1. TITLE PAGE

Full Title: Perturbing the HIV Reservoir with Immune Stimulation

Title of Main Protocol and Protocol #: Perturbing the HIV Reservoir

Local Protocol Number: #160089

DAIDS-ES Document Number: (protocol number provided by DAIDS): 30122

NIAID Funding Mechanism and Principal Investigator:

NIH grant #1R01AI118422, David (Davey) Smith MD, MAS (PI)

Other Collaborators: Susan Little MD, Sara Gianella MD

Pharmaceutical and other Study Product Support Provided by: Not Applicable

IND Number: N/A

Protocol Chair/Co-Chair:

Chair: Dr. Smith, Co-Chairs: Dr. Little, Dr. Gianella

DAIDS Medical Officer: Dr. Lawrence Fox

Version Number: 6.1

Version Date: October 31, 2017

2. SIGNATURE PAGE

I agree to conduct the study in accordance with the relevant, current protocol and will not make changes to the protocol without permission of DAIDS, except when necessary to protect the safety, rights, or welfare of study participants.

I agree to personally conduct or supervise this study.

I will ensure that the requirements relating to obtaining informed consent and Ethics Committee (EC) or Institutional Review Board (IRB) review and approval (insert relevant terms of assurance here, e.g. 45 CFR 46, ICH/GCP, etc.) are met.

I agree to report to the sponsor adverse experiences as per monitoring plan that occur during the course of this study.

I agree to maintain adequate and accurate study records and to make those records available for inspection by DAIDS, DAIDS' authorized representatives, and/or other applicable regulatory entities.

I will ensure that an EC or IRB that complies with the requirements of 45 CFR Part 46 will complete initial and continuing review and approval of the study. I also agree to promptly report to the EC/IRB all changes to the study and all unanticipated problems involving risks to human subjects or others. Additionally, I will not make any changes to the study without DAIDS and EC/IRB approval, except where necessary to eliminate apparent immediate hazards to study participants.

I agree to ensure that all staff members involved in the conduct of this study are informed about their obligations in meeting the above commitments.

Principal Investigator: David Smith, MD Print/Type

Signed: _____ Date: _____ Name/Title

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4. KEY ROLES

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5. LIST OF ABBREVIATIONS

ACTG	AIDS Clinical Trials Group
AE	Adverse Event
AER	Adverse Event Reports
AIDS	Acquired Immunodeficiency Syndrome
ART	Antiretroviral Therapy
AVRC	Antiviral Research Center
CB	Clinical Investigation and Biostatistics (CB) Core
CD4	CD4 lymphocytes
CFAR	Center for AIDS Research
CMV	Cytomegalovirus
CRF	Case Report Form
ddPCR	droplet digital PCR
DSMB	Data Safety and Monitoring Board
EDI	Estimated date of infection
HBsAg	Hepatitis B surface Antigen
HCV	Hepatitis C Virus
HHV	Human Herpesviruses
HIV-1	Human Immuno-deficiency Virus – 1
HDACi	Histone Deacetylase Inhibitors
HPV	Human Papilloma Virus
HRPP	Human Research Protections Program
HRSA	Health Resources and Services Administration
IL	Interleukin
LPS	Lipopolysaccharide
MMR	Measles, Mumps, Rubella

MSM	Men who have sex with men
NA	not applicable
RCT	Randomized Controlled Trial
RT-PCR	Real Time-PCR
PBMC	Peripheral Blood Mononuclear Cells
PCR	Polymerase Chain Reaction
PID	Participant Identifier
SAE	Severe Adverse Reaction
SCA	Single Copy Assay
SDPIRC	San Diego Primary HIV Infection Consortium
TDAP	Tetanus, Diphtheria and Pertussis
UCSD	University of California San Diego
VZV	Varicella Zoster Virus

6. PROTOCOL SUMMARY

Title: Perturbing the HIV Reservoir

Sample Size: 56

Study Population: HIV-infected individuals between 18 and 65 years old who started antiretroviral therapy (ART) during chronic infection (at least one year from estimated date of infection) and remained virally suppressed for at least 48 weeks before enrollment. Participants will have CD4 >250 cells/μl at enrollment and a CD4 nadir >100 cells/μl.

Participating Sites: UCSD's Antiviral Research Center (AVRC)

Study Design: The proposed study is a randomized double-blinded controlled trial conducted over 28 weeks. Randomized interventions will be injections of Influenza vaccine, Pneumococcal vaccine, and Placebo. Each participant will receive each injection but in a randomized order. See Figure 1 below.

Schedule of Evaluations: Study evaluations will be based on three 30 day cycles (Influenza vaccine, Pneumococcus vaccine, Placebo in random order) over 28 weeks of the RCT. *Pre injection*: one paired blood, optional stool and optional genital secretion sample will be collected before each injection. *Post-injection*: Blood will be collected on days 2, 4 and 7 while paired blood and optional genital secretion samples will be collected on days 14 and 30 after each injection.

Table 1. Perturb Study - Schedule of Evaluations

Activity	*Screening*	*Cycle 1 Treatment Period Fluarix /Fluzone, Pneumovax 23 or Placebo* [a]						*Cycle 2 Treatment Period Fluarix/Fluzone, Pneumovax 23 or Placebo* [c]						*Cycle 3 Treatment Period Fluarix/Fluzone, Pneumovax 23 or Placebo* [c]						*Premature Discontinuation*
		Baseline (Day 0)	*Day 2*[b]	*Day 4*[b]	*Day 7*[b]	*Day 14*[b]	*Day 30*[b]	*Week 12 (Day 0)*	*Day 2*[b]	*Day 4*[b]	*Day 7*[b]	*Day 14*[b]	*Day 30*[b]	*Week 24 (Day 0)*	*Day 2*[b]	*Day 4*[b]	*Day 7*[b]	*Day 14*[b]	*Day 30*[b]	
Informed Consent	X																			
Eligibility Checklist	X																			
Medical Release	X																			
Vaccination History	X																			
Randomization	X																			
Blood collection[d]		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Pregnancy test[e]	X																			
Physical Exam		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Medical History, Health Survey	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Concomitant Medications		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
ARV Adherence		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Post-vax Symptoms		X	X	X	X	X	X		X	X	X	X	X		X	X	X	X	X	X
Adverse Events		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Study Drug Injection		X						X						X						
Optional Stool Collection		X						X						X						
Optional Genital Secretion[d]		X				X	X	X				X	X	X				X	X	X
Hematology/Chemistry/ Coagulation Panel		X				X	X	X				X	X	X				X	X	X
Immune responses		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
HIV-1 RNA sequencing		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
CD4+ T-cell Count		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
HIV-1 DNA sequencing		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Archive Plasma		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X

a. Cycle 1 to begin within 30 days of screening visit
b. ± 2 days
c. Minimum 6 week washout period between cycles
d. Paired blood and optional genital secretion sample will be collected **before** each injection
e. Pregnancy Test will be repeated only if pregnancy is suspected.

Study Duration: 240 weeks

Study Regimen/Intervention: This is a double blind RCT of two vaccines (Pneumovax®23 and Fluarix® or Fluzone®) plus placebo (sterile saline injection). Study participants will be followed for 28 weeks after enrollment. During this 28-week period, blood samples will be collected on day 0 and five subsequent time points after each injection (days 2, 4, 7, 14 and 30). Optional genital secretion samples will be collected on day zero and on days 14 and 30 after each injection. Optional stool will be collected at day zero. Injections (vaccine or placebo) will be administered 12 weeks apart and in a random order, to minimize a possible bias due to the order of the vaccines.

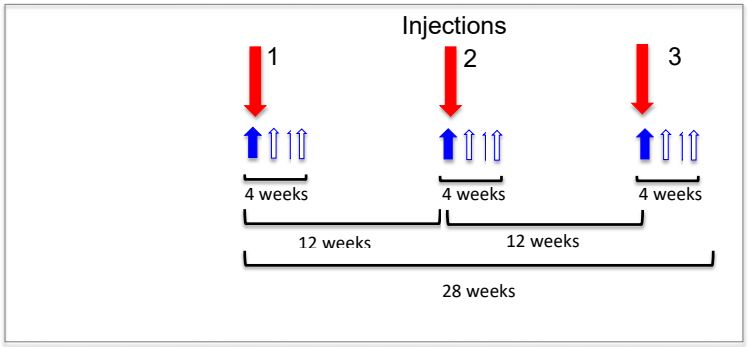
NOTE: Fluarix® quadravalent vaccine is the flu vaccine of choice for this study. Fluzone® quadravalent vaccine may be substituted for Fluarix® only in the event that Fluarix® is unobtainable from the wholesaler/manufacturer.

Primary Objective: To determine the impact of Pneumococcus and Influenza vaccines on the HIV transcriptional activity in individuals who are virologically suppressed for at least 48 weeks on ART.

Primary Outcome: Average change in level of CD4+ T-cell-associated HIV RNA transcription from baseline to day 7 after each injection.

Figure 1. Study Design and sampling

Figure 1. Study Design and sampling



Legend: 56 chronically HIV+ individuals on ART will receive Influenza and Pneumococcus vaccines and Placebo injections in random order over 24 weeks (red arrows). Blood will be collected **before** each injection (blue arrow day 0) and 2, 4, 7, 14 and 30 days **after** each injection (open arrows). Optional genital secretion will be collected on Days 0, 14 and 30. Optional stool samples will be collected before the injection on Day 0.

7. INTRODUCTION

7.1 Background Information

7.1.1 HIV cure strategies

HIV can be effectively treated, but not cured, except for very rare and special circumstances [5]. The recent report of the 'Berlin Patient' [6] has sparked new enthusiasm for such efforts. The first problem with curing HIV is that the virus has a latent stage that persists indefinitely, even during suppressive ART, resulting in recurrence of plasma viremia if ART is stopped. The second problem is identification of specific cells that are latently infected [5]. Most current curative strategies have focused on developing methods that are able to induce the virus from latently infected cells during ART, so that viral proteins are revealed and allow these cellular reservoirs to be cleared by the host immune response, while ART prevents new cells from being infected [7, 8]. Unfortunately, recent interventions to perturb the reservoir, like histone deacetylase inhibitors (HDACi), IL-7, and disulfram, have demonstrated only modest activity [7, 9-13]. It is also becoming increasingly apparent that it is not enough to just induce HIV RNA expression during ART to clear latently infected cells, but activation of HIV-specific immune responses is also needed [7, 8]. Therefore, it seems likely that parallel efforts to augment the HIV-specific immune response may be required [8, 10, 14]. This proposal aims to evaluate two clinically indicated vaccines with longstanding safety records as immune stimulatory methods for perturbing the HIV reservoir and inducing release of virus and the subsequent potential for viral clearance by the activated host immune system.

7.1.2 Vaccines can stimulate the immune system and HIV reservoir

T-cell activation has been shown to effectively reactivate HIV replication in various *in vitro* models [13, 15-17]. Similar effects have been observed with *in vivo* immune activation secondary to current illness [18-26], human herpesvirus (HHV) shedding [27-30] and gut microbial translocation [31-34]. Prophylactic vaccination represents another means of activating the immune system and has been associated with mostly transient increases in levels of cell-free HIV RNA after vaccination for influenza [21, 35-43], pneumococcus [44-46], tetanus [42, 47], hepatitis B [48] and cholera [49]. This stimulation of the immune system and the HIV reservoir probably occurs because standard clinical vaccines activate not only the small subset of antigen specific cells within the lymphocyte population but also induce a bystander activation of non-specific cells. This hypothesis is supported by the measurable increases in various markers of systemic inflammation (e.g. pro-inflammatory cytokines [interleukin (IL)-6, IL-1, C reactive protein, tumor necrosis factor (TNF)], T-cell activation [$CD38^{+}HLA\text{-}DR^{+}$] and proliferation [$Ki67^{+}$]) [50-55] after vaccination. For inactivated vaccines, this inflammation is largely mediated by an adjuvant. Studies of vaccine-associated effects on HIV RNA are mixed however, with several studies demonstrating no significant effect on plasma viral loads (i.e., influenza [56-61], pneumococcus [62-64], tetanus [65], hepatitis A [66, 67], hepatitis B [68], measles/mumps/rubella (MMR) [69], *Haemophilus influenzae* type b [70] and yellow fever [71]). The inconsistent findings in the literature are likely secondary to [72-74]: (1) observational convenience studies with varying sampling times (e.g. 1 week versus 1 month), (2) heterogeneous study populations (e.g. different ART regimens, durations of infection, baseline viral loads and CD4+ T cell counts [36]), and (3) insensitive methods applied to measure how much HIV RNA is being induced from infected cells. The proposed study will attempt to address these issues by: (1) performing a randomized, double-blind, placebo-controlled trial (RCT) (vaccine vs. vaccine placebo) with optimized sampling times post-vaccination, (2) investigating

participants who have received ART for at least 48 weeks, which was started during chronic infection, and (3) using more sensitive methods to measure virus production from cells (single copy assays to measure cell-free HIV RNA and droplet digital PCR (ddPCR) for cell-associated HIV RNA [75].

While no previous study has examined the impact of vaccines on the HIV reservoir, one Spanish RCT of seven vaccines [1] in chronically infected individuals receiving ART did evaluate the hypothesis that transitory increases in HIV replication during ART would strengthen specific responses against HIV and induce a better control of HIV replication after ART interruption. Although increases in HIV-specific T-cell responses and in T-cell activation were observed in the vaccine group compared to the placebo group, interruption of ART was not associated with control of HIV replication. While this study did not show a significant increase in levels of cell-free HIV RNA production in the vaccine vs. placebo arms, it did suggest that enough HIV transcription was induced to increase HIV-specific immune responses. In contrast to the proposed study, this Spanish study only used standard viral load measures at wide intervals (30 days post-injection)[1]. To further evaluate this hypothesis, we generated preliminary data from the Spanish study [1] using more appropriate assays in support of our hypothesis that vaccination can activate proviruses during ART more than any published HDACi compound [7, 9, 12, 13, 76]. In these experiments, we found (see Figure 2 below):

- no difference in cellular HIV RNA levels between vaccine and placebo groups at baseline ($p>0.1$),
- significant increases in HIV transcription following each vaccine (all $p<0.05$),
- mean fold change in cell-associated HIV RNA *gag* transcripts following both Influenza and Pneumococcal vaccines was considerably higher than what has been observed in two clinical studies of the HDACi Vorinostat (Archin et al.[3] and Elliott et al.[4]).

In summary, we acknowledge that the literature is not clear whether or not clinical vaccination can perturb the HIV reservoir, but this is because <u>all previous studies</u>:

- Were observational with a very wide range of sampling times (<1 week to >1 month).
- Had very heterogeneous populations with different ART regimens, ages, duration of infection, baseline viral loads, CD4 T-cell counts, etc. In observational studies that do account for these factors, such heterogeneity can confound any true relationship.
- Used older insensitive methods (i.e. basic standard viral load assays with lower limit of detections between 50 and 400 copies/ml) to measure HIV RNA transcription.

Taken together, we feel that it would be a mistake to discard this line of investigation based on historical, observational studies that were not designed to address the important questions posed in this proposal.

Figure 2. Preliminary Data from Spanish Study

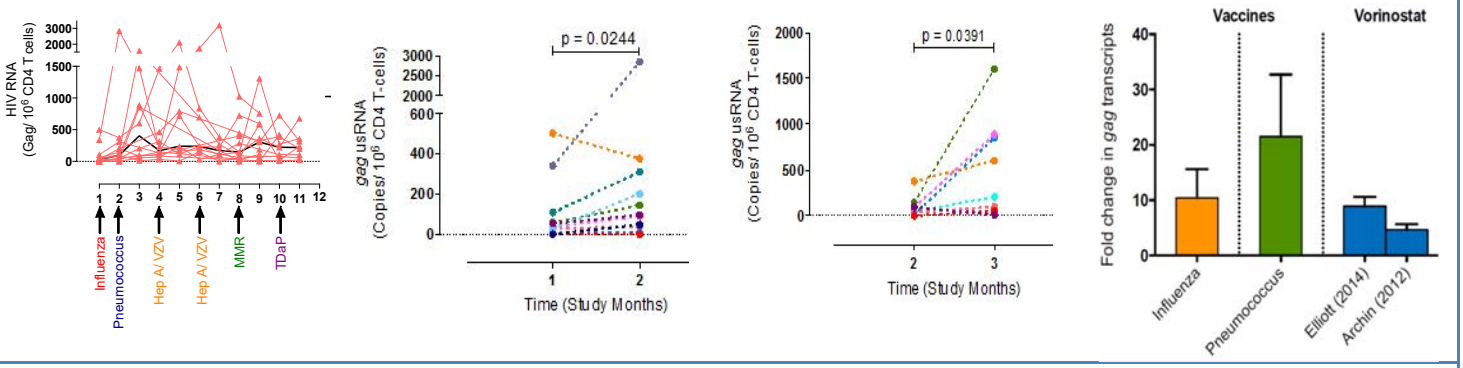
Figure 2. Preliminary Data from Spanish Study [1, 2]

A: Large increases in cell associated HIV RNA after each vaccine administration, but variable across subjects.

B: Cell associated HIV RNA before and after **influenza** vaccine administration.

C: Cell associated HIV RNA before and after **pneumococcus** vaccine administration.

D: HIV transcription induced by influenza, pneumococcus and Vorinostat.



Legend: We used samples shared from the Spanish study **[1]** to measure cell associated HIV RNA in 12 vaccinated and 11 control individuals. At baseline (pre-vaccine), there was no difference in cellular HIV RNA levels between those who would receive the vaccine and those who would receive placebo (controls) (p>0.1). Panel A: After each vaccine we found significant median increases in HIV transcription; like other interventions aimed to perturb the reservoir, there was considerable differences between participants. Panels B and C: Here, we will focus on the evaluation of the two vaccines that demonstrated the highest fold increases in cell associated HIV RNA (Influenza and Pneumococcus). Panel D: The mean fold change (with standard error) in cell-associated HIV RNA *gag* transcripts following both of these vaccines was considerably higher than what has been observed in two studies of *in vivo* administration of Vorinostat (Archin et al.[3] and Elliott et al.[4])

7.1.3 Vaccines for HIV-infected people

HIV-infected individuals are at increased risk for a variety of communicable diseases compared to uninfected people and several vaccines are routinely recommended. In general, vaccines have better efficacy in HIV-infected individuals with CD4 counts >200 cells/μL [77]. However, all vaccines are not created equal [78]. They can differ in immune stimulating mechanism; some induce mostly neutralizing antibodies while others also induce cytotoxic T-cell responses [78-86]. They can also differ in efficacy; for example the human papilloma virus (HPV) vaccine is close to 100% effective while influenza is only ~65% effective [87]. Further, immune responses wane over time and at different rates; the tetanus vaccine requires repeat vaccination every 10 years, while the protection from the smallpox vaccine is likely life-long [87]. Additionally, effective inactivated vaccines often require an adjuvant (Alum, AS04, or MF59), which primes the innate and adaptive immune responses quite differently [78]. This study will evaluate the two standard vaccines that showed the greatest mean increase in HIV RNA transcription following vaccination in the Spanish Trial (see Research Plan). Other vaccines could be evaluated in this proposal, but we chose the Influenza and Pneumococcal vaccines that are commonly used clinically and have a very long safety record.

7.1.4 Incidental Immune Stimulation

Immune stimulation following vaccination has the potential to be confounded by immune activation associated with other specific and non-specific stimuli [28, 88-90]. In an effort to

optimally control for possible confounding within a relatively small RCT, we propose a detailed analysis of the possible contribution of incidental immune stimulation resulting from HHV infections, microbial translocation, drug use and clinical illnesses. For example, we recently showed that CMV replication in seminal plasma of HIV infected subjects on suppressive ART was associated with increased levels of T-cell activation and higher levels of proviral HIV DNA and cellular HIV RNA compared to subjects with no detectable CMV (Figure 2) [30]. Additionally, our group has identified an association between methamphetamine use and increases in both immune activation and HIV DNA levels during suppressive ART [91]. Therefore, we will try to identify these factors and account for their potential effect. Specifically, we will identify and measure: 1) genital shedding of 7 HHV (by RT-PCR), 2) microbial translocation (as measured by lipopolysaccharide [LPS], bacterial 16s rDNA and soluble [s]CD14), 3) active or recent illicit substance use (using validated questionnaires [92-94] and urine toxicology) and 4) recent clinical illnesses (e.g. colds, skin infections, sexually transmitted infections) and clinical events (e.g. surgeries).

7.2 Rationale

Most recent HIV cure efforts have focused on strategies to induce virus from latently infected cells during ART, thus leaving the cellular reservoir vulnerable to the host immune system, while ART prevents new cells from being infected. Unfortunately, such efforts have not demonstrated much activity. On the other hand, it has been documented for almost two decades that routine clinical vaccines can induce viral production from latently infected cells, even during ART, and that these levels of induction are much higher than those seen by recently described interventions (e.g. histone deacetylase inhibitors (HDACi), IL-7, disulfram). Therefore, we propose a RCT with cross-over to determine how two commonly used vaccines (Influenza and Pneumococcus) can stimulate the immune system and induce HIV transcription. In preliminary data, these vaccines demonstrate a higher cell associated HIV RNA transcriptional response even 30 days after vaccination than the HDACi Vorinostat; see Figure 2. In this proposal, we will use a much safer method (vaccination with Influenza and Pneumococcus) and observe timepoints much closer to the time of vaccination to tease out how immune stimulation perturbs the HIV DNA reservoir suppressed by ART.

Rationale for measuring total and replication competent HIV DNA

Currently, much emphasis has been placed on the kick portion of the kick-and-kill HIV cure strategy; however, only modest benefit has been seen with kick interventions and almost none with the killing of infected cells- at least with the current measurements in circulating blood [7, 9-13]. In this study, we will use clinically available influenza and pneumococcus vaccines to stimulate the HIV reservoir and stimulate the immune response. In the prior Spanish Study, described in the Background, it was observed that HIV-specific immune response was enhanced during the 'kick' of the reservoir in the blood after a clinical vaccine. This study did not measure HIV DNA levels or replication competent proviral levels; therefore, we do not know if this HIV-specific immune stimulation had an impact on the HIV reservoir in circulating blood cells. Therefore, in the proposed RCT, we will assess both total HIV DNA levels and replication competent provirus (using an “inducible RNA assay” [95]) among the five participants with the highest cell associated HIV RNA response to the vaccine intervention.

Microbiome Composition

Infection with HIV is associated with dramatic alterations in the gut-associated lymphoid tissue, which occur early in the course of infection and contribute to persistent immune dysfunction and HIV disease progression. Mucosal HIV replication and consequent depletion of gut-associated $CD4^+$ T cells is associated with epithelial barrier damage and increased translocation of bacterial products from the gut lumen into the systemic blood circulation (i.e. microbial translocation). In turn, microbial translocation is associated with systemic immune activation and might play a role in immune response and disease progression in untreated and treated HIV infected individuals.

As part of this study we will determine the effect of the intestinal microbiome composition on the viral and immunologic dynamics before and after vaccine administration.

7.3 Major Hypotheses

(All hypotheses are presented "under the alternative")

Primary Hypothesis:

On average, participants will have a higher absolute increase in levels of cell-associated HIV RNA transcription in CD4+ T-cells seven days after receiving either Pneumococcal or Influenza vaccinations, when compared to seven days after receiving placebo.

Secondary Hypotheses:

- Among the five participants with the largest increases in cell-associated HIV RNA in CD4+ T-cells following active vaccination (maximum across all observed time-points), we will deep sequence cell-associated HIV RNA and DNA populations at 0, 2, 4 7, 14 and 30 days. We hypothesize that active vaccines will *non-selectively* activate cellular transcription of HIV, as evaluated by panmixis tests between sequences of HIV DNA and RNA populations at these sampled timepoints.

- On average, participants will have a greater absolute maximum decrease in levels of total CD4+ T-cell-associated HIV DNA measured 2-30 days after receiving either Pneumococcal or Influenza vaccinations, when compared to 2-30 days after receiving placebo.

- Among the five participants with the largest increases in cell-associated HIV RNA in CD4+ T-cells following active vaccination (maximum across all observed time-points), we will perform inducible RNA assay. We hypothesize that these five participants will have lower levels of replication competent proviruses 30 days after active vaccination, as compared to their levels of replication competent proviruses sampled before this vaccination.

- Participants will have a greater absolute maximum increase in levels of (a) inflammatory cytokines, (b) T-cell activation and proliferation, (c) HIV-specific immune responses, and (d) vaccine-specific immune responses over the 30 days of observation after receiving either active vaccination, when compared to maximum measures taken over the 30 days of observation after receiving placebo.

- Levels of non-antigen specific immune stimulation (cytokines and general T-cell activation) and vaccine-specific immune responses will be positively associated with increased CD4+ T-cell-associated HIV RNA transcription and negatively associated with lower levels of HIV DNA, over the 30 days of observation after receiving either Pneumococcal or Influenza vaccinations.

- Maximum levels of cell-associated HIV RNA over the 30 days of observation following active vaccination will be positively associated with HIV-specific responses 30 days after receiving active vaccinations.

- Genital shedding of human herpesviruses, increased levels of microbial translocation and incident illnesses will stimulate the immune system and induce increased cell-associated HIV RNA transcription from $CD4^+$ T-cells in participants independent of receiving active vaccine or placebo.

- Presence of dysbiosis (for example increased relative frequency of Proteobacteria in stool samples) will be associated with increased microbioal translocation and with increased cell-associated HIV RNA transcription from $CD4^+$ T-cells in participants independent of receiving active vaccine or placebo.

8. OBJECTIVES

8.1 Primary Objective

Determine the impact of Pneumococcus and Influenza vaccines on the HIV transcriptional activity in individuals who are virologically suppressed for at least 48 weeks on ART.

8.2 Secondary Objectives

8.2.1 Aim 1a

Determine the timing and duration of increased HIV transcription following active vaccination.

8.2.2 Aim 1b

Determine if vaccination selectively or non-selectively activates HIV transcription from HIV DNA populations

8.2.3 Aim 2

Determine if active vaccination influences levels of cell associated HIV DNA or replication competent provirus.

8.2.4 Aim 3a

Determine to what degree immune stimulation with an Influenza or Pneumococcal vaccine can induce HIV transcription during ART.

8.2.5 Aim 3b

Determine if immune stimulation associated with active vaccination is associated with reduction in number of HIV DNA populations.

8.2.6 Aim 3c
Determine if HIV-specific, vaccine-specific, or bystander immune stimulation is associated with HIV transcription.

8.2.7 Aim 4
Determine how genital shedding of human herpesviruses, microbial translocation and incident illnesses stimulate the immune system and influence levels HIV transcription.

8.2.8 Aim 5
Determine how dysbiosis contribute to stimulate the immune system and influence HIV transcription.

9. STUDY DESIGN
The proposed study is a randomized double-blinded control trial conducted over 28 weeks. Randomized interventions will be injections of Influenza vaccine, Pneumococcal vaccine, and Placebo. Each participant will receive each injection but in a randomized order.

9.1. Vaccines
Influenza (Fluarix®, GSK or Fluzone®, Sanofi -Pasteur), Pneumococcal (Pneumovax®23, Merck), and placebo (sterile saline injection).

NOTE: Fluarix® quadravalent vaccine is the flu vaccine of choice for this study. Fluzone® quadravalent vaccine may be substituted for Fluarix® only in the event that Fluarix® is unobtainable from the wholesaler/manufacturer.

9.2. Primary endpoint
average change in level of CD4+ T-cell-associated HIV RNA transcription from baseline to day 7 after each injection.

9.3. Study Arms
Each subject will take Influenza (Fluarix®, GSK or Fluzone®, Sanofi -Pasteur), Pneumococcal (Pneumovax®23, Merck), and placebo. Randomization will determine the order in which the subjects receive the injections. There are six possible study arms, one for each order in which someone can receive the injections:

Placebo, Flu, Pneumovax

Placebo, Pneumovax, Flu

Flu, Placebo, Pneumovax

Flu, Pneumovax, Placebo

Pneumovax, Placebo, Flu

Pneumovax, Flu, Placebo

9.4. Study population

This study will enroll 56 HIV-infected individuals between the ages of 18 and 65 who started ART during chronic infection. These subjects will be recruited from both the UCSD AntiViral Research Center and the Owen Clinic. Eligible participants will have CD4 >250 cells/µl at enrollment and a CD4 nadir >100 cells/µl. We chose to limit enrollment to younger individuals with higher CD4 counts to enhance the likelihood of having a more robust immune response to any given vaccine. We limited enrollment to individuals who started ART during chronic infection (at least one-year from estimated date of infection) to enhance the likelihood of having a large replication competent viral reservoir, thus increasing our chances to observe an effect of vaccine administration on HIV RNA transcription [96]. To reduce variability in vaccine stimulation (between antigen naïve versus experienced participants), we will also limit participation to individuals who have documented or reported vaccine history consistent with previous influenza and pneumococcal vaccination.

9.5. Sample collection

Study evaluations will be based on three 30 day cycles (Influenza vaccine, Pneumococcus vaccine, Placebo in random order) over 28 weeks of the RCT. *Pre injection*: one paired blood, optional stool and optional genital secretion sample will be collected before each injection. *Post-injection*: Blood samples will be collected on days 2, 4, 7, 14 and 30 after each injection. Optional genital secretion samples will be collected on days 14 and 30 after each injection; see **Table 1 Schedule of Evaluations**.

9.6. Enrollment and dropout

All participants will be enrolled in the first 36 months of the study to allow for a complete 28 weeks of observation for each subject. To decrease variability, we will only enroll individuals who have remained suppressed with no viral loads >50 copies/ml 48 weeks before enrollment. Viral loads are performed as part of recently funded 'ART NET' study in San Diego (1R01MH100974-01A1, PI Little) every 6 months and in the Owen clinic every 3 months, and we will have access to both measures. Anticipating a 10% dropout, we will enroll 56 subjects into our RCT. We thus conservatively expect to achieve an evaluable sample size of 50 at study end.

9.7 Clinical Site

All study visits will take place at the UCSD Antiviral Research Center (AVRC) located at 220 Dickinson St, San Diego, CA 92103.

10. STUDY POPULATION

10.1 Inclusion/Exclusion Criteria

10.1.1 Participant Inclusion Criteria

10.1.1.1 Documented HIV-1 infection more than 365 days ago (HIV antibody or viral load positive).

10.1.1.2 Capable of signing written informed consent.

10.1.1.3 Documented viral suppression for at least 48 weeks (≤50 copies/mL)

10.1.1.4 Men and women between 18 and 65 years of age.

10.1.1.5 Read and comprehend English.

10.1.1.6 Documented CD4 count at enrollment (>250 cells/µl)

10.1.1.7 Reported CD4 nadir >100 cells/µl.

10.1.1.8 Received seasonal flu vaccination at least 6 weeks prior to first trial injection (and not more recently)

10.1.1.9 Received vaccination for pneumococcal disease at least 12 months prior to first trial injection (and not more recently)

10.1.1.10 Started ART >6 months from estimated date of infection)

10.1.2 Participant Exclusion Criteria

10.1.2.1 Uncontrolled psychiatric condition.

10.1.2.2 Under the influence of drug(s) or alcohol at time of screening.

10.1.2.3 Any condition that, in the opinion of the investigator, would limit follow-up and adequate consent.

10.1.2.4 History of allergic reactions to any of the proposed vaccines or egg allergy.

10.1.2.5 History of Gullian Barre syndrome.

10.1.2.6 Receiving immunosuppressive medications.

10.1.2.7 Pregnancy or lactation.

10.1.3 Co-enrollment Criteria

Co-enrollment in this study and other studies (other than ART NET as discussed above) will be discussed with the protocol team and will be decided on a case-by-case basis.

10.2 Recruitment Process

Subjects will be recruited from the UCSD AVRC and the Owen Clinic. Owen Clinic is an HIV care program affiliated with UC San Diego Health System. It offers HIV primary care in addition to a number of services such as ART therapy, mental health services, medication adherence and substance abuse counseling. Subjects visiting either AVRC or the Owen clinic who are known to be HIV positive and meet the inclusion criteria will be recruited to this study and a screening visit will be scheduled.

10.3 Participant Retention

If a subject meets the inclusion criteria and completes all activities of the screening visit, the study clinician will personally assist the patient in scheduling follow-up visits in order to increase participant retention. The subject will be compensated for each follow up visit according to the following table. Each participant has the opportunity to complete this 30 day cycle a total of three times, one for each study vaccine and placebo. The total amount each participant may be compensated is $570 ($190 for each cycle); see **Table 2** Participant Compensation.

Table 2. Participant Compensation

Study Visit	Laboratory Procedure	Compensation
Baseline	Blood Draw, optional Genital Secretion, optional stool, Intramuscular Injection	$50
Day 2	Blood Draw	$20
Day 4	Blood Draw	$20
Day 7	Blood Draw	$20
Day 14	Blood Draw, Optional Genital Secretion	$30
Day 30	Blood Draw, Optional Genital Secretion	$50

11. INTERVENTIONS

11.1 Biomedical Interventions

11.1.1 Regimen

Each study subject will receive one dose each of the following: Pneumovax®23, Fluarix® (or Fluzone®), and placebo (sterile saline solution). These will be administered at the baseline visit (Day 0) of each cycle and will precede 30 days of follow-up. After the 30 days of each cycle, there is a washout period (minimum 6 weeks) before the next cycle begins. Each study drug will be administered as a one time intramuscular injection.

NOTE: Fluarix® quadravalent vaccine is the flu vaccine of choice for this study. Fluzone® quadravalent vaccine may be substituted for Fluarix® only in the event that Fluarix® is unobtainable from the wholesaler/manufacturer.

11.1.2 Study Product Formulation and Preparation

In order to achieve double blind administration of the vaccines, each drug or placebo will first be transferred to generic, non-labeled syringes by the Study Pharmacist before being delivered to the clinical staff who will administer the injection. This preparation (along with preparation of the sterile saline placebo) will take place in the AVRC pharmacy. No after-market modifications will be made to the formulation of Pneumovax®23 (Merck) or Fluarix® (GSK), or Fluzone® (Sanofi Pasteur) vaccines.

11.1.3 Device Studies: (Not applicable)

11.1.4 Study Product Supply and Accountability

All study product supply will be stored and handled by AVRC pharmacy staff. All pharmacy procedures will be done in accordance with existing verified Standard Operating Procedures (SOPs) that pertain to pharmacy inventory, storage, security and accountability.

11.1.5 Assessment of Participant Adherence with Study Intervention

Since the study intervention (intramuscular injection) will only be administered three times and always with a study clinician present, there is no need to assess participant adherence aside from attending the baseline visit of each study regimen cycle.

11.1.6 Concomitant Medications and Procedure

All concomitant medications will be recorded (including start/stop date, medication type, dose and indications) in a Concomitant Medications Flowsheet (see Appendix A. CRF #CON030). Whenever a concomitant medication or study product is initiated or the dose changed, investigators must review the concomitant medications' and study products' most recent package inserts, investigator's brochure, as well as updated information from DAIDS to obtain the most current information on drug interactions, contraindications, and precautions.

11.1.7 Permitted Medications and Procedures

Because it may interfere with the findings of the study, participants are asked to refrain from receiving routine vaccinations during the study unless otherwise instructed by their primary physician. However, vaccination outside the study protocol will not result in discontinuation of the study. Instead, these instances will be identified and documented, and investigators will control for this instances during analysis. Similarly, any change in any medication, including gain or loss, will be recorded on the Medical History CRF (See Appendix B).

11.1.8 Prohibited Medications and Procedures

Medications that have been suggested to perturb the HIV reservoir or the immune system will require early discontinuation of the study. These medications include: disulfram, histone deacetylase inhibitors, other cancer chemotherapies, biologic immunosuppressives, high dose steroids etc. Other medications or therapies that are taken will be documented during the study, and will be reported to the PI.

11.1.9 Precautionary Medications and Procedures

Other medications that have been suggested to perturb the HIV reservoir or the immune system, like antibiotics, non-steroidal anti-inflammatory drugs, aspirin, or low dose steroids, will be noted and accounted for in the final analyses.

11.1.10 Required Medications

Study participants must be on ART. Moreover, their HIV therapy must remain successful throughout the study (CD4 count >250 cells/ml and nadir >100 cells/ml). The study participants must also have a recent vaccination history including previous seasonal flu vaccination and previous pneumovax vaccination at least 12 months prior to screening visit.

11.2 Behavioral Intervention(s): (not applicable)

12. STUDY PROCEDURES/EVALUATIONS

12.1 Clinical Evaluations and Procedures

12.1.1 HIV Test

An HIV test will be performed to confirm HIV infection if documentation is not available. Acceptable HIV tests include any FDA approved test that is approved for diagnosing HIV infection, such as Oraquick ADVANCE, OraSure Technologies, Inc., INSTI HIV-1/HIV-2 Antibody Test, bioLytical Laboratories Inc., ARCHITECT HIV Ag/Ab Combo, Abbott Laboratories, etc. Participants will receive a finger prick and a few drops of blood will be tested before enrollment.

12.1.2 Pregnancy Test

If the participant is a woman and can become pregnant, a urine pregnancy test will be performed at screening and will be repeated only if pregnancy is suspected.

12.1.3 Medical History and Survey

Participants will be asked about their medical history and will be administered a brief survey of incident illness, which will be recorded on Medical History CRF; See Appendix B.

12.1.4 Vaccination History

Participants will be asked about their vaccination history, specifically, the previous seasonal flu and pneumovax vaccines. A documented or reported pneumovax vaccine will be required at least 12 months prior to the screening visit.

12.1.5 Physical Exam

A brief, symptom-targeted physical exam will be performed and recorded on the Physical History CRF; See Appendix C.

12.1.6 Blood Draw

Approximately 50 mL of blood will be drawn at each visit for the purpose of CD4+ T-cell counts, HIV-1 RNA viral load, HIV genetic testing, and immunologic testing. At baseline, day 14 and day 30 of each cycle, we will collect additional 10mL (60mL total) to perform routine blood chemistry tests, hematology and coagulation (safety tests).

12.1.7 Genital Secretion Collection (optional)

- **For Men:** Men will be asked to provide a semen specimen. In the privacy of their home, or in the privacy of our clinic, they will be asked to give us a semen sample. This will require that they do not have sexual activity for 24 hours prior to the semen collection. They will need to wash their hands and then masturbate using only water or KY jelly as a lubricant and deposit their semen into a plastic container which will be provided to them. If they have collected the specimen at home, they will be asked to bring this container to the clinic within 1 hour after collecting their semen sample. This procedure is optional for participants.

- **For Women:** Women will be asked to self-collect cervical-vaginal secretion swabs. See Appendix D. This procedure is optional for participants.

-

12.1.8 Stool Collection (optional)

Stool specimens will be collected from study participants (See Appendix G) for evaluation of the microbiome composition and its connection to immunologic and viral dynamics. This procedure is optional for participants.

12.1.9 Study Vaccine Injection

Participant will be injected with the study drug. Administration of the intramuscular injection will be blinded. Subjects will complete 1 dose each of Influenza Vaccine, Pneumococcus vaccine and placebo injection every 12 weeks in random order.

- Fluarix (or Fluzone), a vaccine used to prevent infection by the Flu (influenza) virus, or
- Pneumovax 23, a vaccine used to prevent pneumococcal disease, or
- Placebo, a non-active saline solution that contains no vaccine.

12.2 Laboratory Evaluations

All laboratory procedures will be conducted in accordance with Good Clinical Laboratory Practices (GCLP) according to the DAIDS guidelines.

A urine pregnancy test will be performed at screening in women who can become pregnant and will be repeated only if pregnancy is suspected.

A rapid HIV test will be performed if no documentation of previous HIV infection is provided.

Table 3 is a summary table of study assays, methods and time points.

Table 3. Summary of Study Assays and Schedule of Evaluations

	Measurements	Methods	Sample	Sampled Time-points (Days)
Virologic (Aims 1+2)	Cell-associated HIV RNA levels	(dd)PCR	PBMC (CD4+)	0, 2, 4, 7, 14, 30 after each injection
	HIV DNA levels (pol and 2-LTR)	(dd)PCR	PBMC (CD4+)	
	Cell-free HIV RNA levels	SCA	Blood plasma	
	Cell-associated HIV RNA *env* diversity*	NGS	PBMC (CD4+)	0 and time point with highest cell associated HIV RNA level after the active vaccination
	Cell-associated HIV DNA *env* diversity*	NGS	PBMC (CD4+)	
	Replication competent HIV DNA levels*	Inducible RNA assay	PBMC (CD4+)	0 and 30 after each active vaccine
Immunologic	Soluble inflammation markers	ELISA	Blood plasma	0, 2, 4, 7, 14, 30 after each injection

(Aim 3)	Vaccine-specific response	ELISA	Blood plasma	
	Cellular inflammation markers	Flow	PBMC	
	HIV-specific T-cell response	Flow ICC	PBMC	
Incidental stimulation	Microbiome Cmposition	r16s Sequencing	Stool	Day 0
	HHV quantification	qRT-PCR	GS	0, 14, 30 after each injection
	Incident Illness surveys	Survey	--	

Legend: ddPCR: digital droplet PCR, SCA: single copy assay, PBMC: peripheral blood mononuclear cells, ELISA: enzyme linked immunoassay, Flow: flow cytometry, ICC: intracellular cytokine staining, qRT-PCR: quantitative real time PCR. GS: genital secretion. *The 5 participants with the highest sum levels of cell-associated HIV RNA within the 30 days (five measures) following both active vaccinations will undergo these assessments. These same 5 participants will be used for both of these analyses.

12.2.1 Specimen Preparation, Handling and Shipping

All laboratory procedures related to specimen preparation, handling and shipping will be conducted in accordance with Refer to DAIDS Policy Requirements for Laboratories in Clinical Trials, Guidelines for Good Clinical Laboratory Practice Standards and DAIDS Manual of Operational Procedures. The following AVRC Site SOPs are listed below and attached in the Appendix.

- Laboratory SOP “Transporting Specimens within and outside UCSD” (Appendix E)
- Laboratory SOP “Quality Management Plan” (Appendix F)

12.2.2 Biohazard Containment

Transmission of HIV and other blood borne pathogens can occur through contact with contaminated needles, blood, and blood products. Appropriate blood and secretion precautions will be employed by all personnel in the collection of clinical samples and the shipping and handling of all clinical samples and isolates for this study, as currently recommended by the Centers for Disease Control and Prevention in the United States, the WHO internationally and the National Institutes of Health.

All protocol specimens will be shipped using packaging that meets requirements specified by the International Air Transport Association Dangerous Goods Regulations for UN 3373, Biological Substance, Category B, and Packing Instruction 650. Culture isolates, if obtained in this study, are to be shipped as specified for UN 2814 Category A Infectious Substances. All infectious specimens will be transported using packaging mandated in the Federal Code of Regulations, CDC 42 CFR Part 72. Please also refer to individual carrier guidelines, e.g., FedEx, Airborne, for specific instructions.

12.2.3 Total Blood Volume

Participants will have blood drawn at each visit. Approximately 50 or 60 mL of blood will be drawn for the following tests and storage for future research:

- CD4+/CD8+ T-cell Count (3 mL of blood).
- HIV-1 RNA (Viral Load) (6 mL of blood).
- Routine blood chemistry tests, a comprehensive metabolic panel and a complete blood count will be performed to determine overall health at baseline, day 14 and day 30 (we will collect 10 mL of additional blood).
- Blood will be collected for storage (6 mL of blood plasma and all PBMC). Some of the blood will be stored to perform future HIV testing and human genetic testing as well as to assess adherence to ART. Blood will be stored with the usual protectors of identity. The results of these tests will not be shared with the participant since these tests are not used for clinical management of their HIV.

Participants will have approximately 50 or 60 mL of blood drawn at a time with an approximate total of 990 ml (4.5 cups) drawn for this study over the course of 8 months.

12.3 Schedule of Procedures/Evaluations
See Table 1 (page 12) for schedule of evaluations.

12.3.1 Screening
At screening a survey will establish inclusion and exclusion criteria. A screening CRF checklist and a screening survey will be completed by nursing staff. The screening visit will involve a discussion of vaccination history and medical history relevant to the inclusion and exclusion criteria. In addition documentation of plasma HIV-1 RNA and CD4+ T-cell counts will be recorded in the source documents and electronic CRF. A urine pregnancy test will be performed at screening in women who can become pregnant and will be repeated only if pregnancy is suspected.

Written informed consent explained by trained clinical staff will occur before starting screening procedures. The baseline visit (study entry) and administration of the vaccine or vaccine-placebo intervention will be performed within 30 days of the screening visit.

12.3.2 Enrollment
If the participant meets eligibility criteria, a participant identification number (PID) will be assigned to each individual screened and determined to be eligible for the study. PIDs will include a site code and three-digit extension. PIDs may not be reassigned even if the individual fails to enter the study. The PID must be included on every CRF and blood sample. The site must maintain a master list of PIDs in a central location. Participant registration and inclusion/exclusion CRF must be completed. Study entry will occur within 30 days of screening.

The study statistician will provide a randomization list to the pharmacist prior to study start. This list will be used in consecutive order to randomize participants at the time of enrolment.

12.3.3 HIV Counseling and Testing

No HIV testing will occur, as participants must have documented HIV-1 infection more than 365 days prior to enrollment (HIV antibody or viral load positive). Participants are required to be on ART for at least 48 weeks. Counseling can be provided to any participant.

12.3.4 Follow-up

12.3.4.1: Baseline assessments [Day 0 of each cycle]

(These visits will last about 1.5 hours): At study entry the subject should complete all assessments and clinical evaluations with blood sample for banking. Antibody levels for each tested vaccine, cell-associated HIV RNA levels in blood plasma and HIV DNA levels in PBMC should be measured. An optional genital secretion and an optional stool sample will also be collected at baseline assessments. Refusal to provide genital secretion and/or stool sample will not result in termination from the study.

Placebo or vaccine injections: Will be performed by study nursing personnel based on stratification delivered by study pharmacist at baseline.

12.3.4.2 On study evaluations post-vaccination [Days 2, 4, 7, 14 & 30]

(These visits will last about 1 hour): At the time of each injection and five sampling time points afterwards, the following should be measured: cell-associated and cell-free HIV RNA levels, HIV DNA levels, vaccine-specific antibody levels, soluble and cellular inflammatory and proliferation markers, and HIV specific T-cell response. Genital secretion samples will be collection at post-vaccination evaluations 14 and 30 only.

12.3.5 Early Termination Visit

If a subject is unable to complete all of the study visits, they will be asked to return to the clinic to for a study discontinuation visit. The following procedures will be performed:

- brief physical exam and concomitant medication questionnaire
- blood drawn for routine safety tests to check your blood count, liver and kidney function, CD4+cell count, viral load and storage for future testing
- survey about recent illnesses and symptoms
- optional collection of genital secretion

CRITERIA FOR EARLY TERMINATION.

- Active drug or alcohol use or dependence that, in the opinion of the investigator, would interfere with adherence to study requirements.
- The subject becomes pregnant during the course of the study.
- Failure by the subject to attend scheduled clinic visits may result in discontinuation.
- Request by the subject to withdraw from the study.
- The participant’s HIV therapy fails.

- Request of the primary care provider if s/he thinks the study is no longer in the best interest of the subject.
- Clinical reasons believed life threatening by the physician.
- Subject judged by the investigator to be at significant risk of failing to comply with the provisions of the protocol as to cause harm to self or seriously interfere with the validity of the study results.

12.3.6 Pregnancy Visit

Subjects will be screened for pregnancy at the screening visit and at subsequent follow up visits if they are thought to have become pregnant. If a participant becomes pregnant during the course of the study they will be asked to complete the early termination visit as described above in section 12.3.5.

12.3.7 Final Study Visit

The final study visit will take place as the day 30 follow up visit at the completion of the third and final cycle. This visit will have the same procedures and evaluations of all other follow up visits.

13. ASSESSMENT OF SAFETY

13.1 Safety Assessment Overview

Although serious adverse events are not expected in this study, any serious adverse events (SAEs) will be reported in accordance with DAIDS recommendations and local UCSD IRB requirements.

13.2 Adverse Event Procedures and Reporting Requirements

All SAEs will be documented on the SAE Reporting Form within five working days of site awareness of the event and submitted to the Safety Monitoring Committee (SMC).

14. CLINICAL MANAGEMENT

14.1 Clinical Management of Adverse Events

The study investigators will review all adverse events (AEs) from subjects followed as part of the project. Unanticipated and anticipated toxicities will be graded according to the Division of AIDS Table for Grading the Severity of Adult and Pediatric Adverse Events, Corrected Version 2.1 dated July 2017.

Subjects with AE will be followed until the event has resolved or stabilized. Out of range laboratory values will be followed closely until resolved. In every situation, the study investigators will assess if it is safe for the subject to continue in the study. A physician is on-call at the AVRC 24/7 for evaluation and management of study-related emergencies or AEs. Any new, unusual, or unexpected toxicities will be reported to the Human Subjects Committee, other investigators participating in the trial, the FDA, the NIAID/DAIDS, and other regulatory agencies or entities as specified by NIAID, FDA or UCSD policies and procedures. AEs

assessed as related to non-study drugs (concomitant medications) will be handled according to the relevant package inserts and the best medical judgment of the site investigator.

Because all proposed vaccines are FDA-approved for clinical use and clinically recommended for HIV-infected individuals, a low likelihood for study-related adverse events is anticipated. Serious adverse events (SAEs) will be defined using standard FDA guidelines. All SAEs occurring at the clinical site will be reported to the UCSD IRB using standard reporting time frames and IRB requested formats. All higher-grade toxicities will be reported immediately to Dr. Smith (or Dr. Little if Dr. Smith is unavailable) and follow-up monitoring or clinical intervention with the participant's healthcare provider will be performed in consultation with the clinical investigators and nursing staff. Additionally, the clinical investigator team will review safety report summaries from the clinical nursing staff every three months.

14.1.1. Safety Monitoring Committee

Because we are using standard clinical vaccines that are clinically recommended for HIV-infected individuals, the need for a formal DSMB is not anticipated, but the protocol team will convene a safety monitoring committee (SMC) of at least three independent experts including one statistician to perform an interim safety review at least once annually. The SMC will review study progress and data to ensure patient safety, as well as feasibility of completion of the study within the grant-funding period. After the review, the SMC will be asked to make a recommendation for study continuation, modification or conclusion. The SMC is composed of clinical trials experts and a statistician who are not investigators in the specific project and have no conflict of interest with the clinical study. Members of the SMC include Drs. Benson, Karris and Jain, who are all respected experts both in the care and treatment of HIV-infected patients and in the design, conduct, and analysis of clinical trials.

All members of the SMC will receive an introduction to the study trial and will have a clear understanding of how study investigators will approach early study termination for safety reasons. The Safety Monitoring Committee will review the safety and welfare of participants and confidentiality of data. The committee's responsibilities include: reviewing study protocols including plans for data safety monitoring, evaluating the progress of the intervention trial, evaluating participant risk versus benefit, performing assessments of any unexpected factors that can affect the safety of study participants. Following the review, the SMC will provide writing recommendations to the PI, who will distribute these to the study team, local IRB and the DAIDS Program Officer.

14.1.2. Most frequent Adverse Events

Participants will receive two vaccines and a vaccine placebo. This procedure may be associated with pain, bruising, bleeding, infection, dizziness, or fainting. This will be managed by strict adherence to aseptic techniques, and use of experienced clinical nurses administering the injections. The vaccines used in this study are generally safe for HIV infected individuals with CD4 > 200 cells/ul. Occasionally, subjects can develop soreness, swelling, or redness at the injection site. Some vaccines are associated with fever, rash, and achiness. Serious side effects are rare, but may include life-threatening allergic reaction or seizure. Risks associated with each of the specific vaccines used include:

- *Pneumococcal vaccine*: About half of people who get Pneumovax®23 have mild side effects, such as redness or pain where the shot is given. Less than 1% of persons develop a fever, muscle aches, or more severe local reactions. Mild fever, fatigue, headache, chills, and muscle pain have also been reported. Life-threatening allergic reactions are very rare.

- *Influenza vaccine*: Inactivated parental vaccine that is recommended yearly to all HIV-infected individuals. Vaccination is most effective among persons with CD4 of >100 cells/μL and HIV RNA of <30,000 copies/mL, which is in the eligibility requirements for the study. In adults, the most common local and general adverse events were pain and redness at the injection site (20-50%), muscle aches (23%), fatigue (20%), and headache (19%). Vaccination should be avoided by people with known hypersensitivity to any ingredient of the vaccine, and persons previously diagnosed with Guillain-Barré syndrome. Life-threatening allergic reactions from any vaccine are very rare.

14.2 Other Disease Events

Any disease events or complications that occur during study visits will be handled according to the discretion of the study clinician and subsequently subjects will be referred to an appropriate primary physician.

14.3 Pregnancy

A urine pregnancy test will be performed at screening in women who can become pregnant and will be repeated only if pregnancy is suspected. If a subject becomes pregnant during the study, then an early termination visit will be scheduled for them and they will be taken off study.

Reason for excluding pregnant and breastfeeding women: To date, there are still insufficient safety data for the use of pneumococcal and influenza vaccines in pregnant women and its administration is recommended only if clearly indicated for medical reasons. Additionally, as part of this study we will administer the vaccines outside the regular schedule to determine the effect of immune-stimulation on HIV transcription. It is not known if vaccine administration outside the clinically recommended schedule can harm unborn babies or might increase HIV transcription and consequently might affect the risk of vertical HIV transmission. If you are a woman and having sex that could lead to pregnancy, you must agree not to become pregnant

14.4 Treatment Failure

HIV treatment failure will be routinely tested by HIV RNA viral loads. If a participant's HIV treatment fails (two consecutive viral load measurements of >500 copies/ml) or their ART regimen is interrupted, then an early termination visit will be scheduled and the participants will be taken off study.

14.5 Criteria for Discontinuation

- Active drug or alcohol use or dependence that, in the opinion of the investigator, would interfere with adherence to study requirements.

- The subject becomes pregnant during the course of the study.

- Failure by the subject to attend scheduled clinic visits may result in discontinuation.
- Request by the subject to withdraw from the study.
- The participant's HIV therapy fails.
- Request of the primary care provider if s/he thinks the study is no longer in the best interest of the subject.
- Clinical reasons believed life threatening by the physician.
- Subject judged by the investigator to be at significant risk of failing to comply with the provisions of the protocol as to cause harm to self or seriously interfere with the validity of the study results.

15. STATISTICAL CONSIDERATIONS

15.1 Overview and General Design Issues

The proposed study is a randomized double-blind controlled trial conducted over 28 weeks. Randomized interventions will be injections of Influenza vaccine, Pneumococcal vaccine, and Placebo. Each participant will receive each injection but in a randomized order.

Vaccines: Influenza (Fluarix®, GSK), Pneumococcal (Pneumovax®23, Merck), and placebo (sterile saline injection).

Primary endpoint: average level of CD4+ T-cell-associated HIV RNA transcription measured at day 7 after each injection.

Study Arms: Each subject will take Influenza (Fluarix®, GSK), Pneumococcal (Pneumovax®23, Merck), and placebo. Randomization will determine the order in which the subjects receive the injections. There are six potential study arms, one for each possible combination in which someone could receive the injections.

Study population: This study will enroll 56 HIV-infected individuals between the ages of 18 and 65 who started ART during chronic infection. These subjects will be recruited from the UCSD AVRC. Eligible participants will have CD4 >250 cells/μl at enrollment and a CD4 nadir >100 cells/μl. We chose to limit enrollment to younger individuals with higher CD4 counts to enhance the likelihood of having a more robust immune response to any given vaccine. We limited enrollment to individuals who started ART during chronic infection (>365 days from estimated date of infection) to enhance the likelihood of having a large replication competent viral reservoir, thus increasing our chances to observe an effect of vaccine administration on HIV RNA transcription [96]. To reduce variability in vaccine stimulation (between antigen naïve versus experienced participants), we will also limit participation to individuals who have documented or reported vaccine history consistent with previous influenza and pneumococcal vaccination.

Sample collection: Study evaluations will be based on three 30 day cycles (Influenza vaccine, Pneumococcus vaccine, Placebo in random order) over 28 weeks of the RCT. *Pre injection*: one paired blood, optional stool and optional genital secretion sample will be collected before each injection. *Post-injection*: Blood samples will be collected on days 2, 4, 7, 14 and 30 after each injection. Optional genital secretion samples will be collected on days 14 and 30 after each injection

15.2 Study Endpoints

15.2.1 Primary Endpoint

Average change in level of CD4+ T-cell-associated HIV RNA transcription from baseline to 7 days after each injection.

15.2.2 Secondary Endpoints:

1. Average change in level of CD4+ T-cell-associated HIV RNA transcription from baseline to 2, 4, 14 and 30 days after each injection.
2. Average change in levels of HIV DNA from baseline to 2, 4, 7, 14 and 30 days after each injection.
3. Determine selective vs. nonselective HIV RNA production from HIV DNA (by next generation sequencing and panmixis tests) among the five participants with the highest levels of cell associated HIV RNA following active vaccination.
4. Average change in levels of immune activation (soluble and cellular markers) from baseline to 2, 4, 7, 14 and 30 days after each injection.
5. Average change in levels of replication competent HIV DNA (by inducible RNA assay) among the five participants with the highest levels of cell associated HIV RNA following active vaccination.
6. Average change in levels of HIV specific T-cell immune response from baseline to 30 days after each injection.
7. Stimulation of HIV transcription by incidental illness, human herpesviruses replication and microbial translocation over the course of the study.
8. Possible immunemodulatory effect of microbiome composition
9. Vaccine administration will be safe for all included participants.

15.3 Study Objectives and Hypotheses

Primary Objective

Determine the impact of Pneumococcus and Influenza vaccines on the HIV transcriptional activity in individuals who are virologically suppressed for at least 48 weeks on ART.

Secondary Objectives

Aim 1a: Determine the timing and duration of increased HIV transcription following active vaccination.

Aim 1b: Determine if vaccination selectively or non-selectively activates HIV transcription from HIV DNA populations

Aim 2: Determine if active vaccination influences levels of cell associated HIV DNA or replication competent provirus.

Aim 3a: Determine to what degree immune stimulation with an Influenza or Pneumococcal vaccine can induce HIV transcription during ART.

Aim 3b: Determine if immune stimulation associated with active vaccination is associated with reduction in number of HIV DNA populations.

Aim 3c: Determine if HIV-specific, vaccine-specific, or bystander immune stimulation is associated with HIV transcription.

Aim 4: Determine how genital shedding of human herpesviruses, microbial translocation and incident illnesses stimulate the immune system and influence levels HIV transcription.

Aim 5: Determine how dysbiosis might contribute to immune stimulation and influence HIV transcription.

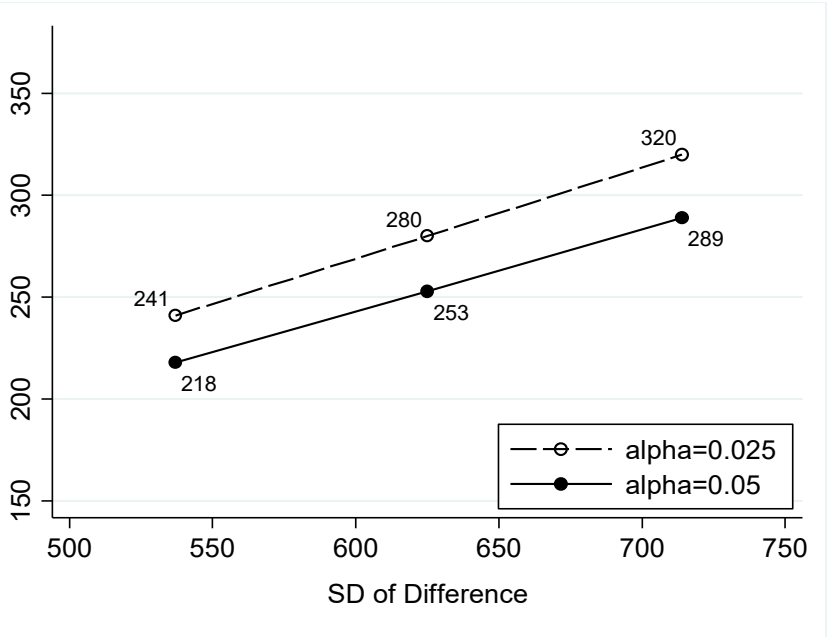
15.4 Sample Size Considerations

Examples of Power:

Primary Hypothesis (stated under the alternative): On average, participants will have a higher absolute increase in levels of cell-associated HIV RNA in CD4+ T-cells seven days after receiving either Pneumococcal or Influenza vaccinations, when compared to seven days after receiving placebo. We have chosen to provide estimates of power on the one sampled time point that is likely to show the largest effect, based on our literature review. All other sampled time points following vaccination will be evaluated as well, but to be conservative, we focus on and present power for one sampled time point for our primary hypothesis.

- Assumption: This is a cross-over study with 56 individuals receiving Placebo, Pneumococcus, and Influenza injections in random order (6 possible orders) with sufficient wash-out period in between (minimum 6 weeks) vaccinations. We expect that 6 individuals drop out, resulting in a fully evaluable sample size of 50 individuals. Pilot data were generated from the previous Spanish Study [1] to provide estimates of standard deviations (SD) of change in cell associated HIV RNA levels. From these data, we used a SD of 537 HIV RNA copies/10^6 CD4 T-cells for Influenza and 714 for Pneumococcus, and a pooled estimate of 625.
- Effect sizes: In Figure 3, we present the effect sizes that we have 80% power to observe at alpha levels of 0.05 (solid line) and 0.025 (dashed line). Since we will compare observations in the same individual, we will use a paired t-test that compares increases in cell-associated HIV RNA in CD4+ T-cells after 7 days for each of the active vaccine injections (Pneumococcus or Influenza) to 7 days after the placebo injection. For example, if the SD of the differences between active and placebo injection measures is 625 HIV RNA copies/10^6 CD4 T-cells, then we will have 80% power at alpha 0.05 to detect an effect size of 253 HIV RNA copies/10^6 CD4 T-cells absolute increase or decrease (two-sided test) in cell associated HIV RNA levels following active vaccination versus placebo, which is well-within the range observed in our preliminary data.

Figure 3 Effect sizes with 80% power to test primary hypothesis
Legend: SD: standard deviation (x axes), effect size (y axes)



Example of Secondary Hypothesis (stated under the alternative): On average, participants will have a greater absolute maximum decrease in levels of total CD4+ T-cell-associated HIV DNA measured 2-30 days after receiving either Pneumococcal or Influenza vaccinations, when compared to 2-30 days after receiving placebo.

- Assumption: This is a cross-over study of three treatments (with 6 possible orders) with a fully evaluable sample size of 50 individuals. We used our data from 199 subjects who had CD4+ T-cell associated HIV DNA levels measured after 48 weeks after virologic suppression on ART to provide estimates of effect sizes. In these samples, we found a stable average level of 41.01 of cell associated HIV DNA copies/10^6 PBMC with a SD of 75.01.
- Effect sizes: In table 4, we present estimates of effect sizes that we have 80% power to observe at an alpha level of 0.05 using a paired t-test that compares maximum decreases (over 2-30 days) in levels of total CD4+ T-cell-associated HIV DNA for each of the active vaccine injections to maximum decreases (over 2-30 days) after the placebo injection, assuming the presented SD. For example, if the SD of the change is similar to the SD from our preliminary data (~75), then we have 80% power to detect an average maximum decrease of 30.3 HIV DNA copies/10^6 PBMC following active vaccination, as compared to after placebo.

Figure 4. Power to see a difference in HIV DNA levels following vaccination versus placebo

Standard deviations of differences in measures of HIV DNA copies/10^6 PBMC	Effect size of average maximum decrease following vaccination if the average value remains 40 after placebo injection
40	-16.2
50	-20.2
60	-24.3
70	-28.3
75	-30.3
80	-32.3
*Assuming the average value remains 40 HIV DNA copies/10^6 PBMC after placebo injection, based on our preliminary data.	

Example of Secondary Hypothesis (stated under the alternative): Participants will have a greater absolute maximum increase in levels of (a) inflammatory cytokines, (b) T-cell activation and proliferation, (c) HIV-specific immune responses, and (d) vaccine-specific immune responses over the 30 days of observation after receiving either active vaccination, when compared to maximum measures taken over the 30 days of observation after receiving placebo.

Assumptions and effect sizes: Assuming 50 fully evaluable participants and that the SD of the differences in increase over days 2-30 in activated CD4+ T-cells is 4.5% (based on data from our CMV studies[97]), then we have 80% power for alpha levels of 0.05 using a paired t-test to compare maximum increases (over 2-30 days) of a 40% increase (or decrease) in levels of activated CD4+ T-cells for each of the active vaccine injections as compared to placebo, which is consistent with changes seen in CD4+ T-cell activation during genital shedding of CMV shedding [97].

15.5 Enrollment/Randomization/Blinding Procedures

Enrollment: A screening survey will establish inclusion and exclusion criteria. A screening CRF checklist and a screening survey will be completed by nursing staff Written informed consent explained by trained clinical staff will occur before starting screening procedures. The baseline visit (study entry) and administration of the vaccine or vaccine-placebo intervention will be completed within 30 days of screening. A PID will be assigned to each individual screened for the study. PIDs will include a site code and three-digit extension. PIDs may not be reassigned even if the individual fails to enter the study. The PID must be included on every CRF and blood sample. The site must maintain a master list of PIDs in a central location. Study entry must be completed within 30 days after participant registration and inclusion/exclusion CRF have been completed.

Randomization: The study statistician will provide a randomization list to the pharmacist prior to study start. Subjects will be considered randomized at the time they have completed enrollment procedures and have received their initial vaccination. Subjects who do not receive any vaccination will not be considered enrolled and will be replaced and will have no follow-up evaluations performed. Subjects who withdraw after receiving the vaccine or vaccine-placebo intervention will be asked to complete the early termination visit.

Blinding: In order to achieve double blind administration of the vaccines, each drug or placebo will first be transferred to generic, non-labeled syringes before being delivered to the clinical staff who will administer the injection using aseptic techniques. This preparation (along with preparation of the sterile saline placebo) will take place in the AVRC pharmacy. Unblinding of the study arms for data analysis of primary and secondary endpoints will occur around 44-48 months (after all participants have completed the entire study follow-up).

15.6 Maintenance of Trial Treatment Randomization Codes

Trial treatment randomization codes will be kept by Study Pharmacist (Dr. Muttera) and Study Biostatistician (Dr. May). Dr. Muttera is located in the AVRC Pharmacy (220 Dickinson Street, Suite A. San Diego, CA 92103) and her office phone number is (619) 543-2688.

15.7 Participant Enrollment and Follow-up

Baseline assessments [Day 0 of each cycle]

At study entry the subject should complete all assessments and clinical evaluations with blood sample for banking. Antibody levels for each tested vaccine, cell-associated HIV RNA levels in blood plasma and HIV DNA levels in PBMC should be measured. An optional genital secretion and optional stool samples will also be collected at baseline assessments.

Placebo or vaccine injections: Will be performed by study nursing personnel using the generic non-labelled syringe delivered by study pharmacist at baseline.

On study evaluations post-vaccination [Days 2, 4, 7, 14 & 30]

At the time of each injection and five sampling time points afterwards, the following will be measured: cell-associated and cell-free HIV RNA levels, HIV DNA levels, HIV levels, antibody levels, soluble and cellular inflammatory and proliferation markers, and HIV specific response. Optional genital secretion samples will be collected at post-vaccination evaluations 14 and 30 only.

15.8 Data and Safety Monitoring

15.8.1 Planned Interim Analyses and Stopping Guidelines

15.8.1.1 Interim safety review:

The study investigators Drs. Smith, Little, Gianella, May and the study nurses will review the individual subject records every 3 months, including consent forms, case report forms (CRFs), supporting source data, laboratory specimen records and medical records (physicians' progress notes, nurses' notes) to ensure protection of study subjects, compliance with the protocol and accuracy and completeness of records. The study investigators will also inspect sites' regulatory files to ensure that regulatory requirements are

being followed and the site pharmacy to review product storage and management. If requested, the site Investigators will make study documents (e.g. consent forms, drug distribution forms, CRFs) readily available for inspection by the local IRB, the site monitors, the FDA, the NIAID, and the OHRP for confirmation of study data.

The study investigators will review all safety events from subjects followed as part of the project. Adverse Events (AE) will be graded using the standard Division of AIDS Table for Grading the Severity of Adult and Pediatric Adverse Events, Corrected Version 2.1 dated July 2017.(). Subjects with AE will be followed until the event has resolved or stabilized. Out of range laboratory values will be followed closely until resolved. In every situation, the study investigators will assess if it is safe for the subject to continue in the study. The clinical investigator team (Drs. Smith and Little) will have a 24-hour call system (using the medical center pager system) so subjects can access a study physician at any time to answer questions or review potential unforeseen consequences.

Because all proposed vaccines are standard clinical vaccines and clinically recommended for HIV-infected individuals, a low likelihood for study-related adverse events is anticipated. Serious adverse events (SAEs) will be defined using standard FDA guidelines. All SAEs occurring at the clinical site will be reported to the UCSD IRB using standard reporting time frames and IRB requested formats. All higher-grade toxicities will be reported immediately to Dr. Smith (or Dr. Little if Dr. Smith is unavailable) and a plan for follow-up monitoring or clinical intervention with the participant's healthcare provider will be performed in consultation with the clinical investigators and nursing staff. Additionally, the clinical investigator team will review safety report summaries from the clinical nursing staff every three months.

15.8.1.2 Safety Monitoring Committee

Because we are using standard clinical vaccines that are clinically recommended for HIV-infected individuals, the need for a formal DSMB is not anticipated, but the protocol team will convene a safety monitoring committee (SMC) of at least three independent experts including one statistician to perform an interim safety review once the study has reached 50% accrual or after approximately one year has passed, whichever occurs first. The SMC will review study progress and data at least annually to ensure patient safety and feasibility of completing the study within the grant-funding period. After the review, the SMC will be asked to make a recommendation for study continuation, modification or conclusion. The SMC is composed of clinical trials experts and statisticians who are not investigators in the specific project and have no conflict of interest with the clinical study. Members of the SMC include Drs. Benson, Karris and Jain, who are all respected experts both in the care and treatment of HIV-infected patients and in the design, conduct, and analysis of clinical trials.

All members of the SMC will receive an introduction to the study trial and will have a clear understanding of how study investigators will approach early study termination for safety reasons. The Safety Monitoring Committee will review the safety and welfare of participants and confidentiality of data. The committee's responsibilities include: reviewing study protocols including plans for data safety monitoring, evaluating the progress of the intervention trial, evaluating participant risk versus benefit, performing assessments of any unexpected factors

that can affect the safety of study participants. The SMC will provide writing recommendations to the PI, who will distribute these to the study team, local IRB and the DAIDS Program Officer

15.8.1.3 Interim Efficacy Review:
A SMC will review trial data when at least 50% of subjects have completed the trail. There will be no formal interim assessment of efficacy. Safety data will be evaluated to determine if the study should continue as planned, should be modified to ensure patient safety or should be stopped.

15.8.2 Analysis Plan
All formal analyses of the data will be performed at the end of the study with no real time clinical decisions based on intermediate results. We will utilize a comprehensive AVRC Data Management System developed through our CFAR that can handle all dynamic aspects of clinical research studies including electronic case report form (eCRF) creation and editing, protocol design interfaces, online data entry and QA workflows, real-time accrual monitoring, multi-study data integration, role-based user permissions, integrated specimen processing workflows, an auditing system to track changes to both forms and data, and data visualization and export tools.

Sample and data collection from study participants will occur from study months 6-36 of the study. Unblinding of the study arms for data analysis of primary and secondary endpoints will occur around 44-48 months (after all participants have completed the entire study follow-up), and the final analysis of the trial will take place between 48 and 60 months.

Analysis of the Primary Hypothesis: We will determine whether average changes in levels of cell-associated HIV RNA in CD4+ T-cells are associated with administration of active vaccine or placebo using two-sided paired t-tests with alpha level of 0.05 for each of the two tests (influenza versus placebo and pneumococcus versus placebo).

Secondary analyses: Since subjects will receive multiple injections, we will determine if changes in cell associated HIV RNA in CD4+ T-cell levels are sustained over the weeks following vaccination using graphical and descriptive statistical methods. We will also explore the effect of the vaccines on the size of cell free and cell associated HIV RNA populations using linear mixed effects models and consider incorporating interactions with time to evaluate the change in effects of the vaccines over time.

Additional analyses are described in the Statistical Analysis Plan (SAP).

16. DATA HANDLING AND RECORDKEEPING

16.1 Data Management Responsibilities
Both archived and prospectively collected and generated data from enrolled subjects will be organized into our central database securely hosted at UCSD, along with all available linked demographic, geographic, clinical, and behavioral data. Confidentiality is maintained by linking data only to the unique ID, and by not storing any personal identifying information in result reporting systems.

16.2 Essential Documents and Access

All essential documents will be maintained by the clinical data manager who will also control permissions and access to the sources documents and essential data.

16.3 Quality Control and Quality Assurance

To ensure an accurate and complete database, key forms will be monitored for discrepancies using automated logical checks. If problems are identified, such as missing clinical data, this will be communicated to study investigators. A Study Status Report including number of participants enrolled or off-study report (list of participants off-study; reasons off-study), SAE report (list of participants; diagnoses), study related AEs ≥ grade 3 will be prepared quarterly. Clinical evaluations and specimen collections will be conducted according to the specifications in the study protocol. The study protocol, including materials and informed consent forms, will undergo review and approval by the UCSD IRB and local Ethics Committee prior to implementation, and all participants will be provided with education, counseling and information, and undergo informed consent for all procedures.

17. CLINICAL SITE MONITORING

The National Institute of Allergy and Infectious Diseases (NIAID) contracted site monitors will visit the clinical research site (AVRC) to review participants records, including informed consent forms, CRFs, medical records (e.g., physicians' progress notes, nurses' notes, individuals' hospital charts), and laboratory records to ensure protection of study participants, compliance with the EC/IRB approved protocol/amendments, and accuracy and completeness of records. The monitors will review sites' regulatory files to ensure that local regulatory requirements, in addition to U.S. Federal regulations, are being followed. They will also inspect sites' pharmacies and review product storage, management and accountability records

18. HUMAN SUBJECTS PROTECTIONS

18.1 Institutional Review Board/Ethics Committee

This protocol and the informed consent document and any subsequent modifications will be reviewed and approved by the UCSD Institutional Review Board responsible for oversight of the study. Written informed consent will be obtained from the subject by a member of the study staff. At the time potential subjects contact us regarding the study, any questions they may have will be answered by a member of the study staff. If the potential subject is still interested in participating, a clinic visit will be scheduled. The subject will be informed of the time that needs to be allotted for their first visit in which the informed consent will be administered.

18.2 Vulnerable Participants

18.2.1 Pregnant women and fetuses: Women who are pregnant will be excluded from the study and women who become pregnant while on the study will be asked to complete an early termination visit and will be removed from follow up.

18.3 Informed Consent

18.3.1 Informed Consent Process

Written informed consent (previously approved by UCSD's IRB) explained by trained clinical staff will occur before starting screening procedures. Study entry and administration of the vaccine or vaccine-placebo intervention will occur within 30 days of completion of screening procedures.

The informed consent will describe the purpose of the study, the procedures to be followed, and the risks, and benefits of participation. This information will be explained to the study subject in a face-to-face setting by the individual who consents the participant. Participants will be encouraged to ask questions throughout the consent process and encouraged to discuss their participation with trusted advisors, such as family members, close friends, etc. Participants will be allotted sufficient time to consider whether or not to participate in the research study. After allowing the potential participant time to read the informed consent the study staff and/or investigator will answer and address any questions or concerns the participant may have. Once all questions and concerns have been addressed and the person wishes to participate, they will be asked to sign the informed consent.

Also, during the consent process, the Health Insurance Portability and Accountability Act (HIPAA) Authorization will addressed. A copy of the consent and HIPAA Authorization form as well as the Notice of Privacy Practices booklet will be given to the subject.

18.3.2 Documentation of Informed Consent

All signed informed consent documents will be kept on file at the AVRC under the supervision of the AVRC regulatory office.

18.3.3 Stored Samples and Associated Data Considerations

18.3.3.1 Data Security: Any data collected as part of this study that is stored at the AVRC and/or is transferred via the internet will follow our data security process as outlined below.

With the fast-developing technology, dependable and comprehensive data security measures are key components to defy the perceived threats of Internet hackers and accidental disclosure of confidential information. In the following we provide a summary of the key features pertinent to this project.

- An anonymous participant identification number is used for all data collection, recording, and submission to the project database.
- Data that contain any participant identifiers (e.g., name or contact information) other than the unique identifier are password protected and accessible only to staff members whose job requires knowledge of such data.

- Laboratories are instructed not to disseminate any participant identifiers in any communications with, or data submissions to, any other AVRC collaborators. Any data transfer over the Internet uses encryption.

- Data transfer and all Web-based utilities use secure access (user and server authentication, 128-bit SSL encryption). This type of encryption is the same as is used for Web-based transactions that involve credit cards or Web banking.

18.3.3.2 Research Laboratory Specimen Identification Policy: All research laboratory specimens leaving the AVRC to an outside laboratory will be de-identified. See the following procedure:

- The Lab Manager will create a study specific AVRC internal lab requisition. The requisition will be saved and accessible via the AVRC internal computer system's shared drive.

- Each research nurse will access and print the study specific requisition(s) via the shared drive.

- Each research nurse will then complete the study specific requisition with the subject's name, DOB, medical record number (MR#), PID, AVRC number and study week.

- The requisition will then be delivered by the research nurse to the AVRC lab.

- The AVRC laboratory staff will then complete the appropriate form for the corresponding laboratory to which the specimen will be sent, using two coded identifiers, the subject's PID number (in the name field) and AVRC number (in the medical record number 's field)

- The AVRC laboratory staff will prepare and label specimen tubes using the same two coded identifiers, the subject's PID number (in the name field) and AVRC number (in the medical record number's field). No personal health identifiers will be included on the specimen label (i.e., no name, initials, DOB, MR#, etc).

- Prior to the blood draw, the phlebotomist will verbally verify the subject's name and DOB. The phlebotomist will confirm the coded specimen tube(s) identifiers with the coded form identifiers.

- Coded specimens are transported to the appropriate lab either by AVRC staff or shipped via FedEx, under IATA regulations.

- All study specific completed AVRC internal lab requisitions will be retained in a locked and secured area for a period of six months and thereafter shredded.

- Samples will be kept frozen and stored for an indefinite length of time.

All stored samples are accessible only to the AVRC laboratory personnel and the appropriate study members. Samples are stored under the coded identifiers as detailed above frozen and in freezers equipped with locks until they are shipped to the central laboratory under contract with the sponsor. The freezers are located in the AVRC and CTF building behind locked doors with cypher or key pad entry.

The stored samples are shipped as outlined above and are then secured under the sponsor's SOPs for storage of human biological samples.

18.4 Risks

Privacy and Confidentiality: Although we will make every effort to protect the subject's privacy and confidentiality it is possible that the subject's status could become known to others. This could cause problems between the subject and the subject's family and/or community and could cause the subject to be discriminated against.

Personal Questions Risks: Subjects will be asked questions about personal issues during this study. There may be questions about their mood, about sexual functioning, about drug use, etc. These types of personal questions may make some subjects uncomfortable.

Reporting Requirements: All cases of HIV, hepatitis B and hepatitis C must be reported to the county public health department. According to California state law, study staff is required to give public health department staff the participant's name, contact information and treatment records for the hepatitis if requested.

Blood Draws: The subject may experience temporary discomfort from the blood draws. The needlesticks may cause local pain, bleeding, bruising and swelling, as well as lightheadedness, dizziness and rarely, blockage of the vein, fainting and/or a local infection.

Vaginal Secretions Collection Risks: Women may have some discomfort or feel embarrassed when they give vaginal fluid sample.

Semen Collection Risks: Men may have some discomfort or feel embarrassed when they give semen sample.

Stool: Subjects may have some discomfort or feel embarrassed when they give stool sample.

Risks of Study Drugs: The drugs used in this study may have side effects, some of which are listed below. Please note that these lists do not include all the side effects seen with these drugs. These lists include the more serious or common side effects with a known or possible relationship. It is very important that subjects tell their study doctor of any changes in their medical condition while taking part in the study. At any time during the study, if subjects believe they are experiencing any of these side effects, they have the right to ask questions on possible and /or known risks. There is a risk of serious and/or life threatening side effects when non-study medications are taken with the study drugs. For their safety, subjects must tell the study doctor or nurse about all medications they are taking before they start the study and must ask approval for taking any new medication while they are on the study.

Risks of Pneumococcal (Pneumovax 23) vaccine: About half of people who get Pneumovax®23 have mild side effects, such as redness or pain where the shot is given. Less than 1% of persons develop a fever, muscle aches, or more severe local reactions. Mild fever, fatigue, headache, chills, and muscle pain have also been reported. Life-threatening allergic reactions are very rare. Serious side effects include: Difficulty breathing, wheezing, rash and hives. These may be signs of an allergic reaction. Participants should contact the study staff immediately if they experience these symptoms.

Risks of Influenza (Fluarix or Fluzone) vaccine: Common side effects include: Low fever, chills, redness or pain where the vaccine was injected, headache, and joint/muscle pain.

*Serious side effects include***:** Severe weakness or unusual feeling in arms or legs, high fever, seizure, convulsions, or unusual bleeding. These may be signs of an allergic reaction. Subjects should contact the study staff immediately if they experience these symptoms.

Inactivated parental vaccine is recommended yearly to all HIV-infected individuals. Vaccination is most effective among persons with CD4 of >100 cells/μL and HIV RNA of <30,000 copies/mL, which is in the eligibility requirements for the study. In adults, the most common local and general adverse events after flu vaccine administration were pain and redness at the injection site (20-50%), muscle aches (23%), fatigue (20%), and headache (19%). Vaccination should be avoided by people with known hypersensitivity to any ingredient of the vaccine, and persons previously diagnosed with Guillain-Barré syndrome. Life-threatening allergic reactions from any vaccine are very rare.

Reproductive Risks: It is not known if drug combinations in this study harm unborn babies. If participants are woman and having sex that could lead to pregnancy, they must agree not to become pregnant.

If participant is a woman participating in sexual activity that could lead to pregnancy, she and/or her male partner must use one form of birth control that they discuss with the study staff. Subjects must start one method of birth control before they start taking the study drugs, while they are taking the study drugs, and for 30 days after stopping study drugs.

- Condoms (male or female) with or without a spermicidal agent. Condoms are recommended because their appropriate use is the only contraceptive method effective for preventing HIV transmission.
- Diaphragm or cervical cap with spermicide
- Intrauterine device (IUD)
- Hormone-based contraceptive

Breastfeeding Risk: It is unknown whether the study drugs pass through breast milk and may cause harm to your infant. You must not breastfeed while you are in this study.

Unknown Risks: In addition to the risks listed above, there are risks that are not known or do not happen often when participants have these procedures performed. Participants will be informed in a timely manner, both verbally and in writing of any new information, findings or changes to the way the research will be done that might influence their willingness to continue participating in this study.

18.5 Social Impact Events

Individuals enrolled in this study may experience personal problems resulting from the study participation. Such problems are termed social impact events. Although we will make every effort to protect participant privacy and confidentiality, it is possible that participants' involvement in the study could become known to others, and that participants may experience stigmatization or discrimination as a result of being perceived as being HIV- infected or at risk for HIV infection. For example, participants could be treated unfairly, or could have problems being accepted by their families and/or communities. Problems may also occur in circumstances in which study participation is not disclosed, such as impact on employment related to time taken for study visits.

In the event that a participant reports a social impact event, every effort will be made by study staff to provide appropriate assistance, and/or referrals to appropriate resources. Social impact events are documented and reviewed on a scheduled basis by the protocol team leadership with the goal of reducing their incidence and enhancing the ability of study staff to mitigate them when possible.

Social impact events that are judged by the designee to be serious, unexpected, or more severe or frequent than anticipated, will be reported to the responsible site's EC/IRB promptly, or otherwise in accordance with the EC/IRB's requirements.

18.6 Benefits

Aside from compensation, the direct benefit to the participant for taking part in this study is provision of free Fluarix (or Fluzone) and Pneumovax vaccinations. Also, the information the investigators obtain from this study may help researchers design studies that could improve treatment for HIV in the future.

18.7 Compensation

Participants will be compensated for each study visit completed after their screening visit for their time and travel. Participants will not be compensated for their screening visit. There are a total of 18 visits after the screening visit (6 visits per cycle, 3 cycles total). A breakdown of subject compensation is as follows:

Screening Visit: No Compensation

Baseline Visit (Weeks 0, 12, 24): $50 for each visit

Cycles 1, 2 & 3 Post-vaccination days 2, 4, 7: $20 for each visit

Cycles 1, 2 & 3 Post-vaccination days 14, 30: $30 and $50 for each visit, respectively.

Total: $570

See **Table 2** for summary of Participant compensation above.

If the participant ends their participation early, he/she will be compensated for every visit they complete as described above. Subjects may receive up to $570 over the course of their approximate 28 weeks participation.

Participants will be able to park free while visiting our clinic in the parking slots allocated for our patients. Should a participant express a need for taxi or bus fare, we will provide transportation to the subject by way of our participant discretionary funds.

18.8 Participant Privacy and Confidentiality

All participant-related information including case report forms, laboratory specimens, evaluation forms, reports, etc., will be kept strictly confidential. All records will be kept in a secure, double locked location and only research staff will have access to the records. Participants will be identified only by means of a coded number specific to each participant. All computerized databases will identify participants by numeric codes only, and will be password-protected. Upon request, participant records will be made available to the study sponsor, the sponsor's monitoring representative, and applicable regulatory entities.

18.9 Critical Event Reporting

All critical events occurring on study will be reported to the UCSD HRPP if they are at least possibly associated or of unknown association to study treatment or procedures. The team will use standard reporting time frames and HRPP requested formats. Critical events will be monitored by the study investigators and reported to the HRPP during annual reviews.

18.10 Communicable Disease Reporting

All cases of newly diagnosed HIV, syphilis, hepatitis B and hepatitis C must be reported to the county public health department. According to California state law, study staff is required to give public health department staff the participant's name, contact information and treatment records for the hepatitis if requested.

18.11 Incidental Findings

Incidental findings that occur during the course of the study will be assessed among the study team, including investigators and NIH staff. Such findings will be reported in the literature and to the research community as appropriate.

18.12 New Findings

Publication of the results of this trial will be governed by NIH policies. Intellectual property and data generated under this project will be administered in accordance with both University and NIH policies, including the NIH Data Sharing Policy and Implementation Guidance of March 5, 2003. Ownership of sole or joint inventions developed under the project will be owned by the institution(s) employing the inventor(s). Inventors shall be determined by U.S. Patent law, Title 35 SC. University and Participating investigators/institutions will disclose any inventions developed under the project and such inventions will be reported and managed as provided by NIH policies. Sole inventions will be administered by the institution employing the inventor. Joint

inventions shall be administered based on mutual consultation between the parties. Similar procedures will be followed for copyrights. Materials generated under the project will be disseminated in accordance with University/Participating institutional and NIH policies. Depending on such policies, materials may be transferred to others under the terms of a material transfer agreement. Access to databases and associated software tools generated under the project will be available for educational, research and non-profit purposes. Such access will be provided using web-based applications, as appropriate. Publication of data shall occur during the project, if appropriate, or at the end of the project, consistent with normal scientific practices. Research data which documents, supports and validates research findings will be made available after the main findings from the final research data set have been accepted for publication. Such research data will be redacted to prevent the disclosure of personal identifiers.

18.13 Study Discontinuation

The study may be discontinued at any time by the EC/IRB, NIAID, or other government entities as part of their duties to ensure that research participants are protected.

18.14 Post-Trial Access

All de-identified data generated through the conduct of this trial will be made openly available to investigators in the field after the primary manuscript has been published.

18.15 Community Advisory Board and Other Relevant Stakeholders

The UCSD AVRC has a standing CAB, which will review the proposed study before its implementation and will receive regular updates on enrollment and any safety issues, as per standard AVRC protocols.

19. ADMINISTRATIVE PROCEDURES

19.1 Protocol Registration

Prior to implementation of this protocol, and any subsequent full version amendments, the protocol and the protocol informed consent form(s) needs to be approved, as appropriate, by the UCSD institutional review board (IRB)/ethics committee (EC) and any other applicable regulatory entity (RE). Upon receiving final approval, the site will submit all required protocol registration documents to the DAIDS Protocol Registration Office (DAIDS PRO) at the Regulatory Support Center (RSC). The DAIDS PRO will review the submitted protocol registration packet to ensure that all of the required documents have been received.

Site-specific informed consent forms (ICFs) *WILL* be reviewed and approved by the DAIDS PRO and sites will receive an Initial Registration Notification from the DAIDS PRO that indicates successful completion of the protocol registration process. A copy of the Initial Registration Notification should be retained in the site's regulatory files.

Upon receiving final IRB/EC and any other applicable RE approval(s) for an amendment, the site should implement the amendment immediately. The site is required to submit an amendment registration packet to the DAIDS PRO at the RSC. The DAIDS PRO will review the

submitted protocol registration packet to ensure that all the required documents have been received. Site-specific ICF(s) *WILL NOT* be reviewed and approved by the DAIDS PRO and sites will receive an Amendment Registration Notification when the DAIDS PRO receives a complete registration packet. A copy of the Amendment Registration Notification should be retained in the site's regulatory files.

For additional information on the protocol registration process and specific documents required for initial and amendment registrations, refer to the current version of the DAIDS Protocol Registration Manual.

19.2 Regulatory Oversight
(pending)

19.3 Study Implementation
(pending)

19.4 ClinicalTrials.gov
This protocol is subject to the Food and Drug Administration Amendments Act of 2007 (FDAAA) and has been registered on ClinicalTrials.gov with the ClinicalTrials.gov unique protocol ID # NCT02707692.

20. PUBLICATION POLICY
Publication of the results of this trial will be governed by NIH policies. Intellectual property and data generated under this project will be administered in accordance with both University and NIH policies, including the NIH Data Sharing Policy and Implementation Guidance of March 5, 2003. Ownership of sole or joint inventions developed under the project will be owned by the institution(s) employing the inventor(s). Inventors shall be determined by U.S. Patent law, Title 35 SC. University and Participating investigators/institutions will disclose any inventions developed under the project and such inventions will be reported and managed as provided by NIH policies. Sole inventions will be administered by the institution employing the inventor. Joint inventions shall be administered based on mutual consultation between the parties. Similar procedures will be followed for copyrights. Materials generated under the project will be disseminated in accordance with University/Participating institutional and NIH policies. Depending on such policies, materials may be transferred to others under the terms of a material transfer agreement. Access to databases and associated software tools generated under the project will be available for educational, research and non-profit purposes. Such access will be provided using web-based applications, as appropriate. Publication of data shall occur during the project, if appropriate, or at the end of the project, consistent with normal scientific practices. Research data which documents, supports and validates research findings will be made available after the main findings from the final research data set have been accepted for publication. Such research data will be redacted to prevent the disclosure of personal identifiers.

21. REFERENCES

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22. APPENDICES

22.1 Appendix A. Concomitant Medications CRF

Appendix A. Concomitant Medication Reco

22.2 Appendix B. Medical History CRF

Appendix B. Medical History CRF.doc

22.3 Appendix C. Physical Exam CRF

Appendix C. Physical Exam CRF.doc

22.4 Appendix D. Collection of Cervical Vaginal Specimens SOP

Appendix D. Collection of Cervical Vagi

22.5 Appendix E. Laboratory SOP Transporting Specimens

Appendix E. Laboratory SOP Transporting

22.6 Appendix F. Laboratory SOP Quality Management Plan

Appendix F. Laboratory SOP Quality Mana

22.7 Appendix G. Stool Specimen Collection SOP

Appendix G. Stool Specimen Collection S