Protocol

Title: Genomics and Epigenomics of the Elderly Response to Pneumococcal Vaccines

NCT number: NCT03104075

Document Date: July 09, 2019

Genomics and Epigenomics of the Elderly Response to Pneumococcal Vaccines

Clinical Protocol

Principal Investigator Clinical Protocol:
George Kuchel, MD
Director, UConn Center on Aging
UConn Health, Farmington, CT

Co-Investigator Clinical Protocol, Principal Investigator Laboratory Analysis
Jacques Banchereau, Ph.D.
Director, Immunological Sciences
The Jackson Laboratory for Genomic Medicine
Farmington, CT

Co-Investigator Clinical Protocol, Laboratory Analysis
Duygu Ucar, Ph.D.
Assistant Professor
The Jackson Laboratory for Genomic Medicine
Farmington, CT

Sponsor: **The Jackson Laboratory**

Funding agency: **National Institute On Aging of the National Institutes of Health under Award Number R01AG052608.**

Key Personnel:
Lisa Kenyon-Pesce, MPH
Study & Recruitment Coordinator
UConn Center on Aging

TABLE OF CONTENTS

Page

PRÉCIS

Study Title: ***Genomics and Epigenomics of the Elderly Response to Pneumococcal Vaccines***

Specific Aims

To complete a 40 subject clinical study with the following aims:

1. To vaccinate healthy elderly with two distinct pneumococcal vaccines, collect longitudinal blood samples and assess pneumococcal-specific antibody responses.

2. To establish the transcriptional and epigenetic profiles of elderly blood immune cells linked with antibody responses to pneumococcal vaccination.

3. To examine the functional status of immune cells in the elderly stratified according to their pneumococcal vaccine responder status.

Design and Outcomes

This is a prospective, single-site, randomized, then open-label study designed to develop a detailed transcriptional and epigenetic profile of the immune response to pneumococcal vaccination with conjugated and non-conjugated polysaccharide vaccines in the senescent immune system of older adults.

Sample Size, Population, Interventions and Duration

In this study, 40 healthy adults ages 60 and older that have never received pneumococcal vaccination, will be randomized in a 1:1 ratio to receive Prevnar-13 (Pfizer), a conjugated 13-valent vaccine or Pneumovax 23 (Merck), a non-conjugated 23-valent vaccine. Following randomized assignment of vaccine, the study will be open-label.

Six (6) study visits will occur over about 70 days, with an optional 7th visit for participants to receive a second vaccination with the other pneumococcal vaccine one to two years after randomization. Participants will provide blood samples for transcriptional, epigenetic and biological analyses pre- and post-vaccination.

1 OBJECTIVES

The goal of our project is to assess the alterations to immune blood cells associated with the response of healthy elderly subjects to two pneumococcal vaccines, unconjugated (PPSV23) and conjugated (PCV13) polysaccharide vaccines. APCs, Tfh and B-cell subsets will be analyzed at different time points after vaccination. Phenotype, function, transcriptome and epigenetic analyses will be carried out on selected high responders and non-responders. These studies would provide us with the unique opportunity to investigate the human immune response of elderly individuals—a primary target for these vaccines—to a TI and TD antigen. Furthermore, a detailed

assessment of circulating immune cells from healthy elderly could enable us to identify the immune deficits that underpin non-responsiveness; such insights could help to improve vaccine efficacy.

Our detailed analyses will include: i) *RNAseq* of whole blood and cell subsets, to generate profiles of both coding RNAs and ncRNAs [1]; ii) *ATACseq,* to assess chromatin state and determine whether the genome is poised towards specific immune responses; and iii) *in vitro cellular immunology* studies to determine the function of APCs, T cells and B cells in vaccine-induced immune responses. We will keep all samples to further assess genotype using high-density SNP chips. Note that this study is not designed to establish baseline phenotypes predictive of vaccine responsiveness.

We expect to find that PPSV23 and PCV13 will activate different pathways leading to pneumococcal-specific antibody responses. Our hypothesis is that the PCV13 response pattern will resemble the response pattern we observed with Fluzone® inasmuch as both vaccines are considered to be TD. Significant differences in responsiveness can, however, be brought by molecular components specific to each vaccine, i.e., the polysaccharide component of PCV13 or the viral nucleic acids that might be included in Fluzone®. Our preliminary data *in vivo* in young adult volunteers and *in vitro* with APC subsets suggest that PPSV23 might trigger different immune pathways to elicit specific antibody responses. While the consensus is that its effects are TI, our observed induction of activated T cells *in vivo* and the presence of mutated IgG and IgA hints to more complex mechanisms, which might include both TD and TI mechanisms of B-cell activation [2].

1.1 Specific Aim 1

To vaccinate healthy elderly with two distinct pneumococcal vaccines, collect longitudinal blood samples and assess pneumococcal-specific antibody responses.

Hypothesis: We hypothesize that PPSV23 and PCV13 differentially activate the circulating immune cells and the blood transcriptome of healthy elderly individuals.

Rationale: Our studies in healthy young adults showed that Fluzone® and pneumococcal vaccines activate different immune cell subsets *in vivo* (data not shown). In addition, we showed that individuals vaccinated with PPSV23 and Fluzone® display a different blood transcriptome at day 1 (**Fig. 1**). Thus, different vaccines might induce the generation of specific antibodies through different mechanisms. Here, our goal is to generate profiles of responses to conjugated and unconjugated polysaccharide-based pneumococcal vaccines and to correlate the elicited immune profiles with antibody responses.

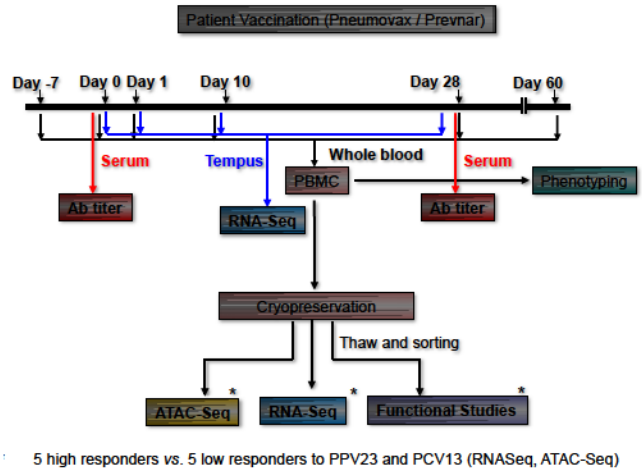

Figure 1. Overview of work.

Approach: The overall flow of the work is illustrated in **Fig. 1.** We will examine whether the induction of pneumococcus

serotype–specific antibodies at day 28 correlates with the activation of T cells (as measured by flow cytometry) at day 10 and the activation of APCs at day 1 (measured by flow cytometry and transcriptomics). In a cohort of 40 healthy elderly naïve to pneumococcal vaccination we will: 1) vaccinate with either PPSV23 or PCV13; 2) longitudinally collect blood, serum and blood cell samples from each group; 3) perform the experiments that require immediate processing, such as flow cytometry analysis; and 4) cryopreserve all the other samples for downstream genomics and cellular immunology studies. Our earlier studies permitted us to draw robust conclusions on the transcriptome profiles in blood cells and on the role of Tfh activation in the generation of Fluzone®-specific antibody responses.

Elderly Populations: We will focus on the healthy adults >60 years of age that meet eligibility criteria (Section 4). 40 healthy community–dwelling elderly from areas surrounding the University of Connecticut Health Center will be accrued. Volunteers will be randomized to receive either the PCV13 or PPSV23 vaccine. CDC currently recommends that healthy older adults with no history of previous pneumococcal vaccination receive PCV13 followed by PPSV23 at least 12 months later. All participants will be offered a boost dose with the other vaccine at one to two years later, at no cost to them.

Rationale for selected time points: We will collect blood (50 ml) at days –7, 1, 10, 28 and 60 and (10 mL) on day 0 prior to vaccination with PPSV23 or PCV13 (**Table 1**).

Table 1. Sample processing details

Day		-7	0	1	10	28	60
Blood Volume		50 ml	10 ml	50 ml	50 ml	50 ml	50 ml
Ab titers			X			X	
Opsonophagocytic Assay			X			X	
Flow cytometry		X	X	X	X	X	X
CBC		X				X	
Cell sorting		X		X		X	X
ATAC-Seq	PBMC	X	X	X	X	X	X
	Monocytes	X		X			
	CD4 T cells	X			X	X	
	B cells	X			X	X	
	CD1c DCs	X		X			
RNA-Seq	Whole blood	X	X	X	X	X	X
	PBMC	X	X	X	X	X	X
	Monocytes	X		X			
	CD4 T cells	X			X	X	
	B cells	X			X	X	
	CD1c DCs	X		X			
In vitro studies	Tfh						X
	B cells						X

Baseline (Day -7 and 0): Blood will be collected on these dates to assess baseline immune status. We will only collect 10ml of blood on day 0 so as to avoid collecting nearly 100ml on two consecutive days. **Day 1** coincides with the peak of the innate response to vaccines in blood. We found that PPSV23 induces a transcriptional signature reflecting the activation of myeloid compartment. We will establish whether this transcriptomic signature is observed in elderly cohorts. **Day 10** is when T-cell responses (including Tfh cells) peak. This time point allows us to determine whether PPSV23/PCV13 also activates a Tfh subset in elderly and whether a lack of Tfh activation correlates with a lack of anti-pneumococcal antibody responsiveness as we showed with Fluzone®. The activation of CD4+ Tfh subsets will be measured as a function of ICOS expression levels in conjunction with CXCR5, CCR6 and CXCR3. In addition, a portion of the blood samples collected at day 10 will be used for transcriptome analysis. **Day 28 (± 3 days)** reflects the peak of the circulating antibody response. We will assess the induction of serotype-specific anti-pneumococcal antibody titers at days 28. **Day 60 (± 5 days)** will provide us with additional blood samples containing memory cells that can be used to eventually confirm the day 28 *in vitro* data. Details of sample processing are given in **Table 1**. We will cryopreserve PBMCs

and sorted cell populations for genetic and cellular immunology experiments at all time points. If cell yield prevents cryopreservation of PBMCs, we will recruit additional subjects to reach our proposed number of 40.

Antibody responses: We will analyze the breadth and magnitude of antibody responses by assessing PPSV23/PCV13 serotype-specific IgG titers at day 0 and 28, as recommended by the World Health Organization, and using the Opsonophagocytic assay. This assay (serotypes 4, 6B, 14 and 23F) will be performed using serotype–specific *S.pneumoniae* incubated with serial-diluted heat-inactivated sera [3]. Newborn rabbit serum is added as a source of complement and differentiated HL-60 cells are added. Sera are tested in duplicate and results obtained using the Opsotiter 1 software. We will use U-scores to rank antibody response by titer, affinity and number of serotypes (as done for antigen-specific T cells in response to DC vaccines [4-6]. Our collaborator, Dr Nahm, will assess serotype-specific anti-polysaccharide responses. His lab will also measure antibody avidity and opsonizing capacity as described [7].

CMV status. Influenza vaccine studies in the elderly have revealed a critical role for CMV status [8]. In a single study, CMV status did not appear to influence antibody responses to PPSV23 vaccine in older adults, yet its role in other aspects of immune responses or in baseline pre-vaccination status cannot be ruled out[9]. As a result, we will obtain CMV status using antibody titers and PCR-based technologies [10], and will include this variable in our analytic plan.

Analytical Approaches:

1. *Multicolor flow cytometry:* 0.5 ml of blood will be used to determine the profile of circulating immune cells. We designed five multi-color monoclonal antibody cocktails that will permit us to establish the detailed phenotype of T-cell subsets, monocytes, DCs and B-cell subsets. We included a set of antibodies to identify NKT cells, as these cells have been reported to contribute to responses to PPSV23 vaccination [11].
2. *Cryopreservation of isolated PBMCs and sorting of purified populations:* We have validated monoclonal antibody cocktails that permit us to sort T-cell subsets, monocytes, DCs and B cells for cryopreservation **(Table 1).** PBMCs will be frozen in 4–5 aliquots (recovering on average $20x10^6$ cells); 1–2 will be used for ATAC-seq and 2–3 for functional studies. After thawing we will sort monocytes, CD1cDCs, CD45RO+CD4+ T cells, and B cells for ATAC-seq and RNA-seq. We aim to collect 15,000–25,000 of each type of cells.
3. *Cryopreservation of whole blood RNA:* Two Tempus tubes (1 ml each) will be stored for further analysis of coding RNA and ncRNAs.
4. *Sample prioritization*: Our preliminary data show that we can perform ATAC-seq on cryopreserved cells. Previous studies [12] show that T/B-cell cultures can be done with cryopreserved and thawed cell samples. If we encounter low cell yields, which might limit the cell culture experiments, we can draw from the blood samples acquired at day 60.

1.2 Specific Aim 2

To establish the transcriptional and epigenetic profiles of elderly blood immune cells linked with antibody responses to pneumococcal vaccination.

Hypothesis: We hypothesize that transcriptome and epigenome profiling of blood and sorted

immune cells will reveal the coding and non-coding regulatory sites associated with different immune responses following vaccination with PPSV23 and PCV13 in healthy elderly.

Rationale: The non-coding regions of the genome contain a variety of RNA species, such as microRNAs (miRNAs) and long non–coding RNAs (lncRNAs), which play fundamental roles in regulating gene expression [1, 13, 14]. The study of these regulatory RNAs is becoming increasingly relevant to the fields of immunology and aging. Recent studies in aging mice and humans reported the up- and downregulation of miRNA expression in PBMCs among other tissues [15, 16], yet little is known about the status of ncRNAs within elderly immune cells, particularly in response to vaccination. In addition to transcriptome differences, it is conceivable that epigenetic remodeling also contribute to decline in the immunity of elderly. Current epigenetic approaches require large numbers of cells, making them impractical for detailed analysis of primary blood–derived human immune cells. In a recent major advance, a transposase-based method to sequence genomes, ATAC-seq, was adapted to interrogate chromatin accessibility. ATAC-seq generates representative chromatin accessibility maps from as few as 500–5,000 cells, making it an ideal approach to investigate genome-wide epigenetic patterns at coding and noncoding regulatory sites in primary human immune cell samples.

To investigate our hypothesis we will apply RNA-seq, rather than DNA microarrays, to generate transcriptional profiles, as this will enable quantitative assessment of both coding RNAs and ncRNAs. We will also use ATAC-seq to resolve the epigenetic landscape of immune cells in healthy elderly in the context of vaccine responses.

Approach:
1. Blood transcriptomics by RNA-seq: We will carry out RNA-seq of whole blood samples at baseline, day 1 and day 10. Samples collected from days 28 and 60 will be kept frozen for future studies. RNA-seq on purified cell subsets will be performed using purified cells from the five lowest and five highest responders for each vaccine. Sequencing will be performed at JAX-GM with an Illumina HiSeq2500.
2. Blood epigenomics analysis by ATAC-seq: We will analyze PBMCs, CD4+ T cells, monocytes, CD1cDCs and B cells ($25x10^3$ cells per assay). We are also developing assays with lower cell numbers to address the scarcity of some populations, and are encouraged by reports of single cell ATAC-seq [17]. We will generate a database describing the DNA accessibility landscape of major immune–cell subsets from elderly individuals at steady state. The data will also include the DNA accessibility landscape of monocytes, T and B cells analyzed at different time points following vaccination with the two pneumococcal vaccines. For these in-depth studies, we will select the five highest and five lowest responders for each vaccine.

Correlative analysis*:* We will correlate the increased levels of day 28 anti-pneumococcal antibodies as defined in Aim 1 with all the parameters collected in this aim. Parameters include: 1) cellular composition at baseline, day 1 and day 10 post-vaccination; 2) transcriptome analysis on blood and purified immune cell subsets at baseline, day 1 and day 10; 3) ATAC-seq data on PBMCs and purified cell subsets at the different time points and 4) pneumococcal colonization at baseline and at day 28. A detailed description of the statistical approaches is given below (see Integrative Data Analysis section).

1.3 Specific Aim 3

To examine the functional status of immune cells in the elderly stratified according to their pneumococcal vaccine responder status.

Hypothesis: We hypothesize that lack of *in vivo* response to PPSV23 and PCV13 depends on alterations to APCs, Tfh cells or B cells.

Rationale: Aging impacts the immune system at steady state as well as its capacity to respond to challenges such as infection or vaccination. In advanced age, there are i) reduced numbers of B cells responding to influenza as well as lower antibody avidity to carbohydrate antigens [18-22] and ii) increased numbers of memory cells occupying tissue niches that is linked with lower migration and maintenance of naïve B and T cells into tissues. These changes, associated with the biased differentiation of CD4+ T-cells into Th17 cells, diminish the overall capacity of CD8+ and CD4+ cells to mount diverse and robust responses to antigens [23, 24]. Nonetheless, major gaps remain in our knowledge of elderly T/B-cell responses. There is also a need to better understand the alterations in function of innate immune cells including DCs and macrophages in advanced age. We propose two sub-aims to further characterize the functions of key immune cell types in the healthy elderly, thereby complementing the genomics and epigenomics data gathered in Aim 2.

Aim 3a: Determine whether a specific immune–cell subset can be linked with the lack of response to pneumococcal vaccines. Here, we will seek to determine whether *ex vivo* responses to vaccines can be related to *in vivo*–derived transcriptional profiles of the blood at steady state and after vaccination as well as to antibody responses. We will analyze PBMCs and sorted monocytes, CD1c+DCs, CD4+ T cells and B cells.

PBMC function*:* We will activate PBMCs from five of each of PPSV23 and PCV13 responders and non-responders with medium alone, PPSV23 or PCV13. PMA plus ionomycin and lipopolysaccharide (LPS) will be used as positive controls. We will measure cell activation by flow cytometry at day 1 and 2. We will measure cytokine production at day 2 (IL-1, IL-6, IL-10, IL-21, TNF and IFNg) post-stimulation. Specific antibody levels will be measured at day 12 as described [3, 20, 25]. Some experiments will be carried out by culturing PBMCs with a few selected polysaccharides from *S. pneumoniae*, such as PP13 and PP23F [18, 20, 21].

Monocyte and CD1c+DC function: we will select baseline monocytes/*CD1c+DCs* from five elderly subjects not responsive to PPSV23 and five not responsive to PCV13 and compare them to monocytes/*CD1c+DCs* from responders. Monocytes/*CD1c+DCs* will be isolated using CD14-beads and cultured in medium, with either of the two pneumococcal vaccines or LPS as an activation control. Before and after stimulation we will:
1) Determine the phenotype of cultured cells by flow cytometry at day 1 post-activation using various markers, including MHC class-II, CD40, 80, 83 and 86.
2) Measure the secretion of cytokines (IL-1, IL-6, TNF and IL-10) by ELISA.
3) Assess the transcriptome of monocytes after six hours of activation using RNA-seq.
4) Analyze monocyte differentiation into either DCs or macrophages following culture with GM-CSF with or without IL-4 [26]. We will measure the differentiation potential of the elderly

monocytes by assessing the phenotype of cultured cells and analyzing their ability to induce a mixed lymphocyte reaction with allogeneic CD4+T cells. We will also measure, after six days of co-culture, the production of cytokines by flow cytometry after activation with PMA and ionomycin [27-29]. Cytokines to be measured include IL-21 and CXCL-13, which are specifically secreted by Tfh cells; IFN-g (Th1); IL-4 (Th2) and IL-17 (Th17). CD1c+DC function will be measured as described for monocytes [30, 31].

B-cell function*:* B cells will be isolated from PBMCs by magnetic sorting and will then be cultured with the vaccines alone or together with SAC (i.e., fixed *Staphylococcus aureus* strain Cowan) and IL-2 [32, 33]. Alternatively we will use a combination of CD40-L and either IL-10 or IL-21, which results in potent cell proliferation and differentiation into plasma cells [34]. Ig secretion will be measured at day 12 by ELISA. Differentiation into plasma cells will be assessed by loss of CD20 and acquisition of CD138 [35].

Aim 3B: Determine whether the CD4+T cells can induce B cells to differentiate into plasma cells secreting anti-pneumococcal antibodies *in vitro*.
For these studies, we will use day 28 and day 60 cells from vaccine responders versus non-responders.

Assess antigen specificity of T-cell subsets: We will use cultures of purified CD4+ T-cells and B cells as we described. Briefly, purified CD4+T cells will be cultured with either of the two vaccines or a mixture of the polysaccharides (ATCC) for which the elderly showed an antibody response. For the PCV13 cohort we will also test the reactivity to protein carrier alone (CRM 197 from LBL). T-cell activation with selected antigens can be measured by induction of CD154 after six hours of culture in the presence of monensin and brefeldin[12]. Cytokine expression will be assessed by flow cytometry after intracytoplasmic staining with anti-IFNg, IL-2, IL-10, IL-17 and IL-21 as described. A second set of experiments will be carried out with separated CXCR5+CD4+CD45RO+ T cells (Tfh cell) or CXCR5-CD4+CD45RO+ T cells (non-Tfh cells).

Assess T-cell subset helper activity in B cells: To analyze the ability of T cells to activate B cells, we will use a dual approach as designed for Fluzone®-vaccinated individuals. Purified T–cell subsets ($5X10^3$ per well) will be cultured with sorted naïve (IgD+CD27-CD3-) or memory (CD27+CD3-CD19+) B cells ($5X10^3$ per well) in the presence of 1ug/ml staphylococcal enterotoxin B to test polyclonal B-cell activation and differentiation. Experiments will also be carried out with purified IgM+ memory B cells. B-cell activation will be monitored by induced expression of CD138 and CD38 after 14 days of culture. Culture supernatants will be harvested at day 14 for measurement of IgM, IgG and IgA by ELISA. For antigen-specific responses, memory B cells will be loaded with the relevant antigen and cultured for six days with the Tfh and non-Tfh CD4+ T-cell subsets as shown earlier. Supernatants will be harvested at day 14 and assessed for anti-pneumococcal polysaccharide antibodies by ELISA.

2 BACKGROUND, RATIONALE AND SIGNIFICANCE

2.1 Background and rationale

Aging, pneumococcal vaccines and S. pneumoniae infection: The declining ability of the aging immune system to combat infection is a major threat to the health, independence and survival of older adults [36-39]. Cellular immunosenescence associated with a hyper-inflammatory state has been linked to many diseases common in the elderly, including infectious diseases [36, 40, 41]. Beyond evidence of poor immune-cell responses with declines in naïve T cells, we know little about the mechanisms of immunosenescence [41-43].

Pneumococcus infection is one common condition in the elderly for which a better understanding of immunosenescence and immune responses to vaccines is urgently needed [8, 44-50]. The polysaccharide pneumococcal vaccine PPSV23 has long remained the only vaccine recommended for prevention of pneumococcal infection in healthy adults >65 and children and adults 2 to 64 years of age with certain health conditions. However, its efficacy was felt to be poor, especially against non-bacteremic pneumonia and in advanced age. Although young and old subjects might demonstrate similar increases in antibody levels post-PPSV23 immunization, the functional activity of these antibodies is lower in the elderly. In contrast, the 13-valent pneumococcal conjugate PCV13 has superior immunogenicity against some serotypes. The CAPiTA trial [51], a randomized placebo-controlled trial of PCV13 involving nearly 85,000 Dutch individuals ≥65 years, indicates that PCV13 has 75% efficacy against invasive pneumococcal infections caused by PCV13 serotypes and 45% efficacy against non-bacteremic variants. In contrast, evaluation of a national program of PPSV23 vaccination in Taiwan [52] in individuals 75 years and older revealed that vaccination in the previous year was associated with a 60% reduction in pneumonia hospitalization, a 76% reduction in invasive pneumococcal disease and >90% reduction in related death in this vulnerable population. In spite of these striking findings, no definitive conclusions can be drawn with regards to the comparative efficacy of the two vaccines, since in both studies PCV13 or PPSV23 were each compared to placebo and not each other. The 2015 CDC Guidelines recommend, for healthy older adults (≥ 65 years) who have not received a pneumococcal vaccine, that PCV13 be administered first followed by PPSV23 ≥ 1 year later, and for those that have received PPSV23, a dose of PCV13 ≥ 1 year later [53]. Thus, the two vaccines may remain complementary to each other, with benefits dependent on which serotypes are most responsible for community-acquired pneumonia in a given population, but many questions as to the nature of (and variability in) vaccine responsiveness remain unanswered.

Even within the landscape of broad late-life vulnerability to infectious diseases, pneumococcal infection assumes an overwhelmingly important role [54]. Thus, preventive strategies such as vaccination remain crucial. It is known that T- and B-cell receptor repertoires become more skewed during aging, and likely contribute to the limited specific immune responses to vaccines or new infections [44]. However, we lack fundamental insight into the transcriptional and epigenetic signatures that could be predictive of immunosenescence and reduced vaccine responsiveness at the personalized level—and could enable us to identify and target individuals most at risk of complications from poor responsiveness to pneumococcal and potentially other vaccines.

Immune responses to vaccination are complex and depend on the coordinated actions of specific B- and T-cell subpopulations. B-cell responses are classified as T-cell-dependent (TD) or T-cell-independent (TI) based on the requirement for T-cell help in antibody production [55]. TD antigens are processed then presented by MHC class-II molecules for recognition by cognate helper T cells. Mouse studies show that B1 cells and splenic marginal zone B cells play major roles in the TI response [56]. In contrast, the human counterpart of mouse B1 cells, the IgM+ memory B cells, remains the object of some controversy [57-61].

Several studies have identified the importance of pneumococcal polysaccharide (PS)-IgM antibodies in generating protective immunity against *S. pneumoniae* [62]. These antibodies are in part produced by human IgM memory B cells [7, 32, 33]. This population of IgM+ memory B cells is reduced in the elderly, splenectomized persons, infants less than two years of age and a subgroup of Common variable–immunodeficiency patients—all of whom respond poorly to PS vaccines and are susceptible to infections with encapsulated microbes [63]. These findings support the concept that IgM+ memory B cells are important in generating responses to TI antigens. It is however unlikely that IgM+ memory B cells are solely responsible for anti-PS antibody production. Indeed, switched memory B cells (CD27+IgM-) secrete higher levels of anti-PS antibody than CD27+IgM+ memory cells following *in vitro* stimulation. This might be due to additional PS-responsive B-cell subsets and/or the versatile role that IgM memory cells play in pneumococcal antibody responses.

Follicular helper T cells (Tfh) play a critical role in the generation of high-affinity memory B cells [12, 64-69]. The memory Tfh cell compartment in human blood is composed of subsets that differentially express the chemokine receptors CXCR3 and CCR6 and display different functions [67]. Recently, we demonstrated a direct correlation between activation of Flu antigen–specific Tfh1 responses and the generation of antibody responses upon vaccination [12](see Preliminary Studies). However, how Tfh cells regulate antibody responses to pneumococcal vaccines in elderly is unknown.

Systems biology of immunity and vaccine responsiveness in humans: We [70] and others [71-76] used systems biology approaches to investigate immune responses to vaccines. We found that young adults mount distinct responses to Flu (Fluzone®) and pneumococcal (PPSV23) vaccines (see Preliminary Studies). Our results provide proof-of-principle that we can detect global immune responses elicited by different vaccines and suggest that comparative analyses of these differences will be critical for understanding the immune mechanisms underpinning successful vaccination. Here, we propose to apply these and other novel immunogenomic approaches to further understand how protective immunity against pneumococcal antigens is established in healthy elderly using two pneumococcal vaccines.

PRELIMINARY STUDIES

I) IMMUNOGENOMICS OF PNEUMOCOCCAL VACCINATION.

Different vaccines elicit distinct transcriptional profiles in blood cells. To investigate the innate and adaptive immune responses to Flu (Fluzone®) and pneumococcal (PPSV23) vaccines, we performed transcriptional profiling of whole blood using DNA microarrays. These studies revealed significant differences in the quality and magnitude of transcriptional responses at different time points after each vaccination. Specifically, we found that influenza vaccination elicited type-I interferon signaling signatures, while PPSV23 vaccination was associated with an acute inflammatory response signature linked with myeloid cells. The day 7 plasmablast response

induced by both vaccines was more pronounced after PPSV23 vaccination. These findings provide proof-of-principle that a transcriptomics approach can be used to identify distinct global immune responses elicited by different vaccines.

Decoding innate responses to PPSV23 vaccine in vitro. In an effort to decipher the mechanisms leading to blood signatures *in vivo*, we assessed the transcriptional profiles of various antigen-presenting cells (APCs) exposed to vaccines *in vitro* [77]. Monocytes, *in vitro*–derived IL-4 dendritic cells (DCs), blood CD1c+ DCs and CD141+ DCs from 4–5 donors were stimulated with vaccines for six hours. Their transcriptional fingerprints yielded 23,060 transcripts. A modular analysis, initially developed to characterize the blood transcriptome [78-81], identified 42 differentially expressed modules, forming four groups of transcriptionally active conditions. We then analyzed 22 modules induced by Fluzone®, PPSV23 or human papilloma virus (HPV) vaccine in IL-4 DCs, monocytes or CD1c+ DCs**.** Importantly for the proposed studies, PPSV23 induced unique modular signatures in monocytes, including IL-1, NF*kB* and type II IFN–related modules. We will further develop these analyses and determine the mechanisms underlying differential responses of APCs to PPSV23 and PCV13. APCs from elderly individuals, responding or not to PPSV23 or PCV13, will be compared to determine whether non-responsiveness can be linked with specific APC alterations.

II) ADAPTIVE IMMUNE RESPONSES TO VACCINATION. To begin to define the cell types that comprise adaptive immune responses to vaccination, we performed detailed phenotypic analyses of T-cell subsets taken from healthy young adults vaccinated or not with Flu or PPSV23 vaccine. We found that vaccination induced ICOS expression almost exclusively on blood $CXCR3^+$ Tfh cells and that the induced $ICOS^+CXCR3^+$ Tfh cell population was enriched with cells specific for Flu antigens. The increase in $ICOS^+CXCR3^+$ Tfh cells in blood at day seven post-vaccination correlated with an increase in antibody titers at Day 28. Isolated $ICOS^+CXCR3^+$ Tfh cells induced antigen-loaded memory B cells to produce Flu-specific Abs *in vitro* through secretion of IL-10 and IL-21. Thus, the analysis of blood Tfh subsets led us to discover biomarkers reflecting vaccine efficacy and provided insights into vaccine mode of action. Further studies in healthy young adults vaccinated with PPSV23 indicated 1) a plasmablast response on days 7 and 10 and 2) the emergence of ICOS+ Tfh cells in blood peaking at day 10. However, the status of Tfh cells in elderly has not been well characterized. We will address this gap by profiling the Tfh cells of healthy elderly vaccinated with either PPSV23 or PCV13.

III) IMMUNOGENOMICS OF BLOOD CELLS FROM OLDER ADULTS. We recently launched a systematic analysis of the status of immune cells in the blood of elderly subjects (age 65 and older; mean=75.3 yrs). Our approach included 1) assessment of cell composition by polychromatic flow cytometry; 2) transcriptional profiling by RNA-seq and 3) assessment of epigenetic landscape of PBMCs as well as sorted subsets by ATAC-seq (Assay for Transposase Accessible Chromatin or ATAC-Seq)[82]. ATAC-seq captures open (i.e., transcriptionally active) chromatin sites and can be used to reveal various categories of epigenetic information, including the genomic locations of open and closed chromatin regions and their interplay with DNA-binding proteins, as well as of individual nucleosomes. Importantly, ATAC-seq generates information *via* a simple two-step protocol that requires only a few thousand cells, making it uniquely suited to study epigenomic profiles of human clinical samples with a systems biology approach, where small cell numbers are a limiting factor.

Reproducibility of ATAC-seq datasets: To determine the feasibility of ATAC-seq for studying the epigenome of immune cells in the elderly, we conducted a pilot study in PBMCs (50,000 cells per experiment) of ten healthy young (20–30 yrs) and ten healthy old (>65 yrs) individuals. Each sample was run in triplicate to confirm reproducibility. ATAC-seq datasets were also generated in FACS-sorted B cells and in naïve and memory CD4+ and CD8+ T cells. We identified ATAC-seq peaks, i.e., open chromatin sites, in each of these PBMC samples. First, we confirmed that our identified ATAC-seq peaks are consistent with PBMC histone modification mark profiles generated by the Roadmap consortium, where ATAC-seq peaks are enriched in active marks (H3K9ac, H3K36me3, H3K4me1) and depleted in inactive marks (H3K9me3 and H3K27me3) (data not shown). Next, we calculated pairwise correlations between ATAC-seq datasets based on read count distribution across the whole genome and clustered the resulting correlation matrix (using hierarchical clustering algorithm) to identify similarities between ATAC-seq datasets globally.

Our analyses showed that ATAC-seq datasets are reproducible and capture cell-type-specific epigenetic landscapes. We found a good match between observed and expected open chromatin sites around cell-type-specific genes, e.g., the CD14 gene promoter had an open chromatin site in CD14+ monocytes (data not shown). We also identified cell-type-specific open chromatin sites in CD4+ and CD8+ cells by comparing memory and naïve ATAC-seq samples. We found that cell-type-specific open sites lose their specificity with aging both in CD4 and CD8 cells, with the change being more dramatic in CD8+ T cells. This preliminary study confirms that our ATAC-seq data generation and analysis workflow yields highly reproducible and high-quality ATAC-seq datasets from PBMCs and sorted cell populations.

ATAC-seq identifies age-associated epigenetic changes in open chromatin sites: In our next set of ATAC-seq analyses, we sought to identify open chromatin sites remodeled with aging in PBMCs, i.e., open chromatin sites closing with age ("young specific") or relatively closed chromatin sites opening with age ("old specific"). To do this we used an algorithm designed to capture differential sites from read count data based on negative binomial distributions; this allowed us to capture a total of ~20,000 differentially open chromatin sites (at 0.05 FDR cut-off, out of 100,000 consensus ATAC-seq peaks) between age groups, where ~11,000 were young specific and ~9000 old specific.

Among these differential sites, we found a remarkable genomic distribution bias between old-specific and young-specific open chromatin sites, whereby young-specific sites were mostly at promoters, whereas old-specific sites were enriched in intergenic and intronic regions. We confirmed that these differentially open chromatin sites separate age groups (i.e., elderly and young PBMCs) using the first two components of a principal component analysis (PCA) (data not shown). Among these differential open chromatin sites, we were further intrigued to find age-dependent closure of chromatin sites at the IL-7R promoter. This decline in chromatin accessibility correlated with a significant decrease in IL7R expression, as measured by RNA-seq. We also confirmed by flow cytometry that IL7R expression is decreased in elderly CD8+ T cells (data not shown). Moreover, among our "young-specific" differentially open chromatin sites, we identified ~60 promoter and intergenic ATAC-seq peaks targeting other genes in the IL7 signaling pathway, which is in agreement with the literature, as alterations in lymphocyte homeostasis related to the IL7/IL7R pathway have been reported in old mice and humans [83-87]. These analyses reveal that the epigenome of the IL7R signaling pathway significantly closes with aging, thereby

uncovering a putative mechanism to explain the decreased ability of elderly individuals to deal with new antigenic challenges. They also further validate our approach using ATAC-seq to identify age-dependent differences in the epigenomes of healthy individuals. Here, we will extend these findings to determine whether vaccine responders and non-responders show differential expression and differential epigenetic profiles of immune-related molecular pathways. We will also analyze whether responders, in contrast to non-responders, reactivate their IL-7R pathways.

Modular interpretation of age-associated epigenetic changes. To systematically interpret the biological meaning of the remodeled open chromatin sites identified via ATAC-seq, we employed the transcriptional immune module analysis described in Fig. 1B, composed of gene sets that are coordinately expressed in PBMCs in a wide range of diseases (28 modules in total from 239 microarray profiles) [79-81, 88]. We developed and used this stable modular framework extensively to interpret immune signatures associated with diverse diseases and immune responses. We first identified putative (i.e., nearest) gene targets for ATAC-seq open chromatin sites. For each module, we then calculated the average log-fold change of read counts per gene between old and young samples for genes imputed to differential open chromatin sites. We next isolated modules whose chromatin landscape significantly changes with age, identifying 12 modules that are differentially open in samples from young individuals, including a module of T-cell regulators, and five modules that are differentially open in samples from elderly, including modules associated with neutrophil and platelet function and inflammation. We also investigated whether we can capture differences in module profiles of individual epigenomes when compared to population averages (i.e., all samples).

We observed systematic and distinct differences in the relative openness of certain modules in samples from elderly versus young individuals, e.g., neutrophils vs. T-cell modules, which helps us to interpret the regulatory implications of epigenetic changes. Module-based functional analysis revealed that i) young-specific open chromatin sites mostly occur around promoters and are associated with modules related to T cell functions and ribosomal proteins; and ii) old-specific open chromatin sites mostly occur at intronic/intergenic sites and are associated with modules related to inflammation and neutrophil and platelet activity. Our modular analyses also helped us to identify outlier samples, including an old individual with a chromatin profile similar to samples from young individuals and vice versa. Modular analysis of immune epigenomes thus reinforced and enhanced our ATAC-seq analyses to provide greater insights into the immunogenomic profiles of elderly individuals. Here, it will be used to interpret epigenetic remodeling changes induced by vaccination in the elderly and to study whether responders and non-responders have different modular profiles before and after vaccination.

ATAC-seq datasets can be obtained from frozen samples. We next performed ATAC-seq on fresh and cryopreserved PBMCs from two different individuals. Our analyses revealed that fresh and frozen samples yield very similar genome-wide read-count profiles (data not shown), with only 44 peaks out of 75,365 total consensus open chromatin sites being different. These results confirm that ATAC-seq profiles can be obtained from frozen samples. This will enable us to retrospectively generate ATAC-seq datasets after vaccination responses are quantified, thereby significantly reducing experimental costs.

Differentially expressed non-coding RNAs in aging. Aging is associated with a complex transcriptional signature that includes changes to ncRNA profiles in addition to coding genes [89-96]. ncRNAs include small transcripts such as microRNAs and lncRNAs [1]. Although many

ncRNAs have been identified, only a few have been fully characterized in terms of their regulatory roles in important cellular processes, including aging. To determine whether ncRNAs are remodeled in elderly human blood, we used limma software to identify differentially expressed ncRNAs between old (n=8) and young (n=10) RNA-seq PBMC samples ($p < 0.05$). This analysis revealed 24 ncRNAs differentially expressed in aging. We will expand our preliminary datasets and analyses to analyze the expression of ncRNAs in aging and determine their relationship to the vaccine response status.

2.2 Significance

Pneumonia due to *Streptococcus pneumoniae* infection is a serious public health challenge among elderly populations. *S. pneumoniae* is the leading cause of community-acquired and in-hospital pneumonia in the United States (US) and globally, and a major cause of morbidity and mortality in the elderly. According to the CDC, an estimated 400,000 hospitalizations from pneumococcal pneumonia occur annually in the US; the case-fatality rate is 5–7% and may be >50% among elderly persons. Although pneumococcus-specific vaccines exist, elderly individuals display reduced responses for reasons that are as yet unclear, leaving many in this group highly vulnerable to infection and the consequences thereof. This project focuses on understanding the immune alterations associated with aging that affect responses to the two available *S. pneumoniae* vaccines, which differ respectively in composition and elicited host immune response. A better understanding of how elderly populations respond to these vaccines could be leveraged to improve overall efficacy and protection in elderly individuals—an outcome of significant public health relevance. Specifically, the proposed study would enable the identification of targets that could be modified to reactivate specific immune cells and/or pathways to improve vaccine response rates. Furthermore, the data generated would represent an important resource for future studies of elderly individuals with chronic diseases.

The declining ability of the aging immune system to combat infection is a major threat to the health, independence and survival of older adults (1-4). Cellular immunosenescence associated with a hyper-inflammatory state has been linked to many diseases common in the elderly, including infectious diseases (1, 5, 6). Beyond evidence of poor immune-cell responses with declines in naïve T cells, we know little about the mechanisms of immunosenescence (6-8).

3 STUDY DESIGN

This prospective, single-site, randomized, then open-label study is designed to develop a detailed transcriptional and epigenetic profile of the immune response to pneumococcal vaccination with conjugated and non-conjugated polysaccharide vaccines in the senescent immune system of older adults. This knowledge may lead to development of more effective vaccines through increased understanding of the effects of immunosenescence on mechanisms of immune response to pneumococcal vaccination in the elderly.

Forty (40) healthy adults ages 60 and older that have never received pneumococcal vaccination, will be randomized in a 1:1 ratio to receive Prevnar-13 (Pfizer), a conjugated 13-valent vaccine or Pneumovax 23 (Merck), a non-conjugated 23-valent vaccine. Following randomized assignment of vaccine, the study will be open-label.

The first six (6) study visits are planned to occur over 67 days at Days -7, 0, 1, 10, 28 (±3 d) and 60 (± 5d). Participants will provide blood samples for transcriptional, epigenetic and biological analyses pre- and post-vaccination.

One to two years after receiving the randomly-assigned vaccination, participants may opt to receive administration of a second pneumococcal vaccine with the vaccine that they did not receive by random assignment at Visit 2 (Day 0). This second vaccine will be provided at no charge to the participant. Administration of this vaccine will occur at an optional Visit 7 for participants who choose to receive the second vaccine and will be scheduled at the participant's convenience one-two years after receiving the first pneumococcal vaccine.

If the participant opts to receive the second vaccine within the study and attends optional Visit 7, blood samples for genomic and biologic analysis will be collected at the visit.

3.1 Characteristics of the Study Population

Number:	40
Age Range:	60 and older
Health Status:	Healthy, as defined by eligibility criteria Have never received pneumococcal vaccination
Duration of Participation:	6 visits* over approximately 70 days

*1 optional additional visit at 1-2 years for vaccination with the other vaccine not provided at Visit 2 (Pneumovax 23 / Prevnar 13, Prevnar 13/ Pneumovax 23). Optional Visit 7 to be scheduled at the convenience of the participant within 1-2 years for second vaccination with the other vaccine at no cost to the participant and for blood sample collection.

3.2 Sampling Plan

The study sample will be drawn from the population of healthy elderly in the catchment area of UConn Health in Farmington, CT.

4 SELECTION AND ENROLLMENT OF PARTICIPANTS

Forty (40) healthy men and women, ages 60 and older, from all ethnic backgrounds, that have never received pneumococcal vaccination will be recruited to this study over a two-year period. The National Health Interview Survey 2013 found that nationally, 40% of adults ages 65 and older have not received pneumococcal vaccination (1), which supports successful recruitment in this population. Recruiting participants aged 60 and up should enhance our recruitment efforts to ensure recruitment goals are met.

Dr. George Kuchel, the Principal Investigator of the clinical protocol, will utilize UConn Center on Aging recruitment resources for this project. The UConn Center on Aging Geriatric Recruitment & Community Outreach Core provides a centralized infrastructure for the recruitment of older research volunteers from the community. Over more than a decade Lisa Kenyon-Pesce, MPH, has managed this core. Such recruitment expertise, together with a

26,000-name database has permitted the successful completion of nearly 100 clinical studies involving older adults. Furthermore, under Dr. Kuchel's leadership, the UConn Center on Aging has an established track record of recruiting more than 145 young and older adults each year for ongoing NIH-funded studies (PO1AG021600, R01AG048023) of the immune response to influenza vaccination in the elderly. Other recruitment methods will include recruitment flyers placed in local clinics, letters mailed to the Center on Aging database, use of broadcast email in the UConn Health system, and newspaper advertisements. Retention will be encouraged by planning visits at convenient times for the participants and by providing financial compensation for study participation.

Vulnerable Populations

Vulnerable populations will not be enrolled in this research study.

Collaborating Sites

All recruitment, enrollment, clinical data and sample collection will occur at the UConn Center on Aging located on the UConn Health campus in Farmington, CT under the direction of Clinical PI Dr. George Kuchel.

All transcriptional, epigenetic and biological analysis of samples will occur at the Jackson Laboratory for Genomic Medicine under the direction of Laboratory Analysis PI, Jacques Banchereau, Ph.D.

4.1 Inclusion Criteria

Participants must meet all of the following inclusion criteria to participate in this study:

- Able and willing to provide written informed consent
- Male or Female, 60 years of age or older
- Willing to receive pneumococcal vaccination with Prevnar 13 (Wyeth/ Pfizer) or Pneumovax 23 (Merck), as randomly assigned.
- Available to attend 6 study visits over 67 days (Visit 7 is optional at Day 365-720).

4.2 Exclusion Criteria

Candidates meeting any of the following criteria will be excluded from study participation:

- Previous pneumococcal vaccination with Prevnar 13 or Pneumovax 23.
- History of anaphylactic/anaphylactoid or severe allergic reaction to any component of Pneumovax 23, Prevnar 13 or any diphtheria toxoid-containing vaccine.
- Established diagnosis of diabetes
- History of receiving Zostavax (shingles vaccine) within previous 4 weeks. (Study entry may be delayed to satisfy a 28-day interval between vaccinations)
- Known history of any of the following co-morbid conditions:
  - Malignancy (participants without a recurrence in the last 5 years will be allowed)
  - Congestive Heart Failure
  - Cardiovascular Disease (unstable ≤ 6 months*)
  - Kidney disease

- Renal failure
- Impaired hepatic function
- Autoimmune disease such as: Rheumatoid Arthritis, systemic lupus erythematosus (SLE), Inflammatory Bowel Disease, etc.
- Use of medicines during past 6 months known to alter immune response such as high-dose corticosteroids
- HIV, AIDS or other Immunodeficiency
- Recent (≤ 3 months) trauma or surgery
- Current substance and/or alcohol abuse

* Unstable disease is defined as a change in therapy or hospitalization for worsening disease.

4.3 Study Enrollment Procedures

Forty (40) healthy men and women, ages 60 and older, from all ethnic backgrounds, that have never received pneumococcal vaccination will be recruited to this study over a two-year period.

Recruitment letters will be prepared, approved by IRB and mailed to potentially eligible individuals from the UConn Center on Aging recruitment database that describe the study and provide contact information to obtain more information from a member of the study team. All persons listed in the recruitment database agreed to be contacted about future research projects during prior interactions with the Center on Aging clinical research team. Other recruitment methods may include recruitment flyers placed in local clinics, use of broadcast email in the UConn Health system, newpaper/print advertisements, outreach to collaborating research clinics in urban areas or through physician referrals to the study.

Interested persons will phone the study team in response to the letter, broadcast email, flyer, referral or other advertisement to complete an IRB-approved preliminary screening questionnaire that asks general and aggregated questions to determine eligibility while minimizing collection of Protected Health Information. Progression through the screening questionnaire will stop when a response is given that renders the caller ineligible. Demographic information (age, gender, race, and ethnicity) will be collected for all callers for transparent reporting of participant selection, but individual identifiers and contact information will only be collected after the preliminary screening questionnaire has been successfully completed, indicating preliminary eligibility. A copy of the IRB approved Informed Consent Form (ICF) will be provided to potential subjects for review prior to the informed consent/screening visit, whenever possible. The ICF may be provided by fax, email, interoffice mail, hand delivery or by US Mail. The participant will then be scheduled for Visit 1, when the informed consent process will be initiated and the ICF signed before full screening or any other study procedures are performed.

5 STUDY INTERVENTIONS

5.1 Interventions

For 2015, the CDC revised recommendations for pneumococcal vaccination of adults age 65 and older that have never received pneumococcal vaccination, to include one dose of PPSV23 (Pneumovax 23) and one dose of PCV13 (Prevnar 13). Their recommendation is to administer PCV13 first followed by PPSV23 ≥ 1 year later[53]. Although pneumococcal vaccine is recommended for healthy adults to receive at age 65 or older, it is routinely given to children and

adults 2 to 64 years of age, with various chronic disease. Subjects who elect to participate in this study would be receiving the vaccination as a healthy adult, at an earlier age.

While PCV13 demonstrated efficacy in reducing incidence of community acquired pneumonia (CAP) due to serotypes of *S. pneumoniae* included in the vaccine, it did not demonstrate efficacy in community acquired pneumonia from any cause with reported vaccine efficacy of 5.1%, 95% CI, (−5.1 to 14.2) (2). PPSV23 includes 11 serotypes that are not included in PCV13 and elicits immune response by a different mechanism than the conjugated vaccine. Since reduced response to pneumococcal vaccination in patients age 65 and older limits the benefits of vaccination, it is critical to develop additional understanding of aging-related immunosenescence to find ways to increase response to vaccination in this population. Each vaccine has potential benefits and limitations. PCV13 and PPSV23 have not been compared directly for effectiveness in reducing incidence or severity of CAP through a randomized, controlled clinical trial (3). Studies to date have used antibody response as outcome measures rather than clinical efficacy and have focused on the 12 serotypes of *S. pneumoniae* that PPSV23 and PCV13 share. This study looks to develop a deeper understanding of the mechanisms of diminished response to both vaccines in the elderly.

To elucidate transcriptional and epigenetic profiles of the mechanisms of response to each vaccine in the elderly, enrolled participants will be randomly assigned to receive pneumococcal vaccination with **Prevnar-13 (PCV13) or Pneumovax-23 (PPSV23).** These vaccines are FDA approved for administration in this population and will be administered by a Registered Nurse at the dose and by route (intramuscular injection), as approved.

Randomized assignment to group will be in a 1:1 ratio utilizing block randomization in blocks of 10. A Randomization Plan will be prepared by the Data Manager at the UConn Center for Aging using Randomization software. Randomization assignments will be revealed to the research nurse when the subject meets eligibility for randomization and is assigned the next available sequential randomization number. Participants will receive the vaccine that was randomly assigned to their sequential randomization number in the study and will be offered a boost dose with the non-assigned vaccine one to two years later.

Participants will be informed of the vaccine that was administered and will be provided with information about the vaccine as provided by the manufacturer. Pneumovax 23 has an FDA-approved Patient Product Information Sheet that will be provided to participants receiving Pneumovax 23. Participants that receive Prevnar 13 will receive a Fact Sheet about the vaccine prepared by Pfizer. Each participant will also be given a pneumococcal vaccine fact sheet from CDC for the vaccine administered.

Summary of Prescribing Information for Prevnar 13 and Pneumovax 23 (4, 5) :

	Prevnar-13	**Pneumovax-23**
Dose	Adults 18 years and older: a single dose 0.5 mL dose.	Adults 50 Single 0.5-mL dose
Route of Administration	Intramuscular injection	Intramuscular injection
Indications	In adults 18 years of age and older, Prevnar 13 is indicated for active immunization for the prevention of pneumonia and invasive disease caused by *S. pneumoniae* serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F and 23F.	Pneumovax 23 is approved for use in persons 50 years of age or older. Pneumovax 23 is indicated for active immunization for the prevention of pneumococcal disease caused by the 23 *S. pneumoniae* serotypes contained in the vaccine (1, 2, 3, 4, 5, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19F, 19A, 20, 22F, 23F, and 33F)
Contraindications	Severe allergic reaction (e.g., anaphylaxis) to any component of Prevnar 13 or any diphtheria toxoid-containing vaccine.	Severe allergic reaction (e.g., anaphylaxis) to any component of Pneumovax 23.
Limitations of Use and Effectiveness	Prevnar 13 does not protect against disease caused by *S. pneumoniae* serotypes that are not in the vaccine.	Pneumovax 23 will not prevent disease caused by capsular types of *S. pneumoniae*other than those contained in the vaccine.
Source	FDA- approved Prescribing Information, Prevnar 13	FDA- approved Prescribing Information, Pneumovax 23

Vaccine Administration

Both Prevnar-13 and Pneumovax 23 will be administered at the dose, by the route and in the population currently approved for marketing by FDA, therefore administered consistent with routine clinical care. Vaccine will be administered by a Registered Nurse using best practices and will be administered by intramuscular injection in the deltoid. Participants will be required

to remain in the clinic for 30 minutes after vaccine administration to be monitored for life-threatening allergic reactions. The nurse will discharge the participant after 30 minutes has elapsed.

5.2 Handling of Study Interventions

The FDA-approved vaccines to be administered in this study, Pneumovax-23 and Prevnar-13, will be ordered and received by the Research Pharmacist at UConn Health, per University policy. Once the study has IRB approval and is ready to initiate, the research pharmacist will dispense all vaccine doses to the UConn Center for Aging research team where they will be stored during the active study period. The UConn Center on Aging Clinical Research unit has a medication refrigerator in which vaccines will be stored until use. Temperature logs will be kept for the refrigerator 24 hours per day, 7 days per week, using an electronic temperature monitoring device that records temperature every 15 minutes. Logs will be downloaded weekly during the study period with a copy of the log added to the study regulatory binder.

Vaccinations will be given by an RN at Visit 2. Randomization and Vaccination Forms will be used by the study team to record the sequential randomization number assigned to each participant when eligibility was confirmed, the participant ID (PID) for the study, the vaccine that was administered, expiration date of the dose, date of administration and the signature of the nurse that administered the vaccine. The sticker from the dose packaging will be added to the form.

Participants will be informed of the vaccine that was administered to them and will be provided with information about the vaccine as provided by the manufacturer. Pneumovax 23 has an FDA- approved Patient Product Information Sheet that will be provided to participants receiving Pneumovax 23. Participants that receive Prevnar 13 will receive a Fact Sheet about the vaccine prepared by Pfizer (Appendix 1). Participants will also receive a handout from CDC about the vaccine that was administered.

Each participant will be given a Documentation of Pneumococcal Vaccine Administration as a record of their vaccination to share with their physician.

-to next page for study schema-

6 STUDY PROCEDURES

6.1 Schedule of Events

Intervention	**Day -7** **Visit 1**	**Day 0** **Visit 2**	**Day 1** **Visit 3**	**Day 10 (± 1 d)** **Visit 4**	**Day 28 (± 3 d)** **Visit 5**	**Day 60 (± 5 d)** **Visit 6**	**Day 365-730 Optional** **Visit 7**
Informed Consent **Prior to study activities**	x						
Screening Form	x						x
Vital Signs (blood pressure, heart rate, temperature)	x	x	x	x	x	x	x
Body weight	x				x	x	x
Height	x						
Medical History	x						
Medical History update					x	x	x
Concomitant Medications	x				x	x	x
Adverse Events	x	x	x	x	x	x	x
Peripheral blood specimen (50 mL)	x		x	x	x	x	x
Peripheral blood specimen (10 mL) (pre-vaccination)		x					
Vaccination with Prevnar 13 or Pneumovax 23 by randomized assignment		x					
Vaccination with Prevnar 13 or Pneumovax 23, Other than received at V1							x

(optional)							

6.2 Description of Study Visits

Visit 1: Day -7. Approximate visit time = 1 hour.

Consent Procedure

At the conclusion of the preliminary phone screening, study personnel briefly explain the study and provide potential subjects a copy of the approved ICF for review, in advance, whenever possible. Subjects will be encouraged to discuss study participation with family members and/or trusted advisors. At Visit 1, the ICF will be reviewed with the subject section by section by a qualified member of the study team in a private area. Subjects will be given the opportunity to ask questions and to have them fully answered. Subjects who elect to enroll will sign and date the consent form. The member of the study team conducting the informed consent discussion will also sign the ICF. A copy of the ICF signed by the consenter and the subject will be provided to the subject. This process will be documented by the Documentation of Consent Form that will be stored in the research record under a unique participant identifier (PID). The original signed ICF will be stored separately from the research record and with other study documents that contain personal identifiers (HIPAA Authorization, W-9, or other).

The HIPAA Authorization form will be provided to the participant to review and sign to authorize the use and disclosure of their Protected Health Information collected for use in this study.

Enrollment

- The participant is considered enrolled and receives a Participant ID (PID) number in the study once the ICF has been signed.

Screening

After informed consent, the following study activities will be performed:

- Medical History Form
- Recording of Concomitant Medications
- Taking and recording of body temperature (°F) oral or ear
- Screening Form completed with subject by interview
- Once all inclusion and exclusion criteria have been reviewed and the subject has been determined to have met all eligibility criteria, the subject proceeds to randomization.

Randomization

- If the subject does not meet all eligibility criteria during the screening process, the subject is not randomized to vaccine and is not assigned a randomization number.
- If study entry is deferred to meet a 28 day interval from receipt of Zostavax, blood will not be drawn at this time. Visit 1 procedures (other than Consent) will be rescheduled and performed when the 28 day interval between vaccinations has been met.
- Once the subject has met all eligibility criteria, they are assigned the next available sequential randomization number on the Randomization Log. The PID is entered in

the space next to the number on the Randomization Log and the Randomization number is entered on the Randomization and Vaccination Form for this PID to be used at Visit 2.

- Visit 2 is scheduled 7 days from Visit 1. There is no window for sample collections in this study.

Baseline measures

- Vital signs (blood pressure, heart rate, and respiration rate), weight and height are recorded.
- **Blood draw of 50 mL:** Blood will be collected peripherally by phlebotomy by qualified research staff using best practices. The tubes will be labeled with PID, Visit number and date of collection. Fifty (50) mL will be drawn and securely transferred to JAX-GM for processing and analysis. Three (3) mL will be sent by JAX-GM to Quest Diagnostics Laboratory or to another JAX campus for a complete blood count (CBC) with differential testing for research purposes only. The results of the CBC blood test will be coded with the PID number only and will not be placed in medical records. Results will be sent back to Jackson Laboratory for use in research testing. Results will not be shared with research participants, however, if the study doctor feels that blood sample shows abnormalities during research testing, he may contact participant and advise them to follow-up with their personal physician.

Visit 2: Day 0: Vaccination Visit. Approximate visit time = 15-30 minutes.

At Visit 2, the following procedures will be performed:

Body Temperature and Blood Draw

- Taking and recording of body temperature °F (oral or ear)
  If the participant has a body temperature of 100.5°F (38° C) or greater, Visit 2 will be deferred and rescheduled for a minimum of 3 days in the future.

  A deferral of vaccination due to fever will not require an additional Visit 1 blood draw 7 days in advance of vaccination as long as vaccination occurs within 14 days of the original Visit 1 blood draw.

  Participants with a temperature below 100.5°F will proceed to blood draw.
- **Blood draw of 10 mL:** Blood will be collected peripherally by phlebotomy by qualified research staff prior to vaccination. The tube will be labeled with PID, Visit number and date of collection and will be securely transferred to JAX-GM for processing and analysis.
- The research nurse reviews the Randomization and Vaccination form and identifies the assigned vaccine from the Randomization Plan for the randomization number.
- The subject is informed of the randomized vaccine assignment and given information about the vaccine.

Data Collection

- Adverse events

- Concomitant medication use

Vaccination

- The research nurse obtains one dose of the assigned vaccine from the refrigerator and prepares the dose per the package insert.
- The vaccine dose label (sticker) is placed in the designated area of the Visit 2 Source Document Form.
- The area of the deltoid where the vaccine will be injected is wiped with alcohol
- The vaccine is injected intramuscularly in the deltoid by the RN.

The participant must remain at the clinic for 30 minutes after vaccine administration to be monitored for life-threatening allergic reactions. Visits are scheduled for study visits 3-6. There is no visit window for follow-up visits. Visits must be performed on the day indicated. The nurse will discharge the participant after 30 minutes has elapsed.

Visit 3: Day 1: Follow-up visit. Approximate visit time = 15 minutes.

Weight, Blood pressure, heart rate, temperature: taken and recorded on source document.

Data Collection

- Adverse events
- Concomitant medication use

Blood Draw

- **Blood draw of 50 mL**: Blood will be collected peripherally by phlebotomy by qualified research staff prior to vaccination. The tube will be labeled with PID, Visit number and date of collection. The sample will be securely transferred to JAX-GM for processing and analysis following the study visit.

Visit 4: Day 10 (± 1 day) : Follow-up visit. Approximate visit time = 15 minutes.

Weight, Blood pressure, heart rate, temperature: taken and recorded on source document.

Data Collection

- Adverse events
- Concomitant medication use

Blood Draw

- **Blood draw of 50 mL**: Blood will be collected peripherally by phlebotomy by qualified research staff prior to vaccination. The tube will be labeled with PID, Visit number and date of collection. The sample will be securely transferred to JAX-GM for processing and analysis following the study visit.

Visit 5: Day 28 (± 3 days): Follow-up visit. Approximate visit time = 15 minutes.

Weight, Blood pressure, heart rate, temperature: taken and recorded on source document.

Data Collection

- Adverse events
- Concomitant medication use
- Medical History Review and update

Blood Draw

- **Blood draw of 50 mL:** Blood will be collected peripherally by phlebotomy by qualified research staff using best practices. The tubes will be labeled with PID, Visit number and date of collection. Fifty (50) mL will be drawn and securely transferred to JAX-GM for processing and analysis. Three (3) mL will be sent by JAX-GM to Quest Diagnostics Laboratory or to another JAX campus for a complete blood count (CBC) with differential testing for research purposes only. The results of the CBC blood test will be coded with the PID number only and will not be placed in medical records. Results will be sent back to Jackson Laboratory for use in research. Results will not be shared with research participants, however, if the study doctor feels that blood sample shows abnormalities during research testing, he may contact participant and advise them to follow-up with their personal physician.

Planning for Optional Visit 7 at Visit 6 or earlier:
To receive vaccination with the other pneumococcal vaccine (Prevnar or Pneumovax) that was not randomly assigned and administered at Visit 2, 1-2 years after administration of randomly assigned vaccine at Visit 2.

- Discuss the option to schedule vaccination with the other vaccine one-two years from when the randomly assigned vaccine was administered.
- Ask the participant if they are interested in receiving the second vaccine at one-two years free of charge and document their choice on the Visit 7 opt-in form.

Participants interested in second vaccination:

- Document participant choice on Visit 7 opt-in form.
- Schedule the visit 365-730 days from date of Visit 2 vaccination.
- Confirm with the participant that they agree to be contacted to be reminded of this visit two weeks prior and again the day before planned Visit 7.
- Participant may change their mind about receiving second vaccine at any time.

Participants not interested in second vaccination:

- Document participant choice on the Visit 7 opt-in form.
- Study participation ends at Visit 6. Complete End of Study form.
- Participants can change their mind before the two-year anniversary of Visit 2 and decide that they would like to receive the other pneumococcal vaccine free of charge. Participants will be instructed to contact the study team if they would like to receive the second vaccine.

Visit 6: Day 60 (± 5 days) : Follow-up visit. Approximate visit time = 15 minutes
Weight, Blood pressure, heart rate, temperature: taken and recorded on source document.

Data Collection

- Adverse events
- Concomitant medication use
- Medical History Review and update

Blood Draw

- **Blood draw of 50 mL**: Blood will be collected peripherally by phlebotomy by qualified research staff prior to vaccination. The tube will be labeled with PID, Visit number and date of collection. The sample will be securely transferred to JAX for processing and

analysis following the study visit.

Visit 7: Day 365-730 (optional) Second vaccine visit Approximate visit time = 30 minutes.

Data Collection

- Adverse events
- Concomitant medication use
- Medical History Review and update
- Screening Form for Visit 7 Vaccination
- Body Temperature. Temperature is taken and recorded on the Visit 7 CRF. If the participant has a body temperature of 100.5°F (38° C) or greater, Visit 7 will be deferred and rescheduled for a minimum of 3 days in the future.

Body Temperature and Blood Draw

- Body Temperature. Temperature is taken and recorded on the Visit 7 CRF. If the participant has a body temperature of 100.5°F (38° C) or greater, Visit 7 will be deferred and rescheduled for a minimum of 3 days in the future. Participants with a temperature below 100.5°F (38° C) will proceed to blood draw.
- **Blood draw of 50 mL:** Blood will be collected peripherally by phlebotomy by qualified research staff prior to vaccination. The tube will be labeled with PID, Visit number and date of collection. The sample will be securely transferred to JAX-GM for processing and analysis following the study visit.

Vaccination

- The research nurse reviews the Randomization and Vaccination form and identifies the vaccine administered at Visit 2. The other vaccine will be administered at Visit 7. (Prevnar at V2 receives Pneumovax at V7, Pneumovax at V2 receives Prevnar at V7.
- The research nurse obtains one dose of the Visit 7 vaccine from the refrigerator and prepares the dose per the package insert.
- The vaccine dose label (sticker) is placed in the designated area of the Vaccination Form for Visit 7.
- The area of the deltoid where the vaccine will be injected is wiped with alcohol
- The vaccine is injected intramuscularly in the deltoid by the RN.
- The participant is given the patient information handout for the vaccine received.
- The RN completes and signs the Vaccination Form for Visit 7.

The participant must remain at the clinic for 30 minutes after vaccine administration to be monitored for life-threatening allergic reactions. The nurse will discharge the participant after 30 minutes has elapsed and note the time on the Vaccination Form for Visit 7.

7 RISKS AND PROTECTIONS

7.1 Potential Risks to Subjects

Risk to Confidentiality: There is a potential risk to confidentiality due to the protected health

information collected and stored in the subject's research record.

Risk from Blood draw: There may be a minor amount of discomfort due to the phlebotomy. There is a minor risk of bruising (< 1%), infection at the phlebotomy site (< 1%) or dizziness following the blood draw (<1%).

Risk from Information on Cytomegalovirus (CMV) Serology and Infection: Given the frequency of CMV positive titers in the aged population and the potential influence on immune responses to pneumococcal vaccines (6), individual samples will be evaluated in the Jackson Laboratory for Genomic Medicine as to CMV serology and infection.

Risk from Vaccination with Prevnar-13

As stated in Prevnar 13 Prescribing Information the following risks are known to occur after vaccination with Prevnar 13:

- In adults aged 50 years and older the commonly reported solicited adverse reactions were pain at the injection site (>50%), fatigue (>30%), headache (>20%), muscle pain (>20%), joint pain (>10%), decreased appetite (>10%), injection site redness (>10%), injection site swelling (>10%), limitation of arm movement (>10%), chills (>5%) or rash (>5%).
- In adults, antibody responses to Prevnar 13 were diminished when given with inactivated trivalent influenza vaccine (TIV).
- Prevnar 13 does not protect against disease caused by *S. pneumoniae* serotypes that are not in the vaccine.

Risk from Vaccination with Pneumovax-23

As stated in Pneumovax-23 Prescribing Information, risks known to occur from vaccination with Pneumovax-23 are as follows:

- The most common adverse reactions, reported in >10% of subjects vaccinated with Pneumovax 23 in clinical trials, were: injection-site pain/soreness/tenderness (60.0%), injection-site swelling/induration (20.3%), headache (17.6%), injection-site erythema (16.4%), asthenia and fatigue (13.2%), and myalgia (11.9%).
- Pneumovax 23 will not prevent disease caused by capsular types of *S. pneumoniae* other than those contained in the vaccine.
- In a randomized clinical study, a reduced immune response to Zostavax® as measured by ELISA was observed in individuals who received concurrent administration of Pneumovax 23 and Zostavax compared with individuals who received these vaccines 4 weeks apart. Consider administration of the two vaccines separated by at least 4 weeks.

Risks from OPTIONAL second vaccination one year after initial vaccine (Visit 7)

- Risk to participants randomized to Prevnar 13 who opt to receive Pneumovax 23 a year after initial vaccination.
  At one year, this sequence is consistent with CDC recommendations for 2015, therefore risk is consistent with that of routine medical care. Data is available only for antibody response to the 12 serotypes that the two vaccines have in common, and for these, the

second vaccination at one year results in similar antibody levels as an initial Prevnar 13 vaccination.

- Risk to participants randomized to Pneumovax 23 who opt to receive Prevnar 13 one year after initial vaccination.
  In patients 60-64 years old, antibody response to the 13 serotypes in Prevnar 13 were found to be lower when given a year after Pneumovax 23 than when Prevnar is given as an initial vaccination. There is no available data for antibody response to all 23 serotypes of *S. pneumoniae* in Pneumovax 23 when followed by Prevnar 13.

7.2 Adequacy of Protection Against Risks

This study will be conducted under the supervision of the Principal Investigator, Dr. Kuchel. Best medical practices will be followed during all procedures. UConn Health emergency care procedures will be followed if an adverse event or medical emergency occurs. The study site is located on the campus of a tertiary care hospital that is available for treatment of medical emergencies.

Protection against Risk to Confidentiality:

All study visits will occur in a private room at the UConn Center for Aging at UConn Health in Farmington, CT.

Research records will be labeled with a participant ID number (PID), an assigned unique identifier that is not derived from any patient identifiers. All contents of the research record will be labeled with the assigned PID. Research records will be stored in a secure area. A complete record of the subject's pertinent history and documentation of the clinical visits will be kept on case report forms and will be stored in a secure area. Research records will be accessible only to the study team directly involved in conduct of the clinical protocol.

Any study documents (Informed Consent Form, HIPAA Authorization, Visit 7 Opt-in form) that contain the participant's name will be kept in a separate file apart from the research record and will be stored in a secure location. A master key that links participant names and PIDs will be maintained in a separate and secure location.

The database manager follows the Data Safety and Security Policy and Confidentiality agreement. These Policies provide procedures to assure that volunteer information remains confidential. All persons in the Recruitment Database have been asked to give permission to leave their name in the database. This policy will be included in this study.

The study database is password protected and secure, thus allowing only certain individuals access to the information. In addition, the database can be monitored in order to track the date, time, and individual who entered the database.
Samples/specimens will be labeled with PID, date, and visit number when they are delivered to the Jackson Laboratory for Genomic Medicine (JAX) for processing, storage and analysis.

A coded clinical data set linked to samples by PID will be provided to JAX for use in analysis. Results of this study when published will not identify subjects by name.

Certificate of Confidentiality:

This research is covered by a Certificate of Confidentiality from the National Institutes of Health. The researchers with this Certificate may not disclose or use information, documents, or biospecimens that may identify you in any federal, state, or local civil, criminal, administrative, legislative, or other action, suit, or proceeding, or be used as evidence, for example, if there is a court subpoena, unless you have consented for this use. Information, documents, or biospecimens protected by this Certificate cannot be disclosed to anyone else who is not connected with the research except, if there is a federal, state, or local law that requires disclosure (such as to report child abuse or communicable diseases but not for federal, state, or local civil, criminal, administrative, legislative, or other proceedings, see below); if you have consented to the disclosure, including for your medical treatment; or if it is used for other scientific research, as allowed by federal regulations protecting research subjects.

The Certificate cannot be used to refuse a request for information from personnel of the United States federal or state government agency sponsoring the project that is needed for auditing or program evaluation by NIH National Institute of Allergy and Infectious Diseases (NIAID) which is funding this project. You should understand that a Certificate of Confidentiality does not prevent you from voluntarily releasing information about yourself or your involvement in this research. If you want your research information released to an insurer, medical care provider, or any other person not connected with the research, you must provide consent to allow the researchers to release it.

The Certificate of Confidentiality will not be used to prevent disclosure as required by federal, state, or local law of information about elder, spousal abuse, reportable communicable diseases. The investigators on this study will report this information to State officials if it becomes known to them. The Certificate of Confidentiality will not be used to prevent disclosure for any purpose you have consented to in this informed consent document.

A description of this clinical study will be available on *http://www.ClinicalTrials.gov*. This Website will not include information that can identify participants. At most, the Web site will include a summary of the results. Participants will be informed in the consent that they can search this Website at any time.

Protection against Risk from Blood Draw:

Blood will be drawn peripherally via venipuncture at all visits. The volume drawn will be 50 mL at Visits 1, 3, 4, 5, 6 & (optional visit) 7 and 10 mL at Visit 2. Blood will be drawn by

experienced, trained research staff in a clinic setting on a hospital campus. The area where the needle will be inserted will be wiped with alcohol before and after the draw. A band-aid will be placed over the site. Emergency treatment is accessible on campus for any severe complications from blood draw.

Protection from Risk from Information on CMV Serology and Infection:

Information about CMV infection will not be shared with individual subjects or their physicians. In addition to the importance of maintaining the "firewall" between our clinical and genomic studies, there is at this time no evidence that a positive CMV serology should result in any specific course of action on the part of the patient or his/her physician. As a result, a decision to share such information would only result in unnecessary anxiety and confusion.

Protection against Risk of Vaccination with Prevnar 13:

A detailed medical history questionnaire will be completed by the participant and reviewed by a qualified member of the study team to identify contraindications to vaccination and to determine study eligibility. For eligible participants, assigned vaccine will be administered by a Registered Nurse as a single dose, given intramuscularly in the deltoid. Participants will be required to remain on site for 30 minutes after vaccine administration so that they can be monitored for adverse events.

Protection against Risk of Vaccination with Pneumovax 23:

In addition to protections listed under vaccination with Prevnar 13, participants will be asked about recent vaccination with Zostavax for shingles within the prior 28 days from date of planned vaccination or if they are planning to receive Zostavax in the 28 days following. In order to optimize response to all vaccines, study entry may be delayed to allow 28 days between vaccine administrations. If study entry is delayed, eligibility criteria will be reviewed and confirmed prior to randomization.

Protection against Risk from optional second vaccination one to two years after initial vaccine (Visit 7):

Participants that opt into a second vaccination with the vaccine not assigned at baseline will receive the benefits of two vaccinations for pneumococcal infection at no cost to them. Both vaccines are FDA approved for healthy adults 65 and older and children and adults 2 to 64 years of age with certain chronic health conditions. Vaccinations are given at the dose and via the route (intramuscular injection) as approved. Medical history will be reviewed and eligibility re-confirmed before the second vaccine is administered by an RN at optional Visit 7.

7.3 Adverse Events and Serious Adverse Events

An **adverse event (AE)** is defined as any unfavorable and unintended diagnosis, symptom, sign, syndrome or disease which either occurs during the study, having been absent at baseline, or if present at baseline, appears to worsen. Adverse events will be collected and recorded at each visit regardless of their relationship to the study intervention or to study participation.

A **serious adverse event (SAE)** is defined as any untoward medical occurrence that results in death, is life threatening, requires inpatient hospitalization or prolongation of existing hospitalization, results in persistent or significant disability/incapacity, or is a congenital anomaly.

7.4 Reporting Procedures

The Clinical Principal Investigator will review all AEs and SAEs to assess causality. The IRB requires reporting of unexpected adverse events that may represent an unanticipated problem involving risks to subjects or others (UPIRSO). Such events are to be reported to UConn Health IRB using the Problem Report Form within the IRIS electronic IRB submission system.

7.5 Treatment for Adverse Events

UConn Health does not provide insurance coverage to compensate for injuries incurred during research. UConn Health does not offer free care. However, treatment for a research related injury can be obtained at UConn Health for the usual fee. Medical care at UConn Health will be available to study participants for treatment of an adverse event at normal cost.

7.6 Data and Safety Monitoring

Data and Safety Monitoring Plan

The Data & Safety Monitoring Plan for this study describes the components of the study that will be monitored by the PI, study coordinator and data manager once annually. Recruitment, drop outs, adverse events, unanticipated problems, data integrity and confidentiality, participant privacy and the general conduct of the study will be reviewed. Minutes of the annual DSMP review will be kept in the regulatory binder and provided to IRB at study continuation. The vaccines given in this study are given at the dose, via the route and in the population for which they are approved by FDA, and study samples are limited to peripheral blood draws in healthy older adults, thus this study poses minimal risk to research subjects. As a result, a Data Safety Monitoring Board (DSMB) is not indicated. Nevertheless, robust procedures are in place to ensure confidentiality of research data.

Procedures in place to ensure confidentiality of research data are as follows:

1. Only authorized individuals have access to any data, used or stored (via electronic format or as hard copy records). Only designated research staff and investigators will be granted access.

2. Logon IDs and passwords for access to the UConn Center on Aging shared network drive and the University's online resources are initially assigned by the Information Technology Department (IT).
3. All data (clinical, recruitment and schedule-based) are stored in password-protected databases on the secured network drive. Only approved personnel have access to these databases and passwords.
4. All data collected on data forms are stored in locked drawers located in the Center on Aging research facility for scanning and verification purposes. Files are stored in locked cabinets in rooms that are locked when not in use. All data are backed up on the shared network drive for UCHC.

8 INTEGRATIVE DATA ANALYSIS

8.1 Statistical Methods

The breadth and magnitude of IgG titers will be analyzed individually and together using best-response and multivariate U-statistic approaches [4, 97]. Differences among groups (e.g., responder status) and/or time of immunological variables (e.g., flow, RNAseq) will be assessed using generalized linear mixed-model analyses with appropriate distributional assumptions (e.g., normal, negative binomial) and link functions (e.g., identity, log). Propensity scores [98] will be adjusted for possible confounds such as gender, age, race and ethnicity, while the Bonferroni correction or Benjamini and Hochberg false discovery rate (FDR) will account for multiple testing [99]. Correlation of clinical and immunological variables will be conducted using the Pearson or Spearman Correlation Coefficient depending on whether or not the assumptions of linearity and homoscedasticity are met. For class prediction, a variety of methods including k-nearest neighbors, radial basis machine, random forests, boosted trees and support vector machines will be employed and validated using leave-one-out cross-validation. Unsupervised analysis of high dimensional data will be conducted using heatmaps, hierarchical cluster analysis and principal component analysis. Lastly, attrition and missing data are issues in longitudinal [100] and elderly studies [101]. "Missingness" will be characterized and multiple imputation applied when appropriate [102-104]. Drs. Churchill (JAX) and Chaussabel (SIDRA) will assist with statistics and bioinformatics as needed.

8.2 Power Calculations

Power calculations are based on the ability to identify an adequate number of differentially expressed genes from RNAseq data to conduct downstream analyses and gain biological insights. Twenty patients per cohort adequately powers (>90%) this study for the detection of at least 6,000 differentially expressed genes. This calculation is established on conservative estimates obtained from RNAseq data of the flu pilot study. This study contained eight high responders and six low responders from the 2011 flu season measured at baseline, day 1, and 7 post vaccination Out of 37,649 genes, 6,412 to 10,915 genes displayed a >2 fold difference between responder status and/or time points Other specific parameters used for the power

calculation include a two-sided two-sample t-test, standard deviation of 0.7 (base 2 log scale), and false discovery rate of 1%. The PASS 2008 statistical package was used for power analysis.

9 DATA COLLECTION AND MANAGEMENT

Data Collected from Human Subjects Specifically for Research Purposes
Medical and medication history provided by the participant: Prior to the first collection of blood at Visit 1, individuals who have consented to participate in the study will be asked to fill out a detailed health history questionnaire with past and current medical conditions with date of diagnosis, including current and prior medication use, and vaccine history.

At all visits, participants will be asked about recent health history (e.g., colds, flu, pneumonia-like symptoms, hospitalizations, infections) and concomitant medication use since the previous review.

Vital signs (blood pressure, heart rate, temperature) and adverse events will be collected by a qualified member of the study team at each visit.

Height at Visit 1 only, weight at all visits.

Participant Identification (PID) number:
A unique participant identifier (PID) not derived from any patient identifiers will be assigned to each participant once written informed consent for study participation has been obtained. All clinical data will be linked to the PID in the database. Participant names or other personal identifiers will not be included in the study data set; all data will be identified only by PID.

Access to individually identifiable private information about human subjects
Access to clinical research records and identifiable study data will be restricted to the study team involved in the conduct of the clinical protocol. Coded clinical data will be provided to the JAX-GM laboratory staff for analysis linked with study samples by the PID. HIPAA personal identifiers will not be included in the clinical dataset provided to JAX-GM for use in analysis. The key linking the PID to participant identifiers will be stored separately and securely from the research records at the UConn Center on Aging. The code key will not be provided to the JAX investigator or staff at any time. JAX Lab staff will have access to coded samples and data only. Dr. Banchereau, as a co-investigator on the clinical protocol may be present at clinical team meetings when identifiable information is present. He will not record or disclose participant identifiers and he will not receive the code key at any time.

9.1 Data Collection Forms (CRF)

Forms to be completed for this study include:

Phone Pre-screening Form:

A partial waiver of consent and HIPAA Authorization will be requested for preliminary phone screening of study participants. No personal identifiers will be added to phone pre-screening forms until the form has been successfully completed. Pre-screening forms for participants that provide informed consent and enter the study will be stored with the identifiable forms in the Informed Consent/HIPAA Authorization Form binder for the study that is kept separate from coded CRF.

The Visit 1 Source Documentation Form

The Visit 1 Source Documentation Form is labeled with the PID and includes the following data:

- Documentation of Informed Consent:
  To confirm content of the informed consent discussion, that signatures/dates were collected and that the participant was given a copy of the signed ICF.
- Screening Form:
  This form lists all eligibility criteria. Participants must meet all inclusion criteria and not have any of the exclusion criteria to be randomized to vaccine.
- Medical History Form including medication use
- Visit 1 Procedures Documentation
- The sequential randomization number assigned per the Randomization Log

Visit 2 Source Documentation Form:

- Checklist for Visit 2 procedures labeled with the PID and date records the members of the study team that contributed to visit procedures.
- Randomization and Vaccination Data:
  The sequential randomization number assigned at Visit 1 the vaccine assigned per the Randomization Plan for that number, the sticker from the vaccine dose administered, date of administration, confirmation of 30 minute waiting period at the clinic after vaccination and the signature of the RN that administered the vaccine.

Visits 3-5: Source Documentation Forms:

These visit forms labeled with the PID and date are identical other than visit number. Members of the study team that collected data at this visit, vital signs and blood draw documentation are recorded on these visit forms.

Visit 7 Opt-in Form:

This form is completed at any time during study participation but must be completed before the end of Visit 6. The participant will indicate their intention to receive the second vaccine (not administered at Visit 2) at the optional Visit 7 as described in study procedures section or will decline the optional visit and second vaccine. The participant will sign and date the form and will be given a copy of the signed form for their records. This form will be stored with the ICF and HIPAA since it has a signature. Visit 7 will be scheduled for participants that choose to attend the optional Visit 7.

Visit 6 Source Documentation Form

This form captures members of the study team that collected data at this visit, vital signs and

blood draw documentation. Completion of Visit 7 Opt-in form is recorded and the selection of the participant made on the form.

Visit 7 (optional visit) Procedures Documentation Form:

- This form is a checklist for Visit 7 procedures for participants that opt for a second vaccination.

- **Vaccination administration detail for Visit 7:**
  The research nurse will identify the original assigned vaccine and the other vaccine to be given at Visit 7 on this form. The sticker from the vaccine dose will be placed on the form. The time and date of vaccine administration and confirmation of the 30 minute post-vaccine observation period with time of dismissal will be recorded by the research RN.

9.2 Data Management

Data Collection Forms (CRF) will be labeled with the Participant ID code, Subject initials and Visit Date and will be stored in the Research Record. Subject initials will be included on forms to aid the research staff in confirming that data is being collected for the correct participant. Participant initials will not be included in the coded dataset that is provided for use at JAX-GM. Clinical data provided to JAX-GM will be labeled only with the PID.

9.3 Quality Assurance

Training

A study initiation meeting will be attended by study staff in which the protocol, visit procedures, secure storage of research records, secure management of electronic databases and sharing of coded data with JAX, sample labeling protocol, and the process for sample transfer to the Jackson Laboratory for Genomic Medicine will be reviewed.

Delegation of Responsibilities

A Delegation of Responsibilities log will be completed at the Initiation Visit for members of the study team that will be entering data on CRF, determining eligibility, labeling samples or administering vaccines. This log will be kept in the study regulatory binder.

9.4 Protocol Deviations

A cumulative Protocol Deviation Log will be kept electronically by the study coordinator with a copy added to the regulatory binder. Deviations from the protocol will be entered on this log with a Note to File labeled with PID and no personal identifiers added to the regulatory binder with a copy to the research record that describes the deviation, date when it was identified, the corrective action taken to prevent recurrence, whether or not the deviation met criteria of an Unanticipated Problem, and the date of IRB notification.

Incidents of non-compliance, defined as any action that is taken or occurs that is not in accordance with an IRB approved study, IRB policies or regulations or failure to follow the requirements and

determinations of the IRB, that is within the control of the study team must be reported to the IRB within 5 days of becoming aware of the occurrence.

9.5 Unanticipated Problems

Per UConn Health policy for the purpose of reporting Unanticipated Problems to IRB, internal adverse events that may also represent an unanticipated problem are defined as those events, experiences or outcomes that are:

1. Unexpected (in terms of nature, severity or frequency) given (a) the research procedures that are described in the protocol-related documents, such as the IRB approved research protocol and informed consent document; and (b) the characteristics of the subject population being studied; and
2. Related or possibly related to participation in the research (i.e., there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures involved in the research). Any internal event meeting these criteria must be reported to the IRB, which will then make the final determination as to whether the research places subjects or others at a greater risk of harm (including physical, psychological, economic or social) than was previously recognized.

10 PARTICIPANT RIGHTS AND CONFIDENTIALITY

10.1 Institutional Review Board (IRB) Review

This protocol and the informed consent form and any subsequent modifications will be reviewed and approved by the UConn Health IRB before use.

10.2 Informed Consent

The informed consent process will begin after a participant successfully completes phone screening and plans to enter the study. A copy of the IRB approved Informed Consent Form (ICF) will be provided to the participant in advance for review, whenever possible.

At Visit 1, a qualified member of the study team will review the ICF, section by section, with the volunteer in a private room prior to any study procedures. Questions from the participant will be encouraged, and will be fully answered. Once the form has been fully reviewed and all questions answered, the volunteer will be asked if they would like to participate in the study. If they agree to participate, they will be asked to sign and date the ICF indicating their consent. The member of the study team obtaining consent will sign the ICF as well and a copy of the form signed by both the subject and the consenter will be provided to the subject. The participant will be asked to verbally confirm continued Informed Consent at each study visit before procedures are performed.

The Informed Consent process will be documented by the consenter on the Documentation of Informed Consent Form labeled with the PID and will be stored in the Research Record. Original signed and dated ICFs will be stored separately and securely away from the research record with other identifiable documents that contain participant identifiers.

10.3 Genomic Data and Sample Sharing

Participants will provide or decline consent within the ICF for sharing of their randomly recoded (new code that is different than the study code) genomic data in public and/or controlled access scientific databases. The study database will include a field for whether consent for genomic data sharing was provided or declined by the participant and if consent provided was for public and/or limited access databases. This information will be included in the dataset when provided to JAX-GM so that it can be provided to dbGAP (NIH database of Genotypes and Phenotypes) at the conclusion of the study to ensure that the wishes of the participant regarding use of their data and samples are respected.

Participants will provide or decline consent for sharing of coded samples that remain after study analysis is completed with other researchers and to be used in other studies. This information will be included as a variable in the coded dataset provided for analysis to JAX-GM.

10.4 HIPAA Authorization

Study participants must provide written authorization at study entry for use and disclosure of their Protected Health Information that is recorded and used in this study in order to participate. Participants may withdraw their Authorization at any time, but will then be withdrawn from the study. Study data will be stored in a password-protected database coded by Participant ID (PID). The key linking PID and participant name will not be shared with the JAX staff performing laboratory analysis. As a co-investigator, Dr. Banchereau will be actively engaged in the conduct of this research and although he will not be provided with the key linking PID and identity of participants, he may have identifiable information disclosed to him during meetings with the clinical study team. Dr. Banchereau will be listed in the HIPAA Authorization form for this reason.

11 REFERENCES

1. **Cech, T.R. and J.A. Steitz,** ***The noncoding RNA revolution-trashing old rules to forge new ones.*** **Cell, 2014. 157(1): p. 77-94.**
2. **Moens, L., et al.,** ***Human memory B lymphocyte subsets fulfill distinct roles in the anti-polysaccharide and anti-protein immune response.*** **J Immunol, 2008. 181(8): p. 5306-12.**
3. **Burton, R.L. and M.H. Nahm,** ***Development and validation of a fourfold multiplexed opsonization assay (MOPA4) for pneumococcal antibodies.*** **Clin Vaccine Immunol, 2006. 13(9): p. 1004-9.**
4. **Wittkowski, K.M., et al.,** ***Combining several ordinal measures in clinical studies.*** **Stat Med, 2004. 23(10): p. 1579-92.**
5. **Banchereau, J., et al.,** ***Immune and clinical responses in patients with metastatic melanoma to CD34(+) progenitor-derived dendritic cell vaccine.*** **Cancer Res, 2001. 61(17): p. 6451-8.**
6. **Palucka, K. and J. Banchereau,** ***Dendritic-Cell-Based Therapeutic Cancer Vaccines.*** **Immunity, 2013. 39(1): p. 38-48.**

7. **Park, S. and M.H. Nahm, *Older adults have a low capacity to opsonize pneumococci due to low IgM antibody response to pneumococcal vaccinations.* Infect Immun, 2011. 79(1): p. 314-20.**
8. **McElhaney, J.E., et al., *The unmet need in the elderly: how immunosenescence, CMV infection, co-morbidities and frailty are a challenge for the development of more effective influenza vaccines.* Vaccine, 2012. 30(12): p. 2060-7.**
9. **O'Connor, D., et al., *The effect of chronic cytomegalovirus infection on pneumococcal vaccine responses.* J Infect Dis, 2014. 209(10): p. 1635-41.**
10. **Leng, S.X., et al., *Relationship between cytomegalovirus (CMV) IgG serology, detectable CMV DNA in peripheral monocytes, and CMV pp65(495-503)-specific CD8+ T cells in older adults.* Age (Dordr), 2011. 33(4): p. 607-14.**
11. **Bai, L., et al., *Natural killer T (NKT)-B-cell interactions promote prolonged antibody responses and long-term memory to pneumococcal capsular polysaccharides.* Proc Natl Acad Sci U S A, 2013. 110(40): p. 16097-102.**
12. **Bentebibel, S.E., et al., *Induction of ICOS+CXCR3+CXCR5+ TH cells correlates with antibody responses to influenza vaccination.* Sci Transl Med, 2013. 5(176): p. 176ra32.**
13. **Atianand, M.K. and K.A. Fitzgerald, *Long non-coding RNAs and control of gene expression in the immune system.* Trends Mol Med, 2014. 20(11): p. 623-31.**
14. **Carpenter, S., et al., *A long noncoding RNA mediates both activation and repression of immune response genes.* Science, 2013. 341(6147): p. 789-92.**
15. **Noren Hooten, N., et al., *Age-related changes in microRNA levels in serum.* Aging (Albany NY), 2013. 5(10): p. 725-40.**
16. **Olivieri, F., A.D. Procopio, and R.R. Montgomery, *Effect of aging on microRNAs and regulation of pathogen recognition receptors.* Curr Opin Immunol, 2014. 29: p. 29-37.**
17. **Cusanovich, D.A., et al., *Epigenetics. Multiplex single-cell profiling of chromatin accessibility by combinatorial cellular indexing.* Science, 2015. 348(6237): p. 910-4.**
18. **Khaskhely, N., et al., *Phenotypic analysis of pneumococcal polysaccharide-specific B cells.* J Immunol, 2012. 188(5): p. 2455-63.**
19. **Kolibab, K., et al., *Immune response to pneumococcal polysaccharides 4 and 14 in elderly and young adults: analysis of the variable heavy chain repertoire.* Infect Immun, 2005. 73(11): p. 7465-76.**
20. **Leggat, D.J., et al., *Pneumococcal polysaccharide vaccination induces polysaccharide-specific B cells in adult peripheral blood expressing CD19(+)CD20(+)CD3(-)CD70(-)CD27(+)IgM(+)CD43(+)CD5(+)/(-).* Vaccine, 2013. 31(41): p. 4632-40.**
21. **Leggat, D.J., et al., *The immune response to pneumococcal polysaccharides 14 and 23F among elderly individuals consists predominantly of switched memory B cells.* J Infect Dis, 2013. 208(1): p. 101-8.**
22. **Schenkein, J.G., S. Park, and M.H. Nahm, *Pneumococcal vaccination in older adults induces antibodies with low opsonic capacity and reduced antibody potency.* Vaccine, 2008. 26(43): p. 5521-6.**
23. **Maue, A.C., et al., *T-cell immunosenescence: lessons learned from mouse models of aging.* Trends Immunol, 2009. 30(7): p. 301-5.**
24. **Nikolich-Zugich, J., et al., *Age-related changes in CD8 T cell homeostasis and immunity to infection.* Semin Immunol, 2012. 24(5): p. 356-64.**

25. **Clutterbuck, E.A., et al., *Pneumococcal conjugate and plain polysaccharide vaccines have divergent effects on antigen-specific B cells.* J Infect Dis, 2012. 205(9): p. 1408-16.**
26. **Steinman, R.M. and J. Banchereau, *Taking dendritic cells into medicine.* Nature, 2007. 449(7161): p. 419-26.**
27. **Banchereau, J., et al., *The differential production of cytokines by human Langerhans cells and dermal CD14(+) DCs controls CTL priming.* Blood, 2012. 119(24): p. 5742-9.**
28. **Klechevsky, E., et al., *Cross-priming CD8+ T cells by targeting antigens to human dendritic cells through DCIR.* Blood, 2010. 116(10): p. 1685-97.**
29. **Klechevsky, E., et al., *Functional specializations of human epidermal Langerhans cells and CD14+ dermal dendritic cells.* Immunity, 2008. 29(3): p. 497-510.**
30. **Yu, C.I., et al., *Human CD141+ dendritic cells induce CD4+ T cells to produce type 2 cytokines.* J Immunol, 2014. 193(9): p. 4335-43.**
31. **Yu, C.I., et al., *Human CD1c+ dendritic cells drive the differentiation of CD103+ CD8+ mucosal effector T cells via the cytokine TGF-beta.* Immunity, 2013. 38(4): p. 818-30.**
32. **Shi, Y., et al., *Regulation of aged humoral immune defense against pneumococcal bacteria by IgM memory B cell.* J Immunol, 2005. 175(5): p. 3262-7.**
33. **Takizawa, M., K. Sugane, and K. Agematsu, *Role of tonsillar IgD+CD27+ memory B cells in humoral immunity against pneumococcal infection.* Hum Immunol, 2006. 67(12): p. 966-75.**
34. **Dullaers, M., et al., *A T cell-dependent mechanism for the induction of human mucosal homing immunoglobulin A-secreting plasmablasts.* Immunity, 2009. 30(1): p. 120-9.**
35. **Xu, W., et al., *Macrophages induce differentiation of plasma cells through CXCL10/IP-10.* J Exp Med, 2012. 209(10): p. 1813-23, S1-2.**
36. **Haq, K. and J.E. McElhaney, *Immunosenescence: Influenza vaccination and the elderly.* Curr Opin Immunol, 2014. 29: p. 38-42.**
37. **High, K.P., A.N. Akbar, and J. Nikolich-Zugich, *Translational research in immune senescence: assessing the relevance of current models.* Semin Immunol, 2012. 24(5): p. 373-82.**
38. **Montecino-Rodriguez, E., B. Berent-Maoz, and K. Dorshkind, *Causes, consequences, and reversal of immune system aging.* J Clin Invest, 2013. 123(3): p. 958-65.**
39. **Pawelec, G. and A. Larbi, *Immunity and ageing in man: Annual Review 2006/2007.* Exp Gerontol, 2008. 43(1): p. 34-8.**
40. **Franceschi, C. and J. Campisi, *Chronic inflammation (inflammaging) and its potential contribution to age-associated diseases.* J Gerontol A Biol Sci Med Sci, 2014. 69 Suppl 1: p. S4-9.**
41. **Tchkonia, T., et al., *Cellular senescence and the senescent secretory phenotype: therapeutic opportunities.* J Clin Invest, 2013. 123(3): p. 966-72.**
42. **Childs, B.G., et al., *Senescence and apoptosis: dueling or complementary cell fates?* EMBO Rep, 2014. 15(11): p. 1139-53.**
43. **Rando, T.A. and H.Y. Chang, *Aging, rejuvenation, and epigenetic reprogramming: resetting the aging clock.* Cell, 2012. 148(1-2): p. 46-57.**

44. **Scott, D.A., et al., *Phase 1 trial of a 13-valent pneumococcal conjugate vaccine in healthy adults.* Vaccine, 2007. 25(33): p. 6164-6.**
45. **Lee, H., M.H. Nahm, and K.H. Kim, *The effect of age on the response to the pneumococcal polysaccharide vaccine.* BMC Infect Dis, 2010. 10: p. 60.**
46. **Drijkoningen, J.J. and G.G. Rohde, *Pneumococcal infection in adults: burden of disease.* Clin Microbiol Infect, 2014. 20 Suppl 5: p. 45-51.**
47. **Jackson, L.A., et al., *Effectiveness of pneumococcal polysaccharide vaccine in older adults.* N Engl J Med, 2003. 348(18): p. 1747-55.**
48. **Shapiro, E.D., et al., *The protective efficacy of polyvalent pneumococcal polysaccharide vaccine.* N Engl J Med, 1991. 325(21): p. 1453-60.**
49. **Weinberger, D.M. and E.D. Shapiro, *Pneumococcal conjugate vaccines for adults: reasons for optimism and for caution.* Hum Vaccin Immunother, 2014. 10(5): p. 1334-6.**
50. **Furman, D., et al., *Cytomegalovirus infection enhances the immune response to influenza.* Sci Transl Med, 2015. 7(281): p. 281ra43.**
51. **Bonten, M.J., et al., *Polysaccharide conjugate vaccine against pneumococcal pneumonia in adults.* N Engl J Med, 2015. 372(12): p. 1114-25.**
52. **Tsai, Y.H., et al., *The 23-valent pneumococcal polysaccharide vaccine is effective in elderly adults over 75 years old-Taiwan's PPV vaccination program.* Vaccine, 2015. 33(25): p. 2897-902.**
53. **Kobayashi, M., et al., *Intervals Between PCV13 and PPSV23 Vaccines: Recommendations of the Advisory Committee on Immunization Practices (ACIP).* MMWR Morb Mortal Wkly Rep, 2015. 64(34): p. 944-7.**
54. **Weinberger, B., et al., *Biology of immune responses to vaccines in elderly persons.* Clin Infect Dis, 2008. 46(7): p. 1078-84.**
55. **Defrance, T., M. Taillardet, and L. Genestier, *T cell-independent B cell memory.* Curr Opin Immunol, 2011. 23(3): p. 330-6.**
56. **Good-Jacobson, K.L. and D.M. Tarlinton, *Multiple routes to B-cell memory.* Int Immunol, 2012. 24(7): p. 403-8.**
57. **Reynaud, C.A., et al., *IgM memory B cells: a mouse/human paradox.* Cell Mol Life Sci, 2012. 69(10): p. 1625-34.**
58. **Weller, S., C.A. Reynaud, and J.C. Weill, *Vaccination against encapsulated bacteria in humans: paradoxes.* Trends Immunol, 2005. 26(2): p. 85-9.**
59. **Weill, J.C., S. Weller, and C.A. Reynaud, *Human marginal zone B cells.* Annu Rev Immunol, 2009. 27: p. 267-85.**
60. **Griffin, D.O., N.E. Holodick, and T.L. Rothstein, *Human B1 cells in umbilical cord and adult peripheral blood express the novel phenotype CD20+ CD27+ CD43+ CD70.* J Exp Med, 2011. 208(1): p. 67-80.**
61. **Griffin, D.O. and T.L. Rothstein, *Human b1 cell frequency: isolation and analysis of human b1 cells.* Front Immunol, 2012. 3: p. 122.**
62. **Westerink, M.A., H.W. Schroeder, Jr., and M.H. Nahm, *Immune Responses to pneumococcal vaccines in children and adults: Rationale for age-specific vaccination.* Aging Dis, 2012. 3(1): p. 51-67.**
63. **Kruetzmann, S., et al., *Human immunoglobulin M memory B cells controlling Streptococcus pneumoniae infections are generated in the spleen.* J Exp Med, 2003. 197(7): p. 939-45.**

64. **Crotty, S., *Follicular helper CD4 T cells (TFH).* Annu Rev Immunol, 2011. 29: p. 621-63.**
65. **King, C., S.G. Tangye, and C.R. Mackay, *T follicular helper (TFH) cells in normal and dysregulated immune responses.* Annu Rev Immunol, 2008. 26: p. 741-66.**
66. **Locci, M., et al., *Human circulating PD-1+CXCR3-CXCR5+ memory Tfh cells are highly functional and correlate with broadly neutralizing HIV antibody responses.* Immunity, 2013. 39(4): p. 758-69.**
67. **Morita, R., et al., *Human Blood CXCR5+CD4+ T Cells Are Counterparts of T Follicular Cells and Contain Specific Subsets that Differentially Support Antibody Secretion.* Immunity, 2011. 34(1): p. 108-121.**
68. **Schmitt, N., et al., *The cytokine TGF-beta co-opts signaling via STAT3-STAT4 to promote the differentiation of human TFH cells.* Nat Immunol, 2014. 15(9): p. 856-65.**
69. **Ueno, H., J. Banchereau, and C.G. Vinuesa, *Pathophysiology of T follicular helper cells in humans and mice.* Nat Immunol, 2015. 16(2): p. 142-52.**
70. **Obermoser, G., et al., *Systems Scale Interactive Exploration Reveals Quantitative and Qualitative Differences in Response to Influenza and Pneumococcal Vaccines.* Immunity, 2013. 38(4): p. 831-844.**
71. **Tsang, J.S., et al., *Global analyses of human immune variation reveal baseline predictors of postvaccination responses.* Cell, 2014. 157(2): p. 499-513.**
72. **Li, S., et al., *Molecular signatures of antibody responses derived from a systems biology study of five human vaccines.* Nat Immunol, 2014. 15(2): p. 195-204.**
73. **Nakaya, H.I. and B. Pulendran, *Vaccinology in the era of high-throughput biology.* Philos Trans R Soc Lond B Biol Sci, 2015. 370(1671).**
74. **Nakaya, H.I., et al., *Systems biology of vaccination for seasonal influenza in humans.* Nat Immunol, 2011. 12(8): p. 786-95.**
75. **Querec, T.D., et al., *Systems biology approach predicts immunogenicity of the yellow fever vaccine in humans.* Nat Immunol, 2009. 10(1): p. 116-25.**
76. **Tan, Y., et al., *Gene signatures related to B-cell proliferation predict influenza vaccine-induced antibody response.* Eur J Immunol, 2014. 44(1): p. 285-95.**
77. **Banchereau, R., et al., *Transcriptional specialization of human dendritic cell subsets in response to microbial vaccines.* Nat Commun, 2014. 5: p. 5283.**
78. **Berry, M.P., et al., *An interferon-inducible neutrophil-driven blood transcriptional signature in human tuberculosis.* Nature, 2010. 466(7309): p. 973-7.**
79. **Chaussabel, D., *Assessment of immune status using blood transcriptomics and potential implications for global health.* Semin Immunol, 2015. 27(1): p. 58-66.**
80. **Pascual, V., D. Chaussabel, and J. Banchereau, *A genomic approach to human autoimmune diseases.* Annu Rev Immunol, 2010. 28: p. 535-71.**
81. **Chaussabel, D., et al., *A Modular Analysis Framework for Blood Genomics Studies: Application to Systemic Lupus Erythematosus.* Immunity, 2008. 29(1): p. 150-164.**
82. **Buenrostro, J.D., et al., *Transposition of native chromatin for fast and sensitive epigenomic profiling of open chromatin, DNA-binding proteins and nucleosome position.* Nat Methods, 2013. 10(12): p. 1213-8.**
83. **Andrew, D. and R. Aspinall, *Age-associated thymic atrophy is linked to a decline in IL-7 production.* Exp Gerontol, 2002. 37(2-3): p. 455-63.**

84. **Bazdar, D.A., M. Kalinowska, and S.F. Sieg,** ***Interleukin-7 receptor signaling is deficient in CD4+ T cells from HIV-infected persons and is inversely associated with aging.*** **J Infect Dis, 2009. 199(7): p. 1019-28.**
85. **Kared, H., X. Camous, and A. Larbi,** ***T cells and their cytokines in persistent stimulation of the immune system.*** **Curr Opin Immunol, 2014. 29: p. 79-85.**
86. **Passtoors, W.M., et al.,** ***Transcriptional profiling of human familial longevity indicates a role for ASF1A and IL7R.*** **PLoS One, 2012. 7(1): p. e27759.**
87. **Sauce, D., et al.,** ***Lymphopenia-driven homeostatic regulation of naive T cells in elderly and thymectomized young adults.*** **J Immunol, 2012. 189(12): p. 5541-8.**
88. **Chaussabel, D. and N. Baldwin,** ***Democratizing systems immunology with modular transcriptional repertoire analyses.*** **Nat Rev Immunol, 2014. 14(4): p. 271-80.**
89. **Harries, L.W., et al.,** ***Advancing age is associated with gene expression changes resembling mTOR inhibition: evidence from two human populations.*** **Mech Ageing Dev, 2012. 133(8): p. 556-62.**
90. **Harries, L.W., et al.,** ***Human aging is characterized by focused changes in gene expression and deregulation of alternative splicing.*** **Aging Cell, 2011. 10(5): p. 868-78.**
91. **Holly, A.C., et al.,** ***Changes in splicing factor expression are associated with advancing age in man.*** **Mech Ageing Dev, 2013. 134(9): p. 356-66.**
92. **Holly, A.C., et al.,** ***Towards a gene expression biomarker set for human biological age.*** **Aging Cell, 2013. 12(2): p. 324-6.**
93. **Pilling, L.C., et al.,** ***Genomics and successful aging: grounds for renewed optimism?*** **J Gerontol A Biol Sci Med Sci, 2012. 67(5): p. 511-9.**
94. **Remondini, D., et al.,** ***Complex patterns of gene expression in human T cells during in vivo aging.*** **Mol Biosyst, 2010. 6(10): p. 1983-92.**
95. **Valdes, A.M., D. Glass, and T.D. Spector,** ***Omics technologies and the study of human ageing.*** **Nat Rev Genet, 2013. 14(9): p. 601-7.**
96. **van den Akker, E.B., et al.,** ***Meta-analysis on blood transcriptomic studies identifies consistently coexpressed protein-protein interaction modules as robust markers of human aging.*** **Aging Cell, 2014. 13(2): p. 216-25.**
97. **Paczesny, S., et al.,** ***Expansion of melanoma-specific cytolytic CD8+ T cell precursors in patients with metastatic melanoma vaccinated with CD34+ progenitor-derived dendritic cells.*** **J Exp Med, 2004. 199(11): p. 1503-11.**
98. **Rosenbaum, P. and D. Rubin,** ***The central role of the propensity score in observational studies for causal effects.*** **Biometrika, 1983. 57: p. 289-300.**
99. **Hochberg, Y. and Y. Benjamini,** ***More powerful procedures for multiple significance testing.*** **Stat Med, 1990. 9(7): p. 811-8.**
100. **Little, R.,** ***Modeling the dropout mechanism in repeated-measures studies.*** **Journal of the American Statistical Association, 1995. 90: p. 1112-1121.**
101. **Hardy, S.E., H. Allore, and S.A. Studenski,** ***Missing data: a special challenge in aging research.*** **J Am Geriatr Soc, 2009. 57(4): p. 722-9.**
102. **Rubin, D.,** ***Multiple imputation after 18+ years.*** **Journal of the American Statistical Association, 1996. 91: p. 473-489.**
103. **Rubin, D.,** ***Formalizing subjective notions about the effect of nonrespondents in sample surveys.*** **Journal of the American Statistical Association, 1977. 72: p. 538-543.**
104. **Hartigan, J.,** ***The dip test of unimodality.*** **Annals of Statistics, 1985. 13: p. 70-84.**