the data. Testing between the two elderly treatment groups will be conducted using unpaired t-test after proper data transformation. In the case that near-normality cannot be achieved with data transformation, we will resort to the Wilcoxon test.

4. Correlation of signatures of generic innate immune responses at days 1, 3 and 7 with serotype specific avidity indexes in a subset of up to 6 vaccinees per vaccine group at day 180 post vaccination with either PPV23 or PCV13 in the elderly.

The analysis will be conducted in each elderly treatment group separately. For traditional immune parameters, we will calculate the correlation coefficient between the immune parameter and the adaptive immune response (serotype specific avidity indexes at day 180 p) the p-values associated with the correlation coefficients will be used to select traditional immune parameters that are associated with the respective adaptive immune responses (serotype specific avidity indexes at day 180). The tentative selection criterion is p-value ≤ 0.01.

For gene expression data, we will use the method Significance Analysis of Microarrays (SAM) with quantitative outcome to identify genes that are significantly associated with the respective adaptive immune responses (serotype specific avidity indexes at day 180). False discovery rate (FDR) will be used as selection criterion. The tentative selection criterion is FDR ≤ 0.1.

5. *Comparison of the adaptive immune responses [Opsonophagocytosis assay (OPA) titers] at day 30 post vaccination with either PPV23 or PCV13 in the elderly.*

Descriptive analysis, boxpots and histograms, will be conducted to present and visually contrast the data. Testing between the two elderly treatment groups will be conducted using unpaired t-test after proper data transformation. In the case that near-normality cannot be achieved with data transformation, we will resort to the Wilcoxon test.

Analyses of exploratory endpoints

1. *Correlation of signatures of generic innate immune responses at days 1, 3 and 7 with adaptive immune responses [Opsonophagocytosis assay OPA titers specifically] at day 30 post vaccination among subjects in different age strata*

First, we will identify innate signatures in each young treatment group, as well as potentially in each age stratum of each elderly treatment group. There are a total of potentially 8 groups: participants between the ages of 25-40 receiving PPV23, participants between the ages of 25-40 receiving PCV13, participants between the ages of 60-69 receiving PPV23, participants between the ages of 60-69 receiving PCV13, participants between the ages of 70-79 receiving PPV23, participants between the ages of 70-79 receiving PCV13, participants between the ages of 80-89

receiving PPV23, participants between the ages of 80-89 receiving PCV13. The analysis will be done using the same method described in the analysis plan of Primary Aim 1.

For correlation analysis, the analysis is similar to primary endpoint 3. Instead of using all the data, we will analyze each age stratum within each treatment group separately.

2. *Comparison of the kinetics and magnitude of select serotype specific plasmablasts (days 7, 14, 30* (with day 30 on a subset of vaccinees only)*) and memory B cells (days 30, 180) post vaccination with either PPV23 or PCV13 in the elderly.*

Summary statistics, boxplots and histograms will be used to summarize and visually contrast the data. We will use t-test or Wilcoxon test to compare each immune parameter (magnitude, kinetics) from D0 to D180 post vaccination between the two treatment groups at the pre-specified time points for serotype specific plasmablasts (days 7, 14, 30 with day 30 on a subset of vaccinees only)) and for memory B cells (days 30, 180).

3. Comparison of the plasmablats repertoire and monoclonal antibodies from plasmablasts in a subset of vaccinees per vaccine group at day 7 post vaccination with either PPV23 or PCV13 in the elderly.

The repertoire and monoclonal antibody levels will be presented as descriptions of the actual values. Summary statistics, boxplots and histograms will be used to summarize the data. Testing between the two treatment groups will be conducted using t-test or Wilcoxon test.

4. Comparison of identified innate signature variables and correlation signature variables between PPV23 and PCV13 recipients

After identifying the innate immune signatures and correlation immune signatures within each elderly treatment group and each young treatment group, there is an interest in comparing the signatures between PPV23 and PCV13 recipients. Here we consider four groups: elderly PPV23, elderly PCV13, young PPV23 and young PCV13. Each group has two signatures – innate signature and correlation signature. For each group, the correlations signature is a subset of the innate signature. For both the innate and the correlation signature, we will make comparisons between:

A-- the two vaccine groups in the same age category: elderly (60-89) receiving PPV23 v.s. elderly (60-89) receiving PCV13 and young (25-40) receiving PPV23 v.s. young (25-40) receiving PCV13),

B-- all the other possible combinations: elderly (60-89) receiving PPV23 v.s. young (25-40) receiving PPV23; elderly (60-89) receiving PPV23 v.s. young (25-40) receiving PCV13; elderly (60-89) receiving PCV13 v.s. young (25-40) receiving PPV23; elderly (60-89) receiving PCV13 v.s. young (25-40) receiving PCV13.

This part of the analysis will depend on the results of the aforementioned analysis and hard to specify at this stage.

As a preliminary consideration, the identified innate signature variables and correlation signature variables can be contrasted between groups using descriptive statistics, such tabulation and Venn diagrams.

5. *Comparison of generic innate immune responses at days 1, 3 and 7 with adaptive immune responses [Opsonophagocytosis assay OPA titers and ELISA] at years 2-3.5 post vaccination with either PPV23 or PCV13.*

For correlation analysis, the analysis is similar to primary endpoint 3. Instead of using all the data, we will analyze data in the elderly.

Enriched biological pathways can be identified using each type of signature in each of the four groups considered above (i.e. elderly (60-89) receiving PPV23, elderly (60-89) receiving PCV13, young (25-40) receiving PPV23 and young (25-40) receiving PCV13), using gene ontology (computer program that generates biological networks from gene lists). Then the enriched pathways can be manually compared between the different treatment groups as per #A and #B above. Biological interpretations of the differences will be made.

9.2.2. Patient populations

For all endpoints, our data analysis will be based on all subjects who are randomized, receive vaccination per label, and for whom there is a blood sample taken at the 2 required time points (Day 0 and Day 30 +/- 7 days) and or Day 0 and Day 180.

9.2.3 Study Participant Baseline Characteristics and Demographics

Summary of descriptive statistics for baseline and demographic characteristics will be provided for all enrolled participants. Demographic data will include age, race, sex, medical history including medication, vaccination history and previous documented Pneumococcal disease will be recorded.

9.2.4 Status of Study Subjects

Status of study subjects (continue in the study vs. left the study and reasons for discontinuation) will be incorporated to the periodic safety reports.

9.3. Interim Analyses

N/A

9.4. Deviations from Statistical Plan

The principal features of the study design and of the plan for statistical analysis of the data are outlined in this protocol. Any changes in these principal features will require a protocol amendment and will be described in the final report. These changes will be subject to review by the IRB, ISM and NIAID.

Although our statistical methods and time-points for measurements will adhere to what is proposed herein, our experience with analyses of similar studies in the past has underscored the need for flexibility and adaptability in trying different statistical approaches to arrive at the most informative results.

As such, we may use alternative approaches such as the gene set enrichment analyses, or other approaches, and run additional statistical analyses of data generated by use of assays at time points other than those stated in the primary, secondary and exploratory endpoints, on an *ad hoc* basis.

10. IDENTIFICATION AND ACCESS TO SOURCE DATA

10.1 Identifying Source Data

The investigator will keep accurate records to ensure that the conduct of the study is fully documented. Data forms are either considered source or protocol-specific CRFs as detailed in the MOPs.

10.2 Updating Source Documentation

Documents describing the safety profile of investigational products, such as the investigator's brochure and the package insert, will be amended as needed by the investigational products manufacturer to ensure that the description of safety information adequately reflects any new clinical findings.

The Principal Investigator (or co-PI) will provide the ISM, the NIAID Medical Officer, and the IRB with the most up-to-date versions of the above documents as soon as the Principal Investigator (or co-PI) becomes aware of any changes. For purchased investigational products, the Principal Investigator (or co-PI) will confirm that there are no changes to the package insert every 3 months. In case of package insert changes, the Principal Investigator (or co-PI) will notify the ISM, the NIAID Medical Officer, and the IRB.

10.3 Permitting Access to Source Data

The investigator will keep accurate records to ensure that the conduct of the study is fully documented. Data forms are either considered source or protocol-specific CRFs as detailed in the MOPs.

The NIAID will provide the ISM and the Principal Investigator with the most up-to-date version of the package insert of PNEUMOVAX®23 AND PREVNAR®13 as soon as it becomes aware of any changes and the Principal Investigator will, in turn inform the IRB.

The investigational site participating in this study will maintain the highest degree of confidentiality for the clinical and research information obtained from the participants in this study. Medical and research records will be maintained at each site in the strictest confidence. However, as a part of the quality assurance and legal responsibilities of an investigation, the investigational site will permit authorized representatives of regulatory authorities and NIAID to examine (and when required by applicable law, to copy) clinical records for the purpose of quality assurance reviews, audits, and evaluations of the study safety and progress. Unless required by the laws that permit copying of records, only the coded identity associated with documents or with other participant data may be copied (and all personally identifying information will be removed). Authorized representatives as noted above are bound to maintain the strict confidentiality of medical and research information that is linked to identify individuals.

11. QUALITY CONTROL AND QUALITY ASSURANCE

The Principal Investigator (or co-PI) will keep accurate records to ensure that the conduct of the study is fully documented. The investigator will ensure that all CRFs and participant study files are legible and complete for every participant.

When the CRFs are complete, they will be reviewed and signed by the Principal Investigator or co-PI. All discrepancies identified by the site monitor or NIAID will be reviewed, and any resulting queries will be resolved with the Principal Investigator (or co-PI) and the CRFs will be amended as needed.

The Principal Investigator (or co-PI), through the use of an internal Quality management Plan and appropriate site quality control and quality assurance monitoring staff will be responsible for the regular review of the conduct of the trial, for verifying adherence to the protocol, and for confirming the completeness, consistency, and accuracy of all documented data and accuracy of source documentation verification. The reports of the internal site monitor will be submitted to the Principal Investigator (or co-PI) and the NIAID Project Manager. NIAID will review these reports.

As per the clinical monitoring plan, site monitoring will be conducted by the independent site monitor in accordance with established Good Clinical Practices (ICH GCP 5.1.1, 5.2, 5.18.1) and the Code of Federal Regulations, as applicable. The overall objectives of site monitoring visits are to ensure:

1. Site compliance with the current version of the approved protocol, consent, documents and local Institutional Review Board requirements.
2. Accuracy and completeness of data entry.
3. Required regulatory documents are current and maintained in the protocol-specific regulatory binder.
4. A designated percent of signed consent forms, inclusion/exclusion criteria, primary, secondary, and tertiary endpoints, safety monitoring parameters, deviations, and serious adverse events are documented.

5. Procedures are in place to administer and monitor study drug / product accountability and documentation of destruction policies.
6. The research staff is adequately trained with respect to GCP and GLP.
7. All observed anomalies or protocol deviations are reported and identify an action plan to minimize study dropouts and non-compliance with defined study procedures.

The results of the site monitoring report will be discussed with the PI, the staff and the NIAID to ensure compliance with the monitor's findings.

12. ETHICAL CONSIDERATIONS AND COMPLIANCE WITH GOOD CLINICAL PRACTICE

12.1 Statement of Compliance

This study was designed to ensure the protection of subjects according to the ethical principles of the Declaration of Helsinki and amendments concerning medical research in human subjects. This clinical study will be conducted using current good clinical practice (cGCP), as delineated in Guidance for Industry: E6 Good Clinical Practice Consolidated Guidance [1], and according to the criteria specified in this study protocol. Before study initiation, the protocol the informed consent documents and the CRFs will be reviewed and approved by NIAID, IRB, as well as any other appropriate health authorities. Any amendments to the protocol or to the consent materials will also be approved by the appropriate bodies listed above prior to implementation.

12.2 Informed Consent and Assent

The informed consent form will provide information about the study to a prospective participant or subject's legal representative to allow for an informed decision about participation in the study. Prospective participant or subject's legal representative must be given ample opportunity to review the informed consent and inquire about the results of the study. All participants (or their legally acceptable representative) must read, sign, and date a consent form prior to study participation. Consent materials for participants who do not speak or read English will be translated into the participants' appropriate language.

The informed consent form will be revised and receive IRB approval whenever important new safety information is available, whenever the protocol is amended, and/or whenever any new information becomes available that may affect participation in the trial.

A copy of the informed consent form will be given to a prospective participant for review. The Principal Investigator or an approved designee will discuss the consent with the prospective participant and answer questions. The prospective participant will be told that being in the trial is voluntary and that he or she may withdraw from the study at any time, for any reason.

12.3 Privacy and Confidentiality

A participant's privacy and confidentiality will be respected throughout the study. Each participant will be assigned a sequential identification number and these numbers rather than names will be used during collection, storage, and reporting of participant information.

13. PUBLICATIONS

Publication of any data from this study must be carried out in accordance with the HIPC publication policy.

14. REFERENCES

1- ICH Expert Working Group. Guidance for Industry: E6 Good Clinical Practice Consolidated Guidance International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use. (1996).

2- Knoll, M.D., *et al.* Standardizing surveillance of pneumococcal disease. *Clin Infect Dis* 48 Suppl 2, S37-48. (2009).

3- Marrie, T.J., Durant, H. & Yates, L. Community-acquired pneumonia requiring hospitalization:5-year prospective study. *Rev Infect Dis* 11, 586-599. (1989).

4- Jackson, L.A. & Janoff, E.N. Pneumococcal vaccination of elderly adults: new paradigms for protection. *Clin Infect Dis* 47, 1328-1338. (2008).

5- Lexau, C.A., *et al.* Changing epidemiology of invasive pneumococcal disease among older adults in the era of pediatric pneumococcal conjugate vaccine. *JAMA* 294, 2043-2051. (2005).

6- Romero-Steiner, S., *et al.* Reduction in functional antibody activity against *Streptococcus pneumoniae* in vaccinated elderly individuals highly correlates with decreased IgG antibody avidity. *Clin Infect Dis* 29, 281-288. (1999).

7- Pilishvili T, Lexau C, Farley MM, Hadler J, Harrison LH, Bennett NM, Reingold A, Thomas A, Schaffner W, Craig AS, Smith PJ, Beall BW, Whitney CG, Moore MR; Active Bacterial Core Surveillance/Emerging Infections Program Network. Sustained reductions in invasive pneumococcal disease in the era of conjugate vaccine. *J Infect Dis* 201(1):32-41. (2010).

8- Khoie T, Tiernan R, deVore N. Prevnar 13™ (PCV13): Pneumococcal 13-valent Conjugate Vaccine (Diphtheria CRM197 Protein). *Applicant: Wyeth Pharmaceuticals Inc. (*2011).

9- Hak E, Grobbee DE, Sanders EA, Verheij TJ, Bolkenbaas M, Huijts SM, Gruber WC, Tansey S, McDonough A, Thoma B, Patterson S, van Alphen AJ, Bonten MJ. Rationale and design of CAPITA: a RCT of 13-valent conjugated pneumococcal vaccine efficacy among older adults. *Neth J Med*.;66(9):378-83. (2008).

10- Querec TD, Akondy RS, Lee EK, Cao W, Nakaya HI, Teuwen D, Pirani A, Gernert K, Deng J, Marzolf B, Kennedy K, Wu H, Bennouna S, Oluoch H, Miller J, Vencio RZ, Mulligan M, Aderem A, Ahmed R, Pulendran B. Systems biology approach predicts immunogenicity of the yellow fever vaccine in humans. *Nat Immunol*.; 10(1):116-25. (2009).

11- Nakaya HI, Wrammert J, Lee EK, Racioppi L, Marie-Kunze S, Haining WN, Means AR, Kasturi SP, Khan N, Li GM, McCausland M, Kanchan V, Kokko KE, Li S, Elbein R, Mehta AK, Aderem A, Subbarao K, Ahmed R, Pulendran B. Systems biology of vaccination for seasonal influenza in humans. *Nat Immunol*. ; 12(8):786-95 (2011).

12- Alonso de Velasco, E., A. F. Verheul, J. Verhoef, and H. Snippe. *Streptococcus pneumoniae*: virulence factors, pathogenesis, and vaccines. *Microbiol. Rev*. 59:591-603. (1995).

13- Antilla, M., M. Voutilainen, V. Jäntti, J. Eskola, and H. Käyhty. 1999. Contribution of serotype-specific IgG concentration, IgG subclasses and relative antibody avidity to opsonophagocytic activity against *Streptococcus pneumoniae*. *Clin. Exp. Immunol*. 118**:**402-407. (1999).

14- Anttila, M., J. Eskola, H. Ahman, and H. Käyhty. Differences in the avidity of antibodies evoked by four different pneumococcal conjugate vaccines in early childhood. *Vaccine*. 17:1970-1977. (1999).

15- Rose, M. A., R. Schubert, N. Strnad, and S. Zielen. Priming of immunological memory by pneumococcal conjugate vaccine in children un responsive to 23-valent polysaccharide pneumococcal vaccine. *Clin. Diagn. Lab Immunol*. 12:1216-1222. (2005).

16- Shapiro, E.D., *et al.* The protective efficacy of polyvalent pneumococcal polysaccharide vaccine. *N Engl J Med* 325, 1453-1460. (1991).

17- Schenkein, J.G., Park, S. & Nahm, M.H. Pneumococcal vaccination in older adults induces antibodies with low opsonic capacity and reduced antibody potency. *Vaccine* 26, 5521-5526. (2008).

18- de Roux A, Schmole-Thoma B, Siber GR, et al. Comparison of pneumococcal conjugate polysaccharide and free polysaccharide vaccines in elderly adults: conjugate vaccine elicits improved antibacterial immune responses and immunological memory. *Clin Infect Dis.*;46(7):1015-1023. (2008).

19- Sen, G., Khan, A.Q., Chen, Q. & Snapper, C.M. In vivo humoral immune responses to isolated pneumococcal polysaccharides are dependent on the presence of associated TLR ligands. *JImmunol* 175, 3084-3091. (2005).

20-Centers for Disease Control and Prevention (CDC); Advisory Committee on Immunization Practices. Updated recommendations for prevention of invasive pneumococcal disease among adults using the 23-valent pneumococcal polysaccharide vaccine (PPSV23). *MMWR Morb Mortal Wkly Rep*.; 59(34):1102-6. (2010).

21-Licensure of 13-Valent Pneumococcal Conjugate Vaccine for Adults Aged 50 years and Older. *MMWR Morb Mortal Wkly Rep*: 61 (21): 395-5. (2012).

21a- Smit et al. Protective efficacy of pneumococcal polysaccharide vaccines. *JAMA* ; 238 (24): 2613-16. (1977).

22- PNEUMOVAX®23 package insert. March, 2013.

23- PREVNAR®13 package insert. January, 2013.

23a- Cordonnier et al. Randomized study of early versus late immunization with pneumococcal conjugate vaccine after allogeneic stem cell transplantation. *Clin Infect Dis*. ;48(10):1392-401. (2009)

23b Crum-Cianflone et al. A randomized clinical trial comparing revaccination with pneumococcal conjugate vaccine to polysaccharide vaccine among HIV-infected adults. *J Infect Dis*.; 202(7):1114-25 (2010)

24-Torling J, Hedlund J, Konradsen HB, Ortqvist A. Revaccination with the 23-valent pneumococcal polysaccharide vaccine in middle-aged and elderly persons previously treated for pneumonia. *Vaccine*; 22:96–103. (2003).

25- Ortqvist A, Hedlund J, Burman LA, Elbel E, Höfer M, Leinonen M, Lindblad I, Sundelöf B, Kalin M. Randomised trial of 23-valent pneumococcal capsular polysaccharide vaccine in prevention of pneumonia in middle-aged and elderly people. Swedish Pneumococcal Vaccination Study Group. *Lancet.* ;351(9100):399-403.(1998).

26-Ortqvist, A., I. Henckaerts, J. Hedlund, and J. Poolman. Non-response to specific serotypes likely cause for failure to 23-valent pneumococcal polysaccharide vaccine in the elderly. *Vaccine* 25:2445-2450. (2007).

27- Manoff, S., C. Liss, M.J. Caufield, R. Marchese, J. Sibbler, J. Boslego, S. Romero-Steiner, G. Rajam, N. Glass, C. G. Whitney, G. M. Carlone and the Pneumococcal Revaccination Study Group. Revaccination with a 23-valent pneumococcal polysaccharide vaccine induces elevated and persistent functional antibody responses in adults $\geq$65 years of age. *J Infect Diseas*. 201:525-533. (2009).

28- de Roux, A., B. Schmöle-Thoma, G.R. Siber, J.G. Hackell, A. Kuhnke, N. Ahlers, S.A. Baker, A. Razmpour, E.A .Emini, P.D. Fernsten, W.C. Gruber, S. Lockhart, O. Burkhardt, T. Welte, and H.M. Lode. Comparison of pneumococcal conjugate polysaccharide and free polysaccharide vaccines in elderly adults: conjugate vaccine elicits improved antibacterial immune responses and immunological memory. *Clin. Infect. Dis*. 46:1015-23. (2008).

29- Jackson, L.A., K.M. Neuzil, C.G. Whitney, P. Starkovich, M. Dunstan, O.Yu, J.C. Nelson, D.R. Feikin, D.K. Shay, J. Baggs, B. Carste, M.H. Nahm, and G. Carlone. Safety of varying dosages of 7-valent pneumococcal protein conjugate vaccine in seniors previously vaccinated with 23-valent pneumococcal polysaccharide vaccine. *Vaccine*. 23:3697-703. (2005).

30- Lazarus R, Clutterbuck E, Yu LM, et al. A randomized study comparing combined pneumococcal conjugate and polysaccharide vaccination schedules in adults. *Clin Infect Dis*.52(6):736-742. (2011).

31- Development Core Team: R: A Language and Environment for Statistical Computing. (2007).

32-Gentleman RC, Carey VJ, Bates DM, Bolstad B, Dettling M, Dudoit S, Ellis B, Gautier L, Ge Y, Gentry J et al: Bioconductor: open software development for computational biology and bioinformatics. *Genome Biol*, 5 :R80. (2004).

33- Hastie T, Tibshirani R, Friedman J: The Elements of Statistical Learning: Data Mining, Inference, and Prediction. New York: Springer. (2009).

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

Appendix 1. Schedule of Events

Day or Year	0	1	3	7 +/-1	14 +/-2	30 +/-7	180 +/- 14	2-3.5
Visit	1	2	3	4	5	6	7	8
Informed consent & HIPAA	X							
Demographic and Medical History (including medication and vaccine history).	X							
Verify eligibility	X							
PID assignment	X							
Vital signs	X							
Focused physical exam (only if indicated based on review of health status)	X	X	X	X	X	X	X	X
Randomization	X							
Vaccination*	X							
Urine Pregnancy Test *****	X							
Record additional pneumococcal vaccines given outside study								X
** Instruction given to study participants on immediate notification of safety events and concomitant medications.	X	X	X	X	X	X		
Assessment of health status *** and concomitant medications after administration of study vaccine		X	X	X	X	X	X	X*****
Blood draw for Innate Assays (microarray, FACS, Luminex) and Adaptive Assays (Plasmablasts, monoclonal Ab, memory B cells, serotype specific ELISA IgG levels, avidity assay, OPA) ****	X	X	X	X	X	X	X	X

Footnotes:

* Any Adverse Event (including – but not only- vaccine reactions and local or systemic Reactogenicity Events) of grade 3 or higher severity or serious adverse event (SAE) occurring after vaccination while the subjects is still at the clinical site will be recorded and reported.

** Instruction includes the following:

1. Participants will be provided (on Day 0 only) with a written description of what represents a local and systemic vaccine reaction of mild, moderate and severe intensity and instructed to call the site to report reactogencity events of grade 3 (severe) or higher severity within 1 week following vaccination.

2. Participants will also be instructed to promptly call the site if he/she develops any of the following:

- Becomes sick or has been treated at the doctor's office or emergency department or has been hospitalized for any illness throughout D180 of the study;
- Develops any adverse event that limits self-care activities of daily living (e.g. bathing, dressing and undressing, feeding self, using the toilet, taking medications, and not bed ridden) even if he/she decides not to get medical care; or
- he/she starts/stops medications during enrollment in the study.
- Becomes pregnant.

*** Assessment of health status includes:

- Evaluation of local REs and systemic REs of grade 3 or higher severity developing after the last visit (only until Day 7 Visit)
- Any adverse event (AE) that limited self-care activities of daily living or any serious adverse event (SAE) which may not have been reported by the participant by calling the site as directed at a prior visit
- Any medications administered after vaccination.

**** Innate assays will not be conducted at days 30 and 180. Adaptive assays will not be conducted at day 1.

***** Eligible female subjects aged 25-40 years will use effective contraception 1 month prior and one month following vaccination.

***** Only for major health problems

Appendix 2. Definitions

"Generic innate immune signatures"=traditional immune parameters measurements + array-based gene expression, which change in response to vaccination.

« Traditional immune parameters » =FACS + Luminex.

« FACS »= Fluorescence Activated Cell Sorting, a method for sorting a heterogeneous mixture of biological cells.

« Luminex »= multiplex analysis technique for measuring of cytokines and chemokines

"Array-based gene expression" =microarray.

"Adaptive immune responses" = OPA + avidity indexes+ ELISA IgG+ studies on plasmablasts.

"Correlation innate immune signatures" =traditional immune parameters measurements + array-based gene expression, which correlate with adaptive immune responses.

"Gene ontology": a set of standard terminology that group gene products based on their biological functions.

Appendix 3. Severity Scale for Local Vaccine Reactions (local reactogenicity events)

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

Note:
Instrumental Activities of Daily Living refer to preparing meals, shopping for groceries or clothes, using the telephone, managing money, etc.
Self-care Activities of Daily Living refer to bathing, dressing and undressing, feeding self, using the toilet, taking medications, and not bedridden.

Appendix 4. Severity Scale for Generalized Vaccine Reactions (systemic reactogenicity events)

	GENERAL ADVERSE REACTIONS		
	Grade		
	1	**2**	**3**
Fatigue/asthenia	Fatigue relieved by rest	Fatigue not relieved by rest; limiting instrumental activities of daily living	Fatigue not relieved by rest, limiting self-care activities of daily living
Body ache/myalgia	Mild pain	Moderate pain; limiting instrumental activities of daily living	Severe pain; limiting self-care ADL
Headache	Mild pain	Moderate pain; limiting instrumental activities of daily living	Severe pain; limiting self-care activities of daily living
Joint pain	Mild pain	Moderate pain; limiting instrumental activities of daily living	Severe pain; limiting self-care activities of daily living
Loss of appetite	Loss of appetite without alteration in eating habits	Oral intake altered without significant weight loss or malnutrition; oral nutritional supplements indicated	Associated with significant weight loss or malnutrition (e.g., inadequate oral caloric and/or fluid intake); tube feeding or intravenous nutrition indicated

Note:
Instrumental Activities of Daily Living refer to preparing meals, shopping for groceries or clothes, using the telephone, managing money, etc.
Self-care Activities of Daily Living refer to bathing, dressing and undressing, feeding self, using the toilet, taking medications, and not bedridden.